FACULTAD DE FARMACIA

DEPARTAMENTO DE CIENCIAS FARMACÉUTICAS

Pharmacological evaluation of plant extracts from the Province of Cuanza Norte (Angola)

Evaluación farmacológica de extractos de plantas de la Provincia de Cuanza Norte (Angola)

DOCTORAL THESIS

PROGRAMA DE DOCTORADO EN FARMACIA Y SALUD

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Salamanca, 2023

P.PORTO

ESCOLA SUPERIOR DE SAÚDE

Pharmacological evaluation of plant extracts from the Province of Cuanza Norte (Angola)

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Salamanca, 24 de julho de 2023

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Num. de Identificação: BI11653340
Data: 2023.07.24 16:41:15+01'00'

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List of publications and communications

The work developed in this PhD Thesis led the following articles, awards, communications (poster and oral), and abstracts:

1. Scientific articles published in indexed journals:

Canga, I., Vita, P., Oliveira, A. I., Castro, M. Á., & Pinho, C. (2022). *In Vitro* Cytotoxic Activity of African Plants: A Review. Molecules (Basel, Switzerland), 27(15), 4989. https://doi.org/10.3390/molecules27154989 (Annex I)

The following article has been submitted for publication in the *Journal of Ethnobiology and Ethnomedicine*:

Isabel Canga, Pedro Vita, Ana Isabel Oliveira, João P. Martins, María Ángeles Castro, Cláudia Pinho. (2023). *In vitro* antioxidant, antibacterial and cytotoxicity activities of three medicinal plants from Angola: *Adansonia digitata*, *Garcinia kola*, and *Gardenia ternifolia*.

2. Scientific articles in journals

Canga ILV, Vita P, Oliveira AI, Pinho C, Castro MÁ. Ethnopharmacological study of medicinal plants from the province of Cuanza Norte (Angola). Rev Contexto & Saúde, 2022;22(46): e13336. https://doi.org/10.21527/2176-7114.2022.46.13336 (Annex II)

3. Communications with Scientific Awards

Isabel Canga, Pedro Vita, Ana Isabel Oliveira, Claudia Pinho, Maria Angeles Castro. *In vitro* antioxidant activity of african medicinal plants: A review. International Conference on Medicinal and Food Plant Research & 3rd Sino-CPLP Symposium on Natural Products and Biodiversity Resources. April 9–10 2021, Hanzhong, China. (Excellent Oral and Poster Presentation Award)

Canga, I., Vita, P., Oliveira, A. I., Castro, M. Á., & Pinho, C. (2022). *In Vitro* Cytotoxic Activity of African Plants: A Review. Molecules (Basel, Switzerland), 27(15), 4989. https://doi.org/10.3390/molecules27154989. (Award granted to publications in high quality scientific journals (Programa de Apoio à Publicação em Revistas Científicas de Elevada Qualidade - PAPRE 2023).

4. Oral Communications in scientific events by invitation

Isabel Canga, Pedro Vita, María Ángeles Castro, Cláudia Pinho. Pharmacological evaluation of plant extracts from the Province of Cuanza Norte (Angola). Oral Communication presented in I Jornadas do CISA-Jovens com ciência, 30 June 2023.

5. Poster communications in scientific events

Canga, I., Vita, P., Pinho, C., Castro, M.A. (2019). A review of ethnobotanical studies in Angola (Africa). Comunicação (Poster) apresentada no XV Colóquio de Farmácia da ESS-IPP, 19 de outubro de 2019.

6. Abstracts in scientific journals/Books

Isabel Canga, Pedro Vita, Ana Isabel Oliveira, Claudia Pinho, Maria Angeles Castro. *In vitro* antioxidant activity of african medicinal plants: A review. International Conference on Medicinal and Food Plant Research & 3rd Sino-CPLP Symposium on Natural Products and Biodiversity Resources. April 9–10, 2021 ISBN: 978-2-88966-548-8

Acknowlegments

Agradecer é reconhecer que a concretização de várias etapas da nossa vida é resultado não só do nosso empenho, mas também do apoio, generosidade, disponibilidade e colaboração de pessoas que nos rodeiam no dia-a-dia. Chegar até aqui, foi sem dúvida um desafio difícil de se ultrapassar. Por momentos incalculáveis quis desistir, pois as coisas não corriam bem, com circunstâncias de tristezas, incerteza, alegrias e de muitos bons resultados pelo caminho, mas apesar de tudo posso dizer que consegui alcançar a meta. Este meu percurso não poderia ter sido realizado sem a ajuda e apoio de todos aqueles que participaram direita ou indiretamente para a concretização de mais uma etapa importante da minha vida.

Agradeço a Deus Pai, Todo-Poderoso e meu tudo, louvado seja o seu nome, hoje e para todo sempre, pois sem ele a vida não valeria o sentido; me protegeu, conduziu e iluminou até ao fim. Porque para Deus nada é impossível.

Assim, gostava de exprimir algumas palavras de agradecimento e profundo reconhecimento, em particular:

À Universidade de Salamanca, em particular à Faculdade de Farmácia, pelo esforço, empenho e dedicação para com os seus doutorandos.

À Escola Superior de Saúde do Instituto Politécnico do Porto (ESS-IPP), pelo convénio com a Universidade de Salamanca. Também um especial agradecimento à Área Técnico Científica (ATC) de Farmácia da ESS e ao Centro de Investigação em Saúde e Ambiente (CISA), pela autorização, material e instalações necessárias à realização deste trabalho.

Um grande agradecimento à minha orientadora de tese, a Prof. Doutora Maria Ángeles Castro González, pelo apoio, compreensão, disponibilidade, paciência e confiança depositada; e acima de tudo, por proporcionar o saber científico partilhado ao longo da realização deste trabalho.

À minha querida professora e orientadora do coração a Prof. Doutora Claúdia Pinho, a quem tenho muita estima. A palavra obrigada não será suficiente para agradecer tudo quanto a professora fez por mim. Foi uma revelação para mim, por impulsionar o meu sucesso, orientando de forma eficiente, e partilhando os seus conhecimentos de forma paciente e confiante. Reforçar que sem o seu incentivo, ensinamento e apoio não manteria a crença quando já eu mesma duvidava, e, por esta razão o meu muito obrigada pela amizade.

Um grande reconhecimento ao meu orientador Prof. Doutor Pedro Vita, que também não fica de fora, e que mesmo distante tem sido o grande catalisador do meu sucesso académico. Como tal, o meu muito obrigado.

Um especial agradecimento ao Prof. Doutor Agostinho Cruz, pelo incentivo e recomendação ao doutoramento. Muito obrigada por acreditar em mim e nas minhas capacidades.

A todos os professores da ESS, em particular à Prof. Doutora Ana Isabel Oliveira, da ATC de Farmácia, pelo suporte e estrutura para a realização das várias fases no laboratório, pela simpatia e ensinamento.

Aos funcionários da ESS, em especial a dona Elisabete pela amizade, ajuda e palavras de ânimo.

Agradeço aos meus pais, Kissungo Kanga e Isabel Samba Manuel, amores da minha vida, pois sem eles não seria possível chegar a esta etapa. Obrigada pelo apoio moral, material, carinho, encorajamento e que dignamente mostram-me o caminho da honestidade, persistência e o amor transmitido durante toda a minha vida. Com a palavra de honra os 3 F de Força, Fé e Foco. Desta forma, a vós devo todo o meu percurso académico.

Aos meus queridos irmãos, Isabel Mampova Manuel Kanga e Feliz Manuel Kanga, que têm sido meus pais, espero que tenham muito orgulho nos meus passos. Vocês são minha referência, e por isso, obrigada por terem acreditado dando-me mais uma oportunidade. Jamais conseguiria chegar até aqui sem o vosso apoio, conselho, força, incentivo, amor, amizade, ânimo, pelos gastos e carinho que me passaram ao longo destes anos. Mesmo sendo a guerreira que sou, e de andar com meus próprios pés, a verdade é que são vocês que fazem acontecer com o lema “nada conseguimos sem que nos esforcemos, pois nada cai dos céus”. O caminho foi longo e difícil, mas sem dúvida que, quando sentimos o apoio incondicional as coisas tornam-se mais simples, e por isso, serei eternamente grata por tê-los na minha vida.

À querida Inês Luzolo, pela companhia. Sem dúvida sou imensamente grata pelo incentivo, força e apoio; ultrapassamos juntas as dificuldades e por isso tenho o orgulho enorme pelas tuas conquistas. Obrigada pela amizade e pelas horas de ideias partilhadas, grata só por te ter na minha vida.

Ao meu noivo Ângelo Luyindula por ter chegado na minha vida, e ter me feito sentir mulher especial, despertando o melhor que há em mim. Sou grata pela companhia, paciência, carinho, respeito, compreensão, força, cumplicidade e pelas nossas infinitas conversas. Na verdade, gostava que tivesses chegado mais cedo, mais assim quis Deus e a ele agradeço a tua existência.

Um particular reconhecimento aos meus familiares, em especial Elsa Manuel Kanga, Ferraz Manuel Kanga, Suzana Kanga, Madalena Kanga, meus sobrinhos (em representação Daniel Kanga) e meus tios Manuel Vemba e João Santos, Daniel Ernesto, António Barbosa, António Manecas, Vawenda Francisco, Moniz Sebastião, Manuel Viegas, Eng. Delminda Lopes, Maria, Ana Conceição, João Lourenço, Lohela, Nsiku Lutete, Ntumba Luyindula, Yann Tamisier e Victor Luvuezo pelo apoio incondicional.

Ao meu querido tio Miguel Bungo "Semplis", pela disposição e o auxílio na aquisição da informação de plantas medicinais, és um exemplo de dedicação e meiguice.

Aos meus colegas de curso, Professora Helena Moreira, Professor Simão Fonseca, assim como aos colegas de laboratório, nomeadamente, a Mariana Vieira, o Alexandre Ribeiro, a Sara Silva, a Inês Viegas, a Daniela Rocha, e o Daniel Carvalho, com quem gostei de trabalhar e a quem direciono os agradecimentos sinceros por tudo aquilo que fizeram, pela disponibilidade e ajuda prestada, pelo cruzar neste caminho de uma jornada bem-sucedida.

Aos meus amigos, irmãos na fé e pessoas preciosas que vou cruzando ao longo da minha vida, vocês preenchem-na de modo particular. Estendo esses agradecimentos para pessoas que sempre estiveram nos momentos bons e menos bons em que pensei desistir e ainda assim, por meio da palavra, abraço e oração, incentivaram a seguir em frente.

À cidade do Porto (Portugal) e à de Salamanca (Espanha), Metz (França), pelo encanto e por todas as coisas boas que me proporcionaram.

A todos, o meu muito obrigada. Deus abençoe!

Bem-haja a todos!

INDEX

Abbreviations, acronyms, and symbols

ABTS	2,2´-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
AMR	Antimicrobial resistance
ASFV	African Swine Fever Virus
ATP	Adenosine Tri-Phosphate
BHT	Butylated hydroxytoluene
CAT	Catalase
CC_{50}	50% cytotoxic concentration
COX	Cyclooxygenase
CUPRAC	Cupric Reducing Antioxidant Capacity
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EC_{50}	50% Effective concentration
EDTAE	EDTA equivalent
EU	European Union
FRAP	Ferric Reducing Antioxidant Power
GA	Garcinoic acid
GAE	Gallic acid equivalent
GB	*Garcinia* biflavanone
GSHpx	Glutathione peroxidase
HAT	Hydrogen Atom Transfer
$HO^{\cdot}$	Hydroxyl radical
H_2O_2	Hydrogen peroxide
HSV-1	Herpes simplex virus type 1
IC_{50}	50% Inhibitory concentration
KV	Kolaviron
LC_{50}	50% Lethal concentration
LPO	Lipid peroxidation
MBC	Minimum Bactericidal Concentration
MDR	Multidrug resistance
MIC	Minimum Inhibitory Concentration
MMP	Matrix metalloproteinases

MRSA	Methicillin-resistant *S. aureus*
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NCI	National Cancer Institute
NO	Nitric oxide
$^1O_2$	Singlet oxygen
$O_2^{\cdot -}$	Superoxide radical
O_2	Molecular oxygen
$ONOO^-$	Peroxynitrite
ORAC	Oxygen radical absorbance capacity
PBMC	Peripheral Blood Mononuclear Cell
RNS	Reactive Nitrogen Species
$ROO^{\cdot}$	Peroxyl radical
ROS	Reactive Oxygen Species
SET	Single Electron Transfer
SOD	Superoxide dismutase
TAA	Total Antibacterial Activity
TAC	Total Antioxidant Capacity
TE	Trolox Equivalent
TEAC	Trolox Equivalent Antioxidant Capacity
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
USA	United States of America
WHO	World Health Organization

List of cancer cell lines

A2780	Ovarian carcinoma
A427	Lung carcinoma
A431	Epidermoid carcinoma
A549	Lung adenocarcinoma
AGS	Gastric adenocarcinoma
Caco-2	Colorectal adenocarcinoma
CADO-ES1	Ewing’s sarcoma
DAN-G	Pancreatic carcinoma
DLD1	Colon adenocarcinoma
H1299	Human non-small cell lung carcinoma
HCT116	Colorectal carcinoma
HeLa	Cervical adenocarcinoma
HepG2	Hepatic carcinoma
HL-60	Acute myeloid leukemia
HT-29	Colorectal carcinoma
Jeko-1	Mantle cell lymphoma
Jurkat E6-1	Human lymphoid
KYSE-70	Esophageal squamous carcinoma
LCLC-103H	Large cell lung carcinoma
LN229	Glioblastoma
LNCaP	Prostate adenocarcinoma
MCF-7	Breast adenocarcinoma
MDA-MBA-231	Breast adenocarcinoma
ME-180	Cervical adenocarcinoma
OE33	Esophageal adenocarcinoma
PC3	Prostate adenocarcinoma
RDES	Ewing’s sarcoma
REH	Leukemia
RT-4	Urinary bladder carcinoma
SiSo	Cervical adenocarcinoma
THP1	Human leukemia monocytic
U87MG	Glioblastoma
U937	Human myeloid leukemia

Abstract

Introduction: Studies on African medicinal plants have been limited to some geographically areas, and even though many native plants are recognized and documented, other valuable medicinal plant species have not been studied. Angola has an important socio-cultural diversity and is one of the richest floristic regions of the world. **Objectives:** This study aims to perform an ethnopharmacological study in the Province of Cuanza Norte (Angola); also, a review of ethnobotanical studies from Angola Provinces; and finally, to perform a pharmacological evaluation (antioxidant, antibacterial and cytotoxic) of plant extracts selected from the Province of Cuanza Norte (Angola). **Methods:** At first it was performed an ethnopharmacological study in order to document the use of medicinal plants from the Province of Cuanza Norte (Angola). The field work was conducted from December 2018 to January 2019 and informants were selected in accordance with community recognition as traditional healers. Medicinal plants were listed along with their popular name, traditional use, part used, and method of preparation. The second part of the work aimed to perform a review of ethnobotanical studies from Angola. To achieve this objective a literature review search was carried out in PubMed, ScienceDirect, and Google Scholar databases, with no data restriction, in Portuguese and English languages. Ethnobotanical studies which collected information based on field work and on different traditional medicinal uses of plants from municipalities and provinces of Angola, were included. Therefore, from the plants listed in the field work performed by us and from the plants listed in the review of ethnobotanical studies, three medicinal plants were selected for further analysis, based on their importance in the country, published studies, and their traditional uses. Finally, the last part of the work aims to evaluate *in vitro* antioxidant, antibacterial and cytotoxicity activities of the selected plants: *Adansonia digitata*, *Garcinia kola*, and *Gardenia ternifolia*. In the experimental study it was used aqueous and methanolic extracts of the three African medicinal plants selected. Total phenolic content (TPC), total flavonoid content (TFC), and *in vitro* antioxidant activity were investigated. Also, their cytotoxic activity against HepG2 cell line, and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, were assessed. **Results:** For the ethnopharmacological study, a total of 131 species of medicinal plants were cited by the informants of Cuanza Norte. Mukumbi (*Lannea welwitschii*), Santa Maria (*Chenopodium ambrosioides*) and Ditumbata (*Boerhavia difusa*) were the most cited species, following by Embondeiro (*Adansonia digitata*), Papaia (*Carica papaya*), and Mbrututu (*Cochlospermum angolense*). Informants were contacted through "snowball" method and through semi-structured interviews. Leaves

were the most frequently material used, and maceration the major form of plants preparation. The main categories of use were infectious and parasitic diseases (e.g., Malaria); undefined pains and illness; diseases of the digestive system; and endocrine, nutritional, and metabolic diseases (e.g., Diabetes). It is also important to inform that some of the traditional indications are supported by data from scientific literature. Regarding the review of ethnobotanical studies performed in Angola, 7 ethnobotanical studies were found from 6 Provinces (Bié, Bengo, Huíla, Cuanza Norte, Namibe, Uíge). In some cases, informants were contacted through "snowball" method and through semi-structured interviews. In general, informants were persons who were known within the community for having high knowledge and experience about the use of plants. In the studies analyzed, the majority of the plants cited by the informants were not endemic to Angola, which may be related to the high deforestation rate in Africa. Some of the predominant families in the studies analyzed were Fabaceae, Asteraceae and Euphorbiaceae. Leaves were the predominant used plant part in the studies and decoction was the most common found method of plants preparation. The majority of the plants are used traditionally for digestive disorders. Some authors showed that few studies have been conducted for some plant species, including for *G. ternifolia*. Some authors also revealed that *A. digitata* can be used for different purposes, showing the high importance for the Angolan population. Therefore, because of the high importance of *A. digitata* in Angola, and the small numbers of studies regarding *G. kola* and *G. ternifolia*, those were the plants selected for further *in vitro* pharmacological studies. Regarding the *in vitro* antioxidant activity of *A. digitata*, *G. kola*, and *G. ternifolia*, IC_{50} values ranged from 6.1 ± 0.1 to 846.0 ± 20.9 µg/mL. *G. kola* methanolic extract showed the best TPC (131.4 ± 5.7 mg GAE/g) and the one of *A. digitata* showed the best TFC (46.0 ± 5.0 mg QE/g). Methanolic extracts showed higher TPC, TFC and antioxidant activity in general, compared to aqueous extracts. Considering the antibacterial activity, methanolic extracts of *G. kola* and *G. ternifolia* had the lowest MIC value of 0.625 mg/mL against Gram-positive *S. aureus*. Regarding cytotoxic activity for HepG2, *A. digitata* and *G. kola* extracts presented no cytotoxicity at any of the tested concentrations (5-500 µg/mL). **Conclusions:** Preserving the ethnobotanical knowledge in order to protect the biodiversity and to discover new therapeutic molecules is crucial in countries like Angola, where the traditional medicine plays an important role in their health care system. The results found in this work highlight the therapeutic potentiality of African flora and provide preliminary knowledge about the selected medicinal plants, partly explaining their use in the traditional medicine practice of Angola and other African countries.

Keywords: Ethnopharmacological studies, African plants, *Adansonia digitata*, *Garcinia kola*, *Gardenia ternifolia*, antioxidant activity, antibacterial activity, cytotoxic activity

Resumen

Introducción: Los estudios sobre plantas medicinales africanas se han limitado a algunas áreas geográficas, y aunque se reconocen y documentan muchas plantas nativas, no se han estudiado otras especies de plantas medicinales valiosas. Angola tiene una importante diversidad sociocultural y es una de las regiones de mayor riqueza florística del mundo. **Objetivos**: Este trabajo tiene como objetivo realizar un estudio etnofarmacológico en la Provincia de Cuanza Norte (Angola); realizar una revisión de los estudios etnobotánicos de las provincias de Angola; y finalmente llevar a cabo una evaluación farmacológica (antioxidante, antibacteriana y citotóxica) de extractos de plantas selecionadas de la Provincia de Cuanza Norte (Angola). **Métodos:** En un primer momento se realizó un estudio etnofarmacológico con el fin de documentar el uso de plantas medicinales de la Provincia de Cuanza Norte (Angola). El trabajo de campo se realizó de diciembre de 2018 a enero de 2019 y los informantes fueron seleccionados de acuerdo con el reconocimiento de la comunidad como curanderos. Las plantas medicinales se enumeraron junto con su nombre popular, uso tradicional, parte utilizada y método de preparación. La segunda parte del trabajo tenía como objetivo realizar una revisión de los estudios etnobotánicos de Angola. Para lograr este objetivo se llevó a cabo una revisión bibliográfica en las bases de datos PubMed, ScienceDirect y Google Scholar, sin restricción de datos, en los idiomas portugués e inglés. Se incluyeron aquellos estudios etnobotánicos que recopilaban información, a partir de trabajo de campo, sobre diferentes usos medicinales tradicionales de las plantas de municipios y provincias de Angola. De las plantas listadas en el trabajo de campo realizado por nosotros y de las plantas enumeradas en la revisión de estudios etnobotánicos, se seleccionaron tres plantas medicinales para su posterior análisis, en función de su importancia en el país, los estudios publicados y sus usos tradicionales. Finalmente, la última parte del trabajo consistió en evaluar *in vitro* las actividades antioxidantes, antibacterianas y de citotoxicidad de las plantas seleccionadas: *Adansonia digitata*, *Garcinia kola* y *Gardenia ternifolia*. En el estudio experimental se utilizaron extractos acuosos y metanólicos de las tres plantas medicinales africanas seleccionadas. Se estudió el contenido fenólico total (TPC), el contenido total de flavonoides (TFC) y la actividad antioxidante *in vitro*. Además, se evaluó su actividad citotóxica frente a la línea celular HepG2 y su actividad antibacteriana contra *Escherichia coli* y *Staphylococcus aureus*. **Resultados**: En nuestro estudio etnofarmacológico se citaron un total de 131 especies de plantas medicinales por parte de los informantes de Cuanza Norte. Mukumbi (*Lannea welwitschii*), Santa María (*Chenopodium ambrosioides*) y Ditumbata (*Boerhavia difusa*) fueron las especies más citadas,

seguido de Embondeiro (*Adansonia digitata*), Papaia (*Carica papaya*), y Mbrututu (*Cochlospermum angolense*). Los informantes fueron contactados a través del método de "bola de nieve" y a través de entrevistas semiestructuradas. Las hojas fueron el material más utilizado y la maceración la principal forma de preparación de la planta. Las principales categorías de uso fueron enfermedades infecciosas y parasitarias (p. ej., paludismo); dolor y enfermedades indefinidos; enfermedades del sistema digestivo; y enfermedades endocrinas, nutricionales y metabólicas (p. ej., diabetes). Es importante mencionar que algunas de las indicaciones tradicionales están respaldadas por datos de la literatura científica. En cuanto a la revisión de los estudios etnobotánicos realizados en Angola, se encontraron 7 estudios etnobotánicos de 6 Provincias (Bié, Bengo, Huila, Cuanza Norte, Namibe, Uíge). En algunos casos, los informantes fueron contactados a través del método de "bola de nieve" y también a través de entrevistas semiestructuradas. Por lo general, los informantes eran personas que se conocían dentro de la comunidad por tener un alto conocimiento y experiencia en el uso de las plantas. En los estudios analizados, la mayoría de las plantas citadas por los informantes no eran endémicas de Angola, lo que puede estar relacionado con la alta tasa de deforestación en África. Algunas de las familias predominantes en los estudios analizados fueron Fabaceae, Asteraceae y Euphorbiaceae. Las hojas fueron la parte de la planta más usada en los estudios y la decocción el método de preparación de plantas más común. La mayoría de las plantas se utilizan tradicionalmente para trastornos digestivos. Algunos autores mostraron que hay especies de plantas para las que se han realizado pocos estudios, entre ellas *G. ternifolia*. También se encontraron estudios que indicaban que *A. digitata* puede ser utilizada con diferentes propósitos, siendo de gran importancia para la población angoleña. Por lo tanto, debido a la gran importancia de *A. digitata* en Angola y a los pocos estudios encontrados sobre *G. kola* y *G. ternifolia*, esas fueron las plantas seleccionadas para posteriores estudios farmacológicos *in vitro*. En cuanto a la actividad antioxidante *in vitro* de *A. digitata*, *G. kola* y *G. ternifolia*, los valores de IC_{50} oscilaron entre 6,1 ± 0,1 y 846,0 ± 20,9 µg/mL. El extracto metanólico de *G. kola* presentó el mejor TPC (131,4 ± 5,7 mg GAE/g) y el de *A. digitata* presentó el mejor TFC (46,0 ± 5,0 mg QE/g). Los extractos metanólicos mostraron mejores valores de TPC, TFC y actividad antioxidante en general, en comparación con los extractos acuosos. En lo quese refiere a la actividad antibacteriana, los extractos metanólicos de *G. kola* y *G. ternifolia* presentaron el valor MIC más bajo de 0,625 mg/mL frente a *S. aureus* grampositivo. En cuanto a la actividad citotóxica frente a HepG2, los extractos de *A. digitata* y *G. kola* no presentaron citotoxicidad en ninguna de las concentraciones ensayadas (5-500 µg/mL). **Conclusiones**: Preservar el conocimiento etnobotánico para proteger la biodiversidad y descubrir nuevas

moléculas terapéuticas es crucial en países como Angola, donde la medicina tradicional juega un papel importante en su sistema de salud. Los resultados de este trabajo destacan la potencialidad terapéutica de la flora africana y proporcionan un conocimiento preliminar sobre las tres plantas medicinales seleccionadas, que justifica en parte su uso en la medicina tradicional de Angola y de otros países africanos.

Palabras clave: Estudios etnofarmacológicos, plantas africanas, *Adansonia digitata*, *Garcinia kola*, *Gardenia ternifolia*, actividad antioxidante, actividad antibacteriana, actividad citotóxica

CHAPTER 1

GENERAL INTRODUCTION

1.1. Relevance and Motivation

Biodiversity contributes significantly towards human livelihood and plays an important role in the well being of the population. Over the years, people have been living in close association with the environment, relying on its flora as a source of food and medicine.

Natural compounds, particularly those of plant origin have been and still are an importantt source of new lead compounds in drug discovery research. It is also known that most pharmaceutical drugs are developed from medicinal plants based on local communities' knowledge and the subsequent extraction and isolation of the main active compounds. Drug discovery from natural sources involves a multidisciplinary approach which combines botanical, phytochemical, biological, and molecular techniques.

Rapid demographic, sociocultural, nutrition and economic transitions are driving an increase in the risk and prevalence of non-communicable diseases (e.g., cardiovascular diseases, diabetes, and cancer), especially in sub-Saharan Africa. Also, communicable diseases in Angola account for more than 50 % of deaths within the population. In the country, half of the population lives in non-urban areas, over one third is below the poverty threshold, and only one half has access to drinking water sources and waste treatment systems. Some of the main problems related to the healthcare system in Angola include the insufficient coverage and poor maintenance of health centers; limited human resources and health technicians, particularly in rural areas; and limited access to safe drinking water, sanitation and energy. Therefore, over 80% of the African population uses traditional medicine as a primary source of health. There are thousands of African medicinal plants that are recognized and used by the population or traditional healers, but the studies documenting this data have been limited to some geographical areas and thus, many valuable medicinal species remain unstudied.

Traditional knowledge related to the health of humans and animals exists in all African countries. This is happening in African countries like Angola, which is one of the richest floristic regions of the world, and where some ethnobotanical studies have been carried out in the south of the country. The studies documenting plant uses in Angola, were conducted mostly in the south of the country, showing completely different vegetation units, or highlighting the savannah plants, or other non-medicinal uses.

Angola had approximately 1,246,700 km^2 and occupies only 4 % of the terrestrial area of Africa but is one of the most diverse countries in terms of ecoregions, revealing the diversity of geographic, climatic, edaphic, and biotic conditions. Therefore, this work intended to document the traditional use of Angolan plants, particularly at the province of Cuanza Norte, allowing us to evaluate the diversity of medicinal species in that province, and also compare

with other regions and with the overall African continent, where about 10 % of the flora is estimated to be used in traditional medicine.

Considering the lack of access to public health care, the preservation of the traditional knowledge and the updated lists of the species occurring in those regions, are an essential tool for managing biological resources, especially given the risk that this knowledge could be lost in future generations. Also, a well-documented knowledge of herbal medicines, may be helpful to decrease the cost and time of natural product development, improving the use of a well-designed strategy for the selection of the plant species for research. Ethnobotanical and ethnopharmacological studies contribute to the discovery of sources of lead compounds for the early stages of drug discovery and development, further highlighting the importance of our study.

The medicinal plants exhibit a broad spectrum of pharmacological activities, and they are used for the treatment and/or prevention of several important diseases, including infectious diseases, cardiovascular and neurodegenerative diseases, diabetes, and cancers. However, many of the plants included in the African traditional medicine are still not fully understood, for example, in terms of chemical composition, pharmacological effect and potential clinical application. Therefore, in our study some potential African medicinal plants were screened for their biological activities based on their reported ethnopharmacological and traditional uses, and also to find unknown applications for the treatment of several disorders.

Based on personal experience, people know the therapeutic potential of medicinal plants without substantiating their effectiveness. Many medicinal plants are also used as food or to feed the animals. Despite this perceived safety by those who prefer to use medicinal plants, there is a need for scientific validation to ensure safety, efficacy and consistent medicinal preparations. Therefore, our team tried to carry out this work also to scientifically confirm some of its traditional uses and study others with relevance in the country. The search for new antioxidant, antimicrobial and anticancer agents are becoming an important objective for scientists. A combination of ethnobotanical, ethnopharmacological, and pharmacological research will provide useful data to encourage the traditional practices, to confirm some indigenous uses, and to find new biological activities related to medicinal plants.

1.2. Outline of the Thesis

This PhD thesis was developed at the Research Centre on Health and Environment hosted at the Escola Superior de Saúde do Instituto Politécnico do Porto (ESS-P.Porto), throughout

the period between April 2019 and May 2023 (experimental work). The field work (ethnopharmacological research) was performed in Angola (in the province of Cuanza Norte) during a period of two months (from December 2018 to January 2019). The doctoral program was conducted through an agreement between two educational institutions: the University of Salamanca and ESS-P.Porto.

The doctoral thesis is organized into five chapters, namely:

- **Chapter 1** (General Introduction) were the relevance, motivation, and outline of the thesis is explained.
- **Chapter 2** (State of the art) where we presented the importance and biodiversity of African medicinal plants; and some reviews related to medicinal plants from Africa and its biological activities, namely, the antioxidant, antibacterial and cytotoxic effects (which are the biological activities explored in the selected medicinal plants).
- **Chapter 3** (Material and Methods) where we described the material and methods used for the two original studies of this thesis and one review, namely (i) to perform an ethnopharmacological study of medicinal plants from the Province of Cuanza Norte (Angola), ir order to identify and document medicinal plants and detailed traditional knowledge on herbal preparations; (ii) to perform a literature review based on ethnobotanical studies described for the Provinces of Angola, in order to document this data, and compare our results with similar studies; and (iii) to evaluate *in vitro* antioxidant, antibacterial and cytotoxicity activities of three medicinal plants from Angola: *Adansonia digitata*, *Garcinia kola*, and *Gardenia ternifolia*.
- **Chapter 4** (Results and Discussion) where the results of the three studies previous reported are presented.
- **Chapter 5 (Conclusion)** where the overall conclusions of the results obtained and the main achievements of this study are revealed, including potential future work.

1.1. Relevancia y Motivación

La biodiversidad contribuye significativamente a la subsistencia humana y desempeña un papel importante en el bienestar de la población. A lo largo de los años, las personas han vivido en estrecha relación con el medio ambiente, dependiendo de su flora como fuente de alimentos y medicinas.

Los compuestos naturales, en particular los de origen vegetal, han sido y siguen siendo una importante fuente de nuevos compuestos en la investigación para el descubrimiento de fármacos. También se sabe que la mayoría de los fármacos se desarrollan a partir de plantas medicinales, basándose en los conocimientos de las comunidades locales y en la posterior extracción y aislamiento de los principales compuestos activos. El descubrimiento de fármacos a partir de fuentes naturales implica un enfoque multidisciplinar que combina técnicas botánicas, fitoquímicas, biológicas y moleculares.

Las rápidas transiciones demográficas, socioculturales, nutricionales y económicas están provocando un aumento del riesgo y la prevalencia de enfermedades no transmisibles (por ejemplo, enfermedades cardiovasculares, diabetes y cáncer), especialmente en el África subsahariana. Además, en Angola las enfermedades transmisibles son responsables de más del 50 % de las muertes de la población. En el país, la mitad de la población vive en zonas no urbanas, más de un tercio está por debajo del umbral de pobreza y sólo la mitad tiene acceso a fuentes de agua potable y sistemas de tratamiento de residuos. Algunos de los principales problemas relacionados con el sistema sanitario de Angola son la cobertura insuficiente y el mal mantenimiento de los centros de salud; la escasez de recursos humanos y técnicos sanitarios, sobre todo en las zonas rurales; y el acceso limitado al agua potable, el saneamiento y la energía.

Por lo tanto, más del 80 % de la población africana utiliza la medicina tradicional como fuente primaria de salud. Existen miles de plantas medicinales africanas reconocidas y utilizadas por la población o los curanderos tradicionales, pero los estudios que documentan estos datos se han limitado a algunas zonas geográficas y, por lo tanto, muchas especies medicinales valiosas permanecen sin estudiar.

En todos los países africanos existen conocimientos tradicionales relacionados con la salud de las personas y los animales. En países africanos como Angola, que es una de las regiones florísticas más ricas del mundo, se han realizado algunos estudios etnobotánicos que documentan el uso de las plantas en Angola, pero esos estudios se realizaron principalmente

en el sur del país, mostrando unidades de vegetación completamente diferentes, o destacando las plantas de la sabana, u otros usos no medicinales.

Angola tiene aproximadamente 1.246.700 km^2 y ocupa sólo el 4 % de la superficie terrestre de África, pero es uno de los países más diversos en términos de ecorregiones, lo que revela la gran diversidad de condiciones geográficas, climáticas, edadológicas y bióticas. Por lo tanto, este trabajo pretende documentar el uso tradicional de las plantas angoleñas, en particular en la provincia de Cuanza Norte, lo que nos permitirá evaluar la diversidad de especies medicinales en esa provincia, y también comparar con otras regiones y con el continente africano en general, donde se estima que alrededor del 10 % de la flora se utiliza en la medicina tradicional.

Teniendo en cuenta la falta de acceso a la sanidad pública, la preservación de los conocimientos tradicionales y las listas actualizadas de las especies que se dan en esas regiones, constituyen una herramienta esencial para la gestión de los recursos biológicos, sobre todo teniendo en cuenta el riesgo de que estos conocimientos se pierdan en las generaciones futuras. Asimismo, un conocimiento bien documentado de las hierbas medicinales puede ser útil para disminuir el coste y el tiempo de desarrollo de productos naturales, mejorando el uso de una estrategia bien diseñada para la selección de las especies vegetales candidatas para la investigación. Los estudios etnobotánicos y etnofarmacológicos contribuyen al descubrimiento de fuentes de compuestos bioactivos “cabezas de serie” para las primeras fases del descubrimiento y desarrollo de fármacos, lo que subraya aún más la importancia de nuestro estudio.

Las plantas medicinales presentan un amplio espectro de actividades farmacológicas y se utilizan para el tratamiento y/o la prevención de varias enfermedades importantes, como las infecciosas, las cardiovasculares y neurodegenerativas, la diabetes y el cáncer. Sin embargo, muchas de las plantas incluidas en la medicina tradicional africana aún no se conocen del todo, por ejemplo, en términos de composición química, efecto farmacológico y potencial aplicación clínica. Por lo tanto, en nuestro estudio se examinaron algunas plantas medicinales africanas potenciales en busca de sus actividades biológicas sobre la base de sus usos etnofarmacológicos y tradicionales declarados, y también para encontrar aplicaciones desconocidas para el tratamiento de diversos trastornos.

Basándose en la experiencia personal, la gente conoce el potencial terapéutico de las plantas medicinales sin que se haya demostrado su eficacia. Muchas plantas medicinales se utilizan también como alimento o para alimentar a los animales. A pesar de esta seguridad percibida por quienes prefieren utilizar plantas medicinales, es necesaria una validación científica para

garantizar la seguridad, la eficacia y la coherencia de los preparados medicinales. Por ello, este equipo trató de llevar a cabo este trabajo también para confirmar científicamente algunos de sus usos tradicionales y estudiar otros con relevancia en el país.

La búsqueda de nuevos agentes antioxidantes, antimicrobianos y anticancerosos se está convirtiendo en un objetivo importante para los científicos. Una combinación de investigación etnobotánica, etnofarmacológica y farmacológica proporcionará datos útiles para fomentar el uso tradicional, confirmar algunos usos tradicionales y encontrar otros nuevos relacionados con las plantas medicinales.

1.2. Esquema de la Tesis

Esta tesis doctoral se desarrolló en el Centro de Investigación en Salud y Medio Ambiente alojado en la Escola Superior de Saúde do Instituto Politécnico do Porto (ESS-P.Porto), a lo largo del periodo comprendido entre abril de 2019 y mayo de 2023 (trabajo experimental). El trabajo de campo (investigación etnofarmacológica) se realizó en Angola (en la provincia de Cuanza Norte) durante un período de dos meses (de diciembre de 2018 a enero de 2019). El programa de doctorado se realizó a través de un convenio entre dos instituciones educativas: la Universidad de Salamanca y la ESS-P.Porto.

La tesis doctoral se organiza en cinco capítulos, a saber:

- **Capítulo 1** (Introducción general) donde se explican la relevancia, motivación, y el esquema de la tesis.
- **Capítulo 2** (Estado de la cuestión) donde se presenta la importancia y la biodiversidad de las plantas medicinales africanas; y algunas reseñas relacionadas con las plantas medicinales de África y sus actividades biológicas, a saber, los efectos antioxidantes, antibacterianos y citotóxicos (que son las actividades biológicas exploradas en las plantas medicinales seleccionadas).
- **Capítulo 3** (Material y Métodos) donde se describen los materiales y métodos usados para los dos estudios originales de esta tesis y una revisión, a saber (i) realizar un estudio etnofarmacológico de las plantas medicinales de la Provincia de Cuanza Norte (Angola), con el fin de identificar y documentar las plantas medicinales y los conocimientos tradicionales detallados sobre los preparados a base de hierbas; (ii) realizar una revisión bibliográfica basada en los estudios etnobotánicos descritos para las provincias de Angola, con el fin de documentar estos datos, comparar nuestros resultados con otros estudios similares; y (iii) evaluar *in vitro*

las actividades antioxidante, antibacteriana y citotóxica de tres plantas medicinales de Angola: *Adansonia digitata*, *Garcinia kola* y *Gardenia ternifolia*.

- **Capítulo 4** (Resultados y Discusión) donde se presentan los resultados de los tres estudios anteriores.

- **Capítulo 5** (Conclusión) donde se establecen las conclusiones generales de los resultados obtenidos y los principales logros de este estudio, incluidos los posibles trabajos futuros.

CHAPTER 2

STATE OF THE ART AND OBJECTIVES

2.1. Traditional knowledge and importance of plants in Africa

Over the years, following the United Nations Conference on Environment and Development held in Rio de Janeiro in 1992, the preservation of biodiversity has become an important aspect of sustainable development worldwide (Najam & Cleveland, 2005). Plants are the basis of both traditional medicines and globally valuable sources against pharmacological targets which include, for example, cancer, neurodegenerative and cardiovascular diseases, microbial infections, inflammation and pain (Sen & Samanta, 2015). According to World Health Organization (WHO), around 80% of the global population still relies on botanical drugs to meet their primary healthcare needs (Hamilton, 2004).

The WHO Traditional Medicine Strategy 2014–2023 stated that traditional treatments, traditional practitioners/healers, and herbal medicines are the main source of health care, if not the only source, for many millions of people (WHO, 2013). Traditional medicine encompasses the use of substances, dosages and practices based on socio-cultural norms and religious beliefs as well as witnessed experiences of a specific group. Traditional medicine studies include ethnomedicine, which involves the practices most used by people living in rural areas and indigenous communities (Reimers et al., 2019). This knowledge is handed down from generation to generation in order to diagnose, prevent or eliminate a physical, social or spiritual imbalance (Diallo & Paulsen, 2000). Ethnopharmacology combines information acquired from people that use medicinal plants with chemical and pharmacological studies, allowing the formulation of hypothesis about the pharmacological activities and compounds responsible for the reported therapeutic effects (Bruhn & Holmstedt, 1981).

Medicinal plant-based therapy is related to fewer side effects (Gaire, 2018), higher autonomy for individuals in caring for their own health, reduced costs, and easy access for people in poor areas with limited or no access to a healthcare system (Albertasse et al., 2010

Studies within the fields of ethnobotany and ethnopharmacology highlight the importance of medicinal plants for indigenous and non-indigenous people in different parts of the world (Giovannini et al., 2011; Jaradat et al., 2017). Furthermore, documentation of ethnobotanical and ethnopharmacological data is crutial for further research in the area of herbal medicine and its implementation in clinical practice (Popović et al., 2016). Unfortunately, with the fast growth in the technical aspects of the world, some of the information related to customs and ethnic cultures, may disappear (Jaradat et al., 2017; Muthu et al., 2006).

In Africa, modern health care and medicine is often available only to a limited number of people because either facilities are too expensive or too few facilities are available for too many

people (Ahmed et al., 2018; Jacob, 2015). Natural products, including plants, continue to play an important role in drug discovery (Newman & Cragg, 2020). Thus, biodiversity represents an unlimited source for novel chemical entities with potential as drug leads and is required to fulfill daily livelihood needs (Maroyi, 2017). Therefore, there is an increase in current research in the identification of active compounds in medicinal plants, their role in the treatment of diseases, drug development, and herbal formulations (Appiah et al., 2018).

The combination of botanical and cultural diversity in Africa, together with local endemism, results in a complex geographical mosaic of indigenous plant use that remains only partially explored from a scientific and commercial perspective (Govaerts, 2001; Van Wyk, 2015). Besides the gradual loss of ethnobotanical knowledge due to lack of documentation, most authors have highlighted the overharvesting of plants from their natural habitat as one of the major threats to the preservation of traditional medicine (Ahrends et al., 2011). Thus, over decades, ethnobotanical studies were conducted in Africa and several handbooks published giving an overview about herbal medicines (Iwu, 2014; Neuwinger, 2000). However, studies on African medicinal plants have in nearly all cases been limited to geographically limited areas (Malterud, 2017), and it is known that many valuable medicinal plant species have not yet been studied or fully characterized**.**

Located in the Atlantic coast of Africa, Angola has one of the poorest health care systems (Shibre, 2020), compared to other sub-Saharan countries (Kagawa et al., 2012). It is estimated that 68% of Angolans live below the poverty line. In rural areas, 94% of households can be categorized as poor (Jacob, 2015). Although several infrastructure measures were undertaken in Angola, development is still slow, especially regarding the public health sector. However, the lack of health infrastructure, especially in rural areas, is a serious problem resulting in the importance of traditional healers and herbal medicines. It is estimated that 70-80% of patients in Africa, are treated by traditional healers and herbal practitioners (Nyika, 2007).

Diseases like malaria, acute respiratory and diarrhoeal diseases, tetanus and malnutrition, combined with poor access to healthcare, damaged infrastructure and lack of trained health professionals, are the main causes of mortality (WHO, 2018). The ratio of traditional healers to population is 1:500 whereas the ratio of medical doctors to population is 1:40 000 (Abdullahi, 2011). Sousa-Figueiredo et al. (2012) detected malnutrition and anaemia as public health problems, in Angola (Sousa-Figueiredo et al., 2012). Smith et al. (2005) documented that the overall prevalence of malnutrition is higher in rural than then in urban areas (Smith et al., 2005).

Angola is regarded as a country with a rich biodiversity covering a high amount of vegetation zones and habitats (Catarino et al., 2019). Therefore, biological resources, which include medicinal plants, can act as a safety net in poor people livelihoods, providing food, medicine, and other resources (Urso et al., 2016). In Angola, more than seventy percent of the population uses herbal medicine to treat various diseases, including parasitic infections (Vahekeni et al., 2020). Thus, ethnobotanical studies represent an attractive approach for applying indigenous knowledge of plant use to modern societies, with the final aim of developing new remedies.

2.2. Biodiversity of Angola

Angola is a country located in the Atlantic coast of Africa. The total surface area of its territory is 1,246.700 km^2 with an estimated population of over 26 million, according to the 2014 population census (Censo, 2016).

The country is known by its diverse geomorphological, geological, climatic and biotic key features (Huntley & Ferrand, 2019), and is divided into eighteen provinces (Bengo, Benguela, Bié, Cabinda, Cuando Cubango, Cuanza Norte, Cuanza Sul, Cunene, Huambo, Huíla, Luanda, Lunda Norte, Lunda Sul, Malanje, Moxico, Namibe, Uíge, Zaire) (Figure 1) (Catarino et al., 2019).

Figure 1. Map with provinces of Angola (De Barros et al., 2015).

Angola has a very rich biodiversity, which results from the combination of a number of factors such as: the vast country extension; its inter-tropical geographic location; the variation of altitude and the high number of ecoregions (Kuedikuenda & Xavier, 2009). The Phytogeografic Chart of Angola (Barbosa, 1970) describes 32 units of vegetation in the country which can be grouped in eight main types of vegetation, namely, dense undeciduous forest; savanas de capim; xerophite savannahs; intermingles; grasslands; steepes; riverside vegetation; and mangroves (Kuedikuenda & Xavier, 2009).

It is now well-established that plants supports critical ecosystem services, which includes: (i) supporting services (e.g., nutrient cycling, and primary production); (ii) regulating services (e.g., climate regulation, and pollination services); (iii) provisioning services (e.g., fuel wood, edible, medicinal, and aromatic plants); and (iv) cultural services (e.g., education, recreational, tourism, or aesthetic value) (Millennium Ecosystem Assessment, 2005).

The country has the highest diversity of biomes and is notable for having representatives of seven of Africa's nine biomes, which can be defined as "vegetation types with similar characteristics grouped together as habitats" (Olson et al., 2001): (1) tropical and subtropical moist broadleaf forests; (2) tropical and subtropical dry broadleaf forests; (3) tropical and subtropical grasslands, savannas, and shrublands; (4) flooded grasslands and savannas; (5) montane grasslands and shrublands; (6) deserts and xeric shrublands; and (7) mangroves (Catarino et al., 2019).

Despite the high diversity of the vascular flora of Angola, with approximately 6850 species native to Angola and a level of endemism around 14.8% (Figueiredo et al., 2009; Huntley & Ferrand, 2019), and the recognized importance of plants for local populations, threats to this flora and their habitats are growing. Deforestation rates in Angola are among the highest in Sub-Saharan Africa (Hansen et al., 2013), which is likely a consequence of wood extraction for firewood and charcoal, slash-and-burn cultivation, urban expansion, and logging (USAID, 2008). Also, information about Angolan biodiversity is scarce, therefore, it is crucial to conserve, document, and study its biodiversity.

Only a few studies tackle the traditional use of plants in Angola, most of them carried out in the south of the country, with different vegetation units (Urso et al., 2016). Eastern and northern provinces are in most need of collecting programmes and botanical documentation (Huntley & Ferrand, 2019). Since the middle of the 20th century to nowadays some documents were published containing ethnobotanical information in different regions, for example, Cuanza Norte, Uíge (Northern Angola), mopane forests (South-western Angola), Bakongo tribes (Northern Angola), and Bié province (Central Angola) (Bossard, 1996; Bruschi et al.,

2017; Costa & Pedro, 2013; Göhre et al., 2016; Gossweiler, 1953; Heinze et al., 2017; Lautenschläger et al., 2018; Mawunu et al., 2016; Novotna et al., 2020; Santos, 1967; Santos, 1989; Urso et al., 2016).

2.3. Medicinal plants from Africa and its biological activities

Angola is rich in medicinal plants whose potential has yet to be explored. Therefore, the rich flora of sub-Saharan Africa suggests enormous potential for the discovery of new secondary plant metabolites with therapeutic value (Moyo et al., 2015).

In this thesis we decided to study and document antimicrobial (namely, antibacterial), antioxidant, and cytotoxic activities of African medicinal plants, in particular because those are activities related to the traditional use of many plants in this continent. Furthermore, the study of antibacterial activity in the selected plant extracts is important due to the efforts that have been made to counteract antibiotic resistance throughout the world and particularly in Africa; and also, because plants with antibacterial activity can contribute to effective management of wound infections (a problem recurrent in African population) (Owusu et al., 2021). Angola is also characterised by poor sanitary conditions that increase the population's vulnerability, currently reflected by the record numbers of microbial infections (Silva et al., 2016). For example, the nosocomial prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in Angola is over 60% and one of the highest in Africa (Conceição et al., 2016).

Regarding antioxidant activity, antioxidants are compounds that inhibit oxidation process by preventing the formation of free radicals that cause damage to healthy cells, thus managing chronic diseases such as cardiovascular diseases, diabetes, and cancer. These substances are also important in wounds, restoring normalcy to injured skin. Some studies have demonstrated that part of the therapeutic value of plant extracts may be explained by their antioxidant activity (Gulumian, Yahaya & Steenkamp, 2018). It is known that the burden of cardiovascular diseases is increasing in most countries of sub-Saharan Africa. For example, in Angola, strokes were responsible for 5.4% and myocardial infarction for 4.7% of all deaths, being in 2015 the fourth and sixth cause of mortality in the country, respectively (Pedro et al., 2018).

Finally, and regarding cytotoxic activity, this is another biological activity focused on our study because in African countries, cancer not only is a growing problem, but also a challenge because available funding and resources are limited. It is also known that some African medicinal plants are traditionally used for the treatment of cancer (Canga et al., 2022).

2.3.1. Oxidative stress and antioxidant activity of African medicinal plants

Metabolism implies oxidative processes, which are vital in cell survival. Oxidative stress alters the oxidant-antioxidant balance (redox homeostasis) that characterizes normal cell functioning, resulting in the occurrence of cytotoxic compounds (malonyl dialdehyde, 4-hydroxynonenal), and it is thought to be associated with the pathogenesis of many diseases (Dudonné et al., 2009). Oxidative stress-induced pathologies include cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, atherosclerosis, and aging (Pisoschi et al., 2016).

2.3.1.1. Mechanisms of Reactive Oxygen Species (ROS) generation

Reactive Oxygen Species (ROS) are byproducts of normal cellular metabolism. Low and moderate amounts of ROS have beneficial effects on several physiological processes including killing of invading pathogens, wound healing, and tissue repair processes. ROS are produced in response to many exogenous agents like ultraviolet radiation, cigarette smoking, alcohol consumption, or ingestion of nonsteroidal anti-inflammatory drugs (Bhattacharyya et al., 2014).

During molecular oxygen stepwise reduction, a series of reactive oxygenated species occur. ROS include both oxygen radicals and certain radicals that are oxidizing agents or can easily converted into radicals, namely, compounds such as superoxide ($O_2^{\cdot -}$), hydroxyl radicals ($HO^{\cdot}$), lipid hydroperoxides, and reactive nonradical compounds including singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hypochlorous acid, chloramines, and ozone (Bedard & Krausem 2007). Reactive radical compounds such as nitric oxide ($^{\cdot}NO$), nitrogen dioxide, and nonradical compounds, e.g., peroxynitrite ($ONOO^-$) and dinitrogen trioxide, are collectively called reactive nitrogen species (RNS). RNS is often linked to ROS, e.g., in the formation of $ONOO^-$ causing nitrosative stress (Bhattacharyya et al., 2014).

2.3.1.2. Antioxidant defense systems

Antioxidants are molecules that can act as chain breakers, scavenging chain initiating radicals like $HO\cdot$, alkoxyl, or peroxyl ($ROO\cdot$), quenching $^1O_2$, decomposing hydroperoxides, and chelating prooxidative metal ions. Natural antioxidants constitute the essential part in the cell's defense mechanisms, and they can be endogenous or exogenous (Pisoschi et al., 2016).

Biological systems have antioxidant mechanisms to control damage of enzymatic and nonenzymatic natures that allow ROS to be inactivated. The endogenous antioxidants are enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHpx), thioredoxins, and peroxiredoxins; or non-enzymatic compounds, such as bilirubin, ferritin, and albumin (Santos Sánchez et al., 2019).

Enzymatic antioxidants are grouped within the primary and secondary defence systems. The primary defence is formed by three enzymes capable of preventing the occurrence or neutralizing free radicals: GSHpx, which donates two electrons that reduce peroxides, CAT that decomposes H_2O_2 into water and molecular oxygen, and SOD that turns $O_2^{\cdot -}$ into H_2O_2. The secondary enzymatic defence includes glutathione reductase and glucose-6-phosphate dehydrogenase. Although the two enzymes do not directly neutralize free radicals, they promote the endogenous antioxidants' activity (Carocho & Ferreira, 2013).

When an organism is exposed to a high concentration of ROS, the endogenous antioxidant system is compromised and may be necessary the use of exogenous antioxidants supplied through food, dietary supplements, or pharmaceuticals. Medicinal plants are rich sources of secondary metabolites that act as natural antioxidant such as phenolic compounds (cinnamic acids, benzoic acids, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans, and lignins), ascorbic acid and carotenoids (Manessis et al., 2020).

2.3.1.3. Analytical methods applied to antioxidant content and antioxidant capacity assessment in plant extracts

There are many methods available for the measurement of antioxidant capacity. Therefore, considering the mechanism underlying the antioxidant–oxidant reaction, they can be divided in hydrogen atom transfer (HAT) and single electron transfer (SET) techniques. The first measure the capacity of an antioxidant to trap free radicals by hydrogen donation, while SET methods rely on the one electron transfer reductive ability of an antioxidant compound versus a radical species. Oxygen Radical Absorbance Capacity (ORAC) is included in HAT methods, while Ferric Reducing Antioxidant Power (FRAP) and Cupric Reducing Antioxidant Capacity (CUPRAC) are SET methods. Some assays are both HAT and SET, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox Equivalent Antioxidant Capacity (TEAC), as the radicals in these cases can be scavenged by either electron reduction or radical quenching that involves hydrogen transfer. Also, the Folin-Ciocalteu method is an electron transfer based assay and

gives reducing capacity, normally expressed as phenolic contents (Prior et al., 2005; Pisoschi et al., 2016).

Examples of *in vitro* methods are DPPH, NO, $OH^{\bullet}$, and $ONOO^{\bullet}$ scavenging activity, H_2O_2 assay, 2,2´-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical cation decolorization assay (also known as TEAC assay), FRAP, phosphomolybdenum method, metal chelating activity, and beta-carotene linoleate bleaching, while the lipid peroxidase, CAT, and GSHpx activity assays are techniques used *in vivo*. Antioxidant activity should not be concluded based on a single antioxidant test model. Therefore, it is common to perform several *in vitro* test procedures to consider the various modes of action of antioxidants, with the samples of interest (Alam et al., 2013).

DPPH is one of the most stable free radical with a deep purple colour and is frequently used in the evaluation of radical scavengers in natural foods. The method is simple and is not only specific to any particular antioxidant, but also applies to the overall antioxidant capacity of the sample. The assay is based on the ability of the stable $DPPH^{\cdot}$ to react with hydrogen donors, leading to loss of color at 515 nm (Moniruzzaman et al., 2011). DPPH is hydrophobic so its reactions must be run in organic solvents (Schaich, et al., 2015).

FRAP is applied to measure the ability of antioxidants to reduce ferric iron, of a number of biological samples and pure substances. This assay is based on the reduction of complexes of 2,4,6-tris(2-pyridyl)-s-triazine with ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), which are almost colourless. The solution will eventually turn slightly brownish forming blue ferrous complexes after complete reduction (Benzie & Strain, 1999).

ORAC assay is a method for quantifying the antioxidant strength of substances, which involves combining the sample to be evaluated (i.e., the antioxidant) with a fluorescent compound as well as a compound that generates free radicals at a known rate. As free radicals are being generated, the fluorescent compound is damaged and subsequently loses its fluorescence (Moniruzzaman et al., 2011). Radicals are generated by heating an azide compound, that decomposes, eliminating nitrogen gas and leaving behind two carbon centred radicals, $R^{\bullet}$. In the presence of oxygen, $R^{\bullet}$ are converted to reactive $ROO^{\bullet}$ which can either attack target molecules that have color or fluorescence or react with antioxidants (Schaich, et al., 2015). This assay is performed using Trolox (a water-soluble analog of vitamin E) as a standard to determine the Trolox Equivalent (TE) (Alam et al., 2013).

ABTS permits the measurement of antioxidant activity of mixtures of substances, helping to distinguish between additive and synergistic effects (Miller et al., 1996, Rice-Evans and Miller, 1995; Rice-Evans et al., 1996). The method measures the loss of colour when an

antioxidant is added to the blue–green chromophore $ABTS^{\cdot+}$. The antioxidant reduces $ABTS^{\cdot+}$ to ABTS and decolorize it (Alam et al., 2013).

Regarding **NO scavenging activity**, the radical $NO^{\bullet}$ is generated in biological tissues by specific NO synthases. The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing $NO^{\bullet}$. Therefore, under aerobic conditions, $NO^{\bullet}$ reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent (Alam et al., 2013).

Finally, in the case of **metal chelating activity**, ferrozine can form a complex with a red colour by forming chelates with Fe^{2+}. This reaction is restricted in the presence of other chelating agents and results in a decrease of the red colour of the ferrozine-Fe^{2+} complexes (Alam et al., 2013).

2.3.1.4. Literature review of antioxidant activity of african medicinal plants

Antioxidants defend the body against diseases such as cancer, cardiovascular and neurodegenerative diseases, arthritis, or diabetes mellitus. Plants are good sources of antioxidants, and their use in diets or in therapeutic reduces the occurrence of such diseases. Some African plants are known by their high content of antioxidant compounds. For example, Saharan plants are rich in phenolic compounds due to the extreme climatic conditions. Table 1 includes articles published between 2017-2021, searched in PubMed database, using the keywords antioxidant assays, *in vitro*, and African plants, which analyze the *in vitro* antioxidant activity of plants collected or purchased in African countries.

Table 1. *In vitro* antioxidant activity of African medicinal plants.

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
1	South Africa	*Adansonia digitata, Artemisia afra, Aloe ferox, Carissa edulis, Crinum macowanii, Elaeodendron croceum, Elaeodendron ransvaalense, Elephantorrhiza elephantina, Euclea natalensis, Helichrysum aureonitens, Heteropyxis natalensis, Lobostemon fruticosus, Moringa oleífera, Peltophorum africanum, Prunus africana, Ricinus communis, Senna petersiana, Sutherlandia frutescens, Terminalia sericea, Ziziphus mucronata* (Leaves)	50% Methanol	DPPH scavenging activity; $ABTS^+$ scavenging assay	**DPPH:** Five extracts exhibited EC_{50} values < 10 µg/mL with *A. digitata* (EC_{50} = 4.64 µg/mL) being the most potent extract, followed by *E. natalensis* (EC_{50} = 5.30 µg/mL). - Results were comparable to the positive control, ascorbic acid (EC_{50} = 2.50 µg/mL). **ABTS:** *E. croceum*, *E. natalensis*, *A. digitata*, in respective order, showed significantly higher $ABTS^+$ reducing power with EC_{50} values < 10 µg/mL. - Extracts has comparable significant EC_{50} values to ascorbic acid, which exhibited good ABTS reducing power with an EC_{50} value of 2.30 µg/mL.	More et al., (2021)
2	South Africa	*Coix lacryma-jobi; Cenchrus ciliaris, Cymbopogon spp., Eragrostis curvula, Imperata cylindrica, Panicum maximum, Setaria megaphylla, Sporobolus africanus, Sporobolus pyramidalis, Vetiveria zizanioides* (Leaves/Roots); *Cynodon dactylon* (whole plant); *Cymbopogon nardus* (Roots/Leaves/Inflorescence)	80% Methanol	DPPH scavenging activity; FRAP assay	**DPPH:** the best EC_{50} values were found for *C. nardus* roots and inflorescence, *Cymbopogon spp*. roots (0.02, 0.04 and 0.04 mg/mL) and for leaves of *C. nardus* and *V. zizanioides* (0.06 mg/mL). - Overall, the evaluated grass species had good antioxidant activity, ranging from 0.02 to 0.11 mg/mL, and comparable with the ascorbic acid with EC_{50} of 0.025 mg/mL. **FRAP:** *S. africanus* (leaves and roots) and *S. pyramidalis* root extracts showed a strong reducing power (slope = 0.124 for roots and slope = 0.086 for leaves of *S. africanus*; slope = 0.094 for roots of *S. pyramidalis*). Results comparable with control (slope = 0.095 for ascorbic acid).	Gebashe et al., (2020)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
3	South Africa	*Eriobotrya japonica* (leaves)	Methanol, acetone, ethyl acetate, hexane	DPPH scavenging activity; FRAP assay	**DPPH:** Hexane extract had the highest IC_{50} value of 1.5422 mg/L while methanol extract had the lowest value of 0.5336 mg/L. The reference standard used ascorbic acid) had an IC_{50} of 0.7155 mg/L. **FRAP:** Methanol extract showed the highest reducing power (IC_{50} = 0.2341 mg/mL). The reference standard used ascorbic acid) had an IC_{50} of 0.3214 mg/L.	Mogole et al., (2020)
4	South Africa	*Teucrium trifidum* (shrubs)	Acetone, water, ethanol, and methanol	DPPH scavenging activity; $ABTS^{+}$ scavenging assay; NO scavenging activity; TAC (phosphomolybdenum assay)	**DPPH:** Best IC_{50} values (mg/mL) was in the following order: rutin (0) > BHT (0.019) > ethanol extract (0.067) > methanol extract (0,017) > acetone extract (0.018) > aqueous extract (0.095). **ABTS:** Best IC_{50} values was in the following order: rutin (0) > BHT (0.005) > ethanol extract (0.012) > methanol extract (0.182) > acetone extract (0.086) > aqueous extract (0.297). - Extracts demonstrated high antioxidant activity in ABTS, DPPH, NO and TAC assays which were comparable to rutin and BHT.	Mazhangara et al., (2020)
5	South Africa	*Heteromorpha arborescens* (leaves)	Acetone, ethanol, and water	DPPH scavenging activity; $ABTS^{+}$ scavenging assay; TAC (phosphomolybdenum assay)	**DPPH:** Ethanolic extracts showed the highest DPPH radical inhibitory activity (IC_{50} = 0.06 mg/mL), compared to the others extracts). **ABTS:** Although the standards showed higher ABTS inhibitory potentials than extracts, the highest inhibitory capacity of the extracts was seen in the ethanol and aqueous extracts (IC_{50} = 0.049 mg/mL). **TAC:** The values IC_{50} of the standards and extracts were in the order: BHT (0.012 mg/mL) > ethanol (0.013 mg/mL) > rutin (0.017 mg/mL) > aqueous (0.024 mg/mL) > acetone (0.046 mg/L).	Abifarin et al., (2020)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
6	South Africa	*Laportea alatipes* and *Obetia tenax* (nettles)	Methanol and dichloromethane	DPPH scavenging activity; FRAP assay	**DPPH:** The extracts showed moderate inhibition of the DPPH radical relative to the known standards, ascorbic acid, and α-tocopherol. The highest antioxidant activity was exhibited by β-carotene (IC_{50} = 339 µg/mL) when compared to the extracts and β-sitosterol. **FRAP:** The methanolic extract of *O. tenax* had the highest reducing ability. Moderate antioxidant activity was observed for the other extracts.	Mahlangeni et al., (2020)
7	South Africa	*Drimia sanguínea* (bulb); *Elephantorrhiza elefantina* (rhyzome); *Helichrysum paronychioides* (whole plant); *Senecio longiflorus* (stem and leaves)	Petroleum ether and 50% methanol	DPPH scavenging activity; β-Carotene-linoleic acid assay	**DPPH:** *E. elephantina* was the most potent extract (EC_{50} = 5.8 ± 0.46 µg/mL); *D. sanguinea* had the least DPPH scavenging activity (92.6 ± 4.34 µg/mL). **β-Carotene-Linoleic Acid Assay:** Plant extracts reduce the coupled oxidation of β-carotene and linoleic acid. - Antioxidant activity for the investigated plants ranged from 64.8% to 84.7% compared to BHT (positive control). - *E. elephantina* displayed the highest activity while *D. sanguinea* had the lowest antioxidant potential at 400 µg/mL.	Asong et al., (2019)
8	South Africa	*Acokanthera opositifolia*, *Plantago lanceolata*, *Conyza canadensis*, *Artemisia vulgaris* (Leaves)	Acetone, ethyl acetate, chloroform, hexane, and water	DPPH scavenging activity	**DPPH:** The *n*-hexane and chloroform extracts of *P. lanceolata* had the best antioxidant activities with IC_{50} of 0.41 µg/mL compared with the positive controls (0.04 ± 0.09 µg/mL - vitamin C; 0.06 ± 0,37 µg/mL - quercetin). - The extracts with the least free radical scavenging activity among those evaluated were the *n*-hexane extracts of *A. vulgaris* (IC_{50} = 3.51 ± 1.22 µg/mL).	Adebayo et al., (2019)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
9	South Africa	*Opuntia stricta (cladodes)*	Water, ethanol, and acetone	DPPH scavenging activity; NO scavenging activity; H_2O_2 scavenging assay; TAC (phosphomolybdenum assay);	**DPPH:** Ethanol extract had the highest scavenging activity of the extracts at 73.79% ± 0.01; - The acetone, aqueous, ethanol, vitamin C and gallic acid had IC_{50} values of 0.511, 0.518, 0.510, 0.436 and 0.439 mg/mL, respectively (lower values compared with the standards used). **NO:** Extracts and standards (vitamin C and gallic acid) show a concentration dependent scavenging activity; Ethanol extract showed the highest antioxidant activity at 52.54% ± 0.1. The IC_{50} values of the extracts (ethanol, 0.97 mg/mL; acetone, 1.04 mg/mL; aqueous, 1.12 mg/mL) were comparable to vitamin C (1.18 mg/mL) and gallic acid (1.18 mg/mL). **H_2O_2: A**queous extract had the highest scavenging activity at 98.63% ± 0.01. **TAC:** the aqueous extract of *O. stricta* exhibited the highest total antioxidant capacity at 67.87% ± 0.004. - The scavenging activity of the three extracts was not significantly different and was higher than that for vitamin C and gallic acid.	Izuegbuna et al., (2019)
10	South Africa	*Schkuhria pinnata* (whole plant without roots)	*n*-hexane, chloroform, dichloromethane, ethyl acetate, acetone, ethanol, and methanol	DPPH scavenging activity; FRAP assay	**DPPH:** Methanol extracts had the greatest antioxidant activity when compared with other extracts at all concentrations, followed by ethyl acetate and acetone extracts. - The lowest scavenging activity was observed with the dichloromethane extracts (0.02 mg/mL). **FRAP:** Results indicated that *S. pinnata* had high ferric reducing power when compared with the positive control.	Masoko & Masiphephethu, (2019)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
11	South Africa	*Cucumis africanus* (whole plant and fruits)	Methanol, acetone, and water	DPPH scavenging activity; $ABTS^+$ scavenging assay, NO scavenging; TAC (Phosphomolybdenum assay)	**DPPH:** Significant antioxidant activity was observed by methanol and acetone extracts even though methanol revealed better hydrogen donating ability. Regarding IC_{50} values of the tested extracts, methanol root and aqueous leaf had the best antioxidant activity (IC_{50} = 0.66 mg/mL and 0.798 mg/mL, respectively). **ABTS:** Scavenging activity of the extracts and standards increased with increasing concentrations. - Regarding IC_{50} values of the tested extracts, aqueous fruit and acetone leaf had the best antioxidant activity (IC_{50} = 0.05 mg/mL and 0.054 mg/mL, respectively). **TAC:** The extracts showed concentration-dependent total antioxidant capacity. - Regarding IC_{50} values of the tested extracts, methanol leaf and acetone leaf had the best antioxidant activity (IC_{50} = 0.187 mg/mL and 0.287 mg/mL, respectively).	Abifarin, et al., (2019)
12	South Africa	*Dianthus thunbergii* (roots), *Hypoxis argentea* (corns)	Ethanol and water	DPPH scavenging activity; $ABTS^+$ scavenging assay; FRAP assay; H_2O_2 scavenging activity; NO scavenging activity	**Ferric-reducing power (FRAP):** Ethanol extracts of both *D. thunbergia* and *H. argentea* showed higher ferric-reducing capacities than the aqueous extracts. **DPPH:** At the maximum concentration tested (0.50 mg/mL), *H. argentea* aqueous extract exhibited the highest DPPH scavenging activity (69.42 ± 5.26%). **ABTS:** Among the extracts, *H. argentea* ethanol extract had the lowest IC_{50} value (0.0125 mg/mL), similar to the standards (vitamin C, BHT, and rutin). **NO:** *D. thunbergia* exhibited a more potent NO inhibitory activity than those of *H. argentea* at higher concentrations. **H_2O_2 scavenging activity:** ethanol extracts possessed higher HO scavenging abilities than that of the aqueous extracts and most of the standards.	Akinrinde et al., (2018)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
13	South Africa	*Coleonema album, Coleonema pulchellum* (leaves)	Petroleum ether, acetone, methanol, ethanol	ORAC method	**ORAC:** Leaf extracts of *C. pulchellum* has higher TE value (1126.7 ± 68.1 µmol TE/g) compared to *C. album* (942.2 ± 34.9 µmol TE/g).	Fajinmi et al., (2017)
14	South Africa	*Seriphium plumosum* (leaves)	Methanol, acetone, and hexane	DPPH scavenging activity; FRAP assay	- The methanol extract showed the best activity among all the extracts in both assays. It exhibited the lowest EC_{50} values of 0.72 mg/mL and 2.31 mg/mL for the DPPH scavenging activity and FRAP, respectively. - These EC_{50} values were lower than those for ascorbic acid which were 1.62 mg/mL and 3.10 mg/mL for the DPPH scavenging activity and the ferric reducing power assay, respectively.	Beseni et al., (2017)
15	Nigeria	*Capsicum annuum* var. *abbreviatum, C. annuum;* var. *acuminatum, C. annuum* var. *grossum, and C. frutescens* var. *baccatum* (mature fruits)	Ethanol and water	DPPH scavenging activity; $ABTS^+$ scavenging assay; NO scavenging; TAC (phosphomolybdenum method)	**DPPH:** *C. annuum* var. *abbreviatum* (ethanolic extracts) had the best IC_{50} value (0.0779 mg/mL). **ABTS:** *C. frutescens* var. *baccatum* (ethanolic and aqueous) were the most potent extracts (IC_{50} = 0.0024 mg/mL and 0.0031 mg/mL, respectively). **NO:** *C. frutescens* var. *baccatum* (ethanolic) had the best IC_{50} value (0.0971 mg/mL). **TAC:** The highest antioxidant capacity was exhibited in *C. frutescens* var. *baccatum* (IC_{50} = 0.0892 mg/mL).	Olatunji, & Afolayan, (2019)
16	Nigeria	*Aerva lanata* (leaves)	Water, ethanol, hydroethanol (50:50)	DPPH scavenging activity; $ABTS^+$ scavenging assay	**DPPH:** The hydroethanol extract exhibited the lowest EC_{50} for the DPPH radical-scavenging ability (2.25 ± 0.04 mg/mL) but was similar to the ethanol extract (2.48 ± 0.01 mg/mL) and significantly higher than the standard, gallic acid (1.25 ± 0.02 mg/mL). **ABTS:** The aqueous extract of *A. lanata* displayed the lowest EC_{50} for the ABTS radical-scavenging abilities (1.79 ± 0.06 mg/mL) compared to the extracts and the standard.	Akanji et al., (2018)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
17	Nigeria	*Irvingia gabonensis* (stem bark)	80% Acetone	Determination of reducing ability; Iron (Fe^{2+}) chelation assay; DPPH scavenging activity; $ABTS^{+}$ scavenging assay	**Reducing ability:** *I. gabonensis* extract (5.94 mg ascorbic acid equivalent, AAE/100 g) reduced Fe^{3+} to Fe^{2+}. **Iron ($Fe2^{+}$) chelating ability:** Fe^{2+} chelating ability of *I. gabonensis* showed an IC_{50} value of 113.10 µg/mL. **DPPH:** The extract showed an IC_{50} value of 19.98 µg/mL. **ABTS:** extract quenched ABTS radical in a concentration-dependent manner (20–100 µg/mL).	Ojo et al., (2018)
18	Nigeria	*Phragmanthera capitata* (leaves)	Acetone, methanol, ethanol, and water	DPPH scavenging activity; $ABTS^{+}$ scavenging assay; FRAP assay; NO scavenging activity; TAC (phosphomolybdenum method)	**DPPH:** The IC_{50} ranged from 24.5 µg/mL in the acetone fraction to 67.2 µg/mL in the aqueous extract. **ABTS:** The IC_{50} values ranged from <5 µg/mL in the acetone and ethanol fractions (1.9 µg/mL) and BHT (4.6 µg/mL), to 22 µg/mL in vitamin C. **Ferric reducing antioxidant power assay:** The IC_{50} obtained for the solvent fractions and standards ranged from 89 µg/mL in Vitamin C to >>400 µg/mL in BHT. **NO:** The scavenging activity as recorded from the IC values is in the order; methanol > vitamin C > acetone > BHT > aqueous > ethanol. **TAC:** The IC_{50} of the solvent fractions and the standard drugs in theorder of decreasing TAC are gallic acid > Vitamin C > acetone > ethanol > methanol > aqueous. - All the solvent fractions showed great antioxidant activities with the acetone fraction having the highest capacity based on ABTS, DPPH, and TAC assays (IC_{50} = < 5 µg/mL, 24.5 µg/mL, and 85 µg/mL, respectively). - The methanol extract however had FRAP and NO antioxidant activities (IC_{50} = 302 µg/mL and < 25 µg/mL, respectively).	Ohikhena et al., (2018)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
19	Algeria	*Peganum harmala*, *Marrubium vulgare*, *Zygophyllum album*, *Anacyclus valentinus*, *Ammodaucus leucotrichus* (aerial parts); *Lupinus albus* (seeds)	Ethanol (0, 50, 80 and 100%)	DPPH scavenging activity	**DPPH:** The highest radical scavenging inhibition activity was achieved with the 100% ethanolic extract of *A. leucotrichus* (81.60% ± 0.56; IC_{50} = of 26.26 µg/mL). The 100% and 80% ethanolic extracts of *M. vulgare* exhibited high activities of 75.84% ± 2.95 and 78.57% ± 1.88, respectively, with IC_{50} values of 24.08 and 19.67 µg/mL, respectively.	Hellal et al., (2020)
20	Algeria	*Heliotropium bacciferum* (aerial parts)	Methanol and chloroform	DPPH scavenging activity; $ABTS^{+}$ scavenging assay	Polyphenol-rich *H. bacciferum* methanol extract showed a significant and concentration dependent free radical scavenging activity both against DPPH (IC_{50} = 70.912 µg/mL) and ABTS (TEAC value of 1.466 mg/mL). *H. bacciferum* chloroform extract were not active as free radical scavengers (IC_{50} > 600 µg/mL and TEAC value of 0.024 and 0.012 mg/mL, respectively).	Aïssaoui et al., (2019)
21	Algeria	*Hertia cheirifolia* (aerial parts)	Methanol (ME) and water (AQ)	DPPH scavenging activity; Ferrous ions chelating activity; FRAP assay; β-Carotene bleaching method	**DPPH:** ME and AQ extracts of *H. cheirifolia* showed a concentration-dependent scavenging activity of DPPH; - ME extract was more active (IC_{50} = 138 µg/mL) than AQ extract (IC_{50} = 197 µg/mL), but lower than BHT (IC_{50} = 44.36 µg/mL), a standard antioxidant. **Ferrous ions chelating activity**: Both extracts chelate ferrous ions in a concentration-dependent manner; - At 300 µg/mL, *H. cheirifolia* AQ extract exerted a strongest chelating effect (99%) (IC_{50} = 61 µg/mL). **FRAP:** ME extract showed a strong reducing power (IC_{50} = 61 µg/mL) compared with AQ extract (IC_{50} = 193 µg/mL) (but lesser activity than BHT (IC_{50} = 17 µg/mL); **β-Carotene:** The inhibition of β-carotene bleaching exerted by 2 mg/mL of methanol and aqueous extracts of *H. cheirifolia* were about 74% and 94%, respectively).	Kada et al., (2017)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraxtion Solvents	Antioxidant Assays	Results	References
22	Cameroon	*Garcinia kola (*leaves, roots, and stem bark) and *Alchornea cordifolia (*leaves, stem bark and twigs)	Hydro-ethanol (1:4 v/v) Methanol	DPPH scavenging activity; $ABTS^+$ scavenging assay; FRAP assay	- **DPPH and ABTS** assays revealed that the extracts exhibited good free radical scavenging activities. The hydroethanolic stem bark of *A. cordifolia* extract exhibited the highest radical scavenging activity (12.606 µ/mL for DPPH; 1.330 µ/mL for ABTS). The hydroethanolic extract presented greater antioxidant potentials than the methanolic extracts.	Djague, et al., (2020)
23	Cameroon	*Sarcocephalus pobeguinii* (leaves, fruits, roots, and bark)	CH_2Cl_2/MeOH (1:2) (fruits); Methanol (roots, leaves and bark)	DPPH scavenging activity; $ABTS^+$ scavenging assay	- Methanol extract from leaves had the best antioxidant capacity among all the extracts that were tested (IC_{50} = 7.98 µg/mL and 15.35 µg/mL for DPPH and ABTS assays respectively). - Methanol extract from leaves was significantly ($p < 0.05$) more potent than all the extracts used.	Mfotie Njoya et al., (2017)
24	Morocco	*Euphorbia resinífera* and *E. officinarum* (aerial parts)	Water	DPPH scavenging activity; NO scavenging activity, superoxide radical scavenging capacity	**DPPH:** With the exception of the ratio 1:100, in the time periods of 30 min and 2 h, *E. resinifera* extracts had lower IC_{50} values (IC_{50} = 0.370 mg/mL). **Superoxide:** Best activity for E. *officinarum* extract (1:100) and after 1 h of extraction (IC_{50} = 0.17 mg/mL). **NO:** The best NO scavenging activity was found for *E. resinifera* (1:100 ratio) and after 2 h of extraction.	Boutoub et al., (2020)
25	Morocco	*Calendula arvenses* (flowers)	Water (AE), hexane (HE), methanol (ME)	DPPH scavenging activity; FRAP assay; $ABTS^+$ scavenging assay; β-carotene-linoleic acid (linoleate) model system	**DPPH:** Radical scavenging effect at all concentrations: gallic acid > ME > AE > HE with EC_{50} of 20.9 ± 5.34 and 33.2 ± 2.12 mg/mL for methanolic and aqueous extracts respectively. **ABTS:** EC_{50} values were lower compared with other studies and comparable with DPPH activity. **FRAP:** Best results shown by *C. arvenses* flowers in the following order ME > AE > HE (methanol solvent = 203.96 ± 1.92 mg AAE/g extract). **β-carotene:** all flower's extracts inhibited the bleaching of β-carotene by scavenging linoleate-derived free radicals; methanolic extract inhibit b-carotene (comparable to BHT).	Abudunia et al., (2017)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
26	Angola	*Julbernardia paniculata* and *Pterocarpus angolensis* (bark and leaves)	Methanol and fractions: n-hexane; diethyl ether, water	DPPH scavenging activity; β-Carotene bleaching method	**DPPH:** barks of *J. paniculata* have the greatest antioxidant activity (IC_{50} = 5.51 ± 0.93 mg/L). The crude methanolic extract of *J. paniculata* barks is the sample that presented the highest value of antioxidant activity index (AAI) (8.21 ± 0.19), corresponding to a "very strong" antioxidant activity. **β-Carotene Bleaching Test:** results also show the greatest antioxidant activity of *J. paniculata* barks, since they presented lower values of IC_{50}, except Fraction 1 (n-hexane) (IC_{50} = 1350.79 ± 287.89 mg/L).	Santos et al., (2020)
27	Ivory Coast	*Macaranga hurifolia*, *Sterculia tragacantha* and *Zanthoxylum gilletii* (leaves and stem barks)	Methanol	DPPH scavenging activity; $ABTS^{+}$ scavenging assay; TAC (phosphomolybdenum assay); FRAP assay; CUPRAC assay; Metal chelating activity	**Metal chelating activity:** Methanolic leaf extracts of the African plants were effective metal chelators; *S. tragacantha* (leaves extracts) was a better metal chelator (64.10±4.66 mg EDTAE/g) followed by *M. hurifolia* (30.39 ± 3.99 mg EDTAE/g) and *Z. gilletii* (25.81 ± 3.53 mg EDTAE/g). **Reducing Power (CUPRAC):** Higher reducing power was shown by the stem barks of *S. tragacantha* (1059.16 ± 7.47 mg TE/g) followed by *M. hurifolia* (966.59 ± 11.65 mg TE/g) and *Z. gilletii* (240.06 ± 7.41 mg TE/g). **ABTS:** Stem barks of *S. tragacantha* have the strongest scavenging ability (943.26 ± 14.29 mg TE/g) while the leaves of *Z. gilletii* is the least effective scavenger (106.34 ± 5.75 mg TE/g). **DPPH:** Leaves of *S. tragacantha* was recognized as the least radical scavenger (30.56 ± 0.99 mg TE/g) as it depends on the natureof radicals. **TAC:** The highest activity was exhibited by the stem bark of *M. hurifolia* (4.50 ± 0.67 mmol TE/g) and the least active was the stem bark extract of *Z. gilletii* (1.91 ± 0.17 mmol TE/g).	Bibi Sadeer et al., (2019)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
28	Sudan	*Abrus precatorius* (seeds); *Acacia nilótica* (pods); *Blepharis linariifolia* (whole plant); *Boswellia papyrifera* (gum); *Cephaelis ipecacuanha* (roots); *Citrullus colocynthis* (seeds); *Cordia sinensis* (leaves); *Cyperus rotundus* (roots); *Cymbopogon proximus* (whole plant); *Geigeria alata* (aerial parts); *Martynia annua* (mature fruit); *Pennisetum glaucum* (grains); *Ruta graveolens* (fruit); *Solenostemma argel* (leaves); *Tinospora bakis* (roots); *Trigonella foenumgraceum* (seeds); *Ziziphus spina-christi* (roots); *Vangueria Madagascariensis* (fruit)	70% Aqueous ethanol and water	DPPH scavenging activity	- Trolox was used as positive control, with an SC_{50} value of 11.35 ± 0.05 µg/mL. - Both extracts of *A. nilotica* exhibited the highest antioxidant activity among extracts (SC_{50} = 4.06 ± 0.09 µg/mL for 70% Ethanol extract; 7.51 ± 0.19 µg/mL for aqueous extracts). - 70% ethanol and water extract of *A. nilotica* were more active than the positive control Trolox. - Approximately, similar scavenging activity as the positive control was shown by the ethanolic extracts of *Z. spina-christi* and *A. precatorius*, with SC_{50} values of 10.75 ± 0.08 and 13.66 ± 0.12 µg/mL, respectively. - Free radical scavenging activity of the water extracts of *A. precatorius*, *Z. spina-christi* and *G. alata*, was less potent with SC_{50} values of 20.93 ± 3.54, 36.01 ± 3.60 and 137.66 ± 1.31 µg/mL, respectively.	Elbashir et al., (2018)
29	Sudan	*Sarcocephalus latifolius* (bark)	Ethanol and solvent fractions: hexane, ethyl acetate, butanol, water, chloroform	DPPH scavenging activity	Ethanolic crude extract exhibited high antioxidant activity with 87 ± 0.03%. The order of the activity (IC_{50} mg/mL) was as follow: hexane (0.098 ± 0.08) > chloroform (0.099 ± 0.029) > butanol (0.104 ± 0.19) > ethyl acetate (0.148 ± 0.33) and water fraction (2.015 ± 0.3).	Osama et al., (2017)

Table 1. *In vitro* antioxidant activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Antioxidant Assays	Results	References
30	Egypt	*Eucalyptus camaldulensis*, *E. camaldulensis var obtusa* and *E. gomphocephala* (leaves)	Methanol	DPPH scavenging activity; *β*-carotene-linoleic acid assay	**DPPH:** The highest total antioxidant activity (%) was found in the methanolic extract of *E. camaldulensis* leaves (90 ± 3.90%), followed by *E. camaldulensis var obtusa* (86.40 ± 2.13%) and *E. gomphocephala* (78 ± 2.20%) in comparison to gallic acid as a standard compound (80 ± 2.12%). ***β*-carotene-linoleic acid assay:** Values were 95 ± 1.2, 89.6 ± 3.2 and 82.6 ± 1.20% for *E. camaldulensis*, *E. camaldulensis var obtusa* and *E. gomphocephala*, respectively.	Elansary et al., (2017)
31	Tunisia	*Juniperus phoenicea* (leaves)	Hexane and methanol	DPPH scavenging activity	- Hexane extract did not show any activity with the DPPH method. However, methanol extract has a potent antioxidant activity (IC_{50} = 28.0 ± 0.1 µg/mL).	Keskes et al., (2017)
32	Tunisia	*Erica multiflora* (leaves), *Cyperus rotundus* (tuberous root), *Teucruim Alopecurus* (aerial parts), *Carduncellus monspelliensium* (roots), *Pituranthos tortuosus* (aerial parts), *T. poluim* (aerial parts), *Lavandula multífida* (leaves and flowers), *Thymus hirtus sp. Algeriensis* (aerial parts), *Nerium oleander* (leaves), *Ruta chalepensis* (aerial parts), *Punica granatum* (peel)	Acetonitrile/water (ACN/W) and water (W)	DPPH scavenging activity	- The extracts at concentrations ranging from 1 to 200 µg/mL exhibited dose-dependent radical scavenging activity. **DPPH:** *P. granatum* (ACN/W and W) showed the best antioxidant activity with IC_{50} values of 7.65 ± 0.05 and 6.78 ± 0.07 µg/mL. - *E. multiflora*, *T. alopecurus*, and *T. hirtus* sp. *algeriensis* are potent scavengers of the DPPH radical, because the EC_{50} values were close to that of the standard BHT (19.3 ± 1.02 µg/mL).	Guesmi et al., (2017)

AAE: Ascorbic acid equivalent; $ABTS^{+}$: 2,2´-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; BHT: Butylated hydroxytoluene; CUPRAC: Cupric Reducing Antioxidant Capacity; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; EC_{50}: 50% effective concentration; EDTAE: EDTA equivalent; FRAP: Ferric reducing antioxidant power; H_2O_2: Hydrogen peroxide; IC_{50}: 50% inhibitory concentration; NO: Nitric oxide; ORAC: Oxygen radical absorbance capacity; SC_{50}: sample concentration required to scavenge 50% of free radicals; TAC: Total antioxidant capacity; TE: Trolox equivalent; TEAC: Trolox Equivalent Antioxidant Capacity.

In the last years, the interest in naturally occurring antioxidants, that can be used to protect from oxidative stress damages, has increased (Akinrinde et al., 2018). Analyzing the studies in Table 1, we can conclude that South Africa is an area rich in medicinal plants and was the predominant country in the studies (14 of 32), followed by Nigeria (4 of 32). In general, plant extracts demonstrated high antioxidant activity in DPPH and $ABTS^{+}$ radical scavenging assays, which were two of the most used methods in the studies.

Some extracts exhibited EC_{50} values < 10 µg/mL in DPPH assay (*A. digitata* = 4.64 µg/mL; *E. natalensis* = 5.30 µg/mL) comparable with positive control (ascorbic acid = 2.50 µg/mL) (More et al., 2021) (Entry 1, Table 1)). Also, the *n*-hexane and chloroform extracts of *P. lanceolata* had IC_{50} values of 0.41 µg/mL, for DPPH (Adebayo et al., 2019) (Entry 8, Table 1). In another study, *P. granatum* (acetonitrile/water and aqueous extracts) showed the best antioxidant activity (IC_{50} = 7.65 ± 0.05 µg/mL and 6.78 ± 0.07 µg/mL, respectively) (Guesmi et al., 2017) (Entry 32, Table 1).

Differences in the antioxidant activity between the extracts from different parts of the plant, may be due to the variation in their chemical composition. Also, different solvents were used especially water, methanol, ethanol, and acetone, due to differences in their polarities. It is known that the choice of extraction solvents can influence the accuracy of measurements of the concentrations of bioactive compounds. Phytochemicals like carotenoids, being lipophilic, are extracted in non-polar solvents, but flavonoids, being hydrophilic, are extracted better in polar solvents (Bae et al., 2012).

In their study, Bea et al. (2012) showed that hexane and methanol were the most efficient solvents in extracting carotenoids and flavonoids, respectively. Also, hexane extracts showed strong DPPH radical scavenging activity, and ethyl acetate and acetone extracts showed the highest reducing power in different pepper cultivars (Bae et al., 2012). As previous reported, Mogole et al. (2020) also showed that hexane extract of *E. japonica* leaves had the highest IC_{50} value (1.5422 mg/L) while methanol extract had the lowest value IC_{50} = 0.5336 mg/L) regarding DPPH scavenging activity (Mogole et al., 2020) (Entry 3; Table 1). In another study performed by Adebayo et al. (2019), using different extraction solvents, the *n*-hexane and chloroform extracts of *P. lanceolata* had the best antioxidant activities with IC_{50} value of 0.41 µg/mL compared with the positive controls (Adebayo et al., 2019) (Entry 8; Table 1). On the other side, Keskes et al. (2017) showed that hexane extract of *J. phoenicea* leaves did not show any activity with the DPPH assay, and methanolic extract has a potent antioxidant activity (IC_{50} = 28.0 ± 0.1) (Keskes et al., 2017) (Entry 31; Table 1).

Methanol has a small molecular weight which enables it to penetrate the plant material more effectively. Also, the extracts obtained with ethanol showed stronger radical scavenging capacity compared to aqueous extracts (Abifarin et al., 2020; Olatunji & Afolayan, 2019; Osama et al., 2017) (Entry 5, 15, 29; Table 1). Acetone it is also known to extract compounds that have a broader spectrum of polarity (Beseni et al., 2017).

Most of the extracts with better antioxidant activity were rich in phenolic compounds, including flavonoids. Pharmacologically, phenolic compounds are responsible for antioxidant activity and correlation between these groups of compounds and their antioxidant capacity is well established (Abuashwashi et al., 2016; Hu et al., 2016). These compounds are powerful antioxidants able to eliminate radicals, chelate metal ions, trigger antioxidant enzymes, and inhibit oxidases (Marin et al., 2004). The antioxidant capacity of phenolic compounds is mainly due to their redox properties, allowing them to act as reducing agents, hydrogen donors, $^1O_2$ quenchers or metal chelators (Wintola & Afolayan, 2011).

For example, in their study Keskes et al. (2017) concluded that the best antioxidant activity for methanolic extract of *J. phoenicea* leaves could in part be attributed to the presence of flavonoid and biflavone compounds identified in fractions from methanolic extract (Keskes et al., 2017) (Entry 31; Table 1). Also, Osama et al. (2017) showed high amount of phenolic and flavonoid contents in hexane and chloroform fractions which could be related to the high antioxidant potential (Osama et al., 2017) (Entry 29; Table 1). The flavonoid moiety acts as terminator of free radicals by rapid donation of a hydrogen atom affording a phenoxy radical intermediate that is relatively stable (Torel et al. 1986; Bors et al. 1990). Also, the antioxidant activity of flavonoids is linked to their function as chelators of metal ions that are capable of catalyzing lipid peroxidation (LPO) (Arora & Nair, 1998). Flavonoids are also water soluble and have been reported to exhibit greater antioxidant activities than vitamins C, E, and carotenoids (Ghasemzadadeh & Ghasemzadadeh, 2011).

Some studies discuss the relationship between the antioxidant activity of flavonoids. Efficient radical scavenging activity requires the 3-hydroxyl (OH) group attached to the 2,3-double bond adjacent to the 4-carbonyl in the C-ring and an *o*-dihydroxy (catechol) structure in the B ring (Figure 2). Also, hydroxyl groups at position 3 on the C ring and 5 and 7 on the A ring provide an increase in antioxidant activity (Keskes et al., 2017). The hydroxyl groups in the B ring are very important in scavenging free radicals, because they can stabilize $HO^{\cdot}$, $ROO^{\cdot}$, and $ONOO^{-}$ radicals through the donation of hydrogen or electrons (Salehi et al., 2020). Flavonols, a class of flavonoids, are a group of poly-hydroxylated phenolics which are highly water soluble as a result of their OH groups (Qian & Nihorimbere, 2004). Other phenolic

compounds like tannins also showed antioxidant activity. Amakura et al. (2002) showed that the antioxidant activity is mostly driven by the gallic and ellagic acids, which are typical low molecular weight polyphenolics (Elansary et al., 2016). In conclusion, African plants seem to be a potent source of antioxidants as deduced from the appreciable values of scavenging activities, described in these studies.

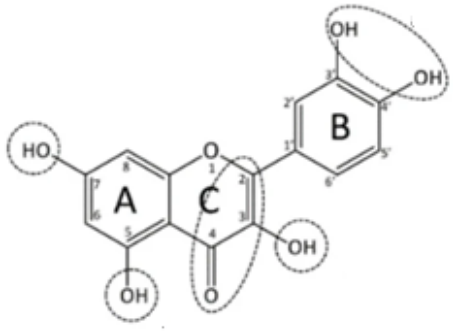


Figure 2. Antioxidant structure and activity relationships of flavonoids (Salehi et al., 2020).

2.3.2. Antimicrobial activity of African medicinal plants

Infectious diseases are a major cause of morbidity and mortality worldwide, including in Africa. The extensive use of antibiotics to control these diseases has led to the emergence of antibiotic-resistant pathogens and, therefore, the need for alternative medicines (Sieberi et al., 2020). Another problem is that some antibiotics have been associated with undesirable side effects, such as, nausea, depression of bone marrow, thrombocytopenia and agranulocytosis (Am et al., 2018), therefore medicinal plants are investigated as possible new sources of antimicrobial agents with possibly new and multiple mechanisms of action and fewer side effects since they have therapeutic relevance in traditional medicine (Hashemi & Davoodi, 2011; Ncube, Finnie & van Staden, 2012).

A study performed in Angola showed that, among the population, the common reasons for antibiotics use were cough and other respiratory symptoms, wounds, flu and body muscle pain, fever, bladder complaints, diarrhea and/or presumed typhoid fever. Also, nearly 40% of the respondents thought that antibiotics should be stopped as soon as people don´t feel sick anymore, showing an almost unacknowledged concept of antimicrobial resistance (AMR) (Cortez et al., 2017).

2.3.2.1. Antimicrobial resistance

Antimicrobial resistance can be defined as the ability of microorganisms to survive and be viable under the influence of antimicrobial agents (Abushaheen et al., 2020).

There are natural, semi-synthetic, and synthetic agents with different mechanisms of action, able to cause major alterations at the metabolic and physiological level, which include modifications in cell wall synthesis such as β-lactams and glycopeptides; protein synthesis inhibition such as macrolides and tetracyclines; metabolic pathway inhibition such as sulfonamides; and interference with deoxyribonucleic acid (DNA) replication and translation such as fluoroquinolones (Abushaheen et al., 2020). However, many pathogenic bacteria have evolved resistance to the main classes of antibiotics, and multidrug-resistant bacteria have caused untreatable infections.

The mechanisms of AMR can be classified as: 1) *Intrinsic Resistance*, when these groups of bacteria normally do not have a target site for the specific antibiotic, thus making them ineffective; 2) *Acquired resistance*, in which the naturally susceptible bacteria can develop resistance against certain antibiotics by receiving genetic codes from other bacterial strains. In this case, there are three major mechanisms of acquired resistance: a) modification of enzymes or inactivation of antimicrobial agents; b) reduced intracellular accumulation of antimicrobial agents (due to either decreased permeability and/or increased efflux); c) alterations at the target sites of the antimicrobial agents (target replacement, target site mutations, target site enzymatic alterations, target site protection, target overproduction or target bypass) (Christaki et al., 2020).

The antibiotic resistance is not only due to the remarkable genetic plasticity of the microorganisms, but also to the inappropriate use or high selective pressures of use or under-use through inaccessibility of drugs, inadequate dosing, poor quality drugs, poor patient compliance and the increased mobility of the world population (Okeke et al., 2007). In recent years, various strategies have been proposed to overcome antibiotic resistance. One of these recommended strategies to achieve this goal involves the combination of molecules, which apparently restores the desirable antibacterial activity (Khameneh et al., 2019).

2.3.2.2. Literature review of African plant extracts with antibacterial activity

Plants, as said before, are a rich reservoir of bioactive compounds that have numerous reported biological activities including antimicrobial properties (Famuyide et al., 2019). Among 109 new antibacterial drugs, approved in the period 1981–2006, 69% originated from

natural products (Newman & Cragg, 2020). The WHO also recognizes the role of plants as a mainstay of primary health for over half of the world's population, especially in countries with few resources (Am et al., 2018). When we have a mixture of plants, we can have a combination of plant species with better biological activities than isolated compounds. Therefore, these could be used to overcome the problem of AMR (van Vuuren & Holl, 2017).

Medicinal plants contain several phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, with antimicrobial properties widely studied for some plants. For example, the crude extracts of basil, cinnamon, ginger, sage, mustard, garlic, and other plants exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria (Alzoreky & Nakahara, 2003).

In Africa, a sizeable number of both the rural and urban population rely on traditional medicine for their primary health care. However, many plants have yet to be fully explored scientifically. The following table (Table 2) includes articles published between 2017 and 2020, searched in PubMed database, using the keywords antibacterial activity, *in vitro*, minimum inhibitory concentration, and African plants, which screened medicinal plants collected in Africa for their *in vitro* antibacterial activities.

Bacterial infections are responsible for a large number of deaths every year worldwide, such as those related to *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Enterobacter species*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* that are resistant to multiple classes of antibiotics (Sieberi et al., 2020). For that reason, in 2017, a comprehensive list of priority pathogens was released by the WHO, including microbes such as *S. aureus*, *A. baumannii, Streptococcus pneumoniae*, *E. coli*, *Klebsiella* spp., *Enterobacter* spp., along with others (Tacconelli et al., 2018). These pathogens have high levels of resistance to most existing antibiotics such as carbapenems, vancomycin, penicillins, ampicillins, and the third-generation antibiotic cephalosporins.

Some bacteria were repeatedly used in the following studies (Table 2), like Gram-positive bacteria *S. aureus* and *Enterococcus faecalis*; and Gram-negative bacteria *E. coli* and *P. aeruginosa*. These are major causes of nosocomial infections in hospitals (Sacho & Schoub, 1993) and are mainly the strains recommended for use by the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 1992).

Table 2. *In vitro* antibacterial activity of African medicinal plants.

Entry	Country	Medicinal Plants	Extraction Solvents	Bacterial Strains	Assays/Results	References
1	South Africa	*Eugenia erythrophylla, Eugenia natalitia, Eugenia woodii, Eugenia untamvunensis, Eugenia zeyheri, Syzygium legatii, Syzygium masukuense subsp. masukuense, Syzygium species A and Syzygium gerrardii* (leaves)	Acetone	*Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium*	- Broth microdilution method with MIC determination; - Plant extracts were active against the tested bacteria with MIC values for the extracts ranging between 0.04–0.31 mg/mL for Gram-negative bacteria and 0.02–1.25 mg/mL for Gram-positive bacteria; - *B. cereus* was the most susceptible of the Gram-positive bacteria (mean MIC of 0.08); Of the Gram-negative bacteria, *S. Typhimurium* and *P. aeruginosa*, (mean MICs of 0.14 mg/mL), and *E. coli* (mean MIC = 0.16 mg/mL) were the most susceptible.	Famuyide et al., (2019)
2	South Africa	*Eugenia erythrophylla, Eugenia natalitia, Eugenia woodii, Eugenia umtamvunensis, Eugenia zeyheri, Syzygium legatii, Syzygium masukuense ssp. masukuense, Syzygium sp.* (a new undescribed species), and *Syzygium gerrardii* (leaves)	Acetone	Six enterotoxigenic *E. coli* and reference *E. coli*	- MIC and total antibacterial activity were performed; - The MIC values for the extracts ranged between 0.04–0.31 mg/m, and for tetracycline ranged from 0.02–0.31 mg/mL. - Of the nine plants, *S. legatii* extract had the best mean MIC (0.05 mg/mL) against all *E. coli* strains investigated in this study followed by *S. masukuense* (MIC = 0.06 mg/mL) and *Syzygium sp*. (MIC = 0.08 mg/mL) extract. - *E. zeyheri* had the highest mean total antibacterial activity of 2.75 L/g (acetone extract of 1 g of acetone crude extract of *E. zeyheri* can be diluted to 2.75 L and still retain the ability to inhibit bacterial growth.	Famuyide et al., (2019a)

Table 2. *In vitro* antibacterial activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Bacterial Strains	Assays/Results	References
3	South Africa	*Hypoxis hemerocalídea* (bulbs)	50% Methanol (MeOH) and petroleum ether (PE)	*Shigella flexneri* and *Bacillus cereus*	- Antibacterial activity determined using the microdilution bioassay (with MIC determinations). - Both 50% MeOH and PE extracts showed considerable inhibitory effects against all bacteria. Noteworthy antimicrobial activity was observed against *Shigella flexneri* (MIC < 1 mg/mL). - MIC values for the extracts against bacterial strains ranged from 0.195 - 1.56 mg/mL. - The presence of phenolics, flavonoids and the other bioactive compounds contributed to the biological activities.	Mwinga et al., (2019)
4	South Africa	*Bidens pilosa* and *Dichrostachys cinerea* (leaves)	Hexane, dichloromethane, ethyl acetate, acetone, and methanol	*Klebsiella pneumoniae, E. coli, Salmonella typhimurium, Shigella boydii, and Vibrio parahaemolyticus*	- MIC assay was performed. - Dichloromethane extracts of both plant species had high antibacterial activity against all the bacterial species tested with an average MIC value of 0.56 mg/mL. - Ethyl acetate extract of *D. cinerea* showed no activity against most of the tested bacteria. *Shigella sp*. was the most susceptible of all the test bacteria with an MIC values of 0.04 mg/mL (ampicillin); - Some of the extracts had high MIC values (1.25–2.5 mg/mL) that is an indication of lack of activity against tested microorganisms. - Terpenoids, steroids and flavonoids were detected in the extracts and these compounds are known to have antibacterial activities.	Shandukani et al., (2018)

Table 2. *In vitro* antibacterial activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Bacterial Strains	Assays/Results	References
5	South Africa	*Schkuhria pinnata* (whole plant excluding roots), *Commelina africana* (leaves), *Dombeya rotundifolia* (leaves) and *Elephantorrhiza elephantina* (leaves)	Acetone	Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*)	- Broth microdilution method with MIC determination. - Combining (1:1:1) *S. pinnata* with *C. africana* and *D. rotundifolia* against *E. coli* (MIC = 0.09 ± 0.04 mg/mL) and *P. aeruginosa* (MIC = 0.06 ± 0.02 mg/mL) showed potent activities. - Ampicillin was used as positive control and its MIC values ranged from 0.02 to 0.08 mg/mL.	Kudumela et al., (2018)
6	South Africa	*Acacia mearnsii* (bark), *Aloe arborescens* (leaves), *Aloe striata* (leaves), *Cyathula uncinulata* (leaves), *Eucomis autumnalis* (bulb), *E. comosa* (bulb), *Hermbstaedtia odorata* (leaves), *Hydnora africana* (tuber), *Hypoxis latifólia* (tuber), *Pelargonium sidoides* (root), *Psidium guajava* (leaves), *Schizocarphus nervosus* (corms)	Acetone	*S. typhi, S. enterica serovar Typhimurium, Shigella flexneri type 1b and Sh. sonnei phase II and typed culture of Escherichia coli, Enterococcus faecalis, Staphylococcus aureus*	- Serial dilution microplate method with MIC determination. - MIC values of the plant extracts ranged from 0.018 mg/mL to 2.5 mg/mL after 24 h of incubation. - Average MIC values varied for the different bacterial species with the lowest value (0.018) against *S. aureus* and *S. flexneri.* - *A. arborescence*, *C. uncinulata*, *E. autumnalis* and *P. guajava* had considerable antibacterial activities with MIC values between 0.018 and 0.078 mg/mL. - Of significance is the antibacterial activity of *A. arborescens* and *P. guajava* against a confirmed extended spectrum betalactamase positive *S. enterica* serovar Typhimurium.	Bisi-Johnson et al., (2017)

Table 2. *In vitro* antibacterial activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Bacterial Strains	Assays/Results	References
7	South Africa	*Hypericum roeperianum* var. *roeperianum*, *Cremaspora triflora*, *Heteromorpha arborescens*, *Bolusanthus speciosus*, *Calpurnia aurea* ssp. *aurea*, *Maesa lanceolata*, *Elaeodendron croceum* and *Morus mesozygia*	Acetone	*Mycobacterium smegmatis*, *Mycobacterium aurum*, and *Mycobacterium fortuitum*	- Serial dilution microplate method with MIC determination. - MIC of activity against *M. smegmatis* ranged from 0.04 to 2.5 mg/mL. *C. triflora* extracts had the best activity (MIC 0.04 mg/mL), while *M. lanceolata* had moderate activity (MIC 0.16 mg/mL); *H. roeperianum* and *M. mesozygia* had weak activity with MIC = 0.63 mg/mL. - MIC values of the extracts against *M. fortuitum* ranged from 0.02 mg/mL to 2.5 mg/mL. *C. triflora* extracts had the best total antimycobacterial activity against *M. fortuitum* with a value of 1008 mL/g. - *M. aurum* was much more resistant to the plant extracts and only *C. triflora* had good activity (MIC = 0.08 mg/mL).	Elisha et al., (2017)
8	South Africa	*Bolusanthus speciosus*, *Calpurnia aurea* ssp. *aurea*, *Maesa lanceolata*, *Elaeodendron croceum*, *Morus mesozygia*, *Hypericum roeperianum* var. *roeperianum*, *Cremaspora triflora*, *Heteromorpha arborescens*, and *Pittosporum viridiflorum*	Acetone	*Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, *Salmonella serotype Typhimurium*, *Proteus mirabilis*, *Enterobacter cloacae and Escherichia coli*	- Broth microdilution method with MIC determination. - MIC values range of the different extracts against *S. maltophilia* was 0.08-0.31 mg/mL, *K. pneumonia* 0.08-0.63 mg/mL, *S. ser. Typhimurium* 0.08-0.63 mg/mL, *P. mirabilis* 0.02-1.25 mg/mL, *E. cloacae* 0.08-0.31 mg/mL and *E. coli* 0.08-0.16 mg/mL respectively. - *C. triflora* extracts had good activity against all the pathogenic egg isolates, with the exception of *P. mirabilis.* - *M. lanceolata* and *E. croceum* had the best total antibacterial activity.	Elisha et al., (2017a)

Table 2. *In vitro* antibacterial activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Bacterial Strains	Assays/Results	References
9	South Africa	*Combretum heroroense*, *Citrus lemon* and *Apodytes dimidiata* (leaves)	Hexane, dichloromethane, methanol, and acetone	*Mycobacterium smegmatis*, *M. tuberculosis*	- MIC determined by the broth dilution method. - Dichloromethane extract of *A. dimidiata* showed good activity against *M. smegmatis* (MIC = 0.1 mg/mL), followed by the dichloromethane and methanol extracts of *C. lemon* with MIC values of 0.3 mg/mL; - Total activity value shown to be highest for the dichloromethane extract of *A. dimidiata*, followed by the methanol extract of *C. lemon* and *C. heroroense*, respectively, against *M. smegmatis.* - The combination of the hexane and acetone, and the dichloromethane and methanol extracts of *C. heroroense* and *A. dimidiata* showed potent activity with MIC value of 0.04 mg/mL (higher than the extract of the plants alone). - The plant with the highest average total activity was *A. dimidiate*, against *M. tuberculosis*.	Komape et al., (2017)

Table 2. *In vitro* antibacterial activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Bacterial Strains	Assays/Results	References
10	Benin	*Piliostigma thonningii* (leaves)	50% Ethanol (EtOH)	*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas. aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, MRSA, and *Enterococcus* species	- Agar well diffusion test and microdilution assay were performed. - Inhibition zones (mm) for a 50% EtOH leaf extract of *P. thonningii* (10 mg/mL stock solution) were found for *S. epidermidis* (17.75 ± 0.43), *S. aureus* (14.75 ± 0.43), MRSA (13.75 ± 0.43), and *E. coli* (12.75 ± 0.43). - *S. epidermidis* and MRSA were then selected to evaluate the MIC of the extract (from 50 µg/mL to 500 µg/mL) against these bacteria; none of these microbial strains showed MIC below 500 µg/mL, suggesting a low antibacterial activity.	Marquardt et al., (2020)
11	Benin	*Combretum Collinum* (leaves)	50% Ethanol (EtOH)	*S. aureus, S. epidermidis*, MRSA, *K. pneumoniae, E. coli* and *Enterococcus sp.*	- Antibacterial activity was evaluated with agar well diffusion and microdilution assays. - Plant extract (10 mg/mL) was potentially effective against *S. epidermidis*, *S. aureus*, MRSA as well as *K. pneumoniae*, with inhibition zones (mm) of 21.75 ± 0.43, 15.75 ± 0.43, 16.25 ± 0.43, and 14.00 ± 0.00, respectively. - *Enterococcus sp*. was resistant against the extract; a low activity against *P. aeruginosa* and *E. coli* was observed, with inhibition zones (mm) of 11.00 ± 0.00 and 11.25 ± 0.43, respectively. - The two most sensitive strains, *S. epidermidis* and MRSA were subsequently selected for MIC determination; MIC values resulted in 275.0 µg/mL for *S. epidermidis* and 385.5 µg/mL for MRSA, respectively.	Marquardt et al., (2020a)

Table 2. *In vitro* antibacterial activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Bacterial Strains	Assays/Results	References
12	Kenya	*Centella asiatica* (whole plant)	Dichloromethane: Methanol DCM:MeOH (1:1)	*Salmonella typhi*, *E. coli*, *Shigella sonnei*, *Bacillus subtilis*, and *S. Aureus*	- Disc Diffusion Method; Determination of MIC by broth microdilution method; and determination of MBC. - Antibacterial effects of tetracycline 30 μg were significantly higher against all bacterial species compared with the *C. asiatica* extract at all concentrations tested (16.625, 31.25, 62.5, 125, 250 and 500 mg/mL) ($p < 0.05$). - Inhibitory activities of the extract (500 mg/mL) were significantly higher against all bacterial species tested compared to the other extract concentrations ($p < 0.05$). - At 500 mg/mL the extract had the following inhibition zones (mm): 12.00, 16.33, 13.00, 9.67, and 15.67 for *S. aureus*, *E. coli*, *S. typhi*, *B. subtlis*, and *S. sonnei*, respectively. - MIC values obtained for the DCM:MeOH extract *of C. asiatica* against *S. typhi* (62.50 mg/mL) and *E. coli* (62.50 mg/mL) were significantly higher compared to those against *S. aureus* (26.04 mg/mL) ($p < 0.05$). - Values of MBCs obtained were not more than 4 times higher than those of MICs on the corresponding test bacteria indicating that the extracts tested had an antimicrobial activity.	Sieberi et al., (2020)
13	Senegal	*Guiera senegalensis* and *Combretum aculeatum*	Water	*Mycobacterium marinum*	- Half inhibitory concentration IC_{50} was evaluated. - Both extracts showed significant activities against intracellular and extracellular *Mycobacterium marinum* growth presenting IC_{50} lower than 0.5 mg/mL compared to the reference drug rifampin (IC_{50} of 0.4 and 7 µg/mL).	Diop et al., (2018)

Table 2. *In vitro* antibacterial activity of African medicinal plants (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Bacterial Strains	Assays/Results	References
14	Sudan	*Calotropis procera* (leaves), *Arestolochia bracteolate* (whole plant), *Xanthium brasilicum* (leaves), *Vernonia amygdalina* (leaves), *Adanosonia digitata* (fruit pulp), *Terminalia laxiflora* (wood), *Terminalia brownii* (wood bark), *Combretum hartmannianum* (wood bark), *Ambrosia maritima* (aerial part), *Euphorbia hirta* (aerial part), *Ricinus communis* (leaves), *Acacia seyal* var. fistula (wood, bark), *Acacia seyal* var. seyal (wood, bark), *Acacia tortilis* (wood, bark), *Cassia acutifolia* (leaves), *Parkinsonia aculeata* (leaves), *Senna italica* (leaves), *Khaya senegalensis* (bark), *Polygonum glabrum* (leaves), *Argemone mexicana* (leaves, seed), *Solanum dubium* (fruits), *Salvadora persica* (leaves, stem), *Tamarix nilotica* (stem), *Tribulus terrestre* (aerial part)	Methanol or 50% ethanol	*Prophyromonas gingivalis*	- MIC determined by the broth dilution method. - Comparatively, methanol extracts displayed better anti-*P. gingivalis* activity than 50% ethanol extracts. - Among 62 plant extracts; 50 extracts exhibited MIC activity at the concentration of 4 mg/mL or less; moderate inhibitory activity (MIC = 1 mg/mL) were found in sixteen plant extracts. - The most potent extracts were methanol extract of *T. laxiflora* (MIC value 0.25 mg/mL) followed by *A. maritima*, A. mexicana (seed), *T. brownii* (wood), *C. hartmannianum* (bark), *Acacia totrtilis* (bark) and 50% ethanolic extract of *T. brownii* (bark) with MIC value 0.5 mg/mL. - The positive control (chlorhexidine) showed a significant inhibitory activity compared to the other extracts.	Mohieldin et al., (2017)

MBC: Minimum Bactericidal Concentration; MIC: Minimum Inhibitory Concentration; MRSA: Methicillin-resistant *Staphylococcus aureus*; TAA = Total antibacterial activity.

The potency of a plant extract may be predicted on the basis of its Minimum Inhibitory Concentration (MIC) in mg/mL. The best plant species to be used is based on the total antibacterial activity (TAA), which is calculated by dividing the mass in mg extracted from one g of dried plant material by the MIC. The TAA indicates the volume (mL) to which the extract obtained from 1 g of plant material can be diluted and still be able to inhibit the bacterial growth (Famuyide et al., 2019a). The total activity of the extracts is a pharmacologically useful measure to compare the efficacy of different plants because it considers not only the antimicrobial activities but also the quantities extracted from different plants (Elisha et al., 2017).

Several authors classified plants according to their antimicrobial activity and the lower the MIC is the better is the activity (Jn, 2004). For example, plant extracts with MIC values lower than 0.1 mg/mL are significantly active/good (can be considered a potent antimicrobial agent); those with MIC values from 0.1 to 0.625 mg/mL are moderately active, and MIC over 0.625 mg/mL have weak or negligible activity (Elisha et al., 2017; Marquardt et al., 2020). Famuyide et al. (2019) studied the antibacterial activity of medicinal plants from South Africa and showed that most of the extracts analyzed had good activity against at least two of the bacteria examined (Famuyide et al., 2019) (Entry 1; Table 2). Also, Famuyide et al. (2019a) showed that MIC of the acetone crude extract of nine of those plants analyzed against the *E. coli* strains ranged from good to moderate (Famuyide et al., 2019a) (Entry 2, Table 2). The antibacterial activity of the medicinal plant extracts varied according to the bacteria tested and as stated for the antioxidant activity, the type of solvent used for the extraction. Extraction is a very important first step in the analysis of medicinal plant properties because the choice of solvent influences the types of compounds that can be extracted and ultimately, the biological activities imparted by the extracted compounds (Shandukani et al., 2018). Regarding all the articles from Table 2, most of the plant extractions were performed with acetone as a solvent (Bisi-Johnson et al., 2017; Elisha et al., 2017; Elisha et al., 2017a; Famuyide et al., 2019; Famuyide et al., 2019a; Komape et al., 2017; Kudumela et al., 2018; Shandukani et al., 2018) (Entries 6, 7, 8, 1, 2, 9, 5, 4; Table 2). Extraction with acetone is considered a good choice because it can extract compounds of a wide range of polarities, it is nontoxic to bioassay systems and easy to remove from extracts (Jn, 2001). The findings in the study of Shandukani et al. (2018) demonstrated that polar solvents such as acetone and methanol produced better antioxidant and antibacterial activities, supporting the traditional use of water as an extractant for preparations of remedies. Secondary metabolites, namely, terpenoids, steroids and flavonoids were detected in the

extracts (Shandukani et al., 2018) (Entry 4, Table 2), and these compounds are known to have antibacterial activities (Compean & Ynalvez, 2014).

The Gram-negative bacteria generally had a higher resistance to the plant extracts than Gram-positive bacteria (Biswas et al., 2013; Boulekbache-Makhlouf et al., 2013). This is attributed to the distinct feature of the morphology of cell walls of Gram-negative bacteria which, in contrast to those of Gram-positive bacteria, comprise a hydrophilic lipopolysaccharide outer layer highly resistant to the penetration of antibacterial agents, as well as the presence of some enzymes in the periplasmic space which break down antibacterial molecules (Djihane et al., 2017). Therefore, in most cases plant extracts have been reported to be more active against Gram-positive pathogens (Vlietinck et al., 1995), and similar observation was found in the study of Bisi-Johnson et al. (2017) but in addition, most of the extracts also had substantial activity against the selected Gram-negative enteric bacteria (Bisi-Johnson et al., 2017) (Entry 6; Table 2). Infections with Gram-negative bacteria are of imminent concern as they are more difficult to treat, and outcome is poor (Elisha et al., 2017).

Since traditional herbal preparations usually comprise of more than one plant, the effect of the combination of different extracts from the same or from different plants was assessed in some studies of the Table 2 for possible synergistic effects. The synergistic effects are often crucial to bioactivity in plant extracts and activity may be lost in some cases, in purified fractions (Komape et al., 2017) (Entry 9; Table 2). Kudumela et al. (2018) demonstrated that combinational therapy may be used to address AMR of Gram-negative strains. They found that when the plant *S. pinnata* was combined with *C. africana* and *D. rotundifolia* in a 1:1:1 combination, potent activities were observed against Gram-negative bacteria *E. coli* (MIC = 0.09 ± 0.04 mg/mL) and *P. aeruginosa* (MIC = 0.06 ± 0.02 mg/mL). Overall, the Gram-negative bacteria were more sensitive to the combinations than the Gram-positive bacteria (Kudumela et al., 2018) (Entry 5; Table 2).

The results obtained in these studies also showed that the plant extracts contained different phytochemicals that included alkaloids, cardiac glycosides, saponins, tannins, flavonoids, terpenoids, and phenols (Sieberi et al., 2020). In general, many studies have already demonstrated the efficacy of plant extracts and their respective compounds in blocking microbial growth. Phenolic compounds have been reported to have an antibacterial activity against *S. aureus* (Saxena et al., 2013), while flavonoids such as quercetin have been reported to completely inhibit the growth of *S. aureus* (Zwenger & Basu, 2008), and catechins have been reported to have *in vitro* activity against *V. cholerae, Streptococcus mutans*, and *Shigella*. Also, quercetin inhibits *E. coli*, while naringenin has been reported to have intensive activity

against MRSA and streptococci (Tapas et al., 2008). Polyphenols, in particular, have an influence on the activity of bacterial enzymes and proteins and on the fluidity of bacterial cell membrane. Thus, they can alter the proton gradient or the ion balance at the cell membrane, which ultimately leads to bacterial cell death (Cushnie & Lamb, 2005)

Mwinga et al., (2019) showed that the antibacterial activity of *H. hemerocallidea* could be attributed to the presence of the bioactive compounds identified in the plant (Mwinga et al., 2019) (Entry 3; Table 2). Compounds such as myrcene, cyclopropene and dotriacontane, which are present in the bulb extracts, have been demonstrated to have antibacterial activities (Silverio et al., 2013).

African countries have been encouraged to look for advancements in herbal medicine use to sustain provision of healthcare and ensure continuity of culture (Marquardt et al., 2020). The study of plant extracts with known antibacterial activities is important in managing various infectious diseases. To promote proper use of herbal medicine and determine their potential as a source of new drugs, it is essential to study medicinal plants that have traditional reputation. Society may be entering a post-antibiotic era with existing antibiotics gradually becoming ineffective due to resistance. This has major threats to health, as well as national security, for example pandemics and bioterrorism (Lowrence et al., 2018).

2.3.3. Cytotoxic activity of African medicinal plants

Cancer is a generic term for a series of malignant diseases that is still a major health problem worldwide. The etiology of carcinogenesis involves distinct levels of regulation. In this process, normal cells acquire genetic and epigenetic changes that result in uncontrolled cell growth and, therefore, cancer. It is also known that ROS are involved in tumor formation through the activation of various oncogenic signaling pathways, DNA mutations, immune escape, the tumor microenvironment, metastasis, and angiogenesis (Kirtonia et al., 2020). Despite all the improvements in cancer therapy due to diagnostic and therapeutic progresses, cancer still has extremely high mortality rates (Mbaveng et al., 2017). Cancer is also a global public health problem and the second leading cause of death in the United States of America (USA) (Siegel et al., 2021). Worldwide, an estimated 19.3 million new cancer cases with almost 10.0 million deaths occurred in 2020 (Sung et al., 2021).

One of the main problems with cancer cells is their ability to escape apoptosis due to unidentified mutations, resulting in cell accumulation, which consequently migrate to distinct parts of the body (Fernald & Kurokawa, 2013). Thus, an effective anticancer drug should target

cancer cells without affecting normal cells, which can be achieved by restoring the apoptosis machinery in the cancer ones (Kumar et al., 2017) and by being able to overcome multidrug resistance (MDR). Cancer cells can rapidly acquire MDR, which can be associated with a variety of mechanisms, including the overexpression of adenosine triphosphate-binding cassette (ABC) efflux transporters (Robey et al., 2018), or the deletion/inactivation of important biomarkers of the tumorigenesis, such as tumor suppressor gene *p53* (Hientz et al., 2017).

2.3.3.1. In vitro cytotoxicity assays

Cell viability can be defined as the number of healthy cells. Cell cytotoxicity assays are generally used for drug screening to detect whether the test molecules have effects on cell proliferation or display direct cytotoxic effects (Adan et al., 2016).

In vitro cytotoxicity assays performed in plant extracts aims to evaluate the toxic potential of chemical and natural materials in cell culture models to help detect the ability of plant extracts to affect cell viability, cellular growth, and cell damage (Gavanji et al., 2023). These assays can provide many advantages and disadvantages, which are summarized in Table 3.

Table 3. General advantages and disadvantages of *in vitro* cytotoxicity assays (Gavanji et al., 2023).

Advantages	Disadvantages
• Lower cost than animal testing; • Reduce number of animal sacrifices; • High precision of the response (lower biological variation than in vivo systems); • Bio-safety and fewer ethical issues; • Rapid screening; • Can be versatile and well-controlled; • Wide availability of various cell types; • Well-suited assays that can be standardized for large-scale screening.	• Cannot monitor the various behavioural responses; • Unable to account for metabolic responses or reactions; • Cannot assess the drug pharmacokinetic effects; • Lack of developed systems to study the interactions among the different cell types; • False positive or false negative test results.

The assays used to measure cell viability or cytotoxicity include the dye exclusion test (e.g., trypan blue, congo red, eosin-nigrosin and erythrosine B); colorimetric assays (e.g., MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide), MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), 2,3-bis (2-methoxy-4-nitro-5-sulphophenyl)−5-carboxanilide-2H- tetrazolium monosodium salt,

2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium monosodium salt, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt, lactate dehydrogenase, sulforhodamine B, neutral red uptake and crystal violet staining); fluorometric assays (e.g., Alamar Blue Assay) and luminometric assays (e.g., Adenosine Tri-Phosphate (ATP) Assay) (Adan et al., 2016; Gavanji et al., 2023, Präbst et al., 2017).

Among the colorimetric assays, (Sylvester, 2011), the MTT assay, developed in 1983 (Mosmann, 1983), is one of the best standard assay for cytotoxicity evaluation, relying on the conversion of tetrazolium component (MTT) into formazan crystals by some specific enzymes in the mitochondria of viable cells. It has the advantage of been suitable for high-throughput screening, but it depends on organic solvents such as isopropanol or DMSO to solubilize the crystals.

2.3.3.2. Literature review of African plant extracts with cytotoxic activity

In Africa, cancer is reported as a critical public health problem (Mbele et al., 2017), with increasing numbers related to aging, population growth, and an increase of risk factors, including smoking, obesity, and sexual and sedentary behaviors. In Africa, an estimated 1.1 million new cases and 711,429 deaths occurred due to neoplasms in 2020. By 2040, the burden of all neoplasms combined is forecasted to increase to 2.1 million new cases and 1.4 million deaths (Sharma et al., 2022). In addition, cancer is a challenge in African countries because, in general, available funding is limited, the lack of resources, and other major health problems (Mbele et al., 2017).

Worldwide, more than 3000 plants have been reported to have anticancer properties (Solowey et al., 2014). Therefore, plants continue to be an important source of new cytotoxic agents due to the structural diversity of phytochemicals, and their use to combat multidrug resistance remains a challenge. The importance of African traditional plants in the prevention and treatment of diseases, including cancer, has already been shown (Mbele et al., 2017). However, despite the traditional use of medicinal plants, research on the cytotoxicity related to African plant extracts against cancer cells is scarce. Therefore, the following table (Table 4) aims to gather the information about the cytotoxic activity, *in vitro*, of African plant crude extracts, describing mechanisms of action and the influence of extraction solvents.

For the information in this table, the published literature from 2017 to 2021 on the African medicinal plants as sources of bioactive compounds with potential cytotoxic activity were

collected from PubMed and ScienceDirect, using the keywords cytotoxic, anti-cancer, antitumor, antiproliferative, cancer cell lines, and African plants were used in combination. Table 4 included articles that evaluated the cytotoxicity of the crude extracts by different assays, from plants collected/purchased fresh in African countries, towards different human carcinoma cell lines. The evaluations of the extract fractions or isolated compounds from medicinal plants were not included.

A total of twenty-three reports, associated to the cytotoxic potential of 105 African medicinal plants, were retrieved from the databases selected. In Africa, many countries still depend on traditional plants for the management of several types of cancers due to the limited access to conventional medicine (Mfengwana et al., 2019). Despite progresses in chemotherapy for the treatment of cancer, other major problems appear (e.g., costs, side effects, and multidrug resistance) particularly in African countries (Makhafola et al., 2020).

Most of the plants came from South Africa (eight studies), followed by Cameroon (six studies), and then Morocco (two studies). Other countries such as Ghana, Ethiopia, Côte d'Ivoire, Egypt, Burkina Faso, Algeria, and Kenya were also included in the studies.

Countries in Africa are facing an increase in the incidence of cancer. The most prevalent in African females is breast cancer (27.7%), followed by cervical cancer (19.6%) (Bahnassy et al., 2020). In South Africa, colorectal cancer occupies the third place for women (GLOBOCON, 2020). Meanwhile, prostate (18.1%), followed by liver (9.7%) and colorectal cancers (6.9%) were the most prevalent among African males (Bahnassy et al., 2020). According to the WHO, it is estimated that there will be an increase in the incidence and mortality rates of other types of cancer for the next two decades (World Health Organization, n.d.). Regarding cell lines used in the analyzed studies, the most selected for the evaluation of cytotoxicity were breast cancer (MCF7 and MDA-MBA-231) and colorectal cancer (HCT-116 or Caco-2) because of the high and increased incidence rates of these cancers.

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines.

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
1	South Africa	*Momordica balsamina* (leaves)	Acetone	Colorectal carcinoma (HT-29)	- Viability of HT-29 cells decreased in a concentration- and time-dependent manner. At concentrations > 50 µg/mL, a significant decrease in cell viability was seen ($p \leq 0.01$). - *M. balsamina* suppressed cell invasion, cell adhesion and cell migration. Cell invasion was associated to downregulation of NF-κB, TNF-α, NF-κB, matrix metalloproteinases (MMP) MMP2, and MMP9, and to the upregulation of TIMP-3 proteins.	Serala et al., (2021)
2	South Africa	*Sutherlandia frutescens* (leaves)	75% (V/V) Ethanol	Colon adenocarcinoma (DLD1)	- Between concentrations of 22.2 µg/mL and 200 µg/mL, all the plant extracts induced cytotoxicity on DLD-1 cells in a dose-dependent manner. - Plants from Colesburg, Zastron, and Gansbaai 1 showed higher potency (IC_{50} values of 158.7, 172.7, and 176.7 µg/mL, respectively) compared to specimens from other localities in South Africa. - Plants from Colesburg had the highest anticancer activity (36.6% viability).	Zonyane et al., (2020)
3	South Africa	*Tulbaghia violacea* (leaves)	Methanol, hexane, butanol	Cervical adenocarcinoma (HeLa and ME-180) Breast adenocarcinoma (MDA-MBA-231 and MCF-7)	- Most cytotoxic extract was the methanol one with the greatest effect on *p53* in all cancers. - Concentration of 15 µM (butanolic extract) was considered optimal for treating cervical and breast cancer cell lines, based on the IC_{50} values in all the extracts. - *T. violacea* extracts inhibit cell proliferation in a cell line- and dose-dependent manner.	Motadi et al., (2020)
4	South Africa	*Opuntia stricta* (cladodes)	Water, acetone, ethanol	Human myeloid leukemia (U937)	- Only acetone extract showed mild cytotoxicity (IC_{50} = 110.1 µg/mL).	Izuegbuna et al., (2019)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
5	South Africa	*Cotyledon orbiculata* (leaves)	Water	Colorectal carcinoma (HCT116); Esophageal adenocarcinoma (OE33); Esophageal squamous carcinoma (KYSE-70)	- *C. orbiculata* extract decreased the viability of KYSE70 (LC_{50} (50% lethal concentration) = 36.9 µg/mL), HCT116 (LC_{50} = 64.9 µg/mL), and OE33 cells (LC_{50} > 100 µg/mL) in a dose-dependent manner. Apoptosis was induced by alternative splicing of hnRNPA2B1 and BCL2L1.	Makhafola et al., (2020)
6	South Africa	*Asparagus laricinus* (cladodes) *Senecio asperulus* (roots)	Water, methanol, methanol:dichloromethane, 1:1 (V/V), dichloromethane, hexane	Breast adenocarcinoma (MCF-7); Prostate adenocarcinoma (PC3)	- Methanol extract of *A. laricinus* showed cytotoxic effect towards MCF-7 cells (IC_{50} = 97.6 µg/mL), and almost no effect on non-cancerous Vero cells. - Dichloromethane extract of *S. asperulus* exhibited cytotoxic effect towards MCF-7 cells (IC_{50} = 69.15 µg/mL). - Methanol:dichloromethane extract of *A. laricinus* and hexane extract of *S. asperulus* were cytotoxic against MCF-7 and PC3.	Mfengwana et al., (2019)
7	South Africa	*Kedrostis africana* (tuber)	Water, ethanol	Cervical adenocarcinoma (HeLa)	- A significant decrease in the number of HeLa cells was not observed by aqueous extract of *K. africana*, at all tested concentrations (50–200 µg/mL). - For aqueous and ethanol extracts of *K. africana*, IC_{50} values are > 200 µg/mL.	Unuofin et al., (2018)
8	South Africa	*Centella asiatica* (leaves) *Curtisia dentata* (leaves) *Warburgia salutaris* (leaves)	Methanol, ethyl acetate, acetone, water	Breast adenocarcinoma (MCF-7); Cervical adenocarcinoma (HeLa); Colorectal adenocarcinoma (Caco-2); Lung adenocarcinoma (A549)	- Methanol and acetone extracts were more active than the ethyl acetate and aqueous extracts. - *C. asiatica*: acetone extract had the most significant activity (IC_{50} = 46.49 ± 0.04 µg/mL, A549 cell line) and aqueous extract was the least active (IC_{50} > 100 µg/mL for A549, Caco-2, and MCF-7 cell lines; and IC_{50} = 76.3 ± 0.06 µg/mL (HeLa)). - *C. dentata*: acetone extracts were the most cytotoxic (IC_{50} = 41.55, 45.13, 57.35 and 43.24 µg/mL against A549, HeLa, CaCo-2, and MCF-7 cell lines, respectively) ($p \leq 0.05$). *W. salutaris*: acetone extracts were the most cytotoxic (IC_{50} = 34.15 µg/mL for MCF-7).	Soyingbe et al., (2018)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
9	Cameroon	*Tetrapleura tetraptera* (fruits)	Dichloromethane-methanol (1:1)	Resistant CEM/ADR5000 and sensitive CCRF-CEM leukemia cells; Colon cancer HCT116 (*p53*+/+) cells and t clone HCT116 (*p53*−/−); Glioblastoma U87MG cells and resistant U87MG.ΔEGFR cells; MDA-MB-231-pcDNA3 breast cancer cells and resistant subline MDA-MB-231-BCRP clone 23 cells; Hepatic carcinoma (HepG2)	- Crude extract of *T. tetraptera* showed cytotoxicity towards all the cancer cell lines (including drug-sensitive and -resistant phenotypes). - Crude extract of *T. tetraptera* showed cytotoxic effects with IC_{50} values ranging from 10.27 µg/mL, in CCRF-CEM leukemia cells, to 23.61 µg/mL, in colon cancer HCT116 (*p53*−/−) cells. - Apoptosis induced by crude extract of *T. tetraptera*, in CCRF-CEM cells, was MMP alteration-mediated and increased reactive oxygen species (ROS) generation.	Mbaveng et al., (2021)
10	Cameroon	*Fagara tessmannii* (bark)	Methanol	Resistant CEM/ADR5000 and sensitive CCRF-CEM leukemia cells; MDA-MB-231-pcDNA3 breast cancer cells and its resistant subline MDA-MB-231-BCRP clone 23 cells; Colon cancer HCT116 (*p53*+/+) cells and their t clone HCT116 (*p53*−/−) Glioblastoma U87MG cells and resistant subline U87MG.ΔEGFR cells; Hepatic carcinoma (HepG2)	- The effect of methanol extract of *F. tessmannii* in models of drug-resistant and drug-sensitive cell lines was studied. - IC_{50} values ranged from 17.34 µg/mL (towards U87MG.ΔEGFR glioblastoma cells) to 40.68 µg/mL (against CCRF-CEM leukemia cells) for crude extract. - Apoptosis induced by *F. tessmannii* bark extract in sensitive CCRF-CEM leukemia cells was mediated by increased ROS production.	Mbaveng et al., (2019)
11	Cameroon	*Ficus elastica* (wood of aerial roots) *Selaginella vogelli* (leaves)	Methanol	Cervical adenocarcinoma (HeLa)	- *S. vogelii* and *F. elastica* extracts had IC_{50} values at 20 µg/mL, for HeLa cell line. - Emetine exhibited an IC_{50} = 0.04 µM. - Extracts of both plants showed low cytotoxic effects on HeLa cell line.	Mbosso Teinkela et al., (2018)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
12	Cameroon	*Sarcocephalus pobeguinii* (Roots, fruits, bark, and leaves)	Methanol (leaves/roots/bark) dichloromethane/methanol (1:2) (fruits)	Breast adenocarcinoma (MCF-7) Cervical adenocarcinoma (HeLa) Colorectal adenocarcinoma (Caco-2) Lung adenocarcinoma (A549)	- Methanol extract from *S. pobeguinii* leaves had the highest cytotoxic activity. - Methanol extract from leaves is active against MCF-7 (IC_{50} = 26.94 µg/mL) and HeLa (IC_{50} = 10.19 µg/mL); methanol extract from bark is only efficient on HeLa cells (IC_{50} = 15.26 µg/mL). - Extract from the fruits was significantly more toxic to non-cancerous (Vero) cells (LC_{50} = 601.42 µg/mL) ($p < 0.05$) than Caco-2 cell line (IC_{50} = 721.03 µg/mL). - Methanol extract (roots) showed the higher IC_{50} values for MCF-7 and Caco-2.	Mfotie Njoya et al., (2017)
13	Cameroon	*Moringa oleifera* (leaves and seeds)	Water	Human lymphoid (Jurkat E6-1) Human leukemia monocytic (THP1)	- Aqueous extract from leaves and seeds had anti-proliferative and pro-apoptotic effects only on cancer cells (not on peripheral blood mononuclear cells - PBMCs). - Pro-apoptotic effect seen with aqueous extract is related with decreased BCL2 levels and sirtuin-1 (SIRT1) protein expression.	Potestà et al., (2019)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
14	Cameroon	*Ananas comosus* (peels) *Arachis hypogaea* (leaves and twigs) *Artocarpus heterophyllus* (leaves) *Camelia sinensis* (leaves) *Citrus sinensis* (fruits) *Cola pachycarpa* (leaves) *Coula edulis* (fruits) *Curcubita pepo* (pericarp) *Curcuma longa* (rhizomes) *Lycopersicon esculentum* (twigs and leaves) *Mangifera indica* (leaves and bark) *Myristica fragrans* (seeds) *Persea Americana* (bark) *Physalis peruviana* (twigs) *Psidium guajava* (bark) *Raphia hookeri* (fruits) *Rubus fellatae* (leaves) *Tristemma hirtum* (leaves)	Methanol	Resistant CEM/ADR5000 and sensitive CCRF-CEM leukemia cells; MDA-MB-231-pcDNA3 breast cancer cells and their transfectant subline MDA-MB-231-BCRP clone 23; Colon cancer HCT116 (*p53*+/+) cells and their knockout clone HCT116 (*p53*−/−); Glioblastoma U87MG cells and their resistant subline U87MG.ΔEGFR	- Doxorubicin (positive control) and 13 out of 21 plant extracts had IC_{50} values < 80 µg/mL, against the sensitive leukemia CCRF-CEM cells. - Six out of the 13 active extracts showed IC_{50} values below 30 µg/mL. - *C. longa* methanol extract: IC_{50} = 6.25 µg/mL (HCT116 *p53*−/−) and IC_{50} = 10.29 µg/mL (MDA-MB-231-BCRP cells). - *L. esculentum* methanol extract: IC_{50} = of 9.64 µg/mL (MDA-MB-231 cells) and IC_{50} = 57.74 µg/mL (HepG2 cells). - *P. guajava*: IC_{50} value = 1.29 µg/mL (CEM/ADR5000 cells and IC_{50} = 62.64 µg/mL (MDA-MB-231 cells). - Each of the six plant extracts showed a degree of resistance (DR) < 1.00 in at least one type of malignant cells. The DR of all extracts were lower than that of doxorubicin in all cancer cells evaluated.	Mbaveng et al., (2018)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
15	Morocco	*Calendula arvensis* (flowers)	Hexane, methanol, water	Human cancer of myeloid cells	- Methanol extract was the most cytotoxic (IC_{50} value = 31 µg/mL), with maximum 89% inhibition at the concentration 100 mg/mL at 24 h ($p < 0.05$). - Methanol and aqueous extracts (flowers) had promising antimyeloid cancer efficacy.	Abudunia et al., (2017)
16	Morocco	*Ormenis eriolepis* (aerial parts)	n-Hexane, methanol	T lymphocyte cell line (Jurkat) Mantle cell lymphoma (Jeko-1) Glioblastoma (LN229) Prostate adenocarcinoma (PC-3)	- Hexanic extract showed high effect against Jurkat, Jeko-1, LN229, and PC-3 cells, but not against normal cells. - Hexanic extract showed IC_{50} values of 19.31 µg/mL (PC-3) and 41.67 µg/mL (LN229) and induced G1 (in Jurkat, Jeko-1, and LN22 cell lines) and G2/M (in PC-3 cell line) phases' cycle arrest.	Belayachi et al., (2017)
17	Ghana	*Aframomum melegueta* (seeds, roots/rhizome) *Alstonia boonei* (leaves, roots) *Baphia nitida* (leaves) *Desmodium adscendens* (leaves, stems) *Ficus asperifolia* (leaves, stem bark) *Mansonia altissima* (stem bark) *Paullinia pinnata* (stem) *Spathodea campanulate* (leaves, stem bark) *Terminalia superba* (leaves, stem bark, roots) *Triplochiton scleroxylon* (leaves, stem bark)	Ethanol–water (1:1)	Hepatic carcinoma (HepG2) Breast adenocarcinoma (MDA-MB-231 and MCF-7) Epidermoid carcinoma (A431) Prostate adenocarcinoma (LNCaP) Lung adenocarcinoma (A549) Gastric adenocarcinoma (AGS) Leukemia (HL-60 and REH) Ewing's sarcoma (CADO-ES1 and RDES)	- From all the plant extracts, only two decreased cell viability in cancer cell lines evaluated. - *A. boonei* extract (leaves) and *P. pinnata* extract (stems) showed IC_{50} values of about 50 µg/mL (IC_{50} = 42.7, 47.5, and 50.9 for leaves of *A. boonei* in A549, MCF-7, and LNCap cell lines, respectively; IC_{50} = 42.8, 43.1, 47.2, and 47.6 for stems of *P. pinnata* in HepG2, MCF-7, LNCap, and AGS-7 cell lines, respectively). - Preliminary TLC investigations showed oligomeric and polymeric proanthocyanidins as the predominant class of phytochemicals in the *P. pinnata* hydroethanolic extract. - The presence of 15-hydroxyangustilobine A (vallesamine-type indole alkaloid) was seen in the *A. boonei* extract and considered as the active principle responsible for the cell cycle arrest of MCF-7 cells at G2/M phase (MCF-7 cells), triggering cells at least partially into apoptosis.	Spiegler et al., (2021)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
18	Ethiopia	*Acmella caulirhiza* (leaves) *Acokanthera schimperi* (leaves) *Ajuga leucantha* (leaves) *Aloe debrana* (roots) *Cineraria abyssinica* (leaves) *Clausena anisate* (leaves) *Clematis simensis* (leaves) *Cleome brachycarpa* (leaves) *Croton macrostachyus* (bark) *Dorstenia barnimiana* (roots) *Euphorbia schimperiana* (roots) *Gnidia involucrate* (roots) *Hydrocotyle mannii* (leaves) *Kalanchoe petitiana* (leaves) *Kniphofia foliosa* (roots) *Leonotis ocymifolia* (leaves) *Pentarrhinum insipidum* (roots) *Rumex nervosus* (roots) *Salvia nilotica* (whole plant) *Sida schimperiana* (roots and leaves) *Thymus schimperi* (leaves) *Vernonia auriculifera* (leaves)	80% Methanol	Breast adenocarcinoma (MCF-7) Lung carcinoma (A427) Urinary bladder carcinoma (RT-4) Cervical adenocarcinoma (SiSo) Large cell lung carcinoma (LCLC-103H) Pancreatic carcinoma (DAN-G) Ovarian carcinoma (A2780) Esophageal squamous carcinoma (KYSE-70) Acute myeloid leukemia (HL-60) Human myeloid leukemia (U-937)	- A first screening was performed where extracts were tested at a concentration of 50 µg/mL towards four cancer cell lines (A427, MCF-7, RT-4, and SiSo). - Four out of 22 plant extracts (*A. schimperi, E. schimperiana, K. foliosa, and K. petitiana*) showed relevant cytotoxic activity and were selected for secondary screening against all the adherent and suspension cell lines. - *A. schimperi* showed potent cytotoxic activity towards all cell lines studied (IC_{50} values ranging from 1.87 to 10.31 µg/mL). - *E. schimperiana* showed potent cytotoxic activity against A427, SiSo, and RT-4 cell lines at concentrations ranging from 1.85 to 3.28 µg/mL. - IC_{50} values presented by *K. petitiana* extracts ranged from 2.09 to 10.41 µg/mL. - *K. foliosa* showed anti-proliferative effect in all cell lines, with IC_{50} values ranging from 14.54 to 27.06 µg/mL. - *E. schimperiana*, *A. schimperi*, *K. foliosa*, and *K. petitiana* extracts demonstrated selective cytotoxicity against suspension cell lines (HL-60 and U-937) compared to PBMC.	Tesfaye et al., (2021)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
19	Côte d'Ivoire	*Bridelia ferruginea* (leaves and stem barks)	Methanol, ethyl acetate, water	Colorectal carcinoma (HCT116)	- A lethality assay was conducted in *Artemia salina* to study the cytotoxicity of the *B. ferruginea* extract. LC50 value from brine shrimp assay was below 2 mg/mL. - Inhibition of HCT116 cell viability was seen in a concentration-dependent manner. - Inhibition of HCT116 cell viability induced by stem bark methanol extract could be related to its rich phenolic content (e.g., catechin fraction).	Mahomoodally et al., (2021)
20	Egypt	*Brassica nigra* (seeds)	50% (V/V) Ethanol	Human non-small cell lung carcinoma (A549 and H1299)	- *B. nigra* extract showed cytotoxic activity against A549 (IC_{50} = 32.02 µg/mL) and H1299 cell lines (IC_{50} = 25.38 µg/mL). - Apoptosis induced by *B. nigra* in a time- and concentration-dependent manner related to increased caspase-3 activity. - Treatment of A549 and H1299 cell lines with *B. nigra* extract result in significant S and G2/M phases' arrest of cell cycle ($p < 0.01$ and $p < 0.001$). - Suppression of migratory and invasive properties of A549 and H1299 by *B. nigra* extract. - *B. nigra* extract downregulated the expression of MMP2 and MMP9 and Snail, and upregulated expression of E-cadherin at mRNA and protein levels.	Ahmed et al., (2020)
21	Burkina Faso	*Lantana ukambensis* (whole plant)	Dichloromethane	Colorectal carcinoma (HCT-116 and HT-29)	- Significant cytotoxic effect towards HCT-116 (20 µg/mL) and HT-29 (80 µg/mL) cell lines was observed with *L. ukambensis* crude extract after 48 h of incubation ($p < 0.0001$). - IC_{50} values = 23.05 µg/mL and 106.81 µg/mL for HCT-116 and HT-29, respectively.	Sawadogo et al., (2020)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
22	Algeria	*Heliotropium bacciferum* (aerial parts)	Chloroform, methanol	Colorectal carcinoma (HCT116) Colorectal adenocarcinoma (DLD1)	- Evaluation of antiproliferative activity of *H. bacciferum* extracts (concentrations ranging from 1.000 to 0.025 mg/mL). - *H. bacciferum* chloroform extract showed an inhibitory effect on the growth of DLD1 (IC_{50} = 62 µg/mL) and HCT116 (IC_{50} = 95 µg/mL) cell lines in a concentration-dependent manner. - *H. bacciferum* methanol extract did not show any cytotoxic effect.	Aïssaoui et al., (2019)
23	Kenya	*Abrus precatorius* *Aeschynomene abyssinica* *Albizia gumífera*, *Aloe volkensii* *Bridelia micrantha* *Conyza sumatrensis* *Croton macrostachyus* *Cyphostemma serpens* *Entada abyssinica*, *Ficus thonningii* *Fuerstia africana*, *Futumia africana* *Harungana madagascariensis* *Ipomoea cairica* *Microglossa pyrifolia* *Momordica foetida* *Moringa oleífera*	Dichloromethane/ methanol (organic) and water	Sensitive and drug-resistant human cancer cell lines: Sensitive CCRF-CEM and multidrug-resistant P glycoprotein-overexpressing CEM/ADR5000; Wild-type HCT116 (*p53*+/+) and knockout HCT116 (*p53*−/−) colon cancer cells; Breast cancer cells transduced with control vector (MDAMB-231-pcDNA3) or with cDNA for the breast cancer resistance protein BCRP (MDA-MB-231-BCRP clone 23)	- Screening results: initially, 34 organic and 19 aqueous extracts tested. - Drug-sensitive CCRF-CEM and multidrug-resistant CEM/ADR5000 cells were inhibited by organic extracts (*H. madagascariensis* and *P. africana*) by more than 80%. - Some organic extracts were more cytotoxic to multidrug-resistant CEM/ADR5000 cells than to sensitive CCRF-CEM cells. - MDA-MB-231 cells exerted collateral sensitivity towards 4 organic extracts (*H. madagascariensis*, *Z. rubescens*, *B. micrantha*, *Z. gilletii*). - Organic extract of *H. madagascariensis* inhibited both wild-type and knockout cell lines by more than 80%. *B. micrantha* and *H. madagascariensis* organic extracts exerted the strongest cytotoxicity towards both U87.MG cell lines. - *P. africana* organic extracts showed the best cytotoxic activity, inhibiting the proliferation of 7 out of 8 tested cancer cell lines (IC_{50} < 40 µg/mL). - Combination treatments: some extracts exhibited enhanced cytotoxicity towards cancer cells, if applied in combination with other extracts.	Ochwang'I et al., (2018)

Table 4. *In vitro* cytotoxicity of extracts from medicinal plants collected from different countries in Africa against different cancer cell lines (Cont.).

Entry	Country	Medicinal Plants	Extraction Solvents	Cancer Cell Lines	Results	Reference
23	Kenya	*Ocimum gratissimum, Olea hotch* *Phyllanthus sapialis, P. fischeri* *Prunus africana* *Psydrax schimperiana* *Rotheca myricoides* *Senna didymobotyra* *Shirakiopsis elliptica, Sida rhombifolia* *Spathodea campanulate* *Synsepalum cerasiferum* *Tragia brevipes, Trichilia emetica* *Triumfetta rhomboidei, Vernonia lasiopus* *Zanthoxylum rubescens, Z. gilletii*	Dichloromethane/ methanol (organic) and water	Wild-type U87MG cells and U87MG glioblastoma multiforme cells transfected with an expression vector harboring an epidermal growth factor receptor (EGFR) gene with a genomic deletion of exons 2 through 7 (U87MG.ΔEGFR)		Ochwang'I et al., (2018)

Different assays were performed to evaluate the cytotoxicity effects of the studied African plant extracts, including a crystal violet cell antiproliferation assay (Tesfaye et al., 2021), resazurin reduction assay (Mbaveng et al., 2019; Mbaveng et al., 2018; Mbaveng et al., 2021; Mbosso Teinkela et al., 2018; Ochwang'I et al., 2028), or tetrazolium-based colorimetric cell proliferation assays such as MTT (Abudunia et al., 2017; Ahmed et al., 2020; Aïssaoui et al., 2019; Izuegbuna et al., 2019; Mahomoodally et al., 2021; Mfotie Njoya et al., 2017; Motadi et al., 2020; Serala et al., 2021; Soyingbe et al., 2018; Spiegler et al., 2021; Tesfaye et al., 2021), MTS (Sawadogo et al., 2020) and WST-1 (Makhafola et al., 2020). There were studies where cell numbers were determined using Hoechst 33342/Propidium Iodide staining (Mfengwana et al., 2019; Unuofin et al., 2018), trypan blue (Potestà et al., 2019), or were based on the quantification of ATP, signaling the presence of metabolically active cells (Belayachi et al., 2017; Zonyane et al., 2020).

According to the American National Cancer Institute USA (NCI), the criteria of cytotoxicity activity for botanicals/crude extracts is IC_{50} < 20 µg/mL or 10 µM upon 48 h or 72 h incubation (Boik, 2001). The NCI considers an IC_{50} upper limit criteria of 30 µg/mL as a promising crude extract for purification (Suffness & Pezzuto, 1990). However, other authors such as Ayoub et al. (2014) consider that higher values and a plant extract could be effective as being cytotoxic at concentrations up to 100 µg/mL (Ayoub et al., 2014). According to results showed in Table 4, some extracts were active below 100 µg/mL, therefore, they can be considered promising sources for the development of novel anticancer drugs.

Regarding plants collected in South Africa, some extracts showed IC_{50} values lower than 100 µg/mL. For example, in the study of Makhafola et al. (2020), the crude extract of *C. orbiculata* decreased cell viability in a dose-dependent manner of HCT116 and KYSE-70 cell lines with LC_{50} values of 64.9 and 36.9 µg/mL, respectively, showing that the KYSE670 esophageal cancer cell line was most susceptible to the extract (Makhafola et al., 2020) (Entry 5, Table 4). In addition, Mfengwana et al. (2019) showed that *A. laricinus* methanol extract (IC_{50} value of 97.6 µg/mL) and *S. asperulus* dichloromethane extract (IC_{50} value of 69.15 µg/mL) showed cytotoxic activity against MCF-7 cancer cells (Mfengwana et al., 2019) (Entry 6, Table 4). Finally, acetone extract from *C. dentata* significantly ($p \leq 0.05$) revealed IC_{50} values of 41.55, 45.13, 57.35, and 43.24 µg/mL against A549, HeLa, CaCo-2, and MCF-7 cell lines, respectively. The acetone extracts from *W. salutaris* showed an IC_{50} value of 34.15 µg/mL against the MCF-7 cell line (Soyingbe et al., 2018) (Entry 8, Table 4). However, some plant crude extracts from South Africa revealed higher IC_{50} values. The studies performed by Serala et al. (2021) (Entry 1, Table 4) and Motadi et al. (2020) (Entry 3, table 4) with *M.*

balsamica and *T. violacea* extracts, respectively, do not determine the exact IC_{50}, but indicate that the extracts affect the viability of the tumoral cell lines in a dose-dependent manner up to the highest concentrations tested (50 and 20 µg/mL, respectively). Other studies revealed IC_{50} values higher than 100 µg/mL. For example, in the study of Zoyane et al. (2020) with *S. frutescens* extracts produced from plants growing at different geographic localities in South Africa, IC_{50} values confirmed the relatively higher potency of plants from Colesburg, Zastron, and Gansbaai 1 (158.7, 172.7, and 176.7 µg/mL, respectively) (Entry 2, Table 4). Furthermore, in the study of Izuegbuna et al. (2019), the acetone-dried extract of *O. stricta* showed mild cytotoxicity (IC_{50} = 110.1 µg/mL) (Entry 4, Table 2), and Unuofin et al. (2018) showed IC_{50} values for aqueous and ethanol extracts of *K. africana* > 200 µg/mL (Entry 7, Table 4).

For plants collected in Cameroon, IC_{50} values below or around 30 µg/mL were shown in nearly all the studies analyzed. Mbaveng et al. (2021) evaluated the cytotoxicity of the fruit's crude extract obtained from *T. tetraptera* on different cancer cell lines. The crude extract displayed IC_{50} values below 20 µg/mL on seven out of nine cancer cell lines tested. The IC_{50} values obtained varied from 10.27 µg/mL (in CCRF-CEM leukemia cells) to 23.61 µg/mL (against HCT116 *p53−/−* cancer colon cells) (Mbaveng et al., 2021) (Entry 9, Table 4). Mbaveng et al. (2019) also determined the cytotoxicity of the *F. tessmannii* methanol bark extract, after 72 h incubation, in seven cancer cell lines. IC_{50} values be-low 20 µg/mL were recorded with the same crude extract in three cancer cell lines (MDA-MB-231-pcDNA, IC_{50} = 19.43 ± 0.88 µg/mL; MDA-MB-231-BCRP, IC_{50} = 18.87 ± 1.16 µg/mL; U87MG. Δ EGFR, IC_{50} = 17.34 ± 1.37 µg/mL) (Mbaveng et al., 2019) (Entry 10, Table 4). In a previous study of Mbaveng et al. (2018), *C. longa* rhizomes and *L. esculentum* leaves displayed IC_{50} values below 20 µg/mL in most cancer cell lines evaluated (Entry 14, Table 4). Additionally, Mbosso Teinkela et al. (2018) showed IC_{50} values of 20 µg/mL for the methanol extract of *S. vogelii* leaves and the wood methanol extract of *F. elastica* aerial roots (Entry 11, Table 4). Mfotie Njoya et al. (2017) also found IC_{50} values below 30 µg/mL on MCF-7 and HeLa cells for the *S. pobeguinii* methanol extracts from leaves and bark (Entry 12, Table 4). Finally, Potestà et al. (2019) studied the antiproliferative activity of boiled and frozen aqueous extracts from *M. oleifera*, a well-known species used for medicinal and nutritional purposes. Both preparations, boiled and frozen, showed EC_{50} values ranging between 10 and 20 µg/mL for THP1 leukemia cells. For Jurkat cells, the boiled preparations had EC_{50} values over 100 µg/mL, although the EC_{50} for the frozen extracts ranged between 1 and 11 µg/mL, showing an interesting antiproliferative and cytotoxic effect (Potestà et al., 2019) (Entry 13, Table 4).

In the two studies performed with plants from Morocco, a methanol extract of *C. arvensis* (flowers) was the most significant anti-myeloid cancer agent, showing an IC_{50} value of 31 µg/mL (Abudunia et al., 2017) (Entry 15, Table 4). Similar IC_{50} values were showed in the study of Belayachi et al. (2017) for PC-3 (19.31 ± 4.88 µg/mL) and for LN229 cells (41.67 ± 1.98 µg/mL) upon treatment with the hexane extract of *O. eriolepis* (Entry 16, Table 4).

For plants collected in other African countries (e.g., Ethiopia, Egypt, Burkina Faso, and Kenya), IC_{50} values up to 30 µg/mL were also seen. Tesfaye et al. (2021) showed the potent activity of two plants out of 22 collected in Ethiopia against all 10 cell lines evaluated, namely *A. schimperi* extract (80% methanol), with IC_{50} values from 1.87 ± 0.4 to 10.31 ± 3.45 µg/mL, and *K. petitiana* extracts which had IC_{50} values from 2.09 ± 0.43 to 10.41 ± 5.59 µg/mL (Entry 18, Table 4). Moreover, *B. nigra* extract, collected from Egypt and evaluated in the study of Ahmed et al. (2020), showed IC_{50} values of 32.02 and 25.38 µg/mL against the A549 and H1299 cell lines, respectively (Entry 20, Table 4). Sawadogo et al. (2020) showed for the *L. ukambensis* crude extract (plant collected from Burkina Faso) an IC_{50} value of 23.05 ± 1.56 µg/mL in the HCT-116 cell line (Entry 21, Table 4). Finally, in the study of Ochwang'I et al. (2018) performed with 35 medicinal plants from Kenya, the highest concentration tested (40 µg/mL) showed an IC_{50} value of 30 µg/mL. For example, the *P. africana* organic extracts showed the best cytotoxic activity, inhibiting the proliferation in seven out of eight tested cancer cell lines (IC_{50} < 40 µg/mL) (Entry 23, Table 4). However, higher IC_{50} values were also seen in other studies. For example, Spiegler et al. (2021) evaluated the hydroethanolic extracts from ten plants collected in Ghana and only the leaf extract from *A. boonei* and the stem extract of *P. pinnata* showed IC_{50} values around 50 µg/mL in some of the cancer cells tested (Entry 17, Table 4). Mahomoodally et al. (2021) studied the pharmacological potential of *B. ferruginea*, collected from Côte d'Ivoire and found an antiproliferative effect against the HCT116 cell line in a concentration-dependent manner up to 200 µg/mL (Entry 19, Table 4). Aïssaoui et al. (2019) described the cytotoxic effects in cancer cell lines of extracts from *H. bacciferum*, a plant collected in Algeria. In this study, *H. bacciferum* chloroform extract showed a concentration-dependent inhibitory effect on the growth of the treated cancer cell lines with IC_{50} values of 95 µg/mL on HCT116 and 62 µg/mL on DLD1 (Entry 22, Table 4).

An ideal anticancer agent should have more of a cytotoxic effect on cancer cell lines than in noncancer cells. Therefore, high selectivity towards cancer cells is a desired property for these agents. In some plants referred in Table 4, this preliminary selectivity was observed. In the study of Mfengwana et al. (2019) with two South African plants, the dose-dependent cytotoxicity effect of the methanol extract of *A. laricinus* and the dichloromethane extract of

S. asperulus were shown to be selective to the MCF-3 and PC3 cell lines, with a negligible effect on Vero cells (Entry 6, Table 4).

Regarding the results from plants collected in Cameroon, Potestà et al. (2019) showed that particularly boiled *M. oleifera* extract showed a specific anti-proliferative activity on cancer cells, but not on the Peripheral Blood Mononuclear Cell (PBMC) (Entry 13, Table 4). In addition, the results of Mfotie Njoya et al. (2017) demonstrated that the methanol extract from leaves of *S. pobeguinii* was selectively cytotoxic to cancer cell lines compared to the normal Vero cells, with the Selectivity Index ranging from 3.15 to 18.28 on the four cancer cells lines (MCF-7, HeLa, Caco-2, and A549), suggesting the potential and antiproliferative effect of this extract (Entry 12, Table 4). Furthermore, in the case of some plants collected from nine districts in Ethiopia, Tesfaye et al. (2021) demonstrated that *E. schimperiana*, *A. schimperi*, *K. foliosa*, and *K. petitiana* extracts (80% methanol) had selective cytotoxicity against suspension cell lines (U-937 and HL-60) when compared to their effect on PBMC. The IC_{50} value of all extracts against PBMC was > 50 μg/mL (Tesfaye et al. 2021) (Entry 18, Table 4).

2.3.3.2.1. Mechanisms of action

Anticancer effects of plants are related to the suppression of cancer-stimulating enzymes, repairing DNA, stimulating the production of antitumor enzymes in cells, increasing body immunity, and inducing antioxidant effects (Kooti et al., 2017). Novel therapeutic strategies against cancer will mediate the induction of the apoptosis pathway or cell cycle arrest. Caspases activation, an increase in ROS production, and matrix metalloproteinases (MMP) disruption have been involved in the induction of apoptosis of several botanicals from African flora (Motadi et al., 2020). Apoptosis is the process of programmed cell death, triggered by physiological and pathological stimuli, in which a cell dies as part of its normal process of development, when the immune system has ordered it to die, or due to a lack of growth factors. However, cancer cells can evade apoptosis, continuing to proliferate and even become resistant to chemotherapy (Mfengwana et al., 2019). Apoptosis has become the most investigated mode of action of cytotoxic drugs and involves an energy-dependent cascade of molecular events. For example, caspases activation and the loss of mitochondrial mem-brane potential are events involved in apoptosis (Mbaveng et al., 2019). In this section, the mechanisms of action described in the previous African plant studies are discussed.

Regarding the plants collected in South Africa, Motadi et al. (2020) showed the involvement of *T. violacea* hexane extracts in the induction of apoptosis, caused by the activation of *p53*,

which can be related to the solvent used in the extraction procedure (Motadi et al., 2020) (Entry 3, Table 4). In some studies, caspase activity has been shown to be activated in methanol extracts (Badmus et al., 2015; Narrima et al., 2014). However, Motadi et al. (2020) observed an increased caspase-3/7 activity, especially in cervical cancer cells treated with the hexane extract of *T. violacea* (Motadi et al., 2020) (Entry 3, Table 4). Moreover, Makhafola et al. (2020) concluded that *C. orbiculata* aqueous extract had an anti-proliferative effect in HCT116, KYSE-70, and OE33 cancer cells, mediated by apoptosis induced by the alternative splicing of hnRNPA2B1 (an RNA-binding protein) and BCL2L1 (an important apoptosis-regulating gene) (Makhafola et al., 2020) (Entry 5, Table 4). It is also known that hnRNPA2B1 plays a significant role in cancer progression, acting as an oncogene in the development of certain types of cancers (Hu et al., 2017). Finally, Mfengwana et al. (2019) observed that *A. laricinus* methanol extract shows cytotoxic effects in MCF-7 cancer cells due to apoptosis when compared with a negative control (medium only) and positive control (melphalan). The authors also found that *S. asperulus* dichloromethane extracts showed cytotoxicity against prostate PC3 cancer cells (IC_{50} values of 69.25 µg/mL) due to cell cycle arrest at the G2 and early mitotic (G2/M) phases (Mfengwana et al., 2019) (Entry 6, Table 4).

In the studies performed with plants from Cameroon, Mbaveng et al. (2021) showed that doxorubicin induced S and G2/M phase cycle arrest in CCRF-CEM cells, whilst crude extract of *T. tetraptera* fruits induced it in the G0/G1 phase. The crude extract induced apoptosis in those cells through MMP alteration and by increasing ROS production (Mbaveng et al., 2021) (Entry 9, Table 4). In another study, Mbaveng et al. (2019) demonstrated that *F. tessmannii* methanol bark extract induced apoptosis in CCRF-CEM cells (Mbaveng et al., 2019) (Entry 10, Table 4). Additionally, in a previous study, Mbaveng et al. (2018) showed that *C. longa* rhizomes, *P. guajava* bark, and *L. esculentum* leaves induced apoptosis via caspase activation and increased ROS generation in CCRF-CEM cells. Additionally, *C. longa* rhizomes and *P. guajava* bark induced mitochondrial membrane potential depletion, which contributed to apoptosis induction too (Mbaveng et al., 2018) (Entry 14, Table 4).

The bioassay-guided fractionation of the *A. boonei* extract, from Ghana, performed by Spiegler et al. (2021), revealed the presence of an alkaloid (15-hydroxyangustilobine A), which, at concentrations ≥ 60 µM, caused an increase in the number of cells in the G2/M phase, which was higher than cells in apoptosis at the same concentration (Spiegler et al., 2021) (Entry 17, Table 4). Moreover, caspase-3 kinetic activity increased in A549, and H1299 cancer cell lines treated with *B. nigra* extract in a time-dependent manner, suggesting that the plant extract collected in Egypt has the ability to induce apoptosis (Ahmed et al., 2020). Ahmed et al.

(2020) also showed that *B. nigra* ethanolic extract significantly delayed and arrested, at the S and G2/M transition, both A549 and H1299 cells in a concentration-dependent manner (Ahmed et al., 2020) (Entry 20, Table 4).

Cancer metastasis is a multi-cascade process involving distinct stages: cell migration, invasion, attachment, and angiogenesis (Li et al., 2018). Extracellular matrix degradation is considered the most crucial step of the metastatic process, being facilitated by MMPs (Zhang et al., 2014) Their expression can also be stimulated by proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukins (IL)-1β, and IL-6 (Chou et al., 2010). In consequence, inhibiting the expression and/or activity of MMP2 and 9 could represent an essential role in the inhibition of cancer metastasis. Serala et al. (2021) showed that *M. balsamina* (a plant collected in South Africa) downregulated, in a significant manner, the expression of MMP2 and MMP9, confirming, therefore, their role in the HT-29 cells invasiveness reduction. *M. balsamina* also downregulated the expression of TNF-α and NF-κB proteins, which suggests that the *M. balsamina* extract might inhibit MMP2 and MMP9 expression at a transcriptional level in addition to inhibiting MMP9 activity through tissue inhibitors, namely TIMP-3 (Serala et al., 2021) (Entry 1, Table 4). Furthermore, the potential migration and invasion inhibitions by the ethanolic extract of the Egyptian *B. nigra* was also evidenced by the downregulation of MMP2, MMP9, and Snail genes, and the upregulation of the E-cadherin gene (hallmark of cancer metastasis) (Ahmed et al., 2020) (Entry 20, Table 4).

Several African medicinal plants have been reported as having antiproliferative properties against MDR cancer cells, which is interesting since novel drugs with activity against tumors that do not respond to established anticancer drugs are urgently needed. Therefore, Ochwang'I et al. (2018) analyzed the ability of plant extracts towards drug resistance mediated by tumor suppressor TP53 functional loss or by the mutational activation of the EGFR oncogene. Results showed collateral sensitivity, where some organic (F. africana, *S. cerasiferum*, *M. pyrifolia*, and *B. micrantha*) and aqueous extracts (*H. madagascariensis*, and Z. gilletii) presented higher cytotoxicity to multidrug-resistant CEM/ADR5000 cells than to sensitive CCRF-CEM cells (Ochwang'I, et al., 2018) (Entry 23, Table 4). Mbaveng et al. (2018) have shown that some Cameroon extracts studied (*C. pachycarpa*, *C. longa*, *L. esculentum*, *P. guajava*, *P. americana*, and *P. peruviana*) could be used against MDR cancer cell lines (Mbaveng et al., 2018) (Entry 14, Table 4). A recent study from Mbaveng et al. (2021) continued to use various models of resistant cancer cells. The hypersensitivity of MDA-MB-231-BCRP and U87MG.ΔEGFR cells compared to their sensitive congeners (MDA-MB-231-pcDNA and U87MG) was observed for

the crude extract of *T. tetraptera* (degree of resistance of 0.28 and 0.63, respectively (Mbaveng et al., 2021) (Entry 9, Table 4).

2.3.3.2.2. Effect of the extract solvents

It is recognized that the choice of the solvent used in an extraction defines the extract's chemical profile and, potentially, influences its cytotoxic effects. In fact, different solvents were used in the studies, especially methanol, water, ethanol, and hexane, with mixed results. Water, taking into consideration its availability, appears as the preferred solvent used by most traditional healers. However, water only extracts polar bioactive compounds. In some studies, performed with South African plants, Soyingbe et al. (2018) showed that aqueous extracts of *C. asiatica*, *W. salutaris*, and *C. dentata* (leaves) were the least active, with IC_{50} values > 100 µg/mL (A549, Caco-2 and MCF-7) and 76.3 ± 0.06 µg/mL (HeLa) (Soyingbe et al., 2018) (Entry 8, Table 4). Comparable results were seen by Unuofin et al. (2018), where *K. africana* aqueous extract presented no significant decrease in cell number in all the studied concentrations (50–200 µg/mL) (Unuofin et al., 2018) (Entry 7, Table 4). For that reason, many authors worked with other solvents to increase the extraction of compounds of varying polarities.

Solvents less predominant in the studies such as ethyl acetate and 1-butanol may have polar molecules, including saponins and polyphenols such as tannins, anthocyanins, phenolic acids, flavonoids, and stilbenes, which are recognized as having preventive and curative anticancer benefits (Sawadogo et al., 2020). Methanol is also an example of a solvent used for most of the authors in Table 4. In some cases, methanol was the most active, as compared to other extracts such as ethyl acetate or aqueous extracts. However, we must consider the plant extract used. For example, in their study, Mbaveng et al. (2021) concluded that a suitable extraction solvent, such as a dichloromethane-methanol (1:1) mixture should be considered if using the fruits of *T. tetraptera* as a cytotoxic agent (Mbaveng et al., 2021) (Entry 9, Table 4). Mahomoodally et al. (2021) suggested the polar extracts from *Bridelia* species as good candidates for future studies aiming to explore *in vivo* anticancer activity (Mahomoodally et al., 2021) (Entry 19, Table 4). Motadi et al. (2020) showed that the methanol extract of *T. violacea* (leaves) showed the highest cytotoxicity of the extracts (Motadi et al., 2020) (Entry 3, Table 4). However, the opposite was observed by Aïssaoui et al. (2019) who demonstrated that *H. bacciferum* methanol extract did not show any cytotoxic effects (Entry 22, Table 4).

The diverse biological properties of plant extracts can also be due to its chemical composition variability. Regarding the studies performed with plants from South Africa, for Zonyane et al. (2020), it is clear that anticancer activity shown for extracts of *S. frutescensare* is not necessarily strongly correlated to the flavonoids. Some other important chemicals found in that extract include sutherlandins isomers, triterpenoids, and cycloartenol glycosides (sutherlandiosides) (Zonyane et al., 2020) (Entry 2, Table 4). In the study performed by Izuegbuna et al. (2019), polyphenols (phenols, flavonoids, flavonols, proanthocyanidins, tannins) were the major compounds found in the several *O. stricta* extracts. However, the non-cytotoxic effect of some extracts of *O. stricta* cladodes against U937 cells may be explained by an insufficient amount of compounds (Izuegbuna et al., 2019) (Entry 4, Table 4). Finally, Soyingbe et al. (2018) showed that *C. asiatica* acetone extract had the most significant activity (IC_{50} = 46.49 ± 0.04 μg/mL) for A549 cells (Entry 8, Table 4). In a study by Naidoo et al. (2017), leukemic THP-1 cells' viability was not significantly altered by *C. asiatica* ethanolic leaf extract (0.2–0.8 mg/mL) as compared to the control.

Analyzing the studies related to plants collected in Cameroon, Mbaveng et al. (2021) showed that the active compounds of the dichloromethane-methanol (1:1) extract of *T. tetraptera* include betulinic acid, naringenin, luteolin, 3-*O*-[6′-*O*-undecanoyl-*β*-D-glucopyranosyl]stigmasterol, ole-an-12-en-3-β-O-D-glucopyranoside, 3-O-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosylurs-12-en-28-oic acid, and 3-O-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-27-hydroxyolean-12-en-28-oic acid (Entry 9, Table 4). In addition, Mfotie Njoya et al. (2017) found the methanol extract from *S. pobeguinii* leaves and methanol extract from *S. pobeguinii* bark presented a high amount of alkaloids in comparison to the two other extracts (methanol extract from roots and CH_2Cl_2/MeOH extract from fruits) and this family of secondary metabolites can also be responsible for the cytotoxic effects (Mfotie Njoya et al., 2017) (Entry 12, Table 4). In fact, several alkaloids isolated from plants are described as having anticancer effects. For example, the evidence based on *in vivo* and *in vitro* models indicated that isoquinoline alkaloids and/or isoquinoline-enriched plants exert significant anti-cancer effects through cell cycle arrest, apoptosis, and autophagy (Yun et al., 2021) Finally, in the study performed by Mbaveng et al. (2019) with *F. tessmannii*, among the different phytochemicals found, it appeared that only benphenanthridines presented cytotoxic effects. Regarding benzophenandrines, the presence of chloride appeared to significantly increase the cytotoxicity. Additionally, the cytotoxic effect may have been influenced by the presence of 8-OH (hydroxyl) and 7-OCH_3 (methoxy) groups instead of two

methoxy groups in C-8 and C-9, within the two chloride-containing benzophenanthridines (Mbaveng et al., 2019) (Entry 10, Table 4).

In traditional medicine, plants are used as mixtures and not as single plants. Therefore, Ochwang'I et al. (2018) studied whether the combination of plant extracts would conduct to increased cytotoxicity against cancer cells. The authors selected aqueous extracts from Kenyan plants, which revealed poor cytotoxicity alone (*H. madagascariensis*, *Spathodea S. campanulate*, *P. africana*), and combined them with other extracts. The combined extracts exhibited stronger cytotoxic effects towards CRRF-CEM (Ochwang'I et al., 2018) (Entry 23, Table 4).

2.4. Objectives

As stated in the Chapter 1 and Chapter 2, in many African countries, primary health care relies on traditional plants. However, many valuable medicinal species remain unstudied as is happening in Angola, one of the richest floristic regions of the world. Ethnobotanical and ethopharmacological studies represent an important approach for applying traditional knowledge of plant use to modern societies, with the final aim of developing new drugs. Therefore, the general objective of this work is:

- To evaluate the potential pharmacological activity of medicinal plants from the Province of Cuanza Norte (Angola).

With this aim, the following specific objectives were proposed:

- To identify and document medicinal plants and detailed traditional knowledge on herbal preparations from the Province of Cuanza Norte (Angola).
- To document ethnobotanical studies from the Provinces of Angola.
- To evaluate pharmacological activities of extracts prepared from selected medicinal plants, namely antioxidant, antibacterial and cytotoxic properties.

2.4. Objetivos

Como se indica en los capítulos 1 y 2, en muchos países africanos la atención primaria de salud se basa en las plantas tradicionales. Sin embargo, muchas especies medicinales valiosas permanecen sin estudiar, como ocurre en Angola, una de las regiones florísticas más ricas del mundo. Los estudios etnobotánicos y etnofarmacológicos representan un enfoque importante para aplicar los conocimientos tradicionales sobre el uso de las plantas a las sociedades modernas, con el objetivo final de desarrollar nuevos fármacos. Por ello, el objetivo general de este trabajo es:

- Evaluar la actividad farmacológica potencial de plantas medicinales de la Provincia de Cuanza Norte (Angola).

Con este fin, se propusieron los siguientes objetivos específicos:
- Identificar y documentar las plantas medicinales y el conocimiento tradicional detallado sobre preparados herbales de la Provincia de Cuanza Norte (Angola).
- Documentar los estudios etnobotánicos de las provincias de Angola.
- Evaluar las actividades farmacológicas de los extractos preparados a partir de las plantas medicinales seleccionadas, en concreto las propiedades antioxidantes, antibacterianas y citotóxicas.

CHAPTER 3

MATERIAL AND METHODS

3.1. Ethnopharmacological study of medicinal plants from the Province of Cuanza Norte (Angola)

To achieve the first specific objective of the work, we perform an ethnopharmacological study of medicinal plants from the Province of Cuanza Norte (Angola), with the following procedures. Thus, we identify and document medicinal plants and detailed traditional knowledge on herbal preparations.

3.1.1. Study area and demographic background

The present study was carried out in the capital N'dalatando (Cazengo), Province of Cuanza Norte (Angola), which belongs to the five Provinces with the lowest population densities in Angola, with a population of about 443.386 inhabitants (300.258 inhabitants livind in urban areas and 143.028 inhabitants living in rural areas) and covering 24.190 km² (Censo, 2016).

3.1.2. Sampling of informants

The field work was conducted during a period of two months (from December 2018 to January 2019) and the ethnobotanical data were collected using semi-structured interviews. Informants were selected through the “snowball” technique (Albuquerque et al., 2010), and a total of 12 traditional informants (6 men and 6 women), agreed to be part of the study and were interviewed independently to avoid bias. The age-group of the informants was between 39 to 69 years old. Informants have lived in N'dalatando since their childhood and were recognized by other members of the community as having traditional botanical experience as healers.

3.1.3. Ethnomedicinal data collection

The survey was conducted through semi-structured, open-ended interviews, in order to preserve the spontaneity of the information. Interviews were held based on a checklist of questions designed to collect data on (i) local names of the plants, (ii) ailments treated by the plants, (iii) used plant part(s), and (iv) preparation methods (Annex III). All species cited for therapeutic purposes were considered in the study. During guided field walk, photographic records were also taken to capture the field sites, plants, and other useful memories. Informants were also informed that the objectives of the research were not for commercial purposes but for academic purposes and all interviews were performed after obtaining voluntary consent from each informant.

3.1.4. Data analysis

The citations for therapeutic purposes were classified in different categories based on the International Classification of Diseases by WHO (WHO, 2019): infectious and parasitic diseases; neoplasms; diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism; endocrine, nutritional and metabolic diseases; mental and behavioural disorders; diseases of the nervous system; diseases of eye and adnexa; diseases of the ear and mastoid process; diseases of the circulatory system; diseases of the respiratory system; diseases of the digestive system; diseases of the skin and subcutaneous tissue; diseases of the musculoskeletal system and connective tissue; diseases of the genitourinary system; pregnancy, childbirth and puerperium; certain conditions originating in the perinatal period; symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified; injury, poisoning and certain other consequences of external causes.

3.2. A Literature review of ethnobotanical studies in Angola (Africa)

To achieve the second specific objective of the work, we perform a literature review based on ethnobotanical studies described for the Provinces of Angola, in order to compare our results with other similar studies. A literature review search was carried out in PubMed, ScienceDirect, and Google Scholar databases, with no data restriction, in Portuguese and English languages. Ethnobotanical studies which collected and analyzed information, based on field work and on different traditional medicinal uses of plants from municipalities and provinces of Angola, were included. Studies regarding specific plant species/botanical families; studies focusing only on plants used in managing animal health; studies only based on the ethno-veterinary and/or fodder plants; and studies which collect only the knowledge of non-food and non-medicinal plants were excluded.

3.3. *In vitro* antioxidant, antibacterial and cytotoxicity activities of three medicinal plants from Angola: *Adansonia digitata, Garcinia kola,* and *Gardenia ternifolia*

To achieve the third specific objective of the work, we selected three African medicinal plants, identified, and documented by the population of N'dalatando (Cazengo), Province of Cuanza Norte (Angola), and we evaluated the antioxidant, antibacterial and cytotoxic activities.

3.3.1. Chemicals and Reagents

Sodium carbonate, ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate, dichloromethane, ethyl acetate, sodium carbonate, sodium nitrite, and iron sulphate (II) were obtained from VWR Chemicals (Leuven, Belgium). Sodium hydroxide was obtained from VWR Chemical (Ohio, USA). Hydrogen peroxide was obtained from Panreac Química SLU (Barcelona, Spain). Ethyl acetate, nutrient broth, and Mueller-Hinton broth (MHB) were obtained from VWR Chemical (Fontenay-Sous-Bois, France). Dimethyl sulfoxide (DMSO) was obtained from Fisher Chemical (Geel, Belgium). Gallic acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), quercetin, sodium chlorite, 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt hydrate (FerroZine™ Iron Reagent), Folin–Ciocalteu, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), clindamycin hydrochloride, resazurin sodium salt, ferric chloride, and Eagle's Minimum Essential Medium (MEM) were purchased from Sigma-Aldrich (St. Louis, USA). Ethanol and hexane were obtained from Fisher Chemical (Waltham, USA). Mueller-Hinton Agar (MHA) was obtained from HiMedia Laboratories (Mumbai, India). Agar was obtained from Labchem (Zelienople, USA). Potassium persulfate was obtained from Biochem Chemopharma (Cosne-Cour-sur-Loire, France). Other chemicals used were of analytical grade.

3.3.2. Plant material

The three plants used in this study were collected in the capital N'dalatando (Cazengo), Province of Cuanza Norte (Angola), from December 2018 to January 2019. Identification of collected plant specimens was performed by specialists, and authenticated plant voucher specimens were deposited (*Garcinia kola* Heckel - n. º (LUAI): 5199; *Gardenia ternifolia* Schumach. & Thonn - n. º (LUAI): 8285; *Adansonia digitata* Linnus - n. º (LUAI): 5023) (Annex IV), as future reference material, at the herbarium of Agostinho Neto University, Luanda (Angola). The plant parts used in this study were fruits from *A. digitata*, seeds from *G. kola* and aerial parts (leaves and stems) *G. ternifolia*.

3.3.3. Preparation of plant extracts

All three freshly collected plants were washed under running tap water, air-dried, and ground using an electric mill to a moderate fine powder (particle size ≤ 0.5 mm). Powdered

material of the different plant parts was submitted to extraction procedures with sequential organic solvents in the increasing polarity order.

Briefly, 10 g of each powder was macerated separately in 100 mL hexane (1:10) with intermittent shaking, at room temperature, for at least 20 h. Then, they were filtered through Whatman no1 filter paper. The residue was further extracted two times using the same fresh solvent and all the filtrates were pooled together. The resulting residue was air dried and further extracted with dichloromethane, followed by ethyl acetate, methanol, methanol-water (80:20 V/V), and boiling water, similarly to the procedure carried out for the hexane extraction. Each filtrate (excluding the aqueous) was concentrated to dryness in a rotary evaporator (VWR, Ika RV8) under reduced pressure and controlled temperature (40 °C) and freeze-dried (FreeZone 4.5 liter benchtop freeze dry system, LabConco®). The lyophilized and freeze-dried extracts were weighed and stored at -20 °C for further analysis.

The yield (%) was calculated using the formula: % yield = W1/W2, where W1 is the weight of extract and W2 is the weight of powder used for extraction. For the different assays performed, methanolic and aqueous extracts were selected.

3.3.4. Parameters related to antioxidant activity

The antioxidant activity of methanolic and aqueous extracts of the three selected plants was measured by the DPPH, ABTS, H_2O_2 and ferrozine assays. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were also determined.

3.3.4.1. Total Phenolic Content determination

The TPC of the samples was determined by using the Folin–Ciocalteu method described by Singleton et al. (1999) with minor modifications. This method involves the reduction of the Folin-Ciocalteu reagent with phenolic compounds in an alkaline medium, which ia accompanied by the formation of a blue-colored complex that has a maximum absorbance at 765 nm (Lamuela-Raventós, 2018). Briefly, 500 µL of the plant extracts (1 mg/mL) was mixed with 2.5 mL Folin–Ciocalteu reagent (1:10 V/V) and 2.5 mL of Na_2CO_3 (7.5% w/V). The mixture was incubated at 50 °C for 10 min with agitation. Afterwards, the sample was cooled at room temperature for 30 min, and the absorbance was measured using a UV/Vis Spectrophotometer (Jenway, 6300) at 765 nm, against a blank without extract (solvent, Folin–Ciocalteu reagent and Na_2CO_3 solution). The calibration curve was prepared using gallic acid as a reference standard in order to obtain a correlation between the sample and standard

concentration (linearity range = 5 – 100 mg/mL; R^2 = 0.9982). The concentration of gallic acid in each extract was calculated from the regression equation using its absorbance. Finally, the results were converted to the TPC as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g) using the following equation: $C = C1 \times \frac{V}{m}$, where C is TPC in mg/g, in GAE (gallic acid equivalent), C1 is the concentration of gallic acid established from the calibration curve in mg/mL, V is the volume of the extract in mL, and m is the weight of the plant extract in grams.

3.3.4.2. Total Flavonoid Content determination (TFC)

The TFC was determined using a colorimetric method performed by Dewanto et al. (2002) with minor modifications described by Heimler et al., (2005). Briefly, to 0.25 mL of the samples (1 mg/mL), 75 µL of $NaNO_2$ solution (5%), 0.150 mL of a freshly prepared $AlCl_3$ solution (10%), and 0.5 mL of NaOH solution (1 M) were added to the mixture. The volume was adjusted to 2.5 mL with deionized water and mixed thoroughly using a vortex. The mixture was incubated at 25 ºC for 5 min, and the absorbance was measured at 510 nm, using a UV/Vis Spectrophotometer (Jenway, 6300), against the same mixture, without the sample, as a blank. The TFC of the sample was expressed as mg of quercetin equivalents (QE) per gram of sample, through the calibration curve of quercetin (R^2 = 0.9916) and using the following equation: C=C1 × V/m , where C is TFC in mg/g, in QE, C1 is the concentration of gallic acid established from the calibration curve in mg/mL, V is the volume of the extract in mL, and m is the weight of the plant extract in grams.

3.3.4.3. DPPH radical scavenging activity assay

The assay was performed according to Lima et al. (2007). Briefly, after addition of different concentrations of extracts (5-1000 µg/mL) to DPPH (90 µM), the percentage of remaining DPPH was determined at different times from the absorbance at 515 nm using a plate reader spectrophotometer (Dynex Technologies MRX II Microplate Reader). As the reaction reached steady state after 1 hour, the percentage of scavenged DPPH was calculated using equation: DDPH scavenging activity (%) = 100 × (Ac – As)/Ac, where Ac is the absorbance of the control and As is the absorbance of the sample. Quercetin was used as a positive control.

3.3.4.4. Determination of metal chelating activity (MCA)

MCA was measured as described by Russo et al. (2005) with minor modifications. Briefly, in each well of the microplate was added 50 µL of the plant extracts at different concentrations (5-1000 µg/mL), 0.12 mM ferrous sulfate solution ($FeSO_4$) (50 µL) and 0.6 mM ferrozine (50 µL). The mixture was shaken and incubated for 10 min, at room temperature. After that, absorbance of the mixture was measured at 562 nm in a microplate reader spectrophotometer (Dynex Technologies MRX II Microplate Reader). Chelating activity was calculated using the following equation: MCA = (Ac – As)/Ac x 100, where Ac is the absorbance of control reaction (without plant extract), and As is the absorbance in the presence of a plant extract. EDTA was used as a positive control.

3.3.4.5. Hydrogen peroxide scavenging assay

The ability of the samples to scavenge H_2O_2 was determined according to the method described by Jayaprakasha et al. (2004). Briefly, 1 mL of each sample extract (1 mg/mL) was diluted in 3 mL of a phosphate buffered saline (PBS, 0.2 M, pH 7.4), followed by the addition of 1 mL of H_2O_2 (40 mM), prepared in the phosphate buffer (pH 7.4). The mixture was incubated at 25 ºC for 10 min, and the absorbance was measured at 230 nm. The percentage of scavenged H_2O_2 was calculated using equation: H_2O_2 scavenging (%) = 100 × (Ac – As)/Ac, where Ac is the absorbance of the control and As is the absorbance of the sample. Ascorbic acid was used as a positive control.

3.3.4.6. ABTS radical scavenging activity

The antioxidant activity of the studied plant extracts against ABTS was determined by the method described by Re et al. (1999). Radical $ABTS^{\bullet+}$ was prepared through oxidation of ABTS by potassium persulfate. Briefly, a mixture (1:1; V/V) of ABTS solution (7 mM) and potassium persulfate solution (4.95 mM) was prepared and kept for 16 h in the dark, at room temperature. Before the assay, the mixture was diluted with PBS until it reached an absorbance value of about 0.70 ± 0.02 at 745 nm, using a UV/Vis Spectrophotometer (Jenway, 6300). Free radical scavenging activity was assessed by mixing 300 µL of test sample with 3.0 ml of ABTS working standard. The percentage inhibition was calculated according to the formula: ABTS scavenging activity (%) = 100 × (Ac – As)/Ac, where Ac is the absorbance of the control and As is the absorbance of the sample. Ascorbic acid was used as a positive control.

3.3.5. Antibacterial susceptibility assays

3.3.5.1. Test bacteria

Two bacterial isolates namely *Staphylococcus aureus* (DSM 346), and *Escherichia coli* (DSM 1576) were obtained from the DSMZ-German Collection of microorganisms and cell cultures GmbH. The above bacteria were maintained on nutrient agar slants at 4 °C until used for the study.

3.3.5.2. Disk diffusion assay

The antibacterial susceptibility was initially assayed by the agar disk diffusion method, described by EUCAST (2020). Disc diffusion is one of the oldest and most frequently used methods for susceptibility testing, because it is applicable to a broad range of agents, and needs no special equipment (Jonasson et al., 2020).

Different concentrations of each plant extract (1 mg/mL, 5 mg/mL, and 10 mg/mL) were prepared. Bacteria cell suspensions were adjusted to 0.5 McFarland turbidity standards to prepare 1×10^8 bacterial/mL inoculum. Each bacterial suspension was inoculated on Mueller-Hinton agar plates (depth level 4.0 ± 0.5 mm), and the plates were then allowed to dry for 5 minutes. Sterile filter paper discs (Whatman No. 1, diameter = 6 mm) containing 20 µL of each prepared extracts were placed on the surface of seeded Petri plates. Plates were incubated for 20 hours at 37 °C. After incubation, diameters of inhibition zones around the well were measured. The diameters of the inhibition zones were expressed by comparing the inhibition zones for test organisms used for this study. Experiments were performed in triplicate. As a reference, antibacterial agent, ciprofloxacin discs (5 µg) were used (Liofilchem, Italy), and solvents-soaked filter paper disk were used as the negative controls.

3.3.5.3. Minimum Inhibitory Concentration (MIC)

Plant extracts that gave a positive result for the disk diffusion assay were used to determine MIC using the broth microdilution assay method of EUCAST (EUCAST, 2003). The MIC values were defined as the lowest concentration (mg/L) at which visible growth of bacteria is prevented under defined growth conditions (Wiegand et al., 2008). Microdilution is a quantitative method that can be used to determine MIC values (Kim et al. 2007), also standardized, accurate, inexpensive to perform, and easy to carry out (Jorgensen & Ferraro 2009).

Initially, serial two-fold dilutions were made directly in a sterile 96-well microplate of the plant extract in the MHB medium covering a broad spectrum of concentrations tested (0.078 to 5 mg/mL) (six serial dilutions of the original extracts). After that, each bacterial suspension in MHB medium previously adjusted to 10^8 CFU/mL was diluted (1:100) and 100 µL of plant extract was added to each well of the 96-well microplate. The final volume of each well was 200 µL with the desired inoculum of 5×10^5 CFU/mL. The plates were then incubated at 37°C for 18–24 h. Clindamycin was used as a positive control for both strains; solvents and plant extracts without bacterial suspension were used as the negative controls.

Resazurin-based 96-well plate microdilution method was used for the determination of MIC. Active bacterial cells reduce the non-fluorescent resazurin (blue) to the fluorescent resorufin (pink) which can be further reduced to hydroresorufin (O'Brien et al. 2000), giving a direct quantifiable measure of bacterial metabolic activity. Resazurin was prepared at 0.015 %, vortexed and sterile filtered (0.22 µm filter) and stored at 4 ºC for a maximum of 2 weeks after preparation. After incubation for 24 h at 37 ºC, resazurin (0.015%) was added to all wells (30 µL per well), and further incubated for 2–4 h for the observation of colour change. Following addition of resazurin, blue coloration indicated pathogen inhibition, and these columns were scored as above the MIC value. A change from blue to red/pink indicated the presence of live microorganisms (Elshikh et al., 2016).

3.3.6. *In vitro* assay for cytotoxic activity

3.3.6.1. Human cell line and culture conditions

HepG2 (hepatocellular carcinoma) cell line were obtained from the American Type Culture Collection (ATCC) and used for cytotoxic screening of the plant extracts. Cells were maintained in culture in 25 cm^2 polystyrene flasks with MEM containing 10% FBS, 1% antibiotic-antimycotic solution, 1 mM sodium pyruvate and 1.5 g/L $NaHCO_3$ under an atmosphere of 5% CO_2 at 37ºC. Once the cells reach 80% confluent, cells were detached by trypsinization and suspended in complete culture medium.

3.3.6.2. MTT assay for cytotoxicity analysis

The MTT assay was conducted based on the method described by Mosmann (1983). Briefly, 0.25% trypsin-EDTA was applied to cells to detach from the surface, and exponentially growing cells were counted using haemocytometer. Cells were seeded in 96-well plates at a

density of 10^5 cells/well in 100 µL culture medium. Following 24 h incubation and attachment, old media was discarded, and cells were treated with different concentrations of plant extracts (from 5 to 500 µg/mL) for 48 h. MTT assay was performed by adding 10 µL of MTT reagent into each well and left incubating for 1 h. After removing the supernatant, 100 µL of DMSO:Ethanol (1:1) was added to each well and then placed on a plate shaker to dissolve formazan crystals. Absorbance (OD) was measured at 570 nm using a microplate reader (Dynex Technologies MRX II Microplate Reader). The results were expressed in percentage by the formula: (As – Ab) / (Ac – Ab) × 100, where As is the absorbance of the sample, Ab is the absorbance of the blank, and Ac is the absorbance of the control. As a blank, DMSO was used. The concentration of extract that decreases by 50% the number of viable cells (IC_{50}) was calculated using the GraphPad program from Prism®.

3.3.7. Statistical analysis

Data were expressed as mean ± standard deviation (SD) determined of, at least, triplicate analysis. Statistical analysis was performed using GraphPad Prism® 8.0 software. The percentages of yield, TPC and TFC were analyzed using a *t*-test or a Mann-Whitney tests. The significant differences in the *in vitro* antioxidant assays were calculated through a one-way ANOVA with Tukey's post hoc multiple comparison test. In the *in vitro* cell assays, one-way ANOVA was employed with Dunnett's multiple comparison test, when comparing each concentration against a control. In all the statistical analysis, differences were considered to be significant at a level of $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Ethnopharmacological study of medicinal plants from the Province of Cuanza Norte (Angola)

Angola's health care system is poor, compared to other sub-Saharan countries (Kagawa et al., 2012). The lack of health infrastructure, especially in rural areas, is a serious problem resulting in the importance of traditional healers and herbal medicines. Angola hosts high levels in terms of the species richness and endemism, but threats to this rich flora and their habitats are emerging. For all the reasons mentioned above, it is imperative to conserve and study its biodiversity. Therefore, ethnobotanical and ethnopharmacological studies represent an attractive approach for applying indigenous knowledge of plant use to modern societies, with the final aim of developing new drugs. Angola is the second richest country with respect to endemic plants in continental Africa. Therefore, the rich flora of sub-Saharan Africa suggests enormous potential for the discovery of new secondary plant metabolites with therapeutic value (Moyo et al., 2015). There are only a few studies documenting plant usage in Angola, which are either carried out in the south of the country, showing completely different vegetation units (Urso et al., 2016) or putting the accent on savannah plants (Göhre et al., 2016). So, in this section, we present and discuss the results obtained from the interviews with the selected informants, with the aim of systematizing the knowledge regarding medicinal plants used by the inhabitants from N'dalatando (Cazengo), in the Province of Cuanza Norte (Angola), recognized for its cultural and biological diversity, and highlight the relevance of the ethnobotanical findings for a more rational use of the plants or for the implementation of phytotherapy programs.

4.1.1. Plant parts used and preparation methods

Primary ethnobotanical information for each species like local name, plant part used, popular use, and method of preparation were collected from the informants and summarized in Table 5. A total of 131 plants were cited for the treatment of various types of ailments. These findings confirmed the existence of a great diversity of plants at N'dalatando, in the Province of Cuanza Norte, used for medicinal purpose and preserved in people's culture. In this study, Mukumbi (*Lannea welwitschii* (Hiern) Engl.), Santa-maria (*Chenopodium ambrosioides* L.) and Ditumbata (*Boerhavia difusa* L.) were the most cited species, following by Embondeiro (*Adansonia digitata*), Papaia (*Carica papaya* L.), and Mbrututu (*Cochlospermum angolense* Welw. ex Oliv.).

Table 5. Plant species used by the N'dalatando community, in the Province of Cuanza Norte (Angola).

Scientific name	**Local name**	**Indication**	**Part used**	**Preparation**
Persea americana Mill.	Abacate	Hypertension, Parasites	Leaf; seed; fruit	Decoction; raw material
Cucurbita pepo L.	Abóbora	Prostate	Seed	Decoction
Curcuma longa L.	Açafrão	Hepatitis	Root	Maceration
Nasturtium officinale R.Br.	Agrião	Pneumonia	Leaf; seed; root	Maceration
Lactuca sativa L.	Alface	Heart problems; diarrhea	Leaf	Raw material
Gossypium sp.	Algodão	Ear pain; diarrhea; nausea	Leaf; seed	Infusion
Allium sativum L.	Alho	Cough; asthma; bronchitis	Bulb	Maceration
Aloe ferox Mill.	Aloé	Healing wounds; alopecia; pneumonia	Leaf	Raw material
Ananas comosus (L.) Merr.	Ananás	Diabetes; Indigestion	Fruit	Raw material
Solidago chilensis Meyen	Arnica do campo	Varicose veins	Leaf	No data
Vernonia polysphaera Baker	Assa-peixe	Respiratory problems	Leaf	Infusion
Unidentified species	Bambe	Respiratory problems	No data	No data
Catharanthus roseus (L.) G. Don	Beijo da mulata	Malaria; Cholera; Diabetes; Typhoid fever	Leaf	No data
Atropa belladonna L.	Beladona	Fungal diseases	Leaf	Infusion
Unidentified species	Bembrequete	Diabetes	No data	No data
Beta vulgaris L.	Beterraba	Anemia	Bulb	Decoction
Unidentified species	Boldo	Nausea; gastritis	Leaf; stem	Maceration
Coffea sp.	Café	Fatigue	Root	Maceration
Anacardium occidentale L.	Caju	Teeth pain; Diarrhea	Leaf; stem; root	Maceration; infusion; decoction
Unidentified species	Camuquina	Undefined pain	No data	Infusion
Unidentified species	Canami	Infectious disease	No data	Maceration
Unidentified species	Capim-de-deus	Respiratory disease	No data	No data

Scientific name	Local name	Indication	Part used	Preparation
Averrhoa carambola L.	Carambola	Anemia; cholesterol	Leaf; fruit	Decoction
Unidentified species	Carapucho	Diabetes	No data	No data
Unidentified species	Casialata	Skin infection	Leaf	Raw material
Unidentified species	Cauya uya	Bleeding problems	Root	Decoction
Unidentified species	Caxetete	Cholera; diarrhea	No data	No data
Allium cepa L.	Cebola	Respiratory problems; bronchitis	Bulb	No data
Cymbopogon citratus (DC.) Stapf	Chá-de-cachimbe	Fever	Leaf	Decoction
Melissa officinalis L.	Cidreira	Gastritis; insomnia	Leaf	Maceration; decoction
Unidentified species	Cipo Kassau	Respiratory problems	No data	No data
Aristolochia cymbifera Mart.	Cipo-mil-homens	Malaria	No data	No data
Unidentified species	Cipo suura	Respiratory problems	No data	No data
Cocos nucifera L.	Coco	Hepatitis	Root	Decoction
Cola acuminata (P. Beauv.) Schott & Endl.	Cola	Pneumonia	Bulb	Raw material
Brassica sp.	Couve-china	Gastritis; typhoid fever	Leaf	Maceration
Unidentified species	Cuanana	Diabetes	No data	No data
Taraxacum officinale (L.) Weber ex F.H. Wigg.	Dente-de-leão	Diabetes; liver diseases	Leaf	Decoction; raw material
Combretum cinereopetalum En gl. & Diels	Dicaxi	Malaria; diabetes; liver diaseases	Stem	Decoction; maceration
Boerhavia diffusa L.	Ditumbata	Malaria; hepatitis; cramps	Stem; leaf; root	Infusion; maceration, raw material
Adansonia digitata L.	Embondeiro	Malaria, diabetes; skin disease; fever	Stem; fruit	Infusion; maceration
Unidentified species	Erva de são Domingos	Malaria	No data	No data
Unidentified species	Espinheiro	Diarrhea	No data	Infusion
Eucalyptus sp.	Eucapilto	Cough; Fever; Diabetes	Leaf	Decoction

Scientific name	Local name	Indication	Part used	Preparation
Artocarpus altilis (Parkinson ex F.A. Zorn) Fosberg	Fruta-pão	Diabetes	No data	No data
Zingiber officinale Roscoe	Gengibre	Nausea; weight loss; respiratory problems	Root	Maceration
Unidentified species	Gihã	Blood problems	Leaf; root	Maceration
Capsicum annuum L.	Gindungo	Pneumonia; typhoid fever	Seed; bulb	Maceration; raw material
Monodora angolensis Welw.	Gipepe	Digestive problems	Seed	Maceration; raw material
Psidium guajava L.	Goiaba	Diarrhea	Leaf; fruit	Raw material
Unidentified species	Guinonga	Malaria	No data	No data
Alternanthera pungens Kunt	Holokosso	Malaria	Leaf	Maceration
Mentha sp.	Hortelã	Nausea; cough	Leaf	Infusion
Unidentified species	Jasmini Amarelo	Diabetes	No data	No data
Unidentified species	Kabuabuata	Malaria	No data	No data
Unidentified species	Kandua grande	Lepra	Root	Maceration
Ocimum gratissimum L.	Kimbuma	Fever; Malaria	Leaf	Infusion
Unidentified species	Kimpuanguele	Lepra	No data	Maceration
Unidentified species	Kingigima	Hepatitis; Malaria	No data	Maceration
Unidentified species	Kintamba	Bronchitis	Root	Decoction
Unidentified species	Kumpidi (pimento)	Diarrhea; fever; bladder pain	Seed	No data
Unidentified species	Leitoso	Anemia	No data	No data
Citrus sp.	Limão	Flu; cough; throat pain	Fruit	Raw material
Unidentified species	Lingua-de-boi	Gastritis	No data	No data
Unidentified species	Lingua-de-cão	Child diseases	Root	Decoction
Unidentified species	Lumpiilu npilu	Wounds	Leaf	Maceration
Unidentified species	Macunde	Diabetes	Leaf	No data
Brillantaisia owariensis P. Beauv	Malemba lemba	Gastritis	Leaf	Maceration
Unidentified species	Malulu	Malaria; hepatitis	Leaf	No data
Unidentified species	Mambuso	Undefined pain	Leaf; root; flower	Maceration
Mangifera indica L.	Manga	Diarrhea; bronchitis	Stem; leaf	Infusion; maceration
Unidentified species	Mansusua nsusua	Bronchitis	Leaf	Infusion

Scientific name	Local name	Indication	Part used	Preparation
Unidentified species	Manua nsongue	Spleen problems	Leaf	Maceration
Cochlospermum angolense W elw. ex Oliv.	Mbrututu	Malaria; hepatitis	Root; stem	Maceration; decoction
Alchornea cordifolia (Schumach. & Thonn.) Müll.Arg.	Mbungi	Teeth pain	Leaf	Decoction
Citrullus lanatus (Thunb.) Matsum. & Nakai	Melancia	Cold	Fruit; seed	No data
Unidentified species	Melva	Lungs; diabetes	No data	No data
Moringa oleifera Lam.	Moringa	Heart; fever; diabetes; typhoid fever	Leaf; seed; root	Infusion; raw; decoction
Dacryodes edulis (G.Don) H.J.Lam	Mubafo	Diarrhea; diabetes	Sap	Raw material
Croton mubango Müll.Arg.	Mubango	Pain; typhoid fever	Leaf	Maceration
Unidentified species	Mubunga wanga	Malaria; diabetes	No data	No data
Unidentified species	Mucuna	Diabetes	No data	No data
Unidentified species	Mudia buinge	Diabetes	No data	No data
Senna occidentalis (L.) Link	Mudianhoca	Malaria; cramps	Leaf, root	Maceration; infusion
Ocimum sp.	Muelele	Fever	Leaf	Infusion
Jatropha curcas L.	Mufulukua	Teeth pain; skin wound	Leaf; root	Decoction
Unidentified species	Mugongonde	Diabetes	No data	No data
Lannea welwitschii (Hiern) Engl.	Mukumbi	Cough; hepatitis; bones	Stem; leaf	Decoction; maceration
Piliostigma thonningii (Schumach.) Milne-Redh.	Mulolo	Infectious diseases; skin allergy; pain	Leaf	Infusion
Unidentified species	Mulongo	Lepra	Stem	Maceration
Gymnanthemum amygdalinum (Delile) Sch.Bip. ex Walp.	Mululu	Malaria; cough; pain; intestinal parasites	No data	Maceration; raw material; infusion
Unidentified species	Mulungo	Diabetes	No data	No data
Unidentified species	Mundende	Lepra	Leaf	Raw material
Unidentified species	Munemuenji	Intestinal cleansing	No data	No data
Unidentified species	Musambela	Gastritis	No data	No data
Momordica charantia L.	Mussequenha	Typhoid fever	Leaf	Maceration; Infusion

Scientific name	Local name	Indication	Part used	Preparation
Unidentified species	Mussunda	Thrombosis; undefined pain	Root	Maceration
Vitex sp.	Muxilo-xilo	Diabetes; fever; lepra	Leaf	Maceration; decoction
Gardenia ternifolia	Ndai	Cough; lepra; inflammation	Stem	Maceration; raw material
Albizia cf. lebbeck (L.) Benth.	Ndendo	Tooth decay; diabetes	No data	No data
Unidentified species	Ndongo	Diabetes	No data	No data
Azadirachta indica A.Juss.	Neem	Malaria; hepatitis; diabetes; hemorrhoids	Leaf	Infusion; maceration
Unidentified species	Nespera	Hypertension; Diabetes	No data	No data
Garcinia kola	Ngadiadia	Pains	No data	No data
Unidentified species	Nganza	Utero problems	Root	Decoction
Unidentified species	Nganzi	Typhoid fever; malaria; pain	Stem	Maceration
Unidentified species	Nhamba nhamba	Teeth pain; Blood disorders	Root	Decoction; infusion
Unidentified species	Nlongua	Back pain	Stem	Maceration
Unidentified species	Nlonlombulo	Bronchitis; bladder pain	Root	Decoction
Unidentified species	Nlono	Bronchitis	Leaf	Infusion
Unidentified species	Nsangu nsangu	Bronchitis	Leaf	Infusion
Unidentified species	Ntontosi	Stomach pain; ear pain	Leaf	Maceration
Unidentified species	Nulunda Michi	Respiratory problems	No data	No data
Unidentified species	Oya	Eye problems	No data	No data
Unidentified species	Paco	Malaria	No data	No data
Unidentified species	Pão tibia	Fatigue	Stem	Maceration
Carica papaya L.	Papaia	Skin wounds; malaria; hepatitis	Leaf; seed; fruit; root	Maceration; decoction
Unidentified species	Pé-de-elefante	Diabetes	No data	No data
Unidentified species	Penga-pinto	Malaria	No data	No data
Opuntia stricta (Haw.) Haw.	Pitela	Diabetes; hepatitis	No data	Infusion
Unidentified species	Repolho	Diabetes; back pain	Leaf	Infusion

Scientific name	Local name	Indication	Part used	Preparation
Punica granatum L.	Romã	Cholera; diarrhea; teeth pain	Fruit	Infusion
Sambucus sp.	Sabugueiro	Cough; asthma; skin allergy; measles	Leaf; flower	Decoction
Chenopodium ambrosioides L.	Santa-maria	Fever, cough; malaria; pain	Leaf	Infusion; maceration
Combretum sp.	Tacange	Teeth pain	No data	Decoction
Tamarindus indica L.	Tambarineiro	Hepatitis; malaria; diabetes	Fruit	Raw material
Unidentified species	Teve-teve	Respiratory problems	No data	No data
Solanum lycopersicum L.	Tomate	Prostate	Bulb	Raw material
Adenia lobata (Jacq.) Engl.	Tonga-tonga	Rheumatism	Leaf	Raw material
Unidentified species	Tuzequieto	Constipation	Leaf	Maceration
Calotropsis procera (Aiton) W. T. Aiton	Umpulukua	Malaria	No data	No data

The present study reveals eight plant parts selected as medicinal materials. Out of the total plant parts, leaves were the most frequently material used (44.4%), followed by underground parts (roots and bulbs with 23.9%), stems (10.3%), seeds and fruits (8.5%), flowers (2.6%), bark and sap (0.9%). It is important to notice that, in some species, more than one part can be used. These results are in accordance with studies conducted in other parts of the world and with studies performed in other Provinces of Angola (Agbodeka et al., 2016; Lautenschläger et al., 2018; Pompermaier et al., 2018), which reported the predominant use of plant leaves. Leaves are the main photosynthetic organs and, therefore, photosynthesize exudates containing bioactive compounds with potential medicinal value. Leaves are also easy to collect and prepare (Chekole et al., 2015). Moreover, collecting leaves for medicinal purpose is usually not a threat to the survival of plants as compared to the use of other parts like roots, and stem barks. Our results contrast with the study of Urso et al. (2016) that showed that underground organs were cited as the main used parts in the preparation of medicinal remedies, by communities living in Mopane woodlands of southern Angola.

In African traditional systems of medicine, plant preparations in the forms of decoctions, concoctions, macerations, or infusions are used to treat a wide range of diseases (Tsobou et al., 2016). In this study, and among several preparation methods, the most frequent was maceration

(37.7%), followed by infusion (23.7%), decoction (21.9%) and raw material (16.7%). It is known that administration methods vary from community to community, from healer to healer and from disease to disease. Therefore, by contrast, the study of Lautenschläger et al. (2018), performed in in the province of Uíge located in the very north of Angola, showed that using a decoction to prepare a remedy was the most frequently found method of preparation.

The oral administration route (65.8%) was the most used in the N'dalatando region for taking the plant preparations, followed by topic application (28.9%) and enema (5.3%). These results are in accordance with other reports (Agbodeka et al., 2016), especially with the study of Lautenschläger et al. (2018) where nearly half of all preparations were administered orally (45%), followed by dermal application (20%) and only 16% was used as enema.

4.1.2. Ailments treated by plants

The population in N'dalatando region traditionally uses plants for the treatment of various diseases. All the recorded ailments were grouped into major ailment categories (Table 6). The main categories of use were certain infectious and parasitic diseases (e.g., Malaria, Lepra, Cholera) (20.3%); diseases of the digestive system (17.3%); endocrine, nutritional, and metabolic diseases (15.3%); symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified (15.3%); and diseases of the respiratory system (14.8%).

The results observed in this study are similar to those of Urso et al. (2016) which demonstrated that medicinal plants were mainly used to treat disorders of the gastrointestinal tract, obstetric and gynecological problems, and respiratory diseases.

To explore the potential clinical application of medicinal plants, it is important to link its traditional use with rigorous evidence-based scientific studies. For some of the species with more citations, the uses cited by the informants showed some similarity to the investigated effects/actions, demonstrating concordance between popular knowledge and academic science.

In the case of Mukumbi (*Lannea welwitschii* (Hiern) Engl.) the informants use this plant for cough, hepatitis, and bone fractures. According to the literature, this plant is found growing in deciduous and secondary forests of Africa from Côte d'Ivoire to Cameroons and extending to Uganda and Angola. Decoction of the leaves is used for the treatment of diarrhea, dysentery, swellings, gout, gingivitis, topical infections, and wounds (Agyare et al., 2009); the roots can be used in nasopharyngeal infections and as emetics; and the stem bark, which contain tannins and saponins, has antidiarrheal property (Olatokunboh et al., 2010).

Table 6. Ailments for which informants use botanical remedies.

Category	Ailment	%
Certain infectious and parasitic diseases	Infections, parasitosis	20.3
Diseases of the digestive system	Constipation, diarrhea, stomach pain, hepatic disorders, digestion; gastritis; Intestinal cleansing	17.3
Endocrine, nutritional, and metabolic diseases	Diabetes, weight loss	15.3
Symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified	Fever, teeth pain, inflammation, pain	15.3
Diseases of the respiratory system	Asthma, cold, cough, sore throat, bronchitis	14.8
Diseases of the skin and subcutaneous tissue	Wounds, skin allergy	4.0
Diseases of the circulatory system	Hypertension, heart disorders; hemorrhoids; varicose veins	3.5
Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism	Anemia; blood disorders	3.0
Diseases of the musculoskeletal system and connective tissue	Rheumatism, back pain, bone fracture	2.0
Diseases of the genitourinary system	Prostate problems, bladder pain menstrual	2.0
Diseases of the ear and mastoid process	Ear pain	1.0
Diseases of the nervous system	Insomnia	0.5
Pregnancy, childbirth, and puerperium	Utero problems	0.5
Diseases of eye and adnexa	Eyes problems	0.5

Regarding Santa-maria (5 citations by the informants) (*Chenopodium ambrosioides* L.) the informants use this plant for fever, cough, malaria, and pain. Cysne et al., (2016) showed that the crude hydroalcoholic extract from the leaves of *C. ambrosioides* exhibited a significant antiplasmodial effect and may be considered as a potential candidate for the development of new antimalarial drugs. Some effects of *C. ambrosioides* have been confirmed, such as its anti-

inflammatory, anti-nociceptive (Calado et al., 2015), and antimicrobial activity against, for example, *Plasmodium falciparum* (Cysne et al., 2016).

The plant Ditumbata (*Boerhavia difusa* L.) was also cited by some informants (5 citations) for the treatment of malaria, hepatitis, and cramps. However, in Ayurvedic literature, this plant is claimed to be a rejuvenating remedy for the urinary system. Experimental studies have also demonstrated its diuretic and possible nephroprotective effects against acetaminophen-induced renal damage (Sawardekar & Patel, 2015).

The stem and fruit (known as mucua) of *Adansonia digitata* (4 citations by the informants) (embondeiro or baobab) was cited for the treatment of malaria, diabetes, skin diseases and fever. Some pharmacological activities related to the plant, namely, hypoglycemic, hypolipidemic, antimicrobial, analgesic, and antipyretic are in accordance with its traditional uses. Furthermore, it is a hepatoprotective agent with anti-inflammatory and antioxidant properties (Silva et al., 2023). This plant is a long-lived tree with over 300 traditional uses recorded and is considered emblematic and essential in traditional medicine in Africa and India. The fruit pulp can be eaten raw, as snack or processed in form of juices, jams, and sweets. A. *digitata* has been identified for domestication through participatory priority setting exercises in Eastern and Central African countries. Domestication of indigenous tree species is recognized as a successful strategy to assure the long-term access of local communities to food trees (Stadlmayr et al., 2020).

Papaia (*Carica papaya*) was another plant cited by informants (4 citations) for skin wounds, malaria, and hepatitis. In Africa, the fruit, leaf, seed, and roots are generally used to treat a variety of diseases such as malaria, cancer, and cardiovascular diseases (Ojo et al., 2017). For example, Oraebosi & Good (2021) recently showed that shor-term co-administration of *C. papaya* and artesunate in *Plasmodium berghei* infected mice is a positive drug-herb combination.

Mbrututu (*Cochlospermum angolense*), a tree native to Africa, was also one of the most cited plant (4 citations) used by informants for malaria and hepatitis. Other studies confirm that the infusion obtained from roots by decoction with water has been traditionally consumed by many African communities for the treatment of malaria and due to its hepatoprotective properties (Pereira et al., 2013; Presber et al., 1992). Despite the potential of this plant for the production of bioactive extracts, studies reported until now have been scarce. The existing studies reported their potential benefits for health, related to their antioxidant, antitumoral, antiviral and neuroprotective properties (Ferreres et al., 2013; Pereira et al., 2014; Presber et al., 1987).

4.2. A literature review of ethnobotanical studies in Angola (Africa)

Angola has 18 provinces (Bengo, Benguela, Bié, Cabinda, Cuando-Cubango, Cuanza Norte, Cuanza Sul, Cunene, Huambo, Huíla, Luanda, Luanda Norte, Luanda Sul, Malange, Moxico, Namibe, Uíge, Zaire), however the ethnobotanical studies found in this work (7 in total) were performed only in 6 provinces (Bié, Bengo, Huíla, Cuanza Norte, Namibe, and Uíge (Table 7).

The results of the review of ethnobotanical studies from Angola provide new insights into the knowledge about medicinal plants from the country. The included studies aim to provide a list of the useful plants from the different provinces, municipalities, and communities with quantitative data about cultural important species. The documented plants are used for diverse applications, which include therapeutic uses, handicrafts, food, and rituals. These studies are important to conserve local knowledge for future generations, and to provide a reliable basis for future ethnobotanical and ethnopharmacological research, in order to identify the pharmacological targets (Göhre et al., 2016; Mawunu et al., 2018).

Approximately 6850 species are native to Angola and the level of endemism is around 14.8%. An additional 230 naturalized species have been recorded, four of which are regarded as highly invasive (Goyder & Gonçalves, 2019). However, despite the high diversity of the vascular flora of Angola only few studies have been performed over the years, regarding the traditional use of plants in the country. Some of the studies performed focused on other applications for plants (sociocultural or economic purposes) (Bruschi et al., 2017) rather than the medicinal use by the population or focused on the medicinal uses of a specific family of plants (Catarino et al., 2019).

Table 7. Ethnobotanical studies from Angola.

Provinces (Municipalities)	Data Collection/Informants	Main Ethnobotanical Results	References
Province: Bié (Municipalities: Kuito; Cuemba)	• Survey undertaken between September and November 2013; • Purposive sampling and snowball method; • Semi-structured interviews (individually); • Most information was obtained in Portuguese; • 10 Informants (herbalists): Kuito (3 men and 2 women), Cuemba (2 men and 3 women); • Age (between 51 and 73 years), with a mean of 60 years of age; • Data collected: local name, plant parts collected, medicinal use, modes of preparation and administration, plant life-form, status of domestication (cultivated or wild), seasonal availability and any other additional information.	• 224 vernacular names of medicinal plants: 114 taxa, with 87 plant species distributed among 57 genera and 36 botanical families; • Fabaceae is the best-represented family (18 species), followed by Phyllanthaceae (6), Apocynaceae (5), Asteraceae (5), Rubiaceae (5), Lamiaceae (4), and Ochnaceae (3); • Plant life-form: trees (31%); shrubs (35%); herbs (21%); sub-shrubs (10%); succulents (2%); vines (1%): • Plant Part Uses: roots (79%); leaves (16%); whole plant (2%); bark (1%), fruit (1%), stem (1%); • Mode of preparation/administration: Decoction (51%); washes/massages (10%). Other types of administration were baths; Infusion; tablets; nose drops; oral ingestion/chewing, vaginal pessary, eye drops, balms, inhalers, rectal suppositories; • Plants with higher use reports (UR): *Securidaca longepedunculata* Fresen (14 UR); *Annona stenophylla* subsp. *nana* N. Robson (14 UR); *Paropsia brazzaeana* Baill. (9 UR); • 280 medicinal use reports: Digestive problems (81 UR); Pregnancy, family planning (e.g.: pain, labour and post-partum disinfection (45 UR); General (e.g.: malaria) (29 UR); • Most frequently cited species: *Securidaca longepedunculata* (10 informants); *Annona stenophylla* subsp. nana (10 informants); followed by *Garcinia huillensis*, *Strychnos cocculoides* Baker and *Holostylon robustum* (Hiern) G. Taylor (6 informants). • From the total of 87 species, ethnomedicinal uses of 26 species have been reported for the first time; a total of 44 species have not yet been tested for their pharmacological potential (some with restricted distribution range).	Novotna et al., (2020)

Table 7. Ethnobotanical studies from Angola (Cont.).

Provinces (Municipalities)	Data Collection/Informants	Main Ethnobotanical Results	References
Province: Huíla (Municipalities: Lubango, Humpata, Chibia, and Gambos)	• Study based on the field records of José Maria Daniel; • Records on the use of plants can be found in the notebooks and field notes performed by José Maria Daniel; • Data presented were based on information provided by the population and by traditional healers; • Data collected: local name, plant parts collected, medicinal use, and modes of preparation;	• 787 records of different plants, corresponding to 158 species, from of which 57 have medicinal use, while the rest were referenced as ornamental, fodder, toxic or poisonous, food and condiments • Medicinal plants constitute about 33% of the total number of registered species, followed by plants used as food (20%), ornamental (18%), forage (16%), toxic/poisonous (12%) and as condiments (1%). • About 787 records of useful plants were made in Huíla Province, highlighting the municipality of Lubango with the highest number of records (86%), followed by Humpata (7%), Chibia (4%) and Gambos (3%). • Fabaceae was the most representative family of the medicinal plants, followed by the families Vitaceae, Passifloraceae, Lamiaceae, Poaceae • Plant Part Uses: leaves, roots and in some cases the use of the whole plant: • Mode of preparation/administration: infusion and decoction are the main forms of preparation of plants:	Gonçalves et al., (2019)

Table 7. Ethnobotanical studies from Angola (Cont.).

Provinces (Municipalities)	Data Collection/Informants	Main Ethnobotanical Results	References
Province: Uíge (13 municipalities)	• Field work: during nine field trips in 13 municipalities, between October 2013 and October 2016; • 162 informants were interviewed (30 of those were interviewed on their own, 132 were interviewed in groups of two to five persons, bringing the total number of 62 interviews); • Informants were persons with experience in traditional medicine; • Two thirds of informants were male, one third female; • All the interviews were conducted with at least one traditional herbalist sometimes accompanied by laypeople; • Semi-structured interviews; • Data sets were requested: local plant name, its usage, used plant part, preparation techniques, and administration techniques.	• 2390 use-reports, listing 358 species in 96 plant families (17 of them only to genus level); • 5% trees, 26% perennial herbs, 16% shrubs, 12% climbers, 10% annuals and less than 1% parasites; • Concurrently, 27% are plants growing in different savannah types, 24% in forests, and 21% in the transition zone connecting these two ecosystems; • The predominant used plant families are Fabaceae (11.7%), Asteraceae (6.1%) and Rubiaceae (5.6%), followed by Apocynaceae, Malvaceae and Euphorbiaceae (4.2%). • Seventy-six percent of the citations collected in the study refer to medicinal uses, 10% to nutritional use and 4% to its use as fodder plant; • Regarding only the medicinal use category, the citations describing the use of leaves was 39% (689 citations, 178 species), and the use of underground organs 32% (582 citations, 137 species); • Ten percent out of 1813 citations for medicinal uses refer to stomach pain (183 citations), 8% to respiratory diseases, 7% to pain and rheumatism, 6% to diarrhoea and 6% to headache and weakness. • Decoction is the most frequently found method of preparation (45%), followed by the manufacture of an ointment (13%), maceration (12%) and the application as raw material, while nearly half of all preparations are administered orally (45%), followed by dermal application (20%) in only 16% is an enema used. • The most important species mentioned for medical uses by women and men, with a percentage of more than 50%, respectively, and the highest numbers of use-reports are: *Aframomum alboviolaceum*, *Dialium englerianum*, and *Jatropha curcas* (for women); *Annona stenophylla* subsp. *cuneata*, *Hymenocardia acida*, and *Securidaca longipedunculata* (men).	Lautenschläger et al., (2018)

Table 7. Ethnobotanical studies from Angola (Cont.).

Provinces (Municipalities)	Data Collection/Informants	Main Ethnobotanical Results	References
Province: Cuanza Norte	• Field work: October and November 2014 and 2015; • Semi-structured interviews; • Ninety-two persons were interviewed; • The majority of them were traditional healers, nominated by local authorities, 53% were male and 47% were female; • The average age is 50 with a range of 25 to 79 years of age; • The 40 data sampling points are located in seven of the ten municipalities, covering nearly all occurring vegetation zones; • Data collected: scientific and vernacular names, their use, application, and plant part used.	• The ethnobotanical investigation documented 533 use reports corresponding to 162 plant specimens representing 58 different plant families; • The predominant families are Fabaceae (11.6%), Asteraceae (5.5%) and Euphorbiaceae (5.5%); • Twenty-eight families are represented by a single species only, indicating high plant diversity in the studied area; • Growth forms: annual and perennial herbs (38.3%), trees (29.6%), shrubs (21.0%), and climbers (11.1%). • Leaves are the most commonly used parts (47.5%), followed by roots (24.3%) and bark (11.1%) while reports about the use of other plant parts were rare; • The informants indicated diverse application methods as well: infusion (27%), chewing (13%) and the use as an ointment (13%); • Stomach pain was mentioned most frequently (112) while specific diseases such as paralysis, appendicitis or typhoid fever are referred just a few times; • The neophytes *Chenopodium ambrosioides* and *Chromolaena odorata* have the highest CI of all collected plants (0.19 and 0.17, respectively); • The majority of plants were referred for just one category (84.1%), only 26 plants show various applications across the applied categories. The use of *Adansonia digitata* shows the highest variety (medicinal use, food); • *Annona muricata*, *Passiflora quadrangularis* and *Ceiba pentandra* are mentioned for three different categories, showing a widespread usage too; • Just two of the 162 collected plants are endemic to Angola (*Cochlospermum angolense* and *Stachytarpheta* cf. *angolensis*.	Heinze et al., (2017)

Table 7. Ethnobotanical studies from Angola (Cont.).

Provinces (Municipalities)	Data Collection/Informants	Main Ethnobotanical Results	References
Province: Uíge (Municipalities: Uíge, Negaje, Quitexe, Mucaba and Ambuíla)	• Field work: conducted in April and May 2014; • semi-structured interviews, free-listing, group discussions: • 41 informant groups made up of 82 individuals between the ages of 23 and 80 years, mainly women); • In total: 32 interviews and 14 field trips with these informant groups; • Informants: farmers (majority), local authorities, traditional healers or midwives, workers or employees, and teachers: • Communication was mainly conducted in Portuguese (or translated into Kikongo); • Data sets collected: vernacular name of the plant in Kikongo and/or Portuguese, usage and the plant parts used as well as preparation and administration techniques;	• The study documents a total of 498 citations for the use of 122 plants from 48 families, 34.0% of which were unknown according to the literature used for comparison; • The examined municipalities are widely dominated by savannah vegetation, severely degraded by frequent fires; • Of the documented plants, 41.3% were shrubs or trees, 9.1 % subshrubs, 24.8% perennial herbaceous plants, 13.2% annuals and 11.6 % annual or perennial climbers; • The most commonly used plant families are Fabaceae (13.1%), Asteraceae (13.1%), Euphorbiaceae (6.6%), Lamiaceae (6.6%) and Malvaceae (5.7%); • On average, 4.1 different use-reports per species were documented, 72.1% of which refer to medical treatments; • The most commonly used plant parts are leaves or fronds (224 citations for 78 species), fruits (73 citations for 35 species, including seeds) and underground organs, such as roots, root tubers and rhizomes (75 citations for 28 species); • The most frequently listed areas of application are abdominal pain (55 use-reports), digestive tract diseases (28 use-reports), childhood diseases (28 use-reports), rheumatic or muscle pain (25 use-reports) and fevers (25 use-reports). • The most frequently cited preparation methods for medical plants are decoctions (72 citations) and freshly crushed material, as it is used for dermal administration or to extract the sap from a tissue (71 citations). • Treatments are mostly accomplished through the enteral route by oral intake (139 citations). Rectal drug administration is also common (68 citations), especially by enema.	Göhre et al., (2016)

Table 7. Ethnobotanical studies from Angola (Cont.).

Provinces (Municipalities)	Data Collection/Informants	Main Ethnobotanical Results	References
Province: Namibe (Communities: Garganta, Haukulu, Assunção, Katuvo, Munhengo, Bibala, Rio d'Areia)	• Field work: conducted in the years 2010–2012; • 66 informants (26 males and 40 females); • Age ranged from 27 to 85 years, with a mean age of 50 (714) years for men and of 57 (713) years for women; • Fifty percent were housewives, 23% charcoal burners, 18% shepherds, 6% farmers and 3% teachers; • Snowball method; • Ethnobotanical uses were mostly collected through semi-structured individual interviews; • Data collected: vernacular names, uses, used plant parts, ways of preparation and administration (in the case of medical remedies), possible mixture with other plants, plant availability in the area (scarce, sufficient, abundant) and harvesting season (rainfall season, dry season, yearlong);	• A total of 1247 citations were recorded, concerning 132 ethnospecies (intended as basic folk taxonomic units corresponding either to botanical species or to different taxa); 104 were identified at different taxonomic levels; • For medicinal purposes: 116 ethnospecies and 20 different uses (650 citations - 52.2% of all the citations) were reported by 65 informants (98.5% of all informants) as used to treat 20 different disease types; • The most cited family of medicinal plants: Aristolochiaceae (58 citations, two ethnospecies), followed by Rutaceae (55, 1), Xanthorrhoeaceae (53, 2) and Euphorbiaceae (51, 5); • All of them are woody plants, as well as most of the identified ethnospecies (trees 34.6%, shrubs 32.7%, perennials 21.2%, annuals 8.7%, others 2.8%); • Besides underground organs, the most used parts are leaves (31 ethnospecies, 111 citations) and barks (28, 137); • Medicinal plants are used to treat disorders of the gastrointestinal tract (52 ethnospecies, 205 citations), obstetric/gynecological troubles (27, 40) and colds and respiratory tract diseases (25, 54). • Most plants (81,61% of the total) were cited by one or two informants; only seven plants (5%) were mentioned by 35 or more: *Adansonia digitata* (60 informants, 88 citations), *Berchemia discolor* (50, 77), *Ximenia americana* var. *americana* (48, 128), *Aristolochia albida* (41, 57), *Ptaeroxylon obliquum* (40, 55), *Sclerocarya birrea* (39, 104), and *Celtis zenkeri* (35, 54). • Most remedies involve the use of a single plant (mixtures are quite rare (6%)); • Plant remedies were mostly prepared as decoctions (274 citations, 62 ethnospecies), followed by the use of raw plant parts (79, 23), and by infusions (51, 20); • Most frequent way of administration was by oral route (406 citations, 98 ethnospecies).	Urso et al., (2016)

Table 7. Ethnobotanical studies from Angola (Cont.).

Provinces (Municipalities)	Data Collection/Informants	Main Ethnobotanical Results	References
Province: Bengo (Municipality: Dande) (Communities: Caxito, Mabubas e Úcua)	• First field work: between May and August 2010; • Samples of plants were collected between the months of December 2011 and February 2012; • 165 informants (87 females, 52,7%); • Age ranged from 16 to 87 years; • Informants were traditional authorities and representatives from local authorities; • The traditional languages most used were Kimbundu, Umbundu, Quicongo and Kioko; • Semi-structured individual interviews; • Data collected: vernacular names, uses, used plant parts, ways of preparation and administration.	• The most cited family of medicinal plants: Fabaceae, Euphorbiaceae, Asteraceae, Anacardiaceae, Lamiaceae and Solanaceae; • The most frequently used mode of preparing herbal remedies was the decoction from leaves, roots, tubers, bark of the tree or even the whole plant (80 citations). • The most mentioned disturbances or disorders: disorders of the gastrointestinal tract (50 in 287 citations, 17%), which included, cramps, stomach pain, constipation, vomiting, diarrhea; • In the first field work: 210 references of medicinal plants were registered corresponding to 79 different traditional names, of which Erva-de-Santa-Maria (*Chenopodium ambrosioides* L.) Kimbuma (*Ocimum viride* Willd.) and Mululu (*Vernonia amygdalina* Delile), were the most mentioned. • In the second field work: 213 plants were collected, corresponding to the same number of references, and 103 different traditional names. Of these, it was possible to identify 42 families and 81 species; • Of the collected and identified plants, the species most referred for medicinal purposes were: *Acanthospermum hispidum* DC. (Holokosso ou Holokosso-ya-diala, Chiholokosso, Solokoto, Ssolokoto-iauú, Ssolokoto-tchambanda ou Shomem); *Vernonia amygdalina* Delile (Mululu); *Senna occidentalis* (L.) Link (Mundianhoca, Pau-de-feijão ou kalongupa); *Carica papaya* L. (Mamoeiro); *Chenopodium ambrosioides* L. (Erva de Santa-Maria); *Ricinus communis* L. (Jimono, Lomôlo, Mumono ou Mimono); *Ocimum viride* Willd. (Kimbuma); and *Boerhavia diffusa* L. (Matrumbata, Ditumbata ou Itumbata).	Costa (2012)

UR: Use reports (one use of a species mentioned by an informant in the use category)

While quite a number of surveys were conducted in Southern Angola, just a few are located in the northern part (Bruschi et al., 2017; Urso et al., 2016). Thus, Costa (2012) performed the ethnobotanical study in the Province of Bengo. Göhre et al. (2016) collected ethnobotanical data in disturbed areas around the city of Uíge. Urso et al. (2016) documented ethnobotanical knowledge in seven communities in a Mopane area of southern Angola (Namibe province). Heinze et al. (2017) conducted the first ethnobotanical studies in the province of Cuanza Norte. Lautenschläger et al. (2018) focused on the traditional knowledge of plant use of local Bakongo communities in the northern province of Uíge. Finally, Gonçalves et al. (2019) documented the ethnobotanical knowledge of Huíla Province.

In Angola, Western medical health care is scarce, especially in rural areas. Although several actions related to infrastructures were undertaken in Angola, development is still slow, especially regarding the public health sector. It is assumed that traditional healers and herbal medicines, especially for rural people, are important and might increase in the coming years. For example, Urso et al. (2016) reported that a common problem in their study area (communities in Namibe province) is the lack of health infrastructures, with the nearest hospital, located at Bibala, 20 - 40 km far from the other studied communities.

In some cases, informants were contacted through "snowball" method. i.e., asking an informant to suggest other informants living in the study area for generations, and holding traditional knowledge about the use of wild plants (Novotna et al., 2020; Urso et al., 2016). Data were collected through semi-structured interviews (individually or in group).

In general, data sets collected in the studies included the vernacular name of the plant, plant parts collected, medicinal use, modes of preparation/administration. Informants were farmers (Göhre et al., 2016; Urso et al., 2016), traditional healers/herbalists/persons with experience in traditional medicine (Göhre et al., 2016; Gonçalves et al., 2019; Heinze et al., 2017; Lautenschläger et al., 2018; Novotna et al., 2020), local authorities (Costa, 2012; Göhre et al., 2016) or others (Göhre et al., 2016; Gonçalves et al., 2019; Urso et al., 2016). In general, informants were persons who were known within the community for having high knowledge and experience about the use of plants.

In some studies, informants were interviewed individually rather than using focus group discussions. Most of the interviewed herbalists perceived their knowledge either as a secret for spiritual or economic reasons. Therefore, they did not wish to share their knowledge with other traditional healers or local people. However, herbalists had no objection in sharing their knowledge with the international scientific community, because they believe their knowledge is not of much use outside their cultural context (Novotna et al., 2020).

In the studies analyzed, the majority of the plants cited by the informants were not endemic to Angola. For example, in the study of Heinze et al., (2017) just two of the 162 collected plants are endemic to Angola (e.g., *Cochlospermum angolense* and *Stachytarpheta* cf. *angolensis*). Also, Lautenschläger et al. (2018) reported that just three out of 358 mentioned species are endemic to Angola, 71 species are naturalized that is equivalent to one fifth, 73% of which are still cultivated. Endemic plants are important for traditional rituals but due to the high deforestation rate in Africa they are threatened (Gurib-Fakim, 2006).

Some of the predominant families in the studies analyzed were Fabaceae, Asteraceae and Euphorbiaceae. Those families belong to the five most common families in Angola (Figueiredo et al., 2009). For example, Fabaceae was the most-represented family in the study of Göhre et al. (2016); Gonçalves et al. (2019), Heinze et al. (2017); Lautenschläger et al. (2018); and Novotna et al. (2020). This family is the third-largest plant family prevailing in mopane woodland vegetation (Timberlake et al., 2010) with the 13.9% of Fabaceae taxa in the flora of Angola (Figueiredo et al., 2009). The Asteraceae family is also one of the largest flowering plant families, with over 1600 genera and 2500 species worldwide (Rolnik & Olas, 2021), and widely distributed in Angola, too. However, the distribution of plant families is difficult to discuss without referring to the occurring vegetation units. Species from Fabaceae and Asteraceae have a high percentage of used savannah plants (> 50%) while the percentage of forest plants increases within the other families (Lautenschläger et al., 2018).

Almost every parts of plants are used in traditional medicine. However, the availability or accessibility influences the preference of the different plant parts. Therefore, leaves were the predominant used plant part in the studies (Göhre et al., 2016; Gonçalves et al., 2019; Heinze et al., 2017; Lautenschläger et al., 2018). These organs are present during the whole year whereas fruits, seeds or flowers occur in a limited period only. Also, roots especially from large trees are additionally more difficult to obtain than leaves (Heinze et al., 2017). In general, the use of the whole plant was mentioned only a few times (Heinze et al., 2017). Roots are also very important for the local healers and cited in some studies as the predominant plant part used (Novotna et al., 2020; Urso et al., 2016). Often, traditional healers characterize roots as the only real medicine, and most of them used the scent of the roots to recognize and distinguish the ethnospecies (Novotna et al., 2020).

Using a decoction to prepare an herbal remedy was the most common method of preparation reported by Costa, 2012; Göhre et al. (2016), Gonçalves et al. (2019), Lautenschläger et al. (2018), Novotna et al. (2020), and Urso et al. (2016). These results are in

line with the most frequently adopted preparations in African traditional medicine (Bruschi et al., 2011; Seleteng Kose et al., 2015).

The high amount of medical use-reports in this review indicates that plants still play a crucial role in rural health care (Göhre et al., 2016). Medicinal plants cited in the studies performed in Angola are used for a variety of diseases or symptoms. However, the majority of the plants are used traditionally for digestive disorders or abdominal/stomach pain (Costa, 2012; Göhre et al., 2016; Heinze et al., 2017; Lautenschläger et al., 2018; Novotna et al., 2020; Urso et al., 2016). These may be related to the fact that abdominal/stomach pain results from various diseases (e.g., constipation, virus, food poisoning, menstrual cramps, and others). Also, contaminated drinking water and food are frequent vectors for virus diarrhoeal diseases or cholera in Angola.

Some plants cited by the informants had no studies published regarding their phytochemical characterization or biological activities. For example, Heinze et al. (2017) showed that eleven of the plants showed no hits by the PubMed search, indicating a high potential for further analysis (*Acacia* cf. *goetzei*, *Eriosema griseum*, *Eriosema* cf. *pauciflorum*, *Hymenostegia laxiflora*, *Hibiscus rhodanthus*, *Perichasma laetificata*, *Ochna* cf. *multiflora*, *Antidesma membranaceum*, *Antidesma venosum*, *Lippia plicata*, *Stachytarpheta* cf. *angolensis*). In contrast, for the majority of the species (58.4%) cited in the study of Göhre et al. (2016), both medicinal and phytochemical studies have already been carried out. However, the authors showed that few studies have been conducted for *Vitex madiensis* subsp. *madiensis*, *Gardenia ternifolia* subsp. *jovis-tonantis* and *Aframomum alboviolaceum*, especially *G. ternifolia* subsp. *jovis-tonantis* and *A. alboviolaceum*By crossing their data with previously published bioactivity studies, Costa (2012) found several associations that may justify the ethnopharmacological use of some species. For example, the use of *Boerhavia diffusa* L., traditionally called as Matrumbata, and reported to treat hepatic disorders, can be supported by several studies demonstrating its hepatoprotective activity. Furthermore, the mentioned effect of *Senna occidentalis* (L.) against malaria and headache is in concordance with the antimalarial and anti-inflammatory effects already proven.

In their study, Göhre et al. (2016) documented a high number of use-reports that were new to the literature used for comparison. Also, the high percentage of unknown plant uses emphasizes the potential of Angola for further ethnobotanical findings. For five plants cited as medicinal by the informants in the study of Urso et al., (2016), no reports were found in the consulted ethnobotanical and ethnopharmacological literature; many uses of several already known medicinal plants were also unrecorded. Urso et al. (2016) showed that for the following

five species (5% of medicinal plants identified), no previous report about therapeutic properties was found in the consulted literature: *Aptosimum gossweileri*, *Buxus benguellensis*, *Ficus tettensis*, *Heteromorpha stenophylla* and *Lannea angolensis*. In the study of Novotna et al. (2020), the following four species were cited only once and their use as medicinal plants cannot be supported by any literature sources: *Lantana angolensis* Moldenke, *Macrotyloma africanum* (Brenan ex R. Wilczek) Verdc., *Pentanisia rubricaulis* (K. Schum.) Kårehed & B. Bremer and *Uapaca gossweileri* Hutch.

In the study of Heinze et al. (2017), the use of *Adansonia digitata* demonstrated the highest variety of uses, from medicinal purposes to construction, nutrition and as a spice paste, showing a high importance for the Angolan population. Urso et al. (2016) showed similar results for *A. digitata*. This tree is native to Africa and therefore plays a crucial role in several traditions over hundreds of years (Wickens & Lowe, 2008). Moreover, products obtained from this plant (e.g., fruits) are sold by people in local markets, playing therefore an important role in the socio-economic sustainability of local communities (Urso et al., 2016). Also, the plant *Chenopodium ambrosioides*, native to Central-America, showed different therapeutic applications, and is common in other provinces as well (Göhre et al., 2016).

The use of medicinal plants is widespread and the occurrence of different vegetation zones in Angola provinces makes this country of particular interest. Also, many medicinal plants found in the studies seem to be particularly interesting for further research. Therefore, more ethnobotanical studies in Angola are needed to evaluate the data with low frequency and to visit the denser forest formations in the north, where studies are scarce.

4.3. Plants selected from the ethnopharmacological and ethnobotanical studies for the evaluation of biological activities

The plant species *A. digitata*, *G. kola* and *G. ternifolia* were the three selected plants for the study of different biological activities. The selection was based on our ethnopharmacological study and on the review of ethnobotanical studies performed over the years in Angola. For example, in our ethnopharmacological study performed in the capital N'dalatando (Cazengo), Province of Cuanza Norte (Angola), *A. digitata* was one of the most cited plants by the informants. In the review of ethnobotanical studies from Angola, *A. digitata* was also mentioned as an important plant which contributes to the livelihood of local communities as a source of food and medicine. It is also known that the different parts of *A. digitata* have been used to treat several diseases, some of them with high prevalence in the country. This plant

was also selected because of the increased scientific community's interest in *A. digitata* after the authorization of the European Union (EU) to place the dried fruit pulp on the market as a novel food ingredient in 2008 (OJEU, 2008), and after the recognition, in 2009, by the U.S Food and Drug Administration of baobab fruit pulp as Generally Recognized as Safe (FDA, n.d.).

Regarding *G. ternifolia* and *G. kola*, and despite their lower citations by informants in our ethnopharmacological study, they are traditionally used in Angola and in other countries to manage and treat important diseases, for example, cancer, diabetes, malaria, hypertension, and other numerous ailments. *G. kola* may include compounds, particular in the seeds, with antioxidant potential and therefore can combat microbial illness. *G. ternifolia* was selected because the studies regarding their traditional uses and biological activities are scarce; and also, because the plant was mentioned in ethnobotanical studies as a promising plant for medicinal uses and further studies.

4.3.1. *Adansonia digitata*

Adansonia digitata L. (Malvaceae), also known as the baobab, monkey-bread tree, upside-down tree, or pharmacist tree, is widely distributed in sub-Saharan Africa (Wickens & Lowe, 2008).

All *Adansonia* species develop large, ovoid or spherical fruits with a woody pericarp, commonly known as capsules; the botanical term is more precisely amphisarcum: a simple, indehiscent fruit with a pericarp differentiated externally into a dry crust and internally into one or more fleshy layers (Stuppy, 2004). The shape of *A. digitata* can vary considerably depending on distribution area. In Angola, the ripe fruits are rather elongated, ranging in length between 30 and 65 cm. The hard-woody fruit shell has a thickness of approx. 6–10 mm. Fruits of *A. digitata* (also referred as mucua in Angola) contain a light beige fruit pulp, which appears quite tough, chalky and crumbles when dried (Kempe et al., 2018) (Figure 3).

The tree is nicknamed as "the small pharmacy tree" with all its parts (fruit, leaves, and bark) reported to be an essential source of food, traditional medicine, shelter and livelihood to the producing communities in Africa (Ismail et al., 2019; Tsetegho Sokeng et al., 2019).

Figure 3. Bisected fruits and position of nine thermocouples in *A. digitata.* Scale bar 50 mm (Kempe et al., 2018).

4.3.1.1. Bioactive compounds

The edible parts of baobab (leaves, seeds, and fruit pulp) are consumed mostly by rural communities who also sell them in local markets (Muthai et al., 2017).

The high concentration of nutrients in the fruit pulp (mainly vitamin) has been recognized on an international level, triggering export from mainly western and southern Africa to the EU and USA (Fischer et al., 2020). Baobab fruit pulp contains more vitamin C than other fruits (over 100 mg/100 g) and it is a good source of calcium, iron, magnesium, and dietary fibre (Muthai et al., 2017).

Few studies are reported about chemical characterization of phenolic compounds of baobab fruit pulp and results showed a high variability between plants from different geographic regions (Braca et al., 2018). Baobab fruit parts have been studied as a source of phenolic compounds, which include flavonoids, phenolic acids, and their glycosides, reported in the pulp (Ismail et al., 2019; Li et al., 2017), seeds (Ismail et al., 2019a; Salih & Yahia, 2015) and also in the leaves (Tsetegho Sokeng et al., 2019).

A study of a Nigerian baobab fruit pulp, performed by Ultra High-Performance Liquid Chromatography with diode-array and high-resolution electrospray ionization mass spectrometry detection (UHPLC-DAD-HR-ESI-MS), isolated hydroxycinnamic acid glycosides, iridoid glycosides, and phenylethanoid glycosides (Li et al., 2017). Tsetegho Sokeng et al. (2019) revealed the presence of procyanidins, phenolic acids, and flavonol glycosides in fruits from Cameroon, by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric (LC-ESI-MS/MS) analysis. Tembo et al. (2017) reported the presence of procyanidin B2, gallic acid, and epicatechin in a Malawi baobab fruit pulp. Finally, Ismail et al., (2019b) detected six phenolic acids in baobab fruit shells, mostly derivatives of hydroxycinnamic and hydroxybenzoic acids (protocatechuic acid, *p*-hydroxybenzoic acid, *p*-

coumaric acid, chlorogenic acid and dihydrocaffeic acid, vanillic acid). The flavonoids identified included sixteen flavonols (kaempferol and quercetin glycosides including rutin and isorhamnetin), eight flavanols, and a flavone (apigenin). Other compounds were also identified and characterized, including organic acids, hydroxy fatty acids, and saponin derivatives (Ismail et al., 2019b).

4.3.1.2. Biological activities, traditional or therapeutic uses

The flour of baobab fruit pulp is popularly used to prepare refreshing drinks, sweets, but also sauces and, recently, ice creams (Braca et al., 2018). In Europe, only the fruit pulp of *A. digitata* is consumed as a food since its authorization as a novel food ingredient by the European parliament and council under the Regulation (EC) No. 258/97 (Commission Decision 2008/575/EC). However, in Africa almost all parts of this tree (fruit pulp, seeds, leaves, flowers, roots, and bark) are used as traditional medicine (Zahra'u et al., 2014) against malaria, tuberculosis, fever, microbial infections (Ramadan et al., 1994), gastrointestinal disorders (constipation and diarrhea), anaemia, and toothache among others (Rahul et al., 2015).

Several extracts from different parts of the plant have been reported to have anti-inflammatory effect, antioxidant properties (Ayele et al., 2013), antiviral (Vimalanathan & Hudson (2009), hepato-protective functions (Adegoke et al., 2017), analgesic (Braca et al., 2018; Owoyele & Bakare, 2018), antipyretic activity (Ramadan et al., 1994), and antidiabetic effects (Braca et al., 2018).

Owoyele & Bakare (2018) investigated the analgesic effect of the aqueous extract of the bark of *A. digitata* using Wistar rats. The findings showed that even at low dosage, *A. digitata* was effective in ameliorating both thermally and chemically (inflammatory) induced pain. For example, animals treated with the plant extract showed significantly ($p < 0.05$) prolonged response time to thermal stimuli (4.42 ± 0.11 s) compared with control group (3.29 ± 0.29 s) in a dose dependent manner. And even at low dosage (25 mg/kg), aqueous bark extract increased the pain threshold of neurons.

The *A. digitata* methanol extract of leaves was evaluated for its antioxidant and anti-inflammatory effects by Ayele et al., (2013). In the ORAC assay, methanolic extract was 10.2 times more potent than vitamin C at eliminating $ROO^{\cdot}$. In 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, methanolic extract also showed a strong reactive oxygen species (ROS) scavenging effect. The extract significantly inhibited NO synthase activity (IC_{50} = 28.6 μg/mL) of lipopolysaccharide-stimulated macrophage Raw264.7 cells.

It has been suggested that medicinal plants could provide protection against different kinds of toxicities with practically little or no side effects. Some African plants are traditionally used in different countries during drug- or toxin-induced toxicities. Adegoke et al. (2017) decided to study the hepatoprotective, LPO and antigenotoxic activities of the methanolic leaf extract of *A. digitata* by comparing the levels of serum aminotransferases, serum alanine and aspartate transferases, in the treated groups and control as an index for hepatotoxicity. Results showed that methanolic leaf extract of *A. digitata* significantly reduce the LPO induced by sodium arsenite in the liver of rats and did not show profound effects on the activities of alanine and aspartate transferases.

Braca et al. (2018) evaluated the extracts of *A. digitata* fruit pulps obtained from three different markets in Mali (Fruits 1, 2, and 3) for their TPC, antioxidant activity and α-glucosidase inhibition. All fruit pulp extracts exerted antioxidant activity (highest for Fruit 3 – from Niamakoro, Segou region) and higher α-glucosidase inhibition than acarbose used as standard. Fruit 3 reported 392.22 ± 28.13 mgTE/g and 799.44 ± 35.96 mgTE/g in DPPH and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assays, respectively. Also, fruit 3 showed the highest reducing power (458.50 ± 23.41 mgTE/g of dried extract) tested by ferric reducing antioxidant power (FRAP).

In another study, the antipyretic activity of *A. digitata* extract (from the fruit pulp) was evaluated on twenty rats, by Ramadan et al. (1994). In this case, hyperthermia was induced by subcutaneous injection of a 12% yeast suspension and the temperature of each rat was monitored. The results showed that after a 4 h treatment period, at a concentration of 800 mg/kg, the rectal temperature of the rats showed a slight decrease (37.3 °C) in comparison to the initial temperature of 38.6 °C, suggesting that baobab extract exhibited antipyretic activity.

Regarding antiviral activity, Vimalanathan & Hudson (2009) studied this activity of *A. digitata* leaves, fruit-pulp and seed extracted with water, DMSO and methanol, against influenza virus, herpes simplex virus and the respiratory syncytial virus. The leaf extract exhibited the most promising activity against the influenza virus with a MIC value ranging from 0.12 µg/mL (DMSO) to 2.8 µg/mL (water).

4.3.1.3. Toxicity

Musila et al. (2013) investigate the toxicity of *A. digitata* traditionally used for the treatment of malaria. Aqueous and organic extracts of *A. digitata* were not toxic to brine shrimp larvae (LD_{50} > 1000 mg/mL). Also, a previous study performed by Ramadan et al. (1994) reported a

LD_{50} > 8000 mg/mL for the aqueous extract of the fruit pulp of *A. digitata* and was categorized as being nontoxic to mice. Therefore, these results explain why most of the plant parts like seeds, fruit pulps and leaves are consumed by many communities (Braca et al., 2018; Bvenura & Sivakumar, 2017).

4.3.2. *Garcinia kola*

Garcinia kola Heckel is a dicotyledonous plant from the Clusiaceae family, which grows about 15 - 17 m high (Dah-Nouvlessounon et al., 2015). It has been recognized as an indigenous and flowering medicinal plant found in the subtropical and tropical rain forest of Central and Western Africa, especially Benin Republic, Sierra Leone, Democratic Republic of Congo, Ivory Coast, Gabon, Ghana, Liberia, Nigeria, Senegal and Cameroon, where it is one of the most valued trees (Ekene & Earnest, 2014). The species of *Garcinia* is sometimes referred to as a "wonder plant" because each of its parts can be used as medicine.

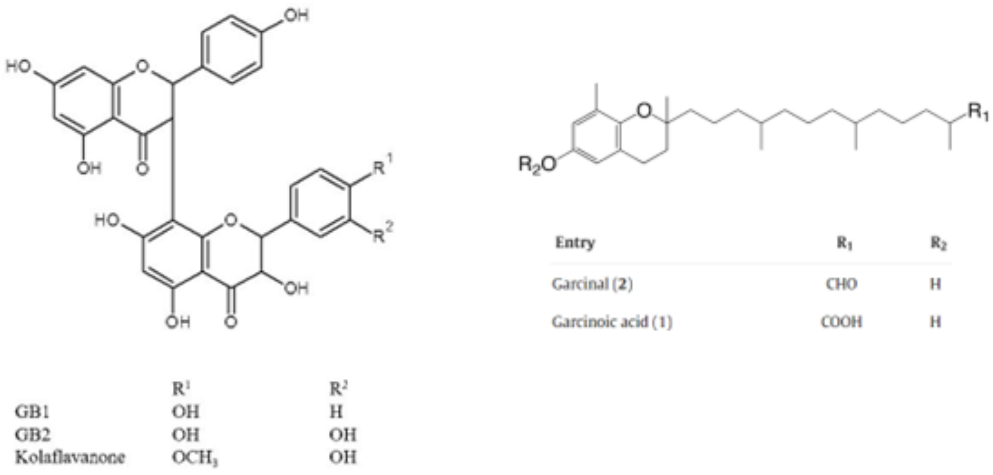
The most valued products are the seeds, commonly chewed by both rural and urban populations to avoid and treat gastric problems or simply for their typical astringent taste (Maňourová et al., 2019; Maňourová et al., 2023). The leaves are simple, 6-14 cm long and 2-6 cm across, shiny on both surfaces and spotted with resin glands. The small flowers are covered with short, red hairs (Iwu, 2014). The fruit is a drupe of 5-10 cm in diameter and weighs 30-50 g. It is usually smooth and contains a yellow-red pulp. The fruit changes colour during maturation from green to orange, and each fruit contains 1-4 seeds (Ekene & Earnest, 2014). It produces a characteristic smooth elliptically shaped seeds, with yellow pulp and brown seed coat (Figure 4).

G. kola seed, also known as "bitter kola" or "false kola", is a highly valued ingredient in African ethno-medicine with an astringent and resinous taste and is used in the management and treatment of several ailments (e.g., coughs, cold, voice hoarseness, aphrodisiac, and liver diseases) (Farombi & Owoeye, 2011; Iwu, 2014). It is also relevant in traditional medicine, cultural and social ceremonies in many parts of West and Central Africa (Farombi & Owoeye, 2011). *G. cola* seems to be an important source of new bioactive compounds with potential therapeutic benefits (Adesuyi al., 2012).

Figure 4. Leaf and fruits (left) and seeds (right) of *Garcinia kola* (Dogara et al., 2022; Onyekwelu et al., 2015).

4.3.2.1. Nutritional and bioactive compounds

The phytochemicals of *G. kola* have attracted much attention in the last years (Icheku et al., 2018). This medicinal plant and other members of the genus *Garcinia* are known to contain in the seeds a complex mixture of phenolic compounds including biflavonoids, xanthones and benzophenones (Farombi, 2003). The high levels of biflavonoid present in the plant have several pharmacokinetic advantages over simple monomeric flavonoids as they survive first-pass metabolism (Iwu & Igboko, 1982). One of the most studied and discussed components in *G. kola* seeds is the kolaviron biflavonoid complex (KV). This complex consists of *Garcinia* biflavanones (GB) GB1, GB2, and kolaflavanone (Figure 5), and has numerous therapeutic effects (Maňourová et al., 2019).



	R^1	R^2
GB1	OH	H
GB2	OH	OH
Kolaflavanone	OCH_3	OH

Entry	R_1	R_2
Garcinal (2)	CHO	H
Garcinoic acid (1)	COOH	H

Figure 5. Biflavonoid complex kolaviron structures (left); garcinoic acid and garcinal (right) (Maňourová et al., 2019).

Even though anti-nutrients such as oxalate and phytate were detected, the seeds are safe for consumption and there are no reports on harmful overdosing so far (Konziase, 2015). Other compounds isolated from *G. kola* include oleoresin, tannin, saponins, alkaloids, and cardiac glycoside. In addition, two new chromanols, i.e., garcinoic acid (GA) and garcinal (Figure 5) together with tocotrienol have been reportedly isolated from bitter kola (Adaramoye et al., 2005; Terashima et al., 2002).

4.3.2.2. Biological activities, traditional or therapeutic uses

G. kola, as a folkloric medicine in Africa, is widely employed for the treatment of liver diseases, diarrhoea, laryngitis, bronchitis (Moneim & Sulieman, 2019), asthma (Okojie et al., 2008) as well as malaria (Ogunkunle et al., 2014). The plant is also well-recognized as aphrodisiac, and a decoction of the leaves and stem bark is traditionally used in the treatment of typhoid fever in Cameroon (Djague et al., 2020). The local people in Central Africa claim that chewing *G. kola* nuts in a small quantity daily has a chemopreventive effect against malaria infection (Tona et al., 1999).

Several studies have reported the biological activities of compounds derived from *G. kola*, including antimicrobial activity (Adegboye et al., 2008), hepatoprotective activity (Adaramoye & Adeyemi, 2006), antioxidant activity (Farombi & Owoeye, 2011); anti-inflammatory activities (Olaleye et al., 2000), α-glucosidase and aromatase inhibitory activities and antiplasmodial activity (Antia et al., 2010; Konziase, 2015).

The presence of tannins in the plant is associated with the use in burns and wounds. The plant's high alkaloid and flavonoid content suggest that they have antioxidant potential (Eleazu et al., 2012).

Kolaviron, a biflavonoid complex extracted from the kola seed, contains GB1, GB2, and kolaflavanone in an approximate ratio of 2:2:1 (Cotterhill et al., 1978). KV has been reported to modulate the hepatotoxicity of carbon tetrachloride (CCl_4), paracetamol, thioacetamide, 2-acetylaminofluorene, aflatoxin B1, and D-galactosamine (Adaramoye, Adeyemi, 2006; Farombi et al., 2005). Adaramoye et al., (2008) confirm that two fractions from KV enhanced recovery from CCl_4-induced hepatotoxicity by decreasing the extent of LPO and also inducing the levels of phase II enzyme glutathione-S-transferase.

Okoko (2009) evaluated the *in vitro* antioxidant and free radical scavenging activities of five fractions from the methanolic extract (ME1-ME5) of *G. kola* seeds. The ME4 fraction, which revealed the presence of four compounds namely garcinia biflavonoids GB1 and GB2,

garcinal and GA, possessed the greatest activities. This fraction strongly inhibited NO production in lipopolysaccharide activated macrophage U937 cells.

Also, Ayepola et al. (2014) evaluated the renal protective effect of KV, in diabetes-induced nephrotoxic rats. The authors concluded that KV treatment of diabetic rats restored the activities of antioxidant enzymes, reduced LPO and increased ORAC and reduced glutathione concentration in renal tissue. Therefore, these beneficial effects may be due to its inhibitory action on oxidative stress, IL-1 production and apoptosis.

Data on anti-inflammatory effects of isolated GA from *G. kola* are rare and until recently its anti-inflammatory potential was merely postulated based on its anti-oxidative effects (Terashima et al., 2002). Data from the study performed by Wallert et al. (2019) revealed that both GA and methanol extract of *G. kola* seeds interfere with anti-inflammatory signaling in lipopolysaccharide-stimulated RAW264.7 macrophages, with GA being slightly more efficient compared to methanolic extract. In other study, Onasanwo & Rotu (2016) explored the mechanisms of action of the antinociceptive and anti-inflammatory potentials of KV and observed that it may be exerting its effects through opioidergic and adrenergic systems instead of the cholinergic system.

The seeds of *G. kola* have broad spectrum antimicrobial activity. Antimicrobial properties of this plant are attributed to benzophenones and flavanones (Konziase, 2015). Although many studies have been performed with this plant, solvents including water, petroleum, butanol and diethyl ether were used which might limit the antimicrobial potentials of plants, since the type of solvent used for plant extraction may have an effect on the nature of compounds extracted and the resulting bioactivity of the extract (Eloff, 1998).

Hioki et al. (2020) evaluated some isolated compounds from the seeds of *G. kola* for their antimicrobial activity against two oral pathogens, *Porphyromonas gingivalis* and *Streptococcus sobrinus*. Among them, (8*E*)-4-geranyl-3,5-dihydroxybenzophenone and δ-GA exhibited antimicrobial activity against both of these microorganisms with a MIC of 31.3-62.5 μM for *P. gingivalis* and 15.6-31.3 μM for *S. sobrinus*. In other study, the authors assessed the bioactivity of *G. kola* seeds on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Plesiomonas shigelloides* and *Salmonella typhimurium.* The inhibition zone diameters of the extracts ranged from 0-24 ± 1.1 mm, while MIC and Minimum Bactericidal Concentration (MBC) values of methanolic extract ranged bet ween 0.04-1.25 mg/mL and 0.081–2.5 mg/mL, respectively. The methanol extract demonstrated a bigger zone diameter of 24 ± 1.1 mm for *S. pyogenes* ($p < 0.05$). *S. pyogenes* and *S. aureus* had the lowest MIC of 0.04 mg/mL (Seanego & Ndip, 2012).

Regarding antimalarial activity, the biflavanoid GB1 present in *G. kola* seeds has been found to exhibit antiplasmodial activity with an IC_{50} value of 1-10 μM (Antia et al., 2010). In his study, Konziase (2015) evaluated the antimalarial potencies *in vitro* and *in vivo* of pure biflavanones from *G. kola*. The biflavanones isolated showed not only potent inhibitory activity *in vitro* against *P. falciparum* proliferation, with GB-1 exhibiting the strongest activity with an IC_{50} of 0.16 μM, but also antimalarial potency through oral administration in mice infected with *Plasmodium berghei* without signs of acute toxicity.

4.3.2.3. Toxicity

Although previous studies have reported the therapeutic uses of various extracts and fractions of *G. cola* seed (Hioki et al., 2020; Konziase, 2015), the consumption of whole seeds especially for long time produce stimulant like effect which could induce some toxicological effects. In their study, Oboh et al. (2018) investigate the effects of *G. cola* seed on survival, locomotion, and oxidative stress markers in *Drosophila melanogaster*. Results from the study revealed that higher (0.5% and 1.0%) dietary inclusions of the plant reduced the survival rate (14.9% and 16.4% reduction in survival, respectively, compared to control) and significantly ($p < 0.001$) reduced fly locomotion after just 5 days of exposure. These results suggest that the seed induced some toxicological effects at these concentrations, which could be attributed to the major phytochemicals in *G. cola* seed, namely, saponins and glycosides that have been reported to be toxic at high concentration. However, dietary inclusion of 0.1% of *G. cola* seed seems tolerable to flies and even produced some percentage increase in flies' survival rate, no significant effect on locomotor performance, and non-impairment in acetylcholinesterase activity compared to the control (Oboh et al., 2018).

4.3.3. *Gardenia ternifolia*

G. ternifolia Schumach. & Thonn. (Rubiaceae) is an evergreen shrub about 5 to 10 m high widely used in African traditional medicine for the treatment of many infectious diseases (Awas et al., 2016; Huxley et al., 1992). The intertwined branches have short twigs which are very hard and thorny (von Maydell, 1986). The genus *Gardenia* comprises of about 140 species recorded in Africa, Madagascar, East and Southeast Asia, western Pacific and Hawaiian Islands (Wong & Low, 2011). The species name "ternifolia" is derived from the Latin word "ternifolius" which means leaves in threes. The bark of *G. ternifolia* is grey to yellowish-brown in color, smooth or slightly rough and peeling off in round pieces in thicker and older trees.

The leaves are usually in whorls of three, clustered near the ends of short rigid branchlets (Figure 6) (Maroyi, 2020).

Figure 6. *Gardenia ternifolia* plant (Agbodjento et al., 2009).

4.3.3.1. Nutritional and bioactive compounds

A wide variety of nutrients associated with different plant parts of *G. ternifolia* imply that the species could be a source of health-promoting nutrients such as calcium, carbohydrates, copper, crude fibre, fat, iron, magnesium, phosphorus, potassium, proteins, sodium and zinc (Maroyi, 2020).

The plant *G. ternifolia* contains bioactive compounds identified from the aerial parts, fruits, leaves, roots and stem bark, which include alkaloids, anthraquinones, flavonoids, phenols, saponins, sterols, tannins, terpenoids, and stereoisomeric neolignans (Aragaw et al., 2020; Maroyi, 2020). According to Awas et al., (2016) phytochemical investigation of surface exudates of the *G. ternifolia* leaves resulted in the characterization of four flavonoids: 3,5,3′-trihydroxy-7,4′-dimethoxyflavone (**1**); 3,5,7- trihydroxy-4′-methoxyflavone (**2**); 5,7-dihydroxy-3,4′-dimethoxyflavone (**3**); 5,4′-dihydroxy-7-methoxyflavanone (**4**); and two triterpenoids: β-sitosterol (**5**) and stigmasterol (**6**) (Figure 7). Also, Tshitenge et al., (2017) isolated and fully structurally characterized eight stereoisomeric 2,3-dihydrobenzo[b]furan neolignans, named gardenifolins A-H (**1a−d** and **2a−d**).

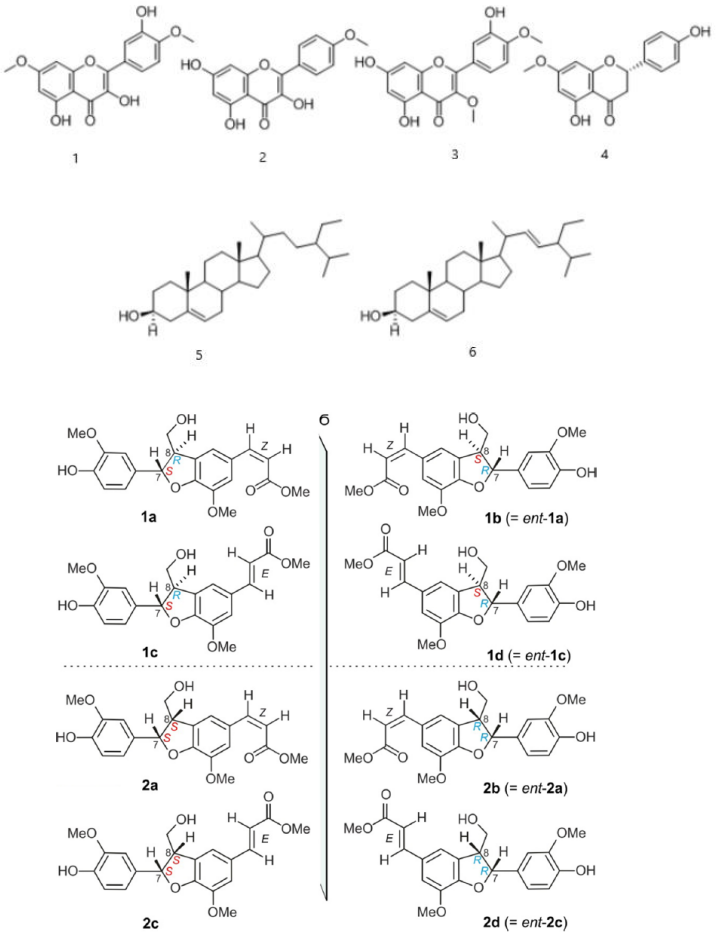


Figure 7. Compounds from the surface exudates of *G. ternifolia* (Awas et al., 2016).

4.3.3.2. Biological activities, traditional or therapeutic uses

Ethnomedical information indicates that stem and root barks of *G. ternifolia* has been used by traditional healers, in different countries, to treat several health conditions. For example, in Ethiopia, the roots of *G. ternifolia* is used to palliate malaria and its related fevers (Giday et al., 2009; Kokwaro, 1993; Nureye et al., 2018; Weenen et al., 1990). In Sudan, its fruits are used in the treatment of malaria (Farah et al., 2018). In Togo, *G. ternifolia* leaves are traditionally used in the treatment of hypertension and diabetes (Karou et al., 2011). In the Guinean pharmacopoeia, all parts of *G. ternifolia* are known to have powerful antibiotic, hypotensive, and antidiabetic properties (Magassouba, et al., 2007). Fresh leaves and edible fruit extracts are also used by tribal healers, in RD Congo, for hemorrhoid lesions. In South Africa and Zimbabwe is used for earache (Maroyi, 2020). Also, the macerated root extract is employed as a laxative and vermifuge for the treatment of stomach aches (Achigan-Dako et al., 2011).

Different parts of this plant have exhibited biological activities including antibacterial (Silva et al., 1996), antiviral (Silva et al., 1997), anti-inflammatory (Larsen et al., 2015; Pompermaier et al., 2018), antiplasmodial (Aragaw et al., 2020; Nureye et al., 2018; Ochieng et al., 2010), and anticancer (Tshibangu et al., 2016).

Regarding antibacterial activity, Silva et al. (1996) tested *G. ternifolia* root maceration and decoction. However, only the latter was active against some organisms, which is consistent with the traditional use of the remedy (hot extract). This extract exhibited activities against *Campylobacter jejuni*, *Campylobacter coli* and *Staphylococcus aureus* with zones of inhibition ranging from 9.0 mm to 14.0 mm. Also, Magassouba et al. (2007) evaluated the antibacterial activities of methanol extract of *G. ternifolia* root bark mixed with those of *Swartzia madagascariensis* Desv., Isoberlinia Doka Craib & Stapf, *Annona senegalensis* Pers., *Terminalia glaucescens* Planch. Ex Benth. and leaves of *Erythrina senegalensis* DC. against *Staphylococcus aureus* using broth dilution method. The extract showed activities against tested pathogens with a Minimum Inhibitory Concentration (MIC) value of 62.5 µg/mL (Magassouba et al., 2007). Another study from Tsobou et al. (2015) showed that ethanol extract of *G. ternifolia* bark exhibited weak activities against *Salmonella typhi* with inhibition zone ranging from 9.5 mm to 11.0 mm, MIC value of 512.0 µl/mL and MBC value of 2048.0 µl/mL (Tsobou et al., 2015).

In 1997, twenty-eight extracts prepared from plants used in Africa were screened by Silva et al., (1997) in order to assay their antiviral activity against Herpes simplex virus type 1 (HSV-1) and African Swine Fever Virus (ASFV). The ethanol extract of *G. ternifolia* roots showed activities against HSV-1 and ASFV exhibiting inhibition effect of 60.0% and 80.0%, respectively (Silva et al., 1997).

Larsen et al. (2015) tested ethanolic extracts of 17 species in a cyclooxygenase (COX)-1 assay, and the extracts of *G. ternifolia*, *Thonningia sanguinea*, *Triumfetta rhomboidea*, and the roots of *Zanthoxylum zanthoxyloides* showed an inhibitory effect over 90% in the final concentration 0.1 µg/µL. Of the four species exhibiting COX-1 inhibition, only *G. ternifolia* has remained in use over the centuries (Larsen et al., 2015). Also, Pompermaier et al. (2018) evaluated the anti-inflammatory activities of methanol extract of *G. ternifolia* seeds at different concentrations (10.0, 50.0 and 100.0 µg/mL) to assess their inhibition of COX-2 expression and NO production inhibition. At a concentration of 10.0 µg/mL and 100.0 µg/mL, inhibition on COX-2 expression was 61.72 ± 4.00% and 73.29 ± 1.44, respectively; and inhibition of NO release ranged from 63.13 ± 2.73% to 91.18 ± 6.30% (Pompermaier et al., 2018).

Some claims made by traditional healers have shown the use of *G. ternifolia* as a remedy for malaria. Nureye et al. (2018) tested the 80% methanolic root bark extract and solvent fractions (chloroform, n-butanol, and water) of *G. ternifolia* agains *Plasmodium berghei*. The chemosuppressive effect exerted by the crude extract and fractions ranged between 30-59% and 14-51%, respectively. Among the fractions, butanol and chloroform fractions exerted a better chemosuppressive effect than aqueous fraction, which can be explained by the presence of alkaloids, flavonoids, and saponins in butanol fraction. A previous study from Ochieng et al. (2010) also demonstrated that the extracts (acetone and methanol) and flavonoids and steroids (quercetin-4,7-O-dimethyl ether, kaempferol-7-O-methyl ether and naringenin-7-O-methyl ether and β-sitosterol) isolated from *G. ternifolia* leaf surface exudates showed an *in vitro* antiplasmodial activity (50% inhibitory concentration, IC_{50}, values ranging from 0.9 to 17.0 μg/mL) against chloroquine-resistant Indochina and chloroquine sensitive to Sierra-Leone strains of *P. falciparum*. More recently, Aragaw et al. (2020) concluded that hydromethanolic crude extract and chloroform fraction of *G. ternifolia* leaves have shown promising antimalarial activity. The antimalarial effects of both hydromethanolic crude extract and chloroform fraction of *G. ternifolia* leaves have higher mean percent parasitemia inhibition, reduction in weight, prevention of anemia, and a rise in mean survival time in days in a dose-dependent manner (Aragaw et al., 2020).

In the last years, a greater emphasis has been given towards the research on medicinal plants and its bioactive compounds related with cancer management. Tshibangu et al. (2016) prepared successive extractions of *G. ternifolia* leaves, using different solvents (petroleum ether, chloroform, ethyl acetate, ethanol and methanol 80%), and evaluated their cytotoxicity against prostate adenocarcinoma (PC-3) and on breast adenocarcinoma (MCF-7). For MCF-7 cell line, the extracts showed values of CC_{50} (50% cytotoxic concentration) of 21.62 μg/mL and 45.44 μg/mL for chloroform and ethyl acetate extracts, respectively. For PC-3 cell line the best CC_{50} values were 9.66 μg/mL, 24.47 μg/mL and 92.10 μg/mL, respectively for chloroform, ethyl acetate and methanol extracts. Tshitenge et al. (2017) demonstrated that individual pure gardenifolin isomers A-H (Figure 7) have different cytotoxic effects against HeLa cells, with 1d and 2a (Figure 7) showing the highest activities, with IC_{50} values of 21.0 and 32.5 μM, respectively. Also, gardenifolin D induces apoptosis on HeLa cells at 25 μM.

Farah et al. (2012) investigated *in vitro* activity of *G. ternifolia* fruits aqueous extract against *Theileria lestoquardi* (which cause malignant ovine theileriosis, a parasitic disease of sheep and goat). The results of the study showed that the *in vitro* activity of the plant extract against *T. lestoquardi* macroschizonts at 250 ppm concentration was 0%, significantly ($p < 0.05$)

increased at 500, 5000 and 10000 ppm being 13, 40 and 60%, respectively. Lethal concentration, 50% and 99% (LC_{50} and LC_{99}) were 6745.28 and 177010.90 ppm, respectively.

4.3.3.3. Toxicity

Despite medicinal uses, few toxicological studies have been conducted on most medicinal plants. Agbodjento et al. (2020) evaluated the toxicological effect of *G. ternifolia* through Artemia salina larvae cytotoxicity and subacute toxicity tests. Larval cytotoxicity data indicate that the studied plants extracts are not cytotoxic ($LC_{50} > 0.1$ mg/mL). The subacute toxicity of *G. ternifolia* was evaluated in male Wistar albino rats at three different doses (200, 400, and 800 mg/kg). Rats treated with *G. ternifolia* showed a non-significant decrease in alanine aminotransferase levels and a significant increase in uremia. Therefore, the results indicated that no alteration of haematological parameters was observed and only a possible renal impairment was reported. In another study, Farah et al. (2018) showed plant fruit safety at 50 mg/kg but altered haematological and biochemical parameters at 500 mg/kg. Nureye et al. (2018) showed that the crude hydroalcoholic extract from root barks of *G. ternifolia* was safe for mice at a single dose of 2000 mg/kg. The toxicity observed may be related to the alkaloids present in the parts of the plant (Agbodjento et al., 2020).

4.4. *In vitro* antioxidant, antibacterial and cytotoxic activities of *A. digitata, G. kola* and *G. ternifolia*

In this study, the plant materials were sequentially extracted with different solvents in increasing polarity order, however, for the evaluation of antioxidant, antibacterial, and cytotoxic activities we used the final extracts (methanolic and aqueous), because they are the solvents that can extract more diversity of compounds, and because water is still used as in the preparation of traditional herbal medicine. This may be related, in part, to the fact that water helps in the diffusion of extractable compounds through plant tissues.

4.4.1. Extraction yield

Different solvents (water and methanol) extracted different quantity of crude extracts obtained from aerial plant parts (leaves and stems), seeds, and fruits. The results showed that aqueous extracts (AE) showed higher percentages of yield compared to methanolic (ME) in all the studied plants (Table 8).

Table 8. Yield percentage of aqueous and methanolic extracts of *A. digitata*, *G. kola* and *G. ternifolia.*

Plant/Extract	Yield (%)	
	AE	ME
Adansonia digitata	4.22 ± 0.31	4.14 ± 0.12
Garcinia kola	3.29 ± 0.24	2.93 ± 0.11
Gardenia ternifolia	5.37 ± 0.35 [a]	4.39 ± 0.10 [b]

AE: aqueous extract; ME: methanolic extract. Comparisons between AE and ME of each plant were performed, and significant differences (p <0.05) are represented with different letters.

However, only for *G. ternifolia* there was a statistically significant difference between extracts (aqueous and methanolic) ($p \leq 0.01$), for which water yielded the highest extractable solids (5.37%) (Table 8).

Solvents commonly used in extraction of medicinal plants are polar solvent (e.g., water,), intermediate polar (e.g., acetone, ethanol, methanol, dichloromethane), and nonpolar (e.g., n-hexane, chloroform). The choice of solvent depends on the type of plant, part used in the extraction, nature of the bioactive compounds to be extracted, and the solvents availability (Altemimi et al., 2017). Solvent type and polarity can affect the extract quality, quantity, extraction velocity, and toxicity (Zhang et al., 2019). In this study, the plant materials were sequentially extracted with different solvents in increasing polarity order. The sequential extraction method ensures the extraction of active compounds from plant material according to its polarity, and also reduces the antagonistic effect of compounds in the extract (Jeyaseelan et al., 2012). Among the extraction solvents used, water and methanol were the solvents selected for the assays performed in this work. On one hand, water is the most polar solvent and is used in the extraction of a wide range of polar compounds. It is also cheap, nontoxic, and nonflammable. On the other hand, methanol is an alcohol, with high polarity, miscible with water (Abubakar & Haque, 2020) whose efficiency in the extraction of phytochemicals, namely phenolic compounds, has been documented (Ezekiel et al., 2009; Njume et al., 2011; Sultana et al., 2009). Flavonoids and their glycosides are easily extracted using ethanol, whereas phenolic acids, catechin, and other polar important compounds are more efficiently extracted using methanol, increasing the total polyphenol content in the extracts obtained (Chirinos et al., 2007; Stalikas, 2007). Boeing et al., (2014) showed that among the pure solvents, methanol was the most efficient solvent for extraction of antioxidant compounds, followed by water, ethanol, and acetone. These may be due to the better solvation of antioxidant compounds present in the fruits and other aerial parts of the plants studied as a result of interactions (hydrogen bonds) between the polar sites of the antioxidant molecules and the solvent.

The results of this study are in line with others confirming methanol as a good extractant of bioactive compounds from plants. However, the extract yield may not be related with its biological activity (Do et al., 2014). For example, the highest yields of extractable solids were $4.22\% \pm 0.31$, $3.29\% \pm 0.24$, and $5.37\% \pm 0.35$ for aqueous extracts of *A. digitata*, *G. kola* and *G. ternifolia,* respectively; but the best results in the assays performed were observed, in general, for methanolic extracts.

In their study, Alrasheid et al. (2019) showed that among the extracts obtained using an alcohol (96% (m/V) ethanol), leaf extracts of *A. digitata* gave a yield of 14.7%, while the bark extract of *A. digitata* gave the lowest yield of 1.9%. The yield for the fruits was 4.64%, which is similar to our results (4.22% for aqueous extract and 4.14% for methanolic extract). Regarding *G. kola* seeds extracts, Ugwuowo et al. (2021) showed a yield of 3.97% for *G. kola* (80 % methanol), Seke et al. (2022) a yield of 25.1% (seeds, 80% methanol for 72 h), and Yakubu & Quadri (2012), a yield of 5.5% (seeds, distilled water for 48 h). These values were higher compared to the yields of *G. kola* seed extracts observed in this study (3.29% for aqueous extract and 2.93% for methanolic extract). The differences in the extract yields might be related to the different availability of extractable compounds, resulting from the diverse chemical composition of plants. Also, the extractable compounds are mainly affected by the extraction procedure and solvent (Sultana et al., 2009). In the case of *G. ternifolia*, Agbodjento et al. (2020) observed that the lowest yield was obtained with roots (< 5%). In our study, we obtained a yield of 4.39% with aerial parts of methanolic extract. In another study, Klotoé et al. (2020) showed that aqueous extract of *G. ternifolia* was more effective in improving the extraction yield (with values higher than hydroethanolic and ethanolic extracts), which is in accordance with our results.

4.4.2. *In vitro* antioxidant activity

Several *in vitro* techniques have been used for preliminary evaluation of the antioxidant potential of plant extracts. Different assay mechanisms including HAT, electron transfer (ET), and metal chelation can be used for the antioxidant activity determination (Gulcin, 2020). Methodologies have different reaction mechanisms, so the results obtained depend on the method used. For this reason, it is recommended to use at least two methods to provide a reliable antioxidant capacity of the sample (Pérez-Jiménez et al., 2008). In this study, four different methods were used to evaluate the antioxidant capacity of the three African medicinal plants: $DPPH^{\bullet}$ assay, metal chelating activity, H_2O_2 scavenging assay, and ABTS assay.

DPPH assay is based on ET and/or HAT from the antioxidants to DPPH free radical. According to the principle, the degree of decolorization of the DPPH solution caused by the neutralization of a comparable number of radicals is proportional to the concentration and potency of the antioxidants (Gulcin, 2020). Metal chelation can also be used for the antioxidant activity determination, since an excess of free irons has been implicated in the induction and formation of free radicals in biological systems. Thus, the African plant extracts in this study were also assessed with a metal chelating assay. Hydrogen peroxide is rapidly decomposed into oxygen and water, and this may produce ·OH that can initiate LPO and cause DNA damage (Sahreen et al., 2011). ABTS radical scavenging assay involves a method that generates a blue/green $ABTS^+$ chromophore via the reaction of ABTS and potassium persulfate (Saeed et al., 2012). The ($ABTS^{\bullet+}$) radical cation-based assays are among the most abundant antioxidant capacity assays, together with the DPPH radical-based assays (Ilyasov et al., 2020).

The results obtained for our extracts are described in Table 9 and varied depending on the method used. IC_{50} values ranged from 42.5 µg/mL to 846.0 µg/mL in the DPPH assay; from 51.5 µg/mL to > 1000 µg/mL in the iron chelating assay; from 6.1 µg/mL to 124.0 µg/mL in the H_2O_2 assay; and from 20.6 µg/mL to 491.4 µg/mL in the ABTS assay.

Regarding DPPH assay, there was a significant difference in the DPPH scavenging activity among the plant extracts, and between the extracts for each plant and the positive standard quercetin ($p < 0.05$). Best results of IC_{50} were observed for methanolic extracts (42.5 ± 0.4 µg/mL for *G. ternifolia*; 46.7 ± 2.9 µg/mL for *A. digitata*; and 68.2 ± 3.5 µg/mL for *G. kola*). These results are in accordance with iron chelating activity where methanolic extracts also showed the lowest IC_{50} values (51.5 ± 2.7 µg/mL for *G. ternifolia*; 139.8 ± 1.8 µg/mL for *G. kola*, and 823.1 ± 12.1 µg/mL for *A. digitata*). However, comparing the extracts with positive control, IC_{50} values for quercetin and EDTA were always significantly lower (1.8 ± 0.2 µg/mL for quercetin, and 1.2 ± 0.0 µg/mL for EDTA).

With regard to H_2O_2 scavenging activity, significant differences were also observed between aqueous and methanolic extract of each plant. Except for *G. kola*, the other plant extracts showed best H_2O_2 scavenging capacity for methanolic extracts. Also, the H_2O_2 scavenging capacity of aqueous extract of *G. kola* (IC_{50} = 6.1 ± 0.1 µg/mL) and methanolic extract of *G. ternifolia* (IC_{50} = 6.5 ± 0.8 µg/mL) were found to be lower than the positive control, ascorbic acid (IC_{50} = 10.0 ± 0.1 µg/mL).

Table 9. *In vitro* antioxidant activities of aqueous and methanolic extract of each studied plant.

Plant species	DPPH• scavenging (IC_{50}, µg/mL)			Iron (II) chelating (IC_{50}, µg/mL)		
	AE	ME	Q	AE	ME	EDTA
Adansonia digitata	846.0 ± 20.9 [a,#]	46.7 ± 2.9 [a,#]		ND	823.1 ± 12.1 [#]	
Garcinia kola	440.0 ± 5.7 [a,#]	68.2 ± 3.5 [a,#]	1.8 ± 0.2 [#]	154.8 ± 4.4 [a,#]	131.1 ± 1.2 [a,#]	1.2 ± 0.0 [#]
Gardenia ternifolia	264.2 ± 13.5 [a,#]	42.5 ± 0.4 [a,#]		420.2 ± 3.3 [a,#]	51.5 ± 2.7 [a,#]	
Plant species	**Hydrogen peroxide assay (IC_{50}, µg/mL)**			**ABTS scavenging (IC_{50}, µg/mL)**		
	AE	ME	AA	AE	ME	AA
Adansonia digitata	124.0 ± 15.1 [a,#]	13.2 ± 1.6 [a]		491.4 ± 0.1 [#]	446.5 ± 45.0 [#]	
Garcinia kola	6.1 ± 0.1 [a]	22.7 ± 1.8 [a]	10.0 ± 0.1 [#]	72.6 ± 6.2 [#]	35.5 ± 1.3	1.57 ± 0.2 [#]
Gardenia ternifolia	23.4 ± 3.2 [a]	6.5 ± 0.8 [a]		211.1 ± 32.3 [a,#]	20.6 ± 2.0 [a]	

AE: aqueous extract; ME: methanolic extract; Q: quercetin; EDTA: ethylenediaminetetraacetic acid; AA: ascorbic acid; ND: not detected at the concentration range tested (1–1000 µg/mL). The IC_{50} values (µg/mL) are expressed as mean ± standard deviation from three independent assays (n = 3). Significant differences ($p < 0.05$) between AE and ME are represented by the same letters; [#] Significantly different compared with control ($p < 0.05$).

Regarding the antioxidant activity quantified by ABTS, again the metanolic extracts showed the best values, particularly methanolic extract of *G. ternifolia* (IC_{50} = 20.6 ± 2.9 µg/mL), with significant differences between the two extracts, although none of them reached the positive control value.

Oxidative stress is one of the major causes of health problems. It is associated to cancer, cardiovascular disorders, and neurodegenerative diseases (Salehi et al., 2020; Velasco et al., 2010). At low or moderate concentrations, reactive oxygen species (ROS) exert beneficial effects on cellular responses and immune function. At high levels, however, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA (Pham-Huy et al., 2008).

Synthetic antioxidants are used routinely in the medicine and food industries. However, these compounds can be associated with adverse effects and potential toxicities (Xu et al., 2021). Therefore, in recent years, there is an increasing interest in finding natural antioxidants derived from plants, because they can inhibit the propagation of free radical reactions, and thereby protect the human body from diseases.

Omisore et al. (2005) considered the cut-off point for antioxidant activity as 50 µg/mL. Therefore, samples with IC_{50} > 50 µg/mL were classified as being moderately active, while samples with IC_{50} < 50 µg/mL were judged as having high antioxidant capacity. Kuete &

Efferth (2010) had a similar classification, where samples were considered to have high or significant antioxidant capacity with IC_{50} < 50 μg/mL (extract) or IC_{50} < 10 μg/mL (compounds), moderate antioxidant capacity with 50 < IC_{50} < 100 μg/mL (extract) or 10 < IC_{50} < 20 μg/mL (compounds) and low antioxidant capacity with IC_{50} > 100 μg/mL (extract) or IC_{50} > 20 μg/mL (compounds). In this study, some extracts showed to have high antioxidant activity. In DPPH assay, methanolic extracts of *G. ternifolia* and *A. digitata* showed IC_{50} values < 50 μg/mL (42.5 ± 0.4 μg/mL and 46.7 ± 2.9 μg/mL, respectively). In H_2O_2 assay, *G. kola* aqueous extract had the lower IC_{50} (6.1 μg/mL). Also, aqueous extract of *G. ternifolia* and methanolic extracts of *G. ternifolia*, *A. digitata*, and *G. kola* had IC_{50} values < 50 μg/mL (23.4 μg/mL, 6.5 μg/mL, 13.2 μg/mL, and 22.7 μg/mL, respectively). Finally, in ABTS assay, only methanolic extracts of *G. ternifolia* and *G. kola* showed significant antioxidant activity (IC_{50} = 20.6 μg/mL and 35.5 μg/mL, respectively).

Alrasheid et al. (2019) reported that the extracts obtained from the fruits of *A. digitata* had higher values of DPPH radical scavenging activity (83.98%), compared to extracts from bark (66.90%) and leaves (15.69%). In the study of Badmus et al. (2014) the methanolic leaf extract of *G. kola* exhibited DPPH scavenging activity with an IC_{50} value of 126.0 μg/mL and $ABTS^{\bullet+}$ was inhibited by the extract in a dose-dependent manner, with an IC_{50} value of 34.3 μg/mL. Popoola et al. (2019) showed that in the DPPH assay, *G. kola* extract (stem bark, 70% ethanol) had IC_{50} values (38.13 ± 2.426 μg/mL) significantly lower than that of ascorbic acid (48.36 ± 0.919 μg/mL). In our study, the methanolic extract of seeds from *G. kola* showed lower IC_{50} values (68.2 ± 3.5 f μg/mL for DPPH and 35.5 ± 1.8 μg/mL for ABTS assay) which showed that the extract can participate in electron transfer, in addition to its H-donating property as found in DPPH radical scavenging. In our study aqueous and methanolic extracts of *G. kola* and *G. ternifolia* efficiently scavenged H_2O_2 (with IC_{50} values < 50 μg/mL), which may be related to the presence of phenolic groups that could donate electrons H_2O_2, thereby neutralizing it into water (Saeed et al., 2012).

In general, a higher polyphenol content is reflected in a higher antioxidant capacity, for this reason, TPC and TFC were also determined, and the results are shown in Tables 10 and 11, expressed as milligram of gallic acid equivalent per gram of extract and milligram of quercetin equivalent per gram of extract, respectively.

In this study, the TPC determination was performed by the well-known Folin-Ciocalteu assay and the best TPC values were 131.4 mg GAE/g ± 5.7, 87.6 mg GAE/g ± 1.8, and 42.8 mg GAE/g ± 2.6, for *G. kola*, *A. digitata* and *G. ternifolia* methanolic extracts, respectively. Also, significant differences were observed in TPC, between aqueous and methanolic extract

of each plant. Regarding the TFC, the results followed a similar tendency with the methanolic extract of *A. digitata* showing the best TFC (46.0 ± 5.0 mg QE/g).

Table 10. Total phenolic content (TPC) of aqueous and methanolic extracts of the studied plants.

Plant species	Total phenolic content (mg GAE/g)	
	AE	ME
Adansonia digitata	17.7 ± 0.6 [a]	87.6 ± 1.8 [b]
Garcinia kola	34.2 ± 2.0 [a]	131.4 ± 5.7 [b]
Gardenia ternifolia	33.3 ± 0.5 [a]	42.8 ± 2.6 [b]

AE: aqueous extract; ME: methanolic extract; GAE: gallic acid equivalents. The results are expressed in mg gallic acid equivalents (GAE) per gram of dried extract, and the values are the mean ± standard deviation from four independent assays (n=4). Comparisons between AE and ME of each plant were performed, and significant differences ($p < 0.05$) are represented with different letters.

Table 11. Total flavonoid content (TFC) of aqueous and methanolic extracts of the studied plants.

Plant species	Total flavonoid content (mg QE/g)	
	AE	ME
Adansonia digitata	12.1 ± 3.5 [a]	46.0 ± 5.0 [b]
Garcinia kola	23.8 ± 2.5 [a]	38.8 ± 1.0 [b]
Gardenia ternifolia	24.3 ± 3.3 [a]	45.4 ± 1.0 [b]

AE: aqueous extract; ME: methanolic extract; QE: quercetin equivalents; The results are expressed in mg quercetin equivalents (QE) per gram of dried extract, and the values are the mean ± standard deviation from four independent assays (n=4). Comparisons between AE and ME of each plant were performed, and significant differences ($p < 0.05$) are represented with different letters.

Consistent with this finding, methanolic extracts showed higher TPC, TFC and antioxidant activity in general, compared to aqueous extracts of the three plants analyzed. In fact, aqueous extract of *A. digitata* showed the lowest antioxidant activity in the DPPH, H_2O_2, and ABTS assays, and had the lowests TPC and TFC (17.7 mg GAE/g and 12.1 mg QE/g, respectively).

In our study, *A. digitata* had a TPC of 17.7 ± 0.6 mg GAE/g of dried extract for aqueous extracts and a value of 87.6 ± 1.8 mg GAE/g of dried extract for methanolic extract. It also has a TFC of 12.1 and 46.0 mg QE/g for the same both extracts. Therefore, methanolic extracts showed higher TPC and TFC, which are in accordance with other published results. For example, Sugandha & Shashi (2017) showed that methanolic extract from the fruit of *A. digitata* was found to have higher flavonoid content followed by aqueous extract. In another study, Klotoé et al. (2020) showed that, in general, aqueous extracts from the plants studied (which included *G. ternifolia*) showed a low total polyphenols content, unlike ethanolic and

hydroethanolic extracts. Finally, Braca et al. (2018) showed values that ranged between 120.1 ± 4.7 and 161.40 ± 2.8 mg GAE/g of dried extract, for n-butanol extracts of baobab fruit pulp.

Several studies substantiate the positive correlation of phenolics quality with DPPH free radical scavenging effect and other antioxidant assays (Liu et al., 2009; Reddy et al., 2021). According to Dudonné et al. (2009), significant correlations were found between DPPH, ABTS, and ferric reducing antioxidant power (FRAP) assays and TPC. Rita et al. (2022) showed that *A. digitata* aqueous extract possesses a strong antioxidant activity and ROS inhibitory capacity. In their study, the authors reported that polyphenolic compounds found in the extract, particularly procyanidins and tannins, could be responsible for its antioxidant activity. In another study, Dzoyem et al. (2014) showed that acetone extract of *A. digitata* (fruit) had the highest antioxidant capacity (IC_{50} values of 8.15 µg/mL and 9.16 µg/mL in the DPPH and ABTS assays respectively) along with the highest amount TPC (237.68 mg GAE/g) and TFC (16.14 mg QE/g).

Antioxidant properties of the plant extracts observed in this study may be attributed to the presence of polyphenol components. Baobab fruit parts are a good source of phenolic compounds (e.g. flavonoids, phenolic acids, and their glycosides). In their study, Ismail et al. (2019b) identified six phenolic acids (protocatechuic acid, *p*-hydroxy-benzoic acid, chlorogenic acid, *p*-coumaric acid, vanillic acid, and dihydrocaffeic acid), sixteen flavonols (e.g. rutin, kaempferol, quercetin, isoquercetin, isorhamnetin), eight flavanols (e.g. D- (+) catechin, (−)-epicatechin, (−)-epiafzelechin), and a flavone (apigenin) in the fruit of the plant. Also, several new compounds such as dihydroflavonols, isorhamnetin, (−)-epiafzelechin, kaempferide, glucosyringic acid, betulinic acid, were reported in baobab fruit (Ismail et al., 2019b).

According to Flora et al. (2016), the methanolic root and stem extracts of *G. kola* contains flavonoids, phenols and tannins. Kolaviron (Figure 5), the biflavonoid from *G. kola* seeds appears to be as effective as butylate hydroxyanisole (BHA) as an *in vivo* natural antioxidant and an effective hepatoprotective agent (Farombi et al., 2000).

In our study, we tryed to identify the main phenolic components present in the extracts, but only the presence of quinic acid, chlorogenic acid, gentisic acid, and 4,5-dicaffeoylquinic acid was detected in the methanolic extract of *G. ternifolia* (data not shown). Further studies are necessary to identy the phenolic compounds in all the extracts. In their study, Kim et al., (2006) identified five new quinic acid derivatives from *Gardenia jasminoides*, which were found to have potent DPPH radical scavenging, superoxide anion scavenging, and LPO inhibition activities. These results are consistent with ours, where *G. ternifolia* methanolic extract showed

IC_{50} values < 50 µg/mL in DPPH, ABTS and H_2O_2 scavenging assays. Chlorogenic acid is a quinic acid conjugate of caffeic acid for which anticancer, antioxidant and anti-inflammatory activities have also been reported (Kumar et al., 2020). Further studies towards the identification of the main components responsible of the activity will be performed in the future.

4.4.3. Antibacterial activity

As shown in Table 12, the methanolic plant extracts showed some antibacterial activity against two tested microorganisms, *E. coli* and *S. aureus*, with the diameters of zone of inhibition ranging between 8.0 and 9.5 mm for *A. digitata*; 7.7 and 9.3 for *G. kola*; and 7.8 and 9.3 mm for *G. ternifolia*. *S. aureus* could be considered as the more susceptible bacteria in this assay; therefore, it was further used to determine the MIC (mg/mL) of *A. digitata*, *G. kola* and *G. ternifolia* extracts.

Table 12. Determination of the diameters of inhibition zones of *A. digitata, G. kola and G. ternifolia* extracts against strains listed. All values are expressed in mm.

Diameters of inhibition zones (mm)								
Plants	**Bacteria**	**AE (mg/mL)**			**ME (mg/mL)**			**CIP (µg)**
		1	**5**	**10**	**1**	**5**	**10**	**5**
A. digitata	*S. aureus*	0	0	0	9.0	9.0	9.5	25.3
	E. coli	0	0	0	8.0	8.4	8.5	38.5
G. kola	*S. aureus*	0	0	0	8.2	9.3	9.3	26.8
	E. coli	0	0	0	7.7	7.8	8.2	38.6
G. ternifolia	*S. aureus*	0	0	0	7.8	8.0	9.3	24.4
	E. coli	0	0	0	8.0	8.0	8.0	37.4

AE: aqueous extract; ME: methanolic extract; CIP: Ciprofloxacin.

Table 13 shows MIC values of the plant extracts on the tested *S. aureus*. It is clear that the plant extracts varied in their efficacy from no effect (aqueous extracts) to the lowest MIC value of 0.625 mg/mL for methanolic extracts of *G. kola* and *G. ternifolia*. The highest MIC value of 1.25 mg/mL was observed for methanolic extract of *A. digitata*.

Table 13. Determination of MIC (mg/mL) of *A. digitata*, *G. kola* and *G. ternifolia* extracts against *S. aureus*.

Plants	MIC (mg /mL) – *S. aureus*		
	AE	**ME**	**Clindamycin**
Adansonia digitata	ND	1.250	1.5625×10^{-5}
Garcinia kola	ND	0.625	
Gardenia ternifolia	ND	0.625	

AE: aqueous extract; ME: methanolic extract; MIC: Minimum Inhibitory Concentration

Infectious diseases are among the 10 leading causes of global disease burden (GBD 2019 Diseases and Injuries Collaborators, 2020), and, in Africa, they account for at least 69% of deaths on the continent (Kariuki et al., 2021). Also, as mentioned in chapter 1, consistent use of synthetic antibiotics is an important cause of resistance to bacteria, which can be related to membrane permeability, mutation of drug target, physiochemical changes, and discharging antibiotics out of cells through an efflux pump (Zhang & Cheng, 2022). Therefore, the search for novel compounds with antibacterial properties and the potential to be used in the development of new treatments is crucial.

Microdilution is a quantitative method that can be used to determine MIC values (Kim et al. 2007). The MIC, which is a key indicator of an antimicrobial agent's potency, is defined as the concentration (mg/L) at which visible growth of bacteria is prevented under defined growth conditions (Wiegand et al., 2008). The microdilution method performed in this work is enhanced through the addition of resazurin dye as a redox indicator (O'Brien et al. 2000).

Generally, the antimicrobial activity of plant extracts is dependent on various factors: the environmental and climate conditions under which the plant grew, the solvent used for the extraction, the extraction method, test concentration, and the method of determination. In the present study, the antibacterial activities were classified according to the diameters of the inhibition zone (DIZ), as follows: not sensitive (DIZ < 8.0 mm), moderately sensitive (8.0 < DIZ < 14.0 mm), sensitive (14.0 < DIZ < 20.0 mm), and highly sensitive (DIZ > 20.0 mm) as described by Li et al. (2018) and Xiao et al. (2019).

In our study, and in general, plant extracts were considered as moderately sensitive. Methanolic extracts of *A. digitata*, *G. kola*, and *G. ternifolia* had diameters of zone of inhibition ranging between 7.0 and 9.6 mm, for *S. aureus*. Results also reported that methanol is an efficient solvent showing MIC values ranging from 0.625 to 1.25 mg/mL. These results may justify certain ethnopharmacological uses of the plants (e.g., diarrhea and wound treatment)

(Diarrassouba et al., 2020). Also, this antibacterial activity can be related to the phenolic compounds present in the extracts, as previously reported. The results observed were better for *S. aureus*, and as it is known, gram-positive bacteria are more sensitive than gram-negative bacteria towards plant extracts. This resistance, in gram-negative is attributed to the cell envelope structure (Diarrassouba et al. 2020). Gram-negative bacteria have an envelope that consists of three layers. The first layer is the outer membrane, a protective and a unique feature responsible for resistance to a wide range of antibiotics (Breijyeh et al., 2020).

Our antibacterial results were consistent with data published by other authors. Thus, El Yahyaoui et al. (2023), showed that methanolic extract (pulp) of *A. digitata* is active against *E. coli* ATCC25922 with an inhibition diameter of 11.5 ± 1.9 mm, as well as *S. aureus* ATCC29213 with inhibition diameter of 11.3 ± 1.4 mm. The methanolic extract of the pulp of *A. digitata* also showed a bactericidal effect on the majority of the bacteria tested, namely E. *coli*, *Pseudomonas aeruginosa*, *S. aureus* and *Enterococcus faecalis*. Seanego & Ndip (2012) found a zone of inhibition of 21 ± 1.1 mm at 50 mg/mL for methanolic extract of *A. digitata* (seeds), with *S. aureus*. Djeussi et al. (2013) reported that extracts of *A. digitata* displayed the most important spectrum of activity, with its inhibitory effects being observed against 81.48% of the bacterial strains (Djeussi et al. 2013). Other authors also corroborate the fact that *S. aureus* has also been reported to be susceptible to *G. kola* extracts (Seanego & Ndip, 2012; Sibanda & Okoh, 2008).

According to Tamokou et al. (2017), a plant extract is considered to be highly active if the MIC < 100 µg/mL; significantly active when 100 ≤ MIC ≤ 512 µg/mL; moderately active when 512 < MIC ≤ 2048 µg/mL; weakly active if MIC > 2048 µg/mL and not active when MIC > 10 000 µg/mL. In the present study, all methanolic extracts presented moderate antibacterial activities (512 < MIC ≤ 2048 µg/mL), on *S. aureus*.

The presence of specific phytochemicals can be related to this antibacterial activity. For example, flavonoids have the ability to complex with extracellular and soluble proteins and to with organisms' cell components. More lipophilic flavonoids may disrupt microbial membranes, inactivate toxins, and inhibit isolated enzymes and complexing activities. The antimicrobial activity of phenolic compounds includes enzyme inhibition, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Aragaw et al., 2020).

Phenolic compounds as (8*E*)-4-geranyl-3,5-dihydroxybenzophenone (benzophenone derivative) and δ-GA exhibited activity against two microorganisms with MICs of 31.3−62.5 µM for *Porphyromonas gingivalis* and 15.6−31.3 µM for *Streptococcus sobrinus* (Hioki et al.,

2020). In case of *G. ternifolia*, five flavonoids (quercetin-4; 7-O-dimethyl ether; kaempferol-7-O-methyl ether; naringenin-7-O-methyl ether; and 4,5-dihydroxy-6,7-dimethoxyflavanone), and two steroids (β-sitosterol and stigmasterol), isolated from the aerial parts, demonstrated to be effective against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* (Ochieng et al., 2010). In another study, at the concentration of 25 mg/ml, 3β,19 α,23,24–tetrahydroxyurs-12-en-28-oic acid (isolated from the roots of *G. ternifolia*) exhibits activity on *S. aureus* to a diameter of 12.2 ± 2 mm (Bernard et al., 2022).

4.4.4. Cytotoxic activity

Over the past decade a number of *in vitro* methods have been evaluated for toxicity testing. The most commonly used methods include MTT, which is converted, by viable cells with active metabolism, into a purple colored formazan product with an absorbance maximum near 570 nm. In the present study, the toxicity of plants extracts was evaluated on HepG2 cell line by the MTT assay. HepG2 cells are commonly employed for liver toxicity investigation due to a series of practical advantages (e.g., almost unlimited life span, stable phenotype, high availability, easy handling, etc.) (Donato et al., 2015).

The results of the MTT assay, carried out on HepG2 cells, after 48 h of exposure to the extracts, are shown in Figure 8, which summarizes cell viability (% to control). In general, the methanolic extract from *G. ternifolia* showed to be more active in decreasing cell viability compared to *A. digitata* and *G. kola*. The IC_{50} concentration is a measure of the effectiveness of a compound/extract in decreasing cell viability. This value was only possible to determine in methanolic extract of *G. ternifolia* (IC_{50} = 228.13 ± 19.15 µg/mL).

The American National Cancer Institute (NCI) guidelines consider a crude plant extract potentially interesting for anticancer research when the IC_{50} for growth inhibition activity falls below 30 µg/mL, following incubations between 48 and 72 hours, while for pure compounds the limit is below 4 µg/mL (Abdel-Hameed et al., 2012; Boik, 2001). Therefore, plant extracts with IC_{50} values ≤ 30 µg/mL are considered pharmaceutically active, which legitimates further investigation on single active constituents (Abdel-Hameed et al., 2012; Boik, 2001; Suffness & Pezzuto, 1990).

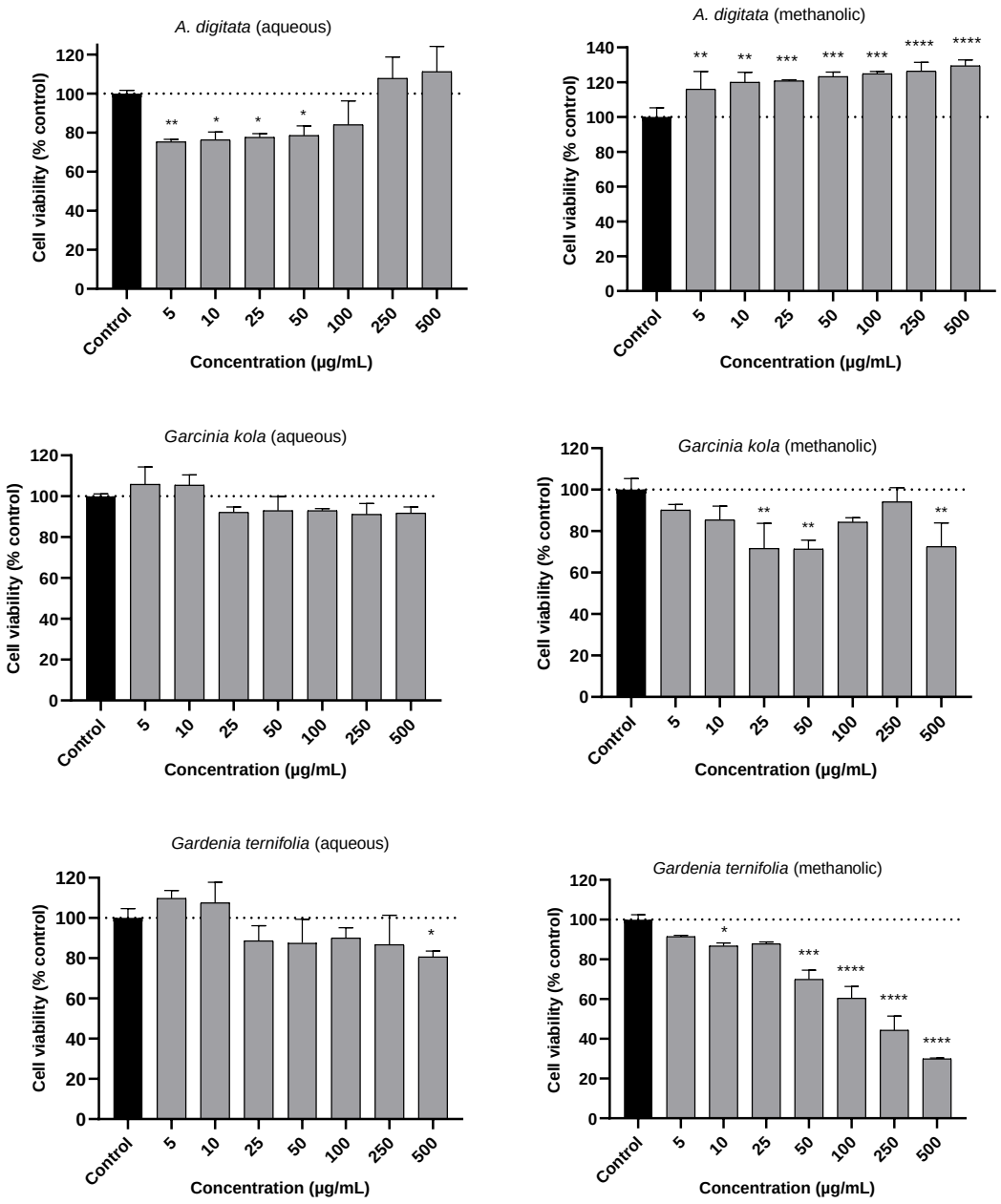


Figure 8. Cytotoxicity of different concentrations of extracts, ranging from 5 to 500 µg/ml, from *A. digitata*, *G. ternifolia*, and *G. kola* (aqueous and methanolic extracts) in HepG2 cell line. Cell viability was determined by MTT assay. Data are presented as mean ± SD of at least three independent experiments, and significant differences as compared with the control group were established at *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001when compared with the control (cells without any compound).

Crude extracts with an IC_{50} below 20 µg/mL are considered highly cytotoxic (Mahavorasirikul et al., 2010). On the basis of this threshold, all extracts tested in our study can be considered as safe. These results provide a support on the safety of its traditional use.

In our study, the extracts of *A. digitata* showed no cytotoxicity in HepG2 cell line. These results were consistent with other reports. For example, according to Ayele et al. (2013) the methanolic extract of *A. digitata* leaves showed no cytotoxic effects against RAW264.7 cells, as determined by MTT assay with $CC_{50} > 100$ µg/mL. In their study, Hanafy et al. (2016) demonstrated the hepatoprotective activity of *A. digitata* extract due to its antioxidant effect which is attributed to its content of vitamin C and the flavonoids that are well known for potent free radical scavenging activity and enhancement of the antioxidant defense system. However, the stem bark of *A. digitata* has many traditional medicinal uses, including the treatment of cancer, but there is currently little scientific evidence to support this. In the study of Adegoke et al. (2021) the methanolic extract of *A. digitata* stem bark induced short-term and long-term (13 to 15 days) cytotoxicity in breast cancer cells in a dose dependent manner and exhibited an IC_{50} of 100 µM, through a mechanism involving the up-regulation of the cell cycle and cell death regulators *p53* and *p21*.

Regarding *G. kola* our results also showed no cytotoxicity against HepG2 cell line at the concentration tested. In fact, KV, the main component of *G. kola* seeds, which comprises biflavanones have been shown to protect the liver from hepatotoxicity caused by a variety of toxins (Chukwudi-Emelike et al., 2022). In another study, one biflavanone isolated from *G. kola*, GB-1 was found to exhibit the strongest *in vitro* antimalarial potency on *P. falciparum* with an IC_{50} of 0.16 µM, whereas it exhibited a very low *in vitro* cytotoxicity on KB 3-1 cells with an IC_{50} of greater than 150 µM (Konziase, 2015).

Finally, in our study the methanolic extract of *G. ternifolia* seemed to be the more potent, showing an IC_{50} value of 228.13 ± 19.15 µg/mL. Similar results were found with other cell lines in other reports. For MCF-7 cell lines, methanolic extracts of *G. ternifolia* leaves showed CC_{50} values higher than 100 µg/mL. For PC-3 cell lines, the CC_{50} of the methanolic extract were 92.10 µg/mL (Tshibangu et al., 2016).

CHAPTER 5

CONCLUSIONS AND FUTURE PERSPECTIVES

Conclusions

Plants have been used as medicines for thousands of years and current research in drug discovery involves an approach that combines different techniques (botanical, phytochemical, biological, and molecular). Many plants are still used in traditional medicine; therefore, they continue to play an important role in the maintenance of health in different parts of the world, especially in Africa. In many parts of African countries, especially in rural areas, traditional healers are the most easily accessible and affordable health resource available to the local community.

With the studies presented in this thesis, the main objective was to increase our knowledge about the medicinal plants used in Angola (because this knowledge could disappear in the future) and to evaluate some of those plants, regarding their biological activities, based on traditional uses and others reported in the literature.

Thus, the work herein presented intended to evaluate the potential pharmacological activity of medicinal plants from the Province of Cuanza Norte (Angola). To achieve that, several specific objectives were considered.

Regarding the first specific objective, which intends to document the use of medicinal plants from the Province of Cuanza Norte (field work), we concluded that people in Angola still depend on biodiversity, and the knowledge of how to use plants in their daily life is fundamental. The ethnopharmacological study demonstrates that N'dalatando (Cazengo) in the Province of Cuanza Norte (Angola), due to its geographical and cultural diversity, has a rich traditional plant knowledge that can be important for treating a wide range of human ailments. A total of 131 species of medicinal plants were cited by local traditional healers, and leaves were the most frequently material used. Also, the major form of plants preparation was maceration, followed by infusion and decoction. Finally, infectious, and parasitic diseases; undefined pains and illness, diseases of the digestive system; and endocrine, nutritional, and metabolic diseases were the problems more cited related to the use of plants by the population. This objective provides an important contribution for conservation and sustainable use of natural resources, and for subsequent pharmacological bioprospection. Although some species cited by the informants are well known scientifically, it is interesting to confirm the purported medicinal properties of others, because studies are scarce. Therefore, further research is necessary to evaluate the pharmacological potential of some promising plants and to improve self-medication practices in the future.

Regarding the next objective of this work, that is, to perform a review of ethnobotanical studies performed in Angola provinces, we found that some of the results from the seven ethnobotanical studies performed in 6 Angolan Provinces (Bié, Bengo, Huíla, Cuanza Norte, Namibe, Uíge) were in accordance with our field work. For example, some informants were contacted through "snowball" method and through semi-structured interviews. Also, many of them were traditional healers, recognized by the community for having high knowledge and experience about the use of plants. Leaves are the predominant used plant part, and the majority of the plant species are used traditionally for digestive disorders. It is known that undernutrition, anemia, and intestinal parasitic infections are public health problems in Angola.

The first two objectives were important for the selection of medicinal plants included in the experimental study. Therefore, because of the high importance of *A. digitata* in Angola, and the small numbers of studies regarding *G. kola* and *G. ternifolia*, those were the plants selected for further studies. Also, some of the traditional uses and biological activities reported in the literarure for those plants are related to important diseases in Africa.

Finally, we evaluated the antioxidant, antibacterial and cytotoxic activities of aqueous and methanolic extracts of those selected African plants. Our results clearly indicate that the biological activities are highly dependent on the extraction solvent used. Although, traditionally, plant extracts are prepared with water as infusions, decoctions, or macerations, given the results obtained in this study, methanolic extracts showed the highest TPC, TFC, and antioxidant activity in most of the assays performed, which indicates that phenolic compounds may be responsible for antioxidant activity. The pharmacological evaluation of the plant extracts in our study, particularly methanolic extracts, indicates a strong antioxidant potential for the investigated plants. As oxidative stress plays an important role in the pathogenesis of chronic diseases such as cardiovascular and neurodegenerative diseases, diabetes, and cancer, these plants can thus be a promising source of bioactive compounds with potential uses in different commercial areas (food industry, cosmetics, pharmaceutical industry).

The results obtained in antibacterial activity indicate the tendency of the extracts to inhibit Gram-positive bacteria more than the Gram-negative and can also justify certain ethnopharmacological uses of the plants. Regarding *in vitro* cell viability assay performed with HepG2 cells and *A. digitata*, *G. kola*, and *G. ternifolia*, our results showed no cytotoxicity in HepG2 cell line, at the concentrations tested, and provide a support on the safety of their traditional use. Previous reports from other authors also demonstrated the hepatoprotective activity of *A. digitata* and *G. kola*.

In general, methanolic extracts comparing with aqueous ones showed increased potential to be used in traditional medicine, and to be explored in a variety of applications. However, for a better understanding, further *in vitro* and *in vivo* studies supported by isolation and structure elucidation of individual bioactive compounds are necessary in order to assess the therapeutical potential and valorization of many African plants that remains poorly explored.

Based on the biological potentials of the extracts, this study proposes that the plants studied can mitigate certain microbial infections as well as reductions in reactive oxidant species, thus giving it the potential for use in the management of oxidative-stress-related diseases as well as infectious diseases. The biological activities observed could partly explain why those plants are used in the traditional medicine practice of Angola, and other African countries.

Future perspectives

Medicinal plants continue to play vital roles in the management of many health problems in African communities. However, it is known that many African countries have not made satisfactory use of their biodiversity and popular knowledge, as regards to the development of new products or drugs, or the integration of African traditional medicine within mainstream healthcare systems.

The popularity of herbal medicines in native populations of Angola, prompted us to identify and document their traditional uses, regarding plants, as well as the pharmacological potential. The plants documented in the ethnopharmacological study should be further explored, especially the more cited ones. Ethnobotany is the study of interrelations between humans and plants, including plants used as food, medicines, and for other economic applications. Therefore, other uses reported by informants in addition to medicinal uses can be collected in future interviews. Also, a phytochemical characterization of the extracts should be performed.

The results observed in the evaluation of antioxidant, antibacterial and cytotoxic activities show the therapeutic potential of *A. digitata*, *G. kola*, and *G. ternifolia*, however, more studies should be conducted and explored (e.g., complementary assays, other solvents, or extraction procedures) to discover new bioactive compounds that can be applied in the prevention and treatment of oxidative stress-associated diseases, infectious diseases, cancer, and others. To confirm the antioxidant effects of the plant extracts, conventional markers of cell oxidative stress should be used, such as lipid peroxidation, glutathione levels and DNA damage. The beta-carotene bleaching activity assay can also be used to complement the antioxidant activity, because an extract capable of retarding or inhibiting the oxidation β-carotene may be described

as a free radical scavenger and primary antioxidant. Also, the *in vivo* antioxidant activity of these extracts needs to be assessed prior to clinical use.

As referred above, methanolic extracts showed the highest TPC, TFC, and antioxidant activity in most of the assays performed, which indicates that phenolic compounds may be related to the antioxidant activity. Plant phenolics (including phenolic acids and flavonoids) constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators; therefore, it is important to identify and characterize the compounds, and relate chemical structure to their biological activities. These may act as chemical markers if a potential extract/fraction/compound is developed into a therapeutic remedy, or as lead compounds for chemical modification to enhance activity. As the analyzed plants showed high values of phenolic compounds, they could be also considered as potential candidates for further evaluation to determine their antioxidant efficacy for food matrix applications.

The observed tendency of the extracts to inhibit Gram-positive bacteria more than the Gram-negative showed that these extracts may contain interesting bioactive compounds capable of attracting scientific attention. However, other biological activities like antifungal and antiparasitic properties can be performed in the future.

In African countries, cancer not only is a growing problem, but also a challenge because available funding and resources are limited. However, in our study, the plant extracts showed no cytotoxicity in HepG2 cell line, at the concentrations tested. Only methanolic extract of *G. ternifolia* showed an IC_{50} = 228.13 ± 19.15 µg/mL. Therefore, further studies with other solvents, cell lines (cancerous and normal), and isolated compounds should be performed. However, and because plant extracts showed no cytotoxicity in HepG2 cells, further studies regarding hepatoprotection (e.g., procedures with co- and pre-incubation of HepG2 cells with a toxic and extracts) should be performed. Particularly because some of the plants used in our study have shown hepatoprotective activity (e.g., *A. digitata* and *G. kola*).

In conclusion, in all the work reported in this thesis, a contribution was made to the elucidation of the biological (antioxidant, antibacterial and cytotoxic) effects *A. digitata*, *G. kola*, and *G. ternifolia*. In addition, the possible application of some extracts in hepatoptotection is suggested. The use of these extracts, especially methanolic extracts, as a therapeutical tool for oxidative stress-related diseases and bacterial infections requires, however, more studies. There is a tremendous potential for developing new products/drugs, but much work remains to be done to continues to generate relevant pre-clinical data and convincing proof of concept through clinical studies.

Conclusión

Las plantas se han utilizado como medicinas durante miles de años y la investigación actual en el descubrimiento de fármacos implica un enfoque que combina diferentes técnicas (botánica, fitoquímica, biológica y molecular). Muchas plantas todavía se siguen utilizando en la medicina tradicional, y por tanto siguen desempeñando un papel importante en el mantenimiento de la salud en distintas partes del mundo, especialmente en África. En muchas partes de países africanos, especialmente en las zonas rurales, los curanderos tradicionales son el recurso sanitario más fácilmente accesible y asequible de que dispone la comunidad local.

Con los estudios presentados en esta tesis, el objetivo principal era aumentar nuestro conocimiento sobre las plantas medicinales utilizadas en Angola (porque este conocimiento puede desaparecer en el futuro) y evaluar algunas de esas plantas, en cuanto a sus actividades biológicas, basándonos en los usos tradicionales y en otros reportados en la literatura.

Así, el trabajo aquí presentado pretendió evaluar la potencial actividad farmacológica de plantas medicinales de la Provincia de Cuanza Norte (Angola). Para ello, se consideraron varios objetivos específicos.

En cuanto al primer objetivo específico, que pretendía documentar el uso de las plantas medicinales de la Provincia de Cuanza Norte (trabajo de campo), concluimos que la población de Angola aún depende de la biodiversidad, y que es fundamental el conocimiento de cómo utilizar las plantas en su vida cotidiana. El estudio etnofarmacológico demuestra que N'dalatando (Cazengo), en la Provincia de Cuanza Norte (Angola), debido a su diversidad geográfica y cultural, posee un rico conocimiento tradicional de plantas que puede ser importante para tratar una amplia gama de dolencias humanas. Los curanderos tradicionales locales citaron un total de 131 especies de plantas medicinales, siendo las hojas el material más frecuentemente utilizado. Además, la principal forma de preparación de las plantas era la maceración, seguida de la infusión y la decocción. Por último, las enfermedades infecciosas y parasitarias; los dolores y enfermedades indefinidos; las enfermedades del aparato digestivo; y las enfermedades endocrinas, nutricionales y metabólicas fueron los problemas más citados relacionados con el uso de las plantas por parte de la población. Este objetivo proporciona una importante contribución para la conservación y el uso sostenible de los recursos naturales, así como para la posterior bioprospección farmacológica. Aunque algunas especies citadas por los informantes son bien conocidas científicamente, es interesante confirmar las supuestas propiedades medicinales de otras, ya que los estudios son escasos. Por lo tanto, es necesario

seguir investigando para evaluar el potencial farmacológico de algunas plantas prometedoras y mejorar las prácticas de automedicación en el futuro.

En cuanto al siguiente objetivo de este trabajo consistene en revisar los estudios etnobotánicos realizados en las provincias de Angola, hemos encontrado que algunos de los resultados de los siete estudios etnobotánicos realizados en 6 provincias angoleñas (Bié, Bengo, Huíla, Cuanza Norte, Namibe, Uíge) estaban de acuerdo con nuestro trabajo de campo. Por ejemplo, algunos informantes fueron contactados mediante el método de "bola de nieve" y mediante entrevistas semiestructuradas. Además, muchos de ellos eran curanderos tradicionales, reconocidos por la comunidad por tener altos conocimientos y experiencia sobre el uso de las plantas. Las hojas son la parte de la planta predominantemente utilizada, y la mayoría de las especies vegetales se emplean tradicionalmente para trastornos digestivos. La desnutrición, la anemia y las infecciones parasitarias intestinales son problemas de salud pública en Angola.

Los dos primeros objetivos fueron importantes para la selección de las plantas medicinales incluidas en el estudio experimental. Por lo tanto, debido a la gran importancia de *A. digitata* en Angola y al escaso número de estudios sobre *G. kola* y *G. ternifolia*, esas fueron las plantas seleccionadas para estudios posteriores. Además, algunas de las actividades biológicas descritas para esas plantas están relacionadas con enfermedades importantes en los países africanos.

Por último, evaluamos las actividades antioxidante, antibacteriana y citotóxica de los extractos acuosos y metanólicos de esas plantas africanas seleccionadas. Nuestros resultados indican claramente que las actividades biológicas dependen en gran medida del disolvente de extracción utilizado. Aunque, tradicionalmente, los extractos de plantas se preparan con agua en forma de infusiones, decocciones o maceraciones, dados los resultados obtenidos en este estudio, los extractos metanólicos mostraron el mayor TPC, TFC y actividad antioxidante en la mayoría de los ensayos realizados, lo que indica que los compuestos fenólicos pueden ser los responsables de la actividad antioxidante. La evaluación farmacológica de los extractos vegetales de nuestro estudio, en particular de los extractos metanólicos, indica un fuerte potencial antioxidante de las plantas estudiadas. Dado que el estrés oxidativo desempeña un papel importante en la patogénesis de enfermedades crónicas como las enfermedades cardiovasculares, la diabetes, las enfermedades neurodegenerativas y el cáncer, estas plantas pueden ser una fuente prometedora de compuestos bioactivos con usos potenciales en diferentes áreas comerciales (industria alimentaria, cosmética, industria farmacéutica).

Los resultados obtenidos en la actividad antibacteriana indican la tendencia de los extractos a inhibir las bacterias Gram-positivas más que las Gram-negativas y también pueden justificar ciertos usos etnofarmacológicos de las plantas. En cuanto al ensayo de viabilidad celular in vitro realizado con células HepG2 y *A. digitata*, *G. kola* y *G. ternifolia*, nuestros resultados no mostraron citotoxicidad en la línea celular HepG2, a las concentraciones ensayadas, y proporcionan un apoyo sobre la seguridad de su uso tradicional. Informes anteriores de otros autores también demostraron la actividad hepatoprotectora de *A. digitata* y *G. kola*.

En general, los extractos metanólicos, en comparación con los acuosos, mostraron un mayor potencial para su uso en medicina tradicional, y para ser explorados en una variedad de aplicaciones. Sin embargo, para comprender mejor el potencial terapéutico y la valorización de muchas plantas africanas que siguen estando poco exploradas, es necesario realizar más estudios *in vitro* e *in vivo*, apoyados por el aislamiento y la elucidación de la estructura de los compuestos bioactivos individuales.

Basándose en el potencial biológico de los extractos, este estudio propone que las plantas estudiadas pueden mitigar ciertas infecciones microbianas, así como reducir especies oxidantes reactivas, lo que les confiere un potencial de uso en el tratamiento de enfermedades relacionadas con el estrés oxidativo, así como de enfermedades infecciosas. Las actividades biológicas observadas podrían explicar en parte por qué esas plantas se utilizan en la práctica de la medicina tradicional de Angola y otros países africanos.

Perspectivas de futuro

Las plantas medicinales siguen desempeñando un papel vital en la gestión de muchos problemas de salud en las comunidades africanas. Sin embargo, se sabe que muchos países africanos no han hecho un uso satisfactorio de su biodiversidad y conocimientos populares, en lo que respecta al desarrollo de nuevos productos o fármacos, o a la integración de la medicina tradicional africana en los sistemas sanitarios convencionales.

La popularidad de las hierbas medicinales en las poblaciones nativas de Angola nos impulsó a identificar y documentar sus usos tradicionales, en lo que respecta a las plantas, así como su potencial farmacológico. Las plantas documentadas en el estudio etnofarmacológico deberían explorarse más a fondo, especialmente las más citadas. La etnobotánica es el estudio de las interrelaciones entre los seres humanos y las plantas, incluidas las plantas utilizadas como alimentos, medicinas y para otras aplicaciones económicas. Por lo tanto, en una futura entrevista se pueden recoger otros usos comunicados por los informantes además de los usos

medicinales. Asimismo, en el futuro debería realizarse una caracterización fitoquímica de los extractos.

Los resultados observados en la evaluación de las actividades antioxidante, antibacteriana y citotóxica demuestran el potencial terapéutico de *A. digitata*, *G. kola* y *G. ternifolia*; sin embargo, se deberían realizar y explorar más estudios (por ejemplo, ensayos complementarios, otros disolventes o procedimientos de extracción) para descubrir nuevos compuestos bioactivos que puedan aplicarse en la prevención y el tratamiento de enfermedades asociadas al estrés oxidativo, enfermedades infecciosas, cáncer y otras. Para confirmar los efectos antioxidantes de los extractos vegetales, deben utilizarse otros marcadores convencionales de estrés oxidativo celular, como la peroxidación lipídica, los niveles de glutatión y el daño en el ADN. El ensayo de actividad blanqueadora del betacaroteno también puede utilizarse para complementar la actividad antioxidante, ya que un extracto capaz de retardar o inhibir la oxidación de β-caroteno puede describirse como un eliminador de radicales libres y antioxidante primario. Asimismo, es necesario evaluar la actividad antioxidante *in vivo* de estos extractos antes de su uso clínico.

Como se ha mencionado anteriormente, los extractos metanólicos mostraron el mayor TPC, TFC y actividad antioxidante en la mayoría de los ensayos realizados, lo que indica que los compuestos fenólicos pueden estar relacionados con la actividad antioxidante. Los compuestos fenólicos de las plantas (incluidos los ácidos fenólicos y los flavonoides) constituyen uno de los principales grupos de compuestos que actúan como antioxidantes, por lo tanto, es importante identificar y caracterizar los compuestos, y relacionar la estructura química con sus actividades biológicas. Éstos pueden actuar como marcadores químicos si un extracto/fracción/compuesto potencial se convierte en un remedio terapéutico, o como compuestos líderes para la modificación química con el fin de mejorar la actividad. Dado que las plantas analizadas mostraron valores elevados de compuestos fenólicos, también podrían ser consideradas como posibles candidatas para una evaluación posterior con el fin de determinar su eficacia antioxidante para aplicaciones en matrices alimentarias.

La tendencia observada de los extractos a inhibir las bacterias Gram-positivas más que las Gram-negativas pone de minifiesto que estos extractos pueden contener interesantes compuestos bioactivos capaces de atraer la atención científica. No se debe descartar, en el futuro llevar a cabo otras actividades biológicas como propiedades antifúngicas y antiparasitarias.

En los países africanos, el cáncer no sólo es un problema creciente, sino también un reto porque la financiación y los recursos disponibles son limitados. Sin embargo, en nuestro

estudio, los extractos vegetales no mostraron citotoxicidad en la línea celular HepG2, a las concentraciones probadas. Sólo el extracto metanólico de *G. ternifolia* mostró una IC50 = 228,13 ± 19,15 µg/mL. Por lo tanto, deberían realizarse más estudios con otros disolventes, líneas celulares (cancerosas y normales) y compuestos aislados. Sin embargo, y dado que los extractos de plantas no mostraron citotoxicidad en las células HepG2, deberían realizarse más estudios relativos a la hepatoprotección (por ejemplo, procedimientos de co-incubación y pre-incubación de las células HepG2 con un tóxico y extractos). Sobre todo, porque para algunas de las plantas utilizadas en nuestro estudio se ha descrito actividad hepatoprotectora (por ejemplo, *A. digitata* y *G. kola*).

En conclusión, todos los trabajos presentados en esta tesis han contribuido a dilucidar los efectos biológicos (antioxidantes, antibacterianos y citotóxicos) de *A. digitata*, *G. kola* y *G. ternifolia*. Además, se sugiere la posible aplicación de algunos extractos en la hepatoprotección. El uso de estos extractos, especialmente los metanólicos, como herramienta terapéutica para enfermedades relacionadas con el estrés oxidativo e infecciones bacterianas requiere, sin embargo, más estudios. Existe un enorme potencial para desarrollar nuevos productos/fármacos, pero aún queda mucho trabajo por hacer para seguir generando datos preclínicos relevantes y pruebas de concepto convincentes mediante estudios clínicos.

CHAPTER 6

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ANNEXES

Annex I - In Vitro Cytotoxic Activity of African Plants: A Review (Article)

molecules

MDPI

Review

In Vitro Cytotoxic Activity of African Plants: A Review

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Abstract: In African countries, cancer not only is a growing problem, but also a challenge because available funding and resources are limited. Therefore, African medicinal plants play a significant role in folk medicine and some of them are traditionally used for the treatment of cancer. The high mortality rate and adverse effects associated with cancer treatments have encouraged the search for novel plant-based drugs, thus, some African plants have been studied in recent years as a source of molecules with proven cytotoxicity. This review aims to discuss the cytotoxic activity, in vitro, of African plant crude extracts against cancer cell lines. For the period covered by this review (2017–2021) twenty-three articles were found and analyzed, which included a total of 105 plants, where the main cell lines used were those of breast cancer (MCF-7 and MDA-MBA-231) and colorectal cancer (HCT-116 and Caco-2), which are among the most prevalent cancers in Africa. In these studies, the plant crude extracts were obtained using different solvents, such as ethanol, methanol, or water, with variable results and IC_{50} values ranging from <20 µg/mL to >200 µg/mL. Water is the preferred solvent for most healers in African countries, however, in some studies, the aqueous extracts were the least potent. Apoptosis and the induction of cell cycle arrest may explain the cytotoxic activity seen in many of the plant extracts studied. Considering that the criteria of cytotoxicity activity for the crude extracts, as established by the American National Cancer Institute (NCI), is an IC_{50} < 30 µg/mL, we conclude that many extracts from the African flora could be a promising source of cytotoxic agents.

Keywords: in vitro; cytotoxicity; African plants; cancer; cell lines

Citation: Canga, I.; Vita, P.; Oliveira, A.I.; Castro, M.Á.; Pinho, C. In Vitro Cytotoxic Activity of African Plants: A Review. *Molecules* **2022**, 27, 4989. https://doi.org/10.3390/molecules27154989

Academic Editors: Sandra Gonçalves and Anabela Romano

Received: 13 July 2022
Accepted: 3 August 2022
Published: 5 August 2022

1. Introduction

Cancer is a generic term for a series of malignant diseases that is still a major health problem worldwide. The etiology of carcinogenesis involves distinct levels of regulation. In this process, normal cells acquire genetic and epigenetic changes that result in uncontrolled cell growth and, therefore, cancer. It is also known that reactive oxygen species (ROS) are involved in tumor formation through the activation of various oncogenic signaling pathways, DNA mutations, immune escape, the tumor microenvironment, metastasis, and angiogenesis [1]. Despite all the improvements in cancer therapy due to diagnostic and therapeutic progresses, cancer still has extremely high mortality rates [2]. Cancer is a global public health problem and the second leading cause of death in the United States [3]. Worldwide, an estimated 19.3 million new cancer cases with almost 10.0 million deaths occurred in 2020 [4].

One of the main problems with cancer cells is their ability to escape apoptosis due to unidentified mutations, resulting in cell accumulation, which consequently migrate to distinct parts of the body [5]. Thus, an effective anticancer drug should target cancer cells without affecting normal cells, which can be achieved by restoring the apoptosis machinery in the cancer ones [6] and by being able to overcome multidrug resistance (MDR). Cancer

Annex II – Ethnopharmacological Study of Medicinal Plants From the Province of Cuanza Norte (Angola) (Article)

Revista Contexto & Saúde
Editora Unijuí
Programa de Pós-Graduação em Atenção Integral à Saúde
ISSN 2176-7114 – v. 22, n. 46, 2022
http://dx.doi.org/10.21527/2176-7114.2022.46.13336
COMO CITAR:
Canga ILV, Vita P, de Oliveira AI de FT, de Pinho CML, Castro MÁ. Ethnopharmacological study of medicinal plants from the province of Cuanza Norte (Angola). Rev Contexto & Saúde, 2022;22(46): e13336

Ethnopharmacological Study of Medicinal Plants From the Province of Cuanza Norte (Angola)

Isabel Lombo Vemba Canga[1]
Pedro Vita[2]
Ana Isabel Oliveira[3]
Cláudia Pinho[3]
María Ángeles Castro[4]

ABSTRACT

Studies on African medicinal plants have been limited to some geographically areas, and even though more than 5400 medicinal plants are recognized and documented, other valuable medicinal plant species have not been studied. Ethnopharmacological studies are important for documenting traditional knowledge associated with the medical use of biodiversity. This study aims to document the use of medicinal plants from the Province of Cuanza Norte (Angola). The field work was conducted from December 2018 to January 2019 and the ethnobotanical data were collected using semi-structured interviews. Informants were selected in accordance with their residence and community recognition as healers. Recorded plants are listed along with their popular name, traditional use, part used, and method of preparation. A total of 131 species of medicinal plants were cited. Mukumbi (*Lonnea welwitschii*), Santa Maria (*Chenopodium ambrosioides*) and Ditumbata (*Boerhavia diffusa*) were the most cited species. Out of the total plant parts, leaves were the most frequently material used. Regarding the mode of preparation for the medicinal materials, the major form of preparation is maceration, followed by infusion and decoction. The main categories of use were infectious and parasitic diseases (e.g., Malaria); undefined pains and illness; diseases of the digestive system; and endocrine, nutritional, and metabolic diseases (e.g., Diabetes). This study revealed the importance of preserving the ethnobotanical knowledge in order to protect the biodiversity and to discover new therapeutic molecules. A comparison of the results with other studies showed that some of the traditional indications are supported by data from scientific literature.

Keywords: Angola; Cuanza Norte; ethnopharmacology; medicinal plants.

ESTUDO ETNOFARMACOLÓGICO DE PLANTAS MEDICINAIS DA PROVÍNCIA DE CUANZA NORTE (ANGOLA)

RESUMO

Os estudos de plantas medicinais na África encontram-se limitados a determinadas áreas geográficas, e, apesar de haver mais de 5.400 plantas medicinais documentadas no país, outras com importante valor medicinal continuam aguardando para serem estudadas. Os estudos etnofarmacológicos documentam o conhecimento tradicional associado ao uso medicinal da biodiversidade. Este estudo tem como objetivo documentar o uso de plantas medicinais pela população da Província de Cuanza Norte (Angola). O trabalho de campo decorreu entre dezembro de 2018 e janeiro de 2019, e os dados etnobotânicos foram recolhidos por intermédio de entrevistas semiestruturadas. A seleção dos informantes teve como critérios a residência e o reconhecimento pela comunidade como curandeiros. Os resultados foram apresentados por meio do nome popular da planta, uso tradicional, parte da planta utilizada e método de preparação. Um total de 131 plantas medicinais foi relatado. As plantas Mukumbi (*Lonnea welwitschii*), Santa-maria (*Chenopodium ambrosioides*) e Ditumbata (*Boerhavia difusa*) foram as mais citadas. De todas as partes das plantas relatadas as folhas foram o material mais utilizado. Quanto ao modo de preparação, a maceração foi o mais usado, seguido da infusão e decocção. As principais categorias de uso das plantas foram: doenças infecciosas e parasitárias (exemplo: Malária); doenças do sistema digestivo; doenças e dores indefinidas; doenças metabólicas, nutricionais e endócrinas (exemplo: Diabetes). Este estudo revelou a importância da preservação do conhecimento etnobotânico para a proteção da biodiversidade e a descoberta de novas moléculas terapêuticas. Comparando os resultados com estudos existentes, observou-se que algumas das indicações de uso tradicionais são suportadas por dados científicos.

Palavras-chave: Angola; Cuanza Norte; etnofarmacologia; plantas medicinais.

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PÁGINAS
1-16

Annex III - Information Collection Form - Ethnopharmacological Study

Escola Superior de Saúde, Politécnico do Porto (ESS-P.Porto)
Universidade de Salamanca (USAL)

FORMULÁRIO

O presente formulário, elaborado no âmbito do Doutoramento em Farmácia e Saúde (Universidade de Salamanca em convénio com a Escola Superior de Saúde do Politécnico do Porto) e intitulado: Estudo Etnofarmacológico de Plantas Medicinais de Angola, tem como principal objetivo o registo, pela população, dos usos populares de plantas aromáticas e medicinais angolanas, assim como a colheita de amostras para estudos fitoquímicos, e a realização posterior de estudos farmacológicos para caracterizar a bioatividade das amostras recolhidas.

Obrigado pela colaboração!

DECLARAÇÃO DE CONSENTIMENTO INFORMADO

Conforme a lei 67/98 de 26 de outubro e a "Declaração de Helsínquia" da Associação Médica Mundial (Helsínquia 1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996; Edimburgo 2000; Washington 2002; Tóquio 2004; Seul 2008; Fortaleza 2013)

Estudo Etnofarmacológico de Plantas Medicinais de Angola

Eu, abaixo-assinado_____

Fui informado que o Estudo de Investigação acima mencionado se destina ao registo, pela população, dos usos populares de plantas aromáticas e medicinais angolanas.

Sei que neste estudo está prevista o preenchimento de um formulário tendo-me sido explicado em que consiste e qual o seu fim.

Foi-me garantido que todos os dados relativos à identificação dos Participantes neste estudo são confidenciais e que será mantido o anonimato.

Sei que posso recusar-me a participar ou interromper a qualquer momento a participação no estudo, sem nenhum tipo de penalização por este facto.

Compreendi a informação que me foi dada, tive oportunidade de fazer perguntas e as minhas dúvidas foram esclarecidas. Aceito participar de livre vontade no estudo acima mencionado.

Também autorizo a divulgação dos resultados obtidos, no meio científico, garantindo o anonimato.

A autora principal do estudo (Isabel Canga):

Data	Assinatura
__/__/_____	_____

Annex III - Information Collection Form - Ethnopharmacological Study

Escola Superior de Saúde, Politécnico do Porto (ESS-P.Porto)
Universidade de Salamanca (USAL)

FORMULÁRIO DE RECOLHA DE INFORMAÇÃO
– ESTUDO ETNOFARMACOLÓGICO

1. Caracterização sócio-demográfica do Participante no estudo

Idade: _____

Localidade onde reside: _____

Género:

☐ Masculino ☐ Feminino

Habilitações Literárias:

☐ Não escolarizado(a)
☐ 1º Ciclo (1.º ao 4.º ano)
☐ 2º Ciclo (5.º e 6.º ano)
☐ 3º Ciclo (7.º ano 9.º ano)
☐ Ensino secundário (10.º ao 12.º ano)
☐ Ensino Superior

Annex III - Information Collection Form - Ethnopharmacological Study

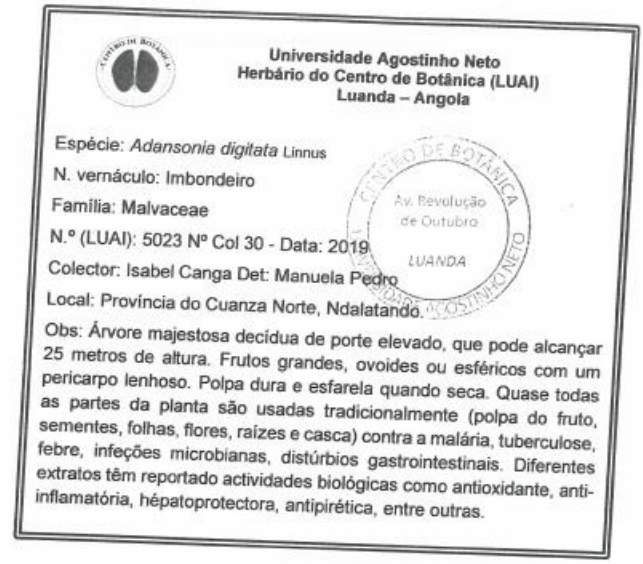
Escola Superior de Saúde, Politécnico do Porto (ESS-P.Porto)
Universidade de Salamanca (USAL)

2. Recolha de Informação das preparações naturais utilizadas pelos participantes:

Nome Popular	Usos Populares	Modo Preparação	Forma Administração	Parte Planta Usada	Observações*

*Observações: (Exemplo – referir se as plantas são usadas frescas ou secas; se também são tóxicas; se são usadas em mistura com outras plantas, se usada na alimentação com um fim específico, ...)

Annex IV – Vouchers (*A. digitata*; *G. kola*; *G. ternifolia*)



Universidade Agostinho Neto
Herbário do Centro de Botânica (LUAI)
Luanda – Angola

Espécie: *Adansonia digitata* Linnus
N. vernáculo: Imbondeiro
Família: Malvaceae
N.º (LUAI): 5023 Nº Col 30 - Data: 2019
Colector: Isabel Canga Det: Manuela Pedro
Local: Província do Cuanza Norte, Ndalatando.

Obs: Árvore majestosa decídua de porte elevado, que pode alcançar 25 metros de altura. Frutos grandes, ovoides ou esféricos com um pericarpo lenhoso. Polpa dura e esfarela quando seca. Quase todas as partes da planta são usadas tradicionalmente (polpa do fruto, sementes, folhas, flores, raízes e casca) contra a malária, tuberculose, febre, infeções microbianas, distúrbios gastrointestinais. Diferentes extratos têm reportado actividades biológicas como antioxidante, anti-inflamatória, hépatoprotectora, antipirética, entre outras.

Universidade Agostinho Neto
Herbário do Centro de Botânica (LUAI)
Luanda – Angola

Espécie: *Garcinia kola* Heckel
N. vernáculo: Ngadiadia
Família: Clusiaceae
N.º (LUAI): 5199 - Nº Col 23 - Data: 2019
Colector: Isabel Canga Det: Manuela Pedro
Local: Província do Cuanza Norte, Ndalatando.

Obs: Árvore com cerca de 15 - 17 m de altura, folhas simples, com 6-14 cm de comprimento e 2-6 cm de diâmetro, brilhantes em ambas as faces e com glândulas de resina. As pequenas flores são cobertas por pêlos curtos. O fruto é uma drupa de 5-10 cm de diâmetro.

Universidade Agostinho Neto
Herbário do Centro de Botânica (LUAI)
Luanda – Angola

Espécie: *Gardenia ternifolia* Schumach. & Thonn
N. vernáculo: Ndai
Família: Rubiaceae
N.º (LUAI): 8285 - Nº Col 9 - Data: 2019
Colector: Isabel Canga Det: Manuela Pedro
Local: Província do Cuanza Norte, Ndalatando.

Obs: arbusto perene com cerca de 5 a 10 metros de altura e muito utilizado na medicina tradicional africana para o tratamento de doenças infeciosas, hemorroidas, febres e malária. A casca da planta é lisa ou ligeiramente áspera, enquanto as folhas são geralmente em espirais de três, agrupadas perto das extremidades de pequenos ramos rígidos.