Faculty of Sciences

Department of Biological Sciences and Biotechnology

ASSESSMENT OF ANTIOXIDANT, ANTIMICROBIAL, AND ANTICANCER PROPERTIES OF *KIGELIA AFRICANA* (LAM.) BENTH. AND *PTEROCARPUS ANGOLENSIS*.

by

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LIST OF ABBREVIATIONS

ADMET –Absorption, Distribution, Metabolism, Excretion, and Toxicity

ANOVA – Analysis of Variance

ATCC – American Type Culture Collection

CFU – Colony Forming Units

CYP – Cytochrome P450

DE – Dry extract

DNA – Deoxyribonucleic acid

DMSO – Dimethyl sulfoxide

DPPH – 2,2-Diphenyl-1-picrylhydrazyl

EGFR – Epidermal Growth Factor Receptor

FDA – Food and Drug Association

FRAP – Ferric Reducing Antioxidant Power

g – Gravity

GAE – Gallic Acid Equivalence

Gen – Gentamicin

Amp B – Amphotericin B

IC_{50} – Half maximal inhibitory concentration

INT – p-iodonitrotetrazolium

MHA – Mueller Hinton Agar

MHB – Mueller Hinton Broth

MIC – Minimum Inhibitory Concentration

NF-*k*B – Nuclear Factor-*k*B

Pdbqt – Protein Data Bank, Partial Charge (Q), and Atom Type (T)

PI3K/Akt/mTOR – Phosphoinositide 3-Kinase/protein kinase B/mammalian target of rapamycin

QE – Quercetin Equivalence

PDB – Protein Data Bank

PM – Plant Material

RNA - Ribonucleic acid

Sdf – Spatial Data File

STDs – Sexually Transmitted Diseases

STIs – Sexually Transmitted Infections

TAC – Total Antioxidant Power

TCM – Traditional Chinese Medicine

TMS – Traditional Medicine System

TMP – Traditional Medicinal Plants

TPC – Total Phenolic Content

TFC – Total Flavonoid Content

WHO – World Health Organization

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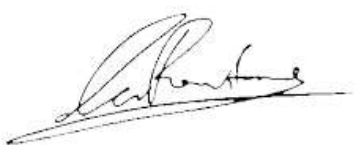
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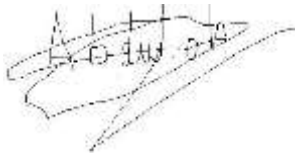
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CERTIFICATION

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Assessment of antioxidant, antimicrobial, and anticancer properties of *Kigelia africana* (lam.) Benth. and *Pterocarpus angolensis*, in the fulfilment of the requirements for the degree of Master of Science *Biological Sciences and Biotechnology* of the BIUST.





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I, the candidate's **primary supervisor,** hereby confirm that I have inspected the above-titled thesis, and, to the best of my knowledge, it is based on the original work of the candidate.

Signed:  Date: _____29 MAY 2023____

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I thank God Jehovah (Yaweh) for the peace that He gave me through faith in Jesus Christ (son of God), a peace that is not achieved through material gain or human approvals. For no matter how many promises God has made, they are 'Yes' in Christ. And so, through Christ, the 'Amen' is spoken by us to the glory of God (Ephesians 1:20).

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ABSTRACT

The incidence of drug resistance is an expanding global issue that necessitates the search for novel drug substitutes. Humans have used traditional medicinal plants (TMPs) for thousands of years to treat a variety of diseases, such as cancer and microbial infections. The scientific validation of therapeutic effects of TMPs aided in the development of complementary and alternative therapies for a variety of medical conditions. The therapeutic nature of TMPs can be attributed to phytochemicals. *Kigelia africana* (*K. africana*) and *Pterocapus angolensis* (*P. angolensis*) are medicinal plants found throughout Southern Africa. They are used in these countries to treat a variety of diseases such as rheumatic conditions, stomach ailments, infertility, cancer, inflammation, dysentery, and sexually transmitted infections (STIs). In this study, we extracted phytochemicals (as crude extracts) from *K. africana* and *P. angolensis* in Botswana. We then assessed their antioxidant, antimicrobial, and anticancer properties. The crude extracts from leaves, barks, roots, and fruits of the two plants were prepared through maceration using distilled water (aqueous), methanol, ethyl acetate, and hexane. Total phenolic content (TPC) and total flavonoid content (TFC) of the crude extracts were determined using the Folin-Ciocalteu (F-C) and aluminum chloride (AlCl3) colorimetric assays respectively. The antioxidant activity and capacity of the crude extracts were evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, and Phosphomolybdenum inhibition total antioxidant capacity (TAC) assay. The Kirby Bauer Disc diffusion method and the serial micro-dilution method were used to assess the antimicrobial activity of crude extracts. *In-silico* analysis was used to investigate the anticancer properties of *K. africana* and *P. angolensis*. Protein-ligand molecular docking was used to analyze the site-specific binding interaction of *K. africana* and *P. angolensis* phytochemicals with the epidermal growth factor receptor (EGFR). Drug-likeness analysis and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME/Tox) predictions were used to further assess the safety of the top-performing phytochemicals. A significantly higher TPC was observed in *P. angolensis* bark aqueous extract (556.2 ± 3.7 µg gallic acid equivalent (GAE)/mg dry extract (DE)), while a significantly higher TFC was observed in *K. africana* leaves ethyl acetate extract (445.1 ± 1.6 µg quercetin equivalence (QE)/mg (DE)). *Pterocarpus angolensis* bark methanol extract exhibited significantly higher DPPH radical scavenging

activity (87.2 ± 0.084 % and DPPH IC_{50} of 3 ± 3 µg/mL), FRAP activity (1042.2 ± 133.2 µg GAE/mg DE) and Phosphomolybdenum inhibition TAC (2206.5 ± 14 µg GAE/mg DE). TPC, TFC, and antioxidant activity were positively correlated. Both plants exhibited antimicrobial activity. *Kigelia africana* crude extracts exhibited broad spectrum antimicrobial activity. Yohimbine, Digoxigenin and Lupeol phytochemicals showed high affinity to EGFR (≥ 8.7 kcal/mol) and they showed promising drug-like and ADME/Tox properties.

CHAPTER 1.0: INTRODUCTION

The occurrence of drug resistance during the treatment of different human diseases is a rising global problem. Hence, the need for development of treatment alternatives. Current treatment and preventive measures are biased toward the use of synthetic drugs since they are more cost and time-effective and require less stringent product and quality assessment in production compared to drugs isolated from medicinal plants (Veeresham, 2012; Betzler *et al.*, 2016). However, synthetic drugs are limited by their unavailability (particularly in developing and resource-limited countries), and undesirable side effects compared to their counterpart medicinal plants (Karimi *et al.*, 2015; Betzler *et al.*, 2016). Medicinal plants are defined as plants that exert beneficial therapeutic effects on the human or animal body (Sofowora *et al*., 2013; Namdeo, 2018). On the other hand, herbal medicine or phytomedicine refers to the use of medicinal plant parts such as leaves, stembark, roots, flowers, fruit, seeds, or whole plant for the prevention, treatment, and mitigation of diseases (Sofowora *et al*., 2013; Nagalingam, 2017; World Health Organization [WHO], 2019). Since ancient times, medicinal plants have been used as traditional, and natural remedies for the treatment of multiple human health conditions such as cancer, sexually transmitted infections (STIs), cardiovascular diseases, and inflammatory disorders among others (Petrovska, 2012; Kurhekar, 2021). Medicinal plants have also been the main component in traditional medicine systems (TMS) developed by different cultural groups around the world. TMS is the overall rationalized and non-rationalized knowledge, services, and procedures based on the indigenous concepts, experiences, and beliefs of different cultures and used in healthcare for prevention, diagnosis, enhancement, or treatment of physical and mental illnesses (Petrovska, 2012). Knowledge from TMS was conveyed to successive generations through writing, oral communication, and multicultural comparisons through horizontal transmission as determined by communal heritage, geographical locations, and environments (WHO, 2019). Developed TMS including Traditional Chinese Medicine (TCM), Ayurveda, Siddha, Amchi, Unami and Naturopathy use medicinal plants to treat major and minor health conditions such as arthritis, inflammation, asthma, and common flu (Patwardhan *et al.*, 2005; Petrovska, 2012). These TMS are still being practiced and have been incorporated into the overall healthcare system of many developed countries in Europe and Asia (WHO, 2009; Moshi and Mhame, 2013; Miranda, 2021).

Approximately, 60 % of the world population uses medicinal plants for therapeutic benefits and 80 % of the population in developing countries rely on medicinal plants for primary health care (Khan and Ahmad, 2019; WHO, 2019). Over 80 000 plant species around the world are used for medicinal purposes (Chen *et al*., 2016). An estimation of 5 000 plant species in Africa are used for medicinal purposes (Mahomoodally, 2013). *Citrus limon* (lemon), *Capsicum annuum* (red chili pepper), *Eucalyptus spp*., *Aspalathus linearis* (rooibos), *Curcuma longa* (turmeric), *Matricaria recutita* (chamomile), *Aloe vera*, *Harpagophytum procumbens* (Devil's claw), *Vaccinium macrocarpon* (cranberry), *Zingiber officinale* (ginger), *Moringa olifera* (moringa), *Mentha pulegium* (penny-royal), and *Allium sativum* (garlic) are some of the commercialized and most used medicinal plants around the world (Gunjan *et al*., 2015; Che *et al*., 2017; Alabi *et al*., 2020; Anywar *et al*., 2021). They have scientific validation against different diseases including cancer, inflammation, and microbial infections (Petrovska, 2012; Tamokou *et al*., 2017; Sánchez *et al.*, 2020). These plants are mainly used in different households as flavourings, and as a form of self-medication against conditions such as skin problems, urinary-tract infections, stomach disorders, respiratory problems, insomnia, and others. Rooibos, devil's claw, turmeric, garlic, ginger, cranberry, chamomile, and moringa have been scientifically validated for their therapeutic effectiveness against hypoglycemia, cancer, microbial infection, and rheumatoid arthritis (Andersen *et al*, 2004; Grant *et al*., 2007; Gopalakrishnan *et al*., 2016; Kumar *et al.*, 2017; Kuruppu *et al*., 2019).

Furthermore, the use of medicinal plants remains an integral part of the African TMS and common practice in disease treatment. In Africa, the use of medicinal plants is mainly prescribed by traditional healers using various holistic practices in treatments involving the body, mind, and spirituality (Danley, 2006; Mahomoodally, 2013; Ozioma and Chinwe, 2019; Anywar *et al.*, 2021). Traditional healers' knowledge or experience in disease treatment is from retaining cultural practices passed down from one generation to another. Other traditional healers account their knowledge and experience to ancestral spirit guidance (Danley, 2006; Ozioma and Chinwe, 2019; Anywar *et al.*, 2021). The treatment of diseases is usually based on targeting the symptoms experienced by the patient. Different plants are used to treat minor and major diseases. In Southern Africa (South Africa, Botswana, Namibia, and Zimbabwe) plants such as *Gloriosa superba*, *Centella asiatica*, *Catharanthus roseus*, *Xylopia parviflora*, *Harpagophytum procumbens*, and *Amaranthus spinosus* are used in the traditional treatment of

diseases such as HIV/AIDS, infertility, headache, malaria, eczema, diarrhoea, wounds, and stomach problems (Motlhanka, 2012; Mahomoodally, 2013; Widowati *et al.*, 2013). *Cyclopia genistoides, Cyclopia burtonii,* and *A. linearis* are commercialized tea plants native to South Africa. These plants are traditionally used as expectorant and treatment for stomach ailments, and tuberculosis (Mahomoodally, 2013; Widowati *et al.*, 2013). TMPs used in Northern and Western Africa include *Acacia senegal*, *Afzelia africana*, *Bridelia ferruginosa*, and *Artemisia herba-alba.* Different plant parts of *A. Senegal* are used in Nigeria to treat gonorrhoea, ulcers, diarrhoea, leprosy, and bronchitis while *Afzelia africana*, *Bridelia ferruginosa*, and *Artemisia herba-alba* are used in Morocco, Sudan, Uganda, and Senegal for traditional treatment against malaria, gonorrhoea, arthritis, and body aches (Mahomoodally, 2013; Widowati *et al.*, 2013). *Moringa Olifera* is native to countries in South, West, and East Africa, where it has traditionally been used to treat diseases such as hypertension, HIV/AIDS, and diabetes (Mahomoodally, 2013; Ozioma and Chinwe, 2019; Omara *et al.*, 2020).

A growing number of these medicinal plants are being documented and scientifically validated (Agyare *et al*., 2013; Fatima *et al*., 2017; Khan and Ahmad, 2019). For example, *A. Senegal,* A. *herba-alba, and P. sidoides* were scientifically proven to have antimicrobial properties against *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Penicillium digitatum*, *Geotrichum citri-aurantii*, *Streptococcus pyogenes*, *Phytophthora citrophthora*, *Staphylococcus aureus and Pseudomonas aeruginosa* (Mahomoodally, 2013). *Harpagophytum procumbens* was scientifically proven to have anti-inflammatory and analgesic effects in animals and humans (Andersen *et al.*, 2004; Grant *et al.*, 2007). *Bredelia retusa*, *X. parviflora*, *M. olifera*, *Bridelia montana*, *C. roseus,* and *K. pinnata* were shown to have anticancer properties (Moudi *et al*., 2013; Alabi *et al.*, 2020; Omara *et al.*, 2020).

In Botswana, herbal medicine is often practiced alongside ancestral spirit communications or consultation. It still plays a big part in the health of several communities in Botswana due to retained cultural beliefs and practices (Danley, 2006). Approximately 80% of Botswana's population in rural areas reportedly use medicinal plants, mostly for the treatment of parasitic infections (Hedberg and Staugard, 1989; Majinda and Motswaledi, 1998). In addition, most Batswana who use these remedies recognize their effectiveness in treating minor (e.g., colds and constipation) and major (e.g., STIs, cancer, diabetes, and malaria) health conditions

(Majinda and Motswaledi, 1998). The administration is usually by traditional healers, herbalists, or spiritualists, and treatment is targeted toward the symptoms expressed by patients (Magora, 2003; Danley, 2006). Different plant parts such as roots, stems, bark, leaves, flowers, or fruit may be used, and patients can be given one or as a concoction of different plants. The plant parts are usually prepared by boiling and administered orally. Occasionally treatment can also be administered through topical application, smoke inhalation, and rectal route (Danley, 2006; Motlhanka and Nthoiwa, 2013). Indigenous plants commonly used for medicinal purposes in Botswana include *Harpagophytum procumbens*, *Cassia abbreviata*, *Acacia senegal*, *Moringa olifera*, *Ziziphus mucronata*, *Clerodendrum ternatum*, *Vahlia capensis*, *Euclea undulata*, *Grewia flava*, *Ximenia americana*, *Jatropha seineri*, *Solanum incanum*, *Solanum nigrum*, *Argemone mexicana*, *Combretum hereroense*, *Cassia italica*, *Peltophorum africana*, *Myrothamnus flabellifollius*, *Albizia harveyi*, *Aloe ferox*, *Asclepias fruticosa*, *Mimusops zeyheri*, *Lippia scaberrima*, *Vernonia amygdalina*, *Schinus molle*, *Withania somnifera*, *Datura innoxia*, *Physalis minima*, *Kigelia africana* (**Figure 1**) and *Pterocarpus angolensis* (**Figure 2**). There are many testimonials from local people on the effectiveness of these medicinal plants. However, only a few medicinal plants have been scientifically evaluated for their phytochemical and therapeutic effects against different health conditions (Hedberg and Staugard, 1989; Motlhanka and Nthoiwa, 2013).

Kigelia africana (Syn. *Kigelia pinnata*), also known as the sausage plant, is locally known as Moporota in Botswana. It is widely distributed across southern, western, and central Africa in sub-tropical regions (Saini *et al.*, 2009; Khan *et al*., 2012; Atawodi and Olowoniyi, 2015). In Botswana, *K. africana* is widely distributed throughout the northern and eastern region of the country (Bosekeng, 2021). The plant belongs to the family Bignoniaceae and is a deciduous tree (Figure 1A) that can grow up to 20 m tall. It is characterized by long woody sausage-like fruits (Figure 1B) that hang from the limbs of the tree with long rope like stalks. The matured fruits can be found on the tree throughout the year, and they produce numerous obovate-shaped seeds (Nabatanzi *et al.*, 2020). *Kigelia africana* is also characterized by dark-red bell-shaped flowers and pinnate leaves (Figure 1C) with oblong leathery leaflets (Atawodi and Olowoniyi, 2015). *Kigelia africana* is traditionally used in different parts of the world for the treatment of different diseases (**Table 1**) such as rheumatoid arthritis, malaria, uterine cancer, fever, and

high blood pressure. The different parts of the plant are used alone or in a concoction to target different diseases.

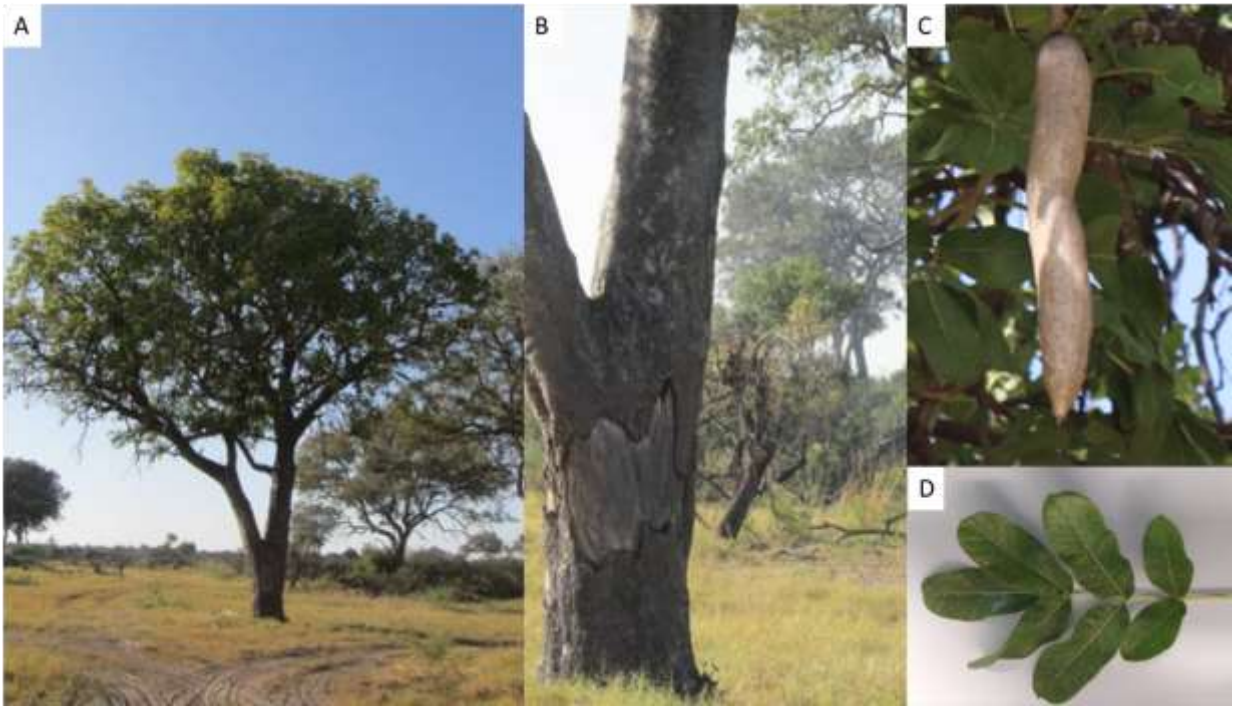


Figure 1: *Kigelia africana* tree and plant parts. Whole tree (A), tree trunk with haversted bark (B), fruit with a long and sausage-like shape (C), trunk (B), and bark (E).

Pterocarpus angolensis is commonly known as blood wood or African teak. It is called Mukwa or Morotomadi in the vernacular which translates to 'bloody urine' in Botswana. The plant is widely distributed throughout northern Botswana (Mmolotsi *et al*., 2012; De Cauwer *et al*., 2014). It is a large deciduous tree (Figure 1A) that can grow up to 20 m tall. The name Morotomadi was given due to the characteristic, red-colored sap (Figure 1 B). The plant belongs to the Fabaceae or Papilionoideae family of flowering plants. It is characterized by a spreading flat-top canopy composed of compound imparipinnate compound leaves (Figure 1 C) and odd shaped brown papery pods (Mojeremane and Lumbile, 2016). It is usually found in southern tropical African countries including Botswana, South Africa, Tanzania, Namibia, Mozambique, and Angola. Due to the plant's highly durable woody stem, it is highly used as building and furniture material. In addition to its uses as building and furniture material, *P. angolensis* is used as a traditional medicinal plant in countries such as Angola, Botswana, Zimbabwe, and

Tanzania (**Table 1**). In these countries, the different parts of *P. angolensis* are used to treat diseases including diabetes, eczema, bilharzia, and diarrhoea (**Table 1**).

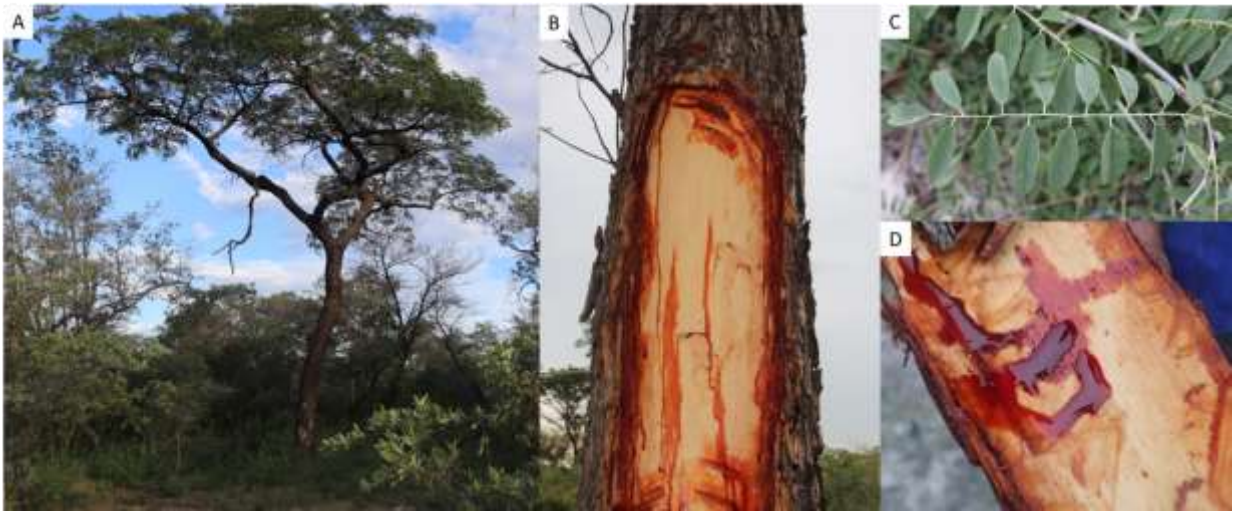


Figure 2: *Pterocarpus angolensis* tree and plant parts. Whole plant (A) stem and bark (B), leaf (C) and blood-like showing the red-coloured sap collected on bark (D).

Table 1: Traditional medicinal uses of different parts of *K. africana* and *P. angolensis*.

Plant species	**Plant part**	**Country**	**Traditional medicinal uses**	**References**
K. africana	Leaves	Uganda, South Africa, Benin, Ivory coast, Namibia	High blood pressure, STIs, jaundice, diarrhoea, abortifacient, aphrodisiac, snakebite antidote, and impotence	Anaduaka *et al*. (2014); Bello *et al*. (2016); Nabatanzi *et al*., (2020)
	Flower	Cameroon, Nigeria,	Sore eyes, fertility in women	Bello *et al*. (2016); Nabatanzi *et al*. (2020)
	Bark	Botswana, South Africa, Nigeria,	Aphrodisiac, syphilis, fungal infections, snake bite antidote, rheumatism,	Saini *et al*. (2009); Atawodi and Olowoniyi (2015);

		Ethiopia, Cameroon	stomach ailments, and dysentery	Nabatanzi *et al*. (2020)
	Stem and root bark	Ethiopia, South Africa, Botswana	Epilepsy, rheumatisms, edema, venereal diseases, sores, wounds, uterine cancer, gynecological conditions, hemorrhoids, and tapeworm infections	Oliver-Bever (1986); Bello *et al*. (2016); Singh *et al*. (2018); Nabatanzi *et al*. (2020)
	Stem bark and leaves decoction	Namibia, Cameroon, South Africa	Herpes, syphilis, skin cancer, abortifacient, boils, filariasis cataract, and eczema	Akhtar and Haqqi (2012); Agyare *et al*. (2013); Bello *et al*. (2016); Nabatanzi *et al*. (2020)
		Zimbabwe	Malaria and fever	Bello *et al*. (2016); Nabatanzi *et al*. (2020)
	Roots	Botswana	Fibroids, post-natal disorders, tapeworm infections, and gastrointestinal disorders	Saini *et al*. (2009); Bello *et al*. (2016); Singh *et al*. (2018)
	Fruit	Botswana,	Psoriasis, leprosy, fungal infections, boils, ulcers, rheumatism, wounds, abscesses, acne, skin cancer, wound healing, menorrhagia, and febrile jaundice.	Saini *et al*. (2009); Agyare *et al*. (2013); Saini *et al*. (2013); Atawodi and Olowoniyi (2015); Bello *et al*. (2016)

		South Africa, Zimbabwe, Kenya	Eczema, infertility, worm infestation, malaria, skin melanoma, toothache, dysentery, and diabetes	Singh *et al*. (2018); Nabatanzi *et al*. (2020)
		India, Africa	Diarrhoea, kidney stones, leprosy, penile and breast development, and skin cancer	Bello *et al*. (2016); Nabatanzi *et al*. (2020)
		India, South Africa, Botswana,	Penile, and breast enlargement	Bello *et al*. (2016); Nabatanzi *et al*. (2020)
	Bark and fruit infusion	Botswana, Nigeria, Ghana	Ulcers, pneumonia, body aches, wounds, malignant tumors, gynecological complaints, stomach problems in children	Saini *et al*. (2009); Agyare *et al*. (2013); Bello *et al*. (2016)
	Bark and fruit	Cameroon, Congo, Nigeria	Asthma	Sonibare and Gbile (2008); Noumi (2010); Bello *et al*. (2016)
	Seeds	South Africa, Kenya, Nigeria, and Tanzania	Waist pain, pneumonia, fungal infections, malaria, eczema, penile enlargement, aphrodisiac, and diabetes	Saini *et al*. (2009); Bello *et al*. (2016); Nabatanzi *et al*. (2020)
P. angolensis	Leaves	South Africa (Venda)	Bilharzia, piles, venereal diseases, amenorrhea, and hematuria	Mulaudzi *et al*. (2011)

	Bark and sap	Botswana, Zimbabwe, South Africa, Angola	Nose bleeds, mouth, and corneal ulcers, diarrhoea, ringworm, blackwater fever, urinary tract infections, menstrual complaints, malaria, skin problems, corneal ulcers, schistosomiasis, urinary tract infections, and eye cataracts	Ndamba *et al*. (1994); Samie *et al*. (2009); Mojeremane and Lumbile (2016)
	Root	Angola, Tanzania, South Africa	Gonorrhoea, corneal ulcers, bilharzia, malaria, abdominal pains, blackwater fever, and diarrhoea	Sigidi *et al*. (2016); Zininga *et al*. (2017)
	Ground seed powder	South Africa	Wound dressing	Sigidi *et al*. (2016); Zininga *et al*. (2017); Santos *et al*. (2020)

Medicinal plants and plant foods contain phytochemicals, which are the bioactive compounds responsible for therapeutic effects (Koche *et al.*, 2016; Forni *et al.*, 2019). Scientists extract and purify these bioactive compounds for use to treat different health conditions and their underlying causes. Different phytochemicals have shown antioxidant, antimicrobial, and anticancer properties. For example, essential oils, plant peptides, and sterols have broad-spectrum antimicrobial activity and are used in cosmetic, pharmaceutical, and drug products (Barbieri *et al.*, 2017; Salehi *et al.*, 2020; Nkwe *et al.*, 2021). Among these compounds, phenols and flavonoids have high antioxidant activity and are effective in reducing or preventing oxidative damage. They are used for skin cosmeceuticals, food preservation, and other applications (Škrovánková *et al.*, 2012; Fagbohun *et al.*, 2020). Moreover, different alkaloids,

plant peptides, and polyphenols have anticancer properties. For example, the Vinca alkaloids isolated from Catharanthus roseus have great anticancer activity against different cancer types such as lung, bladder, and breast cancers (Moudi et al., 2013).

1.1. Justification

Drug resistance occurring during the treatment of diseases such as cancer and microbial infections are a growing worldwide problem. Furthermore, synthetic drugs used in disease treatment are associated with deleterious side effects on patients. Medicinal plants have been presented as treatment alternatives as they have lower side effects compared to synthetic drugs (Karimi *et al.*, 2015; Betzler *et al.*, 2016). They have also been shown to have multitargeted phyto-therapeutic effects. Therefore, the treatment of one disease using a medicinal plant can help prevent another. Local medicinal plants are also easily accessible and less costly to local people in developing countries where modern medical resources are limited due to economic reasons. However, the use of medicinal plants is low compared to synthetic drugs due to their uncertain efficacy and safety. Inherent toxic effects and toxicities from contaminants in medicinal plants may also cause adverse health effects associated with medicinal plants. Furthermore, with little evidence on the efficacy of medicinal plants, there are limited registered medicinal plant products. The scientific validation of these medicinal plants is thus critical to ensuring the safety of medicinal plant extracts and phytochemicals used in the development of modern drugs.

Scientific assaying and validation of medicinal plants will ensure that the plant extract or separated phytochemical can be successfully utilized as potential pharmaceutical alternatives for diseases such as cancer and microbial infections. This will also increase the possibility of medicinal plants being incorporated into the healthcare system and being used as treatment alternatives after or to prevent the occurrence of drug resistance. The registration of these medicinal plants will also help in promoting the commercial exportation of medicinal plants and generating income for locals. Scientific assaying and a condition-oriented approach will also allow for the best method of administration to be chosen, and medicinal plants can then be processed into a more appropriate and palatable dosage form.

Although the use of *K. africana* and *P. angolensis* against different diseases has recognized effectiveness among locals and traditional healers, their scientific validation in the treatment of

all these diseases has not been fully established. Effective and safe doses during administration have not been established to avoid undesired effects such as poisoning. The types of active medicinal compounds have not been fully identified and the effects of different extraction solvents on the antioxidant activity, total flavonoid content, total phenolic content, and antimicrobial activity of the plant extracts have not been assessed. Therefore, in this study, we assessed the antioxidant, antimicrobial, and anticancer medicinal properties of *K. africana* and *P. angolensis*.

1.2. General objective

i. To assess the antioxidant, antimicrobial and anticancer properties of *K. africana* and *P. angolensis.*

1.3. Specific objectives

i. To extract and quantify bioactive compounds from *K. africana* and *P. angolensis*.

ii. To determine the plant parts that possess the highest quantity of bioactive compounds.

iii. To assess the influence of solvent choice on the quantity and or quality of bioactive compounds.

iv. To establish optimal antimicrobial activity, and minimum inhibitory concentrations of the plant extracts on 5 different microbial species.

v. To assess EGFR protein inhibition anticancer drug potential of identified *K. africana* and *P. angolensis* phytochemicals using *in silico* analysis.

CHAPTER 2.0: LITERATURE REVIEW

2.1. History of medicinal plants

Herbal medicine is a common human practice worldwide and its existence dates to the earliest stage of human development of the Paleolithic period about 2.5 million years ago (Hardy, 2021). During this period, herbal medicine started as an instinctive act since there was not enough information on the cause of diseases, thus treatment of diseases using plants was based

on experience. The consumption of plants for food also offered protective medicinal properties (Petrovska, 2012; Liu *et al.*, 2013). The oldest recorded evidence of herbal medicine usage was found on recovered Sumerian clay slabs from Nagpur, approximately 5 000 years ago. The slabs had some prescriptions of 250 medicinal plants used in 12 combinations for medicinal preparations (Petrovska, 2012). During the 18th century, herbalists, traditional healers, and midwives around the world were still using herbal medicine for the treatment of disease (Ozioma and Chinwe, 2019; Anywar *et al.*, 2021). Selected medicinal plants during these periods evolved through human culture and were integrated into traditional medicine systems (TMS) across different cultures.

Medicinal plants have been an integral part in the theoretical and conceptual foundation of cultural TMS created by ancient sages and physicians. For example, in Indian TMS such as Ayurveda, Siddha, Amchi, Unami, Tibetan, and Naturopathy, the use of medicinal plants is popular (Che *et al.*, 2017; Kapoor, 2018). The most practiced treatment system in India is the Ayurveda, and it has been practiced since 6000 BC. It entails the use of up to 10 000 medicinal plants (Kumar *et al.*, 2017). Ayurvedic herbs and spices such as turmeric, cumin, ginseng, cinnamon, sandalwood, and black pepper are used to treat wounds, sores, and boils. *Allium sativum* (garlic), *Allium cepa* (onion), and *Withania somnifera* (Indian ginseng) are among the commonly used medicinal plants in Ayurveda to treat prostate and colon cancer (S. Kumar *et al.*, 2017; Kuruppu *et al.*, 2019). In China, Traditional Chinese Medicine (TCM) involves the use of over 11 000 medicinal plants referred to as Chinese Herbal Medicine (CHM) (Zhang and Yang, 2012; Pelkonen *et al.*, 2014). The TCM utilizes the basic principle of formulating herbal medicine described as the system of "Jun Chen Zuo Shi" (Chief, Deputy, Assistant, Envoy) where each medicinal plant performs an essential purpose within the formula (Xu and Yang, 2009; Che *et al.*, 2017). TCM has been practiced for over 2 000 years and it was integrated into other Asian TMS including Kampo, and Hanja from Japan and Korea respectively (WHO, 2010; Che *et al.*, 2017).

The African TMS is mainly based on medicinal plants and a prescription usually consists of a concoction of medicinal plants used to treat different symptoms experienced by an individual (Moshi and Mhame, 2013; Anywar *et al.*, 2021). The Ebers Papyrus from Egypt dates to circa 1550 BC, and it contains herbal knowledge on a collection of 800 prescriptions used in herbal

therapy. Medicinal plants used in the prescriptions include garlic, onion, pomegranate (*Punica granatum*), castor oil plant (*Ricinus communis*), *Aloe vera*, and senna (Petrovska, 2012; Kurhekar, 2021). The use of medicinal plants in these TMSs was helpful in the treatment of minor and major diseases including infertility, liver disease (hepatitis and cirrhosis), cancer, insomnia, colds and flu, menstrual pain, irregular menstruation, sexual dysfunction, and STIs. The continuity of TMS shows the recognition of the historic and evolutionary therapeutic efficiency of herbal medicine in humans.

2.2. Phytochemicals

The therapeutic effectiveness of medicinal plants is on account of the presence of phytochemicals. Phytochemicals are biologically active compounds that naturally occur in plants (Arendt and Zannini, 2013; Diep *et al.*, 2015). These bioactive compounds are classified according to their role in plant metabolism and their biogenesis or biosynthetic origin; as primary or secondary metabolites (Koche *et al.*, 2016; Webb, 2019). The primary metabolites are essential for the plant's photosynthetic or respiratory metabolism and include plant polysaccharides, proteins, amino acids, purines, pyrimidines, and lipids (**Figure 3**) (Sangwan *et al.*, 2018). On the other hand, secondary metabolites are responsible for the plant's therapeutic efficiency and are not necessary for the plant's survival. Secondary metabolites play different important roles in plants including protection of plants against abiotic (e.g., drought, salinity, low or high temperatures, and other environmental extremes) and biotic (e.g., microbial infections, pests, parasites, diseases, and weeds) stresses. They have high chemical structural diversity which influences their biological activity and classification as phenolic compounds, terpenes, terpenoids, lipids, nitrogen-containing compounds, and others (**Figure 3**) (Wink, 2016; Sangwan *et al.*, 2018).

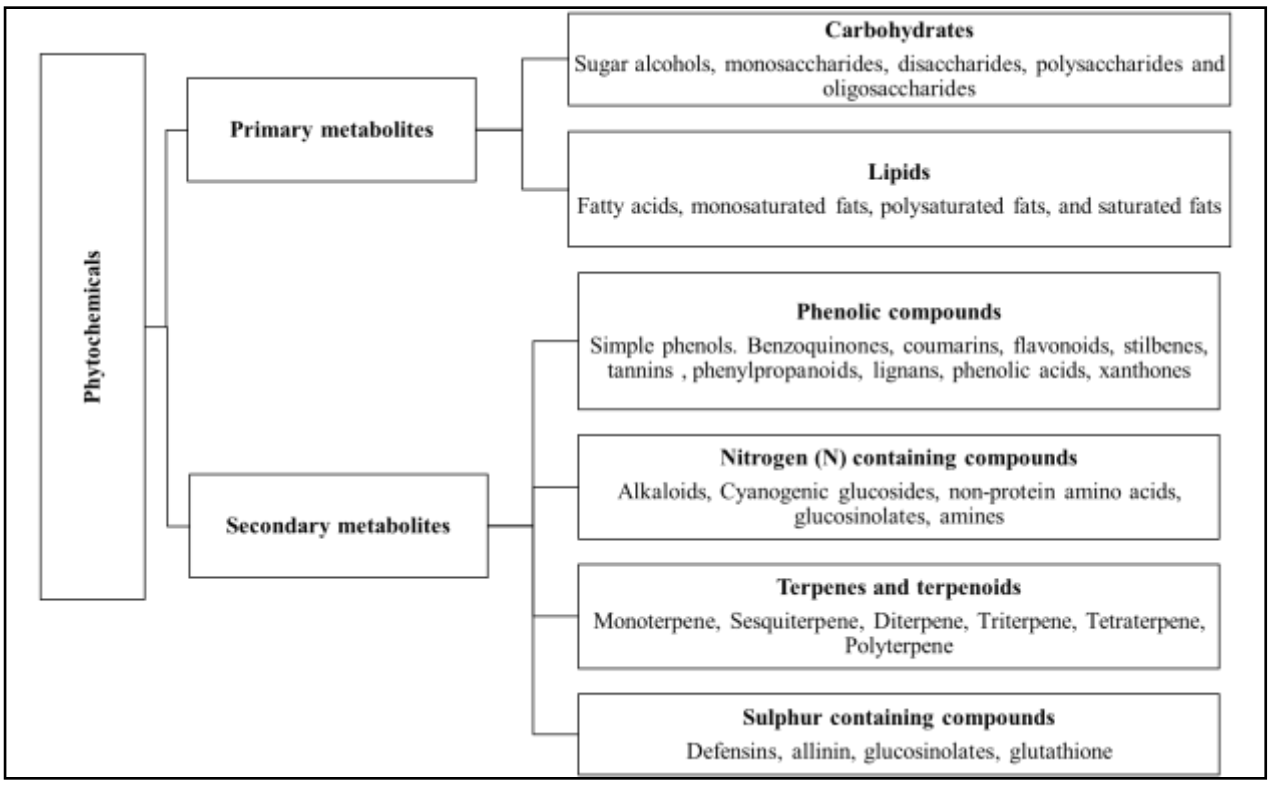


Figure 3: Phytochemical classification chart showing primary metabolites (carbohydrates and lipids) and secondary metabolites (phenolic, nitrogen-containing, sulphur containing, terpenes, and terpenoid compounds). Classification is according to the metabolites' role in plant metabolism and their biogenesis or biosynthetic origin. This chart was designed based on information from Huang *et al*. (2016), Koche *et al*. (2016), and Webb (2019).

The phytochemical composition of different medicinal plants is influenced by geographical location, environmental conditions (e.g., altitude, temperature, illumination, precipitation, humidity, and soil type), and natural or anthropogenic disturbances (Jan and Abbas, 2018; Ukwueze *et al.*, 2019). The phytochemical composition of a plant species also differs across its parts (i.e., leaves, bark, stem, and roots) (Ukwueze *et al.*, 2019; Stankov *et al.*, 2020). Thus, a specific part of the plant can have higher biological activity than the other. The biological capacity of different phytochemicals is based on their effective abilities on antioxidant activity, effect on cell differentiation, increased detoxifying enzyme activity, DNA metabolism, DNA repair conservation, increased cancer cell apoptosis, decreased cell proliferation, and antimicrobial activity (Pandey and Tripathi, 2014; Forni *et al.*, 2019).

2.2.1. Phytochemicals and drug discovery

According to WHO (2009), approximately 25% of modern drugs are phytochemicals. The development of phytochemicals into modern clinical drugs involves initially their extraction and purification. Plant bioactive compounds (as crude extracts or isolated compounds) can be evaluated and confirmed for pharmacological properties through *in-vitro* and *in-vivo* experimental analysis. *In-vitro* experimental analysis is usually a start point and it involves the use of microorganisms, animal or human cells in culture as models to provide researchers with insight into how cells respond to a novel drug in a controlled, isolated environment. The *in-vivo* analysis involves the use of living animals, plants, or whole cells (Sirisuth and Eddington, 2002; Mattes, 2020). The assessed and successfully purified compounds can then be taken through clinical trials (*in-vivo* experimental analysis using humans) and approved by the medical health associations such as Food and Drug Association (FDA) and WHO. In contrast, *in-silico* analysis involves the use of computational models to predict the molecular interaction of small molecules (ligands or bioactive compounds) with macromolecules, pharmacokinetic properties, and safety of different bioactive compounds (Wilson and Lill, 2011; Roy *et al.*, 2015). Nowadays, the *in-silico* analysis approach is regularly carried out as part of the early stage of drug discovery before *in-vitro* and *in-vivo* analysis (Cheng *et al*., 2013). This is done to narrow down the search and selection of potential lead compounds from large compound databases. This approach is eco-friendly, and it reduces the costs and time used in drug discovery.

Molecular docking is an example of a computational modelling technique used to predict how a macromolecule (protein) interacts with small molecules, nucleic acids, or other proteins. It focuses on the macromolecule-small molecule complex formed and the non-covalent bonds (e.g., hydrogen bonds, van der Waals forces, hydrophobic interaction, etc.) involved and whether that might, in turn, inhibit the biological function of the macromolecule (Roy *et al.*, 2015). Computational modelling of absorption, distribution, metabolism, and excretion, (ADME), toxicity (T), and drug-likeness analysis is also considered during drug discovery. ADMET and drug-likeness analysis predict the pharmacokinetic properties of a drug compound determining how it reaches its protein target, and how long it will stay in the body (Cheng *et al.*, 2013; Wang *et al.*, 2015). The predictions can also help in the evaluation of genetic toxicology, carcinogenicity, cardiotoxicity, hepatotoxicity, and other properties of the drug.

Many drug candidates fail the late clinical trial stage primarily due to failure to meet the required pharmacokinetic profile (Wang *et al.*, 2015).

2.2.2. Extraction of phytochemicals

The selection, development of an effective extraction, and isolation method are crucial for accurate, safe, and high yield of bioactive compounds. The different methods considered during extraction have been based on the duration, cost, and quality of compounds of interest. The solvent extraction technique is usually preferred over distillation, pressing, and sublimation extraction (Zhang *et al.*, 2018; Gasser and Rahman, 2021). The solvent extraction technique involves the removal of a compound from a combination or mixture utilizing its property of greater solubility in a particular solvent (Zhang *et al.*, 2018). The technique has higher quality and yield of product (bioactive compounds) (Altemimi *et al*., 2017; Dhanani *et al*., 2017). During extraction, it is also important to consider the extraction solvent, the particle size of the plant sample, solvent-to-sample ratio, and extraction temperature. Extraction solvents with high to medium polarities like ethanol, methanol, acetone, ethyl acetate, and dichloromethane are usually considered during extraction and investigation because they are more likely to dissolve more phytochemicals than solvents with a low polarity like hexane (Iloki-Assanga *et al.*, 2015; Altemimi *et al.*, 2017). Solvent extraction methods such as percolation, maceration, decoction, and Soxhlet (reflux) extraction are more conventional methods while pressurized liquid extraction (PLE), ultrasound sonication extraction, supercritical fluid extraction (SFC), and microwave-assisted extraction (MAE) are modern, and environmentally friendly methods (Azmin *et al.*, 2016; Dhanani *et al.*, 2017; Zhang *et al.*, 2018). Modern methods have improved compound extraction yield more than conventional methods. Maceration extraction is suitable for the extraction of different phytochemicals including thermolabile compounds as compared to extraction techniques like Soxhlet and microwave-assisted extraction (Ingle *et al.*, 2017; Abubakar and Haque, 2020).

2.2.3. Phytochemical identification

Identification of phytochemicals in plant extracts is important for their isolation and structural property elucidation. Compounds isolated from plants can be examined for their biological activity and their efficacy, and their potential for further development into clinical drugs (Jan

and Abbas, 2018; Oyedepo and Palai, 2021). The toxicity of the compounds can be assessed using wet-lab and *in-silico* techniques. Techniques involved in the separation and identification of compounds in plant extracts are chromatography, electrophoresis, and spectroscopy (Sasidharan *et al.*, 2011; Altemimi *et al.*, 2017). Thin-layer chromatography (TLC), Capillary liquid chromatography (u-LC), Gas chromatography (GC), and High-resolution liquid chromatography (HPLC) are examples of chromatographic techniques used. The separation of chemical compounds by these techniques is based on their characteristics such as charge, hydrophobicity, binding capacity, and size or shape (Waksmundzka and Sherma, 2010; Banu and Cathrine, 2015; Altemimi *et al.*, 2017; Sangwan *et al.*, 2018). Electrophoresis techniques separate compounds through their differential migration in an electrical field. Electrophoresis techniques include Thin-layer electrophoresis (TLE), Isotachophoresis (ITP), and Capillary electrophoresis (CE) (Zhou *et al.*, 2018). Spectrophotometry techniques are usually used to elucidate the chemical structure of the present phytochemicals. Nuclear magnetic resonance spectroscopy (NMR), UV spectroscopy, Infrared spectroscopy (IR), Near-infrared spectroscopy (NIR), and Mass spectroscopy (MS) are examples of spectrophotometry techniques (Banu and Cathrine, 2015; Sangwan *et al.*, 2018).

2.2.4. Antioxidant activity of phytochemicals

The antioxidant activity of phytochemicals helps in the instigation of cellular antioxidant defense mechanism, moderation, and prevention of oxidative stress (Zhang *et al.*, 2015; Lin *et al.*, 2016). Oxidative stress is an outcome of excessive production of oxidants in the human body that lead to an imbalance and oxidative damage to biological macromolecules such as lipids, DNA, and proteins (Pizzino *et al.*, 2017). Oxidative stress is one of the leading causes of human diseases including hypertension, ischemia injury, diabetes mellitus, atherosclerosis, cardiovascular diseases, neurodegenerative diseases, and cancer (Zhang *et al.*, 2015; Salehi *et al.*, 2020). Plants respond to environmental stress by producing antioxidants such as polyphenols. Consumption of food plants or medicinal plants will ultimately provide therapeutic benefits provided by such antioxidants. Studies have proven that phenolic compounds are the most potent antioxidants compared to other metabolites as they have free radical scavenging ability and can act as reducing agents (hydrogen and electron donors), metal chelators, and singlet oxygen quenchers (Kumar and Goel, 2019; Muflihah *et al.*, 2021).

The antioxidant property of bioactive compounds is based on their ability to scavenge and neutralize reactive oxygen species (ROS). These phytochemicals achieve this by acting as reducing agents, transferring hydrogen atoms or electrons, and acting as singlet oxygen quenchers and metal chelators to ROS (Shankar and Mehendale, 2014; Ortiz *et al.*, 2017). Thus, different assays are employed when evaluating the antioxidant properties of phytochemicals. This helps in classifying the bioactive compounds as primary, and secondary antioxidants (Ito *et al.*, 2002; Tolinski, 2015). Chemical-based antioxidant assays used to characterize hydrogen transferring compounds include the Total Oxyradical Scavenging Capacity (TOSC) assay, the Hydroxyl Radical Antioxidant Capacity (HORAC) assay, the Total Peroxyl Radical Trapping Antioxidant Parameter (TRAP) assay, and Oxygen Radical Absorption Capacity (ORAC) assay. Assays based on electron transferring compounds include the Ferric Reducing Antioxidant Power (FRAP) assay, Cupric Reducing Antioxidant Power (CUPRAC) assay, and the Folin–Ciocalteu assay (also used for phenolic compounds quantification). Assays including the 2,20 -Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay and the [2,2-di(4-tert-octyl phenyl)-1-picrylhydrazyl] (DPPH) assay characterise both transfers of a hydrogen atom and electron (Barreca, 2020; Munteanu and Apetrei, 2021). More recent studies have demonstrated that antioxidant activity can be exhibited through modulation of key protein expression and activity, modulation of gut microbiota, interactions with intracellular signaling cascade prominent proteins, and epigenetic manipulating mechanisms (Wang *et al.*, 2017; Hrelia and Angeloni, 2020). These antioxidant mechanism properties are assessed using cell-based bioassays.

2.2.5. Antimicrobial activity of phytochemicals

Antimicrobial activity is the ability of a chemical compound to inhibit the growth of a microorganism, inhibit the formation of microbial colonies, and or kill microorganisms (Monga and Sharma, 2020). Plant primary metabolites such as peptides and lectins have exhibited antimicrobial properties. Their mechanism of action was shown to be through the formation of ion channels in the cell membrane of microbial cell leading to cell membrane disruption and cell death (Nawrot *et al.*, 2014; Tam *et al.*, 2015; Li *et al.*, 2021). Secondary metabolites such as alkaloids, glucosinolates, and terpenoids have exhibited antimicrobial activity against gram-negative and gram-positive bacterial strains. This is through a down-regulating expression of

virulence factors such as toxins, biofilms, cell surface capsules, glyco- and lipoprotein (Rahman, 2015; Barbieri *et al.*, 2017; Leitão, 2020). Phenolic and alkaloid compounds contribute to antimicrobial properties of plant extracts. For example, the two quinolone alkaloids isolated from *Evodia rutaecarpa* fruit, 1-methyl-2-[(Z)-8-tridecenyl]-4-(1H)-quinolone and 1-methyl-2-[(Z)-7-tridecenyl]-4-(1H)-quinolone, were found to have selective antibacterial activity against a strain of *Helicobacter pylori* (Hamasaki *et al*., 2018). Alkaloids from different plant species including *Dalbergia scandens*, *Eugenia dysenterica*, and *Pouteria ramiflora* exhibited antifungal activity and their mechanism of action was shown to be through inhibition of cell wall formation, RNA, DNA, and protein synthesis, interfering with mitochondrial function and plasma membrane disruption (Serpa *et al.*, 2012; da Costa *et al.*, 2014; Da *et al.*, 2019).

The use of phytochemicals as a treatment against microbial infections gained interest due to the global rise in antibiotic resistance in microorganisms. Antibiotic resistance allows the microorganism to survive leading to persistent microbial infection (de Sousa Oliveira *et al.*, 2016; Wester *et al.*, 2017). Methods used in the testing of antimicrobial activity of bioactive compounds in-vitro include the disc diffusion method, broth dilution, and agar dilution method (Sandle, 2016; Christenson *et al.*, 2018; Tenover, 2019). The disk diffusion method, also known as Kirby Bauer's Disk diffusion method, is an agar diffusion method that evaluates the susceptibility of a fast-growing microorganism to a bioactive compound. This is done by observing the inhibition zone proportional to the microbe's susceptibility to the test compound (Christenson *et al.*, 2018). Broth dilution and agar dilution methods are considered when determining the MIC of the test sample required for observable growth inhibition of the microorganism being tested (Michael *et al.*, 2002; Sandle, 2016; Tenover, 2019).

2.2.6. Anticancer activity of phytochemical

Phytochemicals have the potential as anticancer compounds to prevent, inhibit, or stop the development of cancer cells (Ogawa, 1997; Vincenzi *et al.*, 2016). Anticancer bioactive compounds in plants are alkaloids, saponins, terpenoids, flavonoids, coumarins and lactones, anthraquinones, tannins, cardiac glycosides, phenols, and phytosterols (Gavamukulya *et al*., 2014; Yang *et al*., 2015). The anticancer mechanism of different phytochemicals has been demonstrated through *in-vitro*, *in-vivo*, and *in-silico* experimental methods; and was shown to

be through mechanisms like induction of cell membrane disruption, cell cycle arrest, inhibition of signalling pathways, suppression of metabolic enzymes, transcription regulation, and modulation of autophagy (Khan *et al.*, 2019; Nkwe *et al.*, 2021).

Different studies have demonstrated anticancer properties of *Catharanthus roseus* known as Madagascar periwinkle. It has anticancer alkaloids (vinblastine and vincristine), which are derived from stems and leaves. The plant showed high anticancer activity against different human cancer cell lines including cervical, breast, lung, and prostate (Widowati *et al.*, 2013; Le Roux and Guéritte, 2017; Taher *et al.*, 2019). The compounds form an essential component of standard chemotherapy treatments (Moudi *et al.*, 2013; Sprangers *et al.*, 2020). The alkaloids (also known as vinca alkaloids) bind to tubulin, inhibiting polymerization and assembling of microtubules, thus causing meta-phase arrest during mitosis. Another plant with proven anticancer properties is *Annona muricata* (soursop) (Pieme *et al*., 2014; Najmuddin *et al*., 2016). Studies by Pieme *et al*. (2014) and Najmuddin *et al*. (2016) showed the anti-proliferative and induction of apoptosis on cancer by *A. muricata* plant extracts. The plant has high anticancer activity against many human cancers (Najmuddin *et al.*, 2016; Wahab *et al.*, 2018; Hadisaputri *et al.*, 2021).

2.3. Scientific study of medicinal plants in Botswana

An increasing number of scientific studies focusing on the medicinal properties of Botswana plants are being performed. Some of the plants that have been scientifically studied include *Kigelia africana* (moporota), *Pterocarpus angolensis* (mukwa), *Grewia flava* (moretlwa), *Ozoroa paniculosa* (monokane), *Colophospermum mopane* (mophane), *Sanseviera scabrifolia* (mosokelatsebeng), *Myrothamnus flabellifolius* (galalatshwene), *Combretum hereroense* (mokabi), *Ximenia americana* (moretologa wa pudi), *Ximenia caffra* (moretologa wa kgomo), *Euclea undulata* (motlhakola), *Ziziphus mucronata* (mokgalo), *Terminalia sericea* (mogonono), *Urginea sanguinea* (sekaname), *Physalis minima*, *Solanum nigrum*, *Withania somnifera* (khusubele), *Schinus molle* (pepere), *Vernonia amygdalina* (moqune), *Elephantorrhiza burkei* (ntolane), *Ocimum gratissimum*, *Hypoxis hemerocallidea* (tshukudu-ya-poo), *Momordica balsamina* (motangtang), *Ficus sansibarica* (mochaba), *Sclerocarya birrea* (morula), *Artemisia afra* (lengana), *Hoodia gordonii*, *Plumbago zeylanica* (masigomabe), *Clerodendrum ternatum* (sedupapula), *Solanum incanum* (tholwana-e-tona),

*C*assia *abbreviata* (monepenepe), *Peltophorum africana* (mosetlha), *Croton megalobotrys* (motshebe), *Aloe ferox* (mokgwapha), *Mimusops zeyheri* (mmopudu), *Cassia sieberiana* (mororwe), *Cassine transvaalensis* (monomane), and *Lippa scaberrima* (musukujane) (Majinda and Motswaledi, 1998; Motlhanka and Makhabu, 2011; Setshogo and Mbereki, 2011; Motlhanka and Nthoiwa, 2013; Tietjen *et al*., 2016).

Table 2: Medicinal plants of Botswana and their scientifically validated medicinal properties.

Plant species	**Plant part**	**Medicinal properties**	**References**
Cassia abbreviata	Leaves	Antioxidant	Padmaja *et al*. (2016)
	Stembark	Antidiabetic	
		Antioxidant	Padmaja *et al*. (2016)
	Roots	*In vitro* HIV-1c replication inhibition	Leteane *et al*. (2012)
Cassia sieberiana	Roots	*In vitro* HIV-1c and HIV-$1_{NL4.3}$ replication inhibition	Leteane *et al*. (2012); Tietjen *et al*. (2016)
Plumbago zeylanica	Roots	*In vitro* HIV-1c replication inhibition	
Croton megalobotrys		*In vitro* HIV-$1_{NL4.3}$ replication inhibition	Tietjen *et al*. (2016)
Vitex doniana		*In vitro* HIV-$1_{NL4.3}$ replication inhibition	Tietjen *et al*. (2016)

Ozoroa paniculosa	Leaf	Antioxidant	Motlhanka (2008); Motlhanka (2009); Motlhanka and Mathapa (2012)
	Stem	Antioxidant	
	Roots	Antioxidant	
Colophospermum mopane	Seeds	Antioxidant	(Motlhanka (2008)
Myrothamnus flabellifolius	Shoot	Antioxidant, antidiabetic	Motlhanka and Mathapa (2012); Kwape *et al.* (2016)
Combretum hereroense	Leaf	Antimicrobial	Majinda and Motswaledi (1998)
	Root	Antimicrobial	
Ximenia americana	Fruit	Antimicrobial	Majinda and Motswaledi (1998)
Ximenia caffra	Fruit	Antimicrobial	Majinda and Motswaledi (1998)
Sanseviera scabrifolia	Leaf	Antimicrobial	Majinda and Motswaledi (1998)
Grewia flava	Roots	Antimicrobial	Majinda and Motswaledi (1998)
	Fruit	Antioxidant	Masisi *et al.* (2021)
Euclea undulata	Roots	Antimicrobial	Majinda and Motswaledi (1998)
Ziziphus mucronata	Leaves	Antimicrobial	Majinda and Motswaledi (1998)
	Roots	Antimicrobial	

Terminalia sericea	Roots	Antimicrobial	Majinda and Motswaledi (1998)
Urginea sanguinea	Bulbs	Antimicrobial	Majinda and Motswaledi (1998)
Elephantorrhiza spp.	Rhizomes	Antimicrobial	Majinda and Motswaledi (1998)
Ocimum gratissimum	Aerial	Antioxidant, antidiabetic	Keneilwe *et al.* (2018)
Momordica balsamina	Aerial	Antioxidant, antidiabetic	Keneilwe *et al.* (2018)
Hypoxis hemerocallidea	The underground stem (corms)	Antioxidant, antidiabetic	Keneilwe *et al.* (2018)
Lippia scaberrima	Leaves	Antioxidant, antidiabetic	Keneilwe *et al.* (2018)
	Stem	Antioxidant, antidiabetic	
Cassine transvaalensis	Roots	Antioxidant	Motlhanka *et al.* (2006)
Harpagophytum procumbens	Shoots	Antioxidant	Motlhanka (2012)
	Tubers	Antioxidant	
	Roots	Antioxidant	Muzila *et al*. (2016)

		Anti-inflammatory	
Ficus sansibarica	Ripe fruit	Antioxidant	Motlhanka and Topo (2012)
Physalis minima	Leaves	Antioxidant	Fatima *et al.* (2017)
	Fruit	Antioxidant	
Solanum nigrum	Leaves	Antioxidant	Fatima *et al.* (2017)
	Fruit	Antioxidant	
Withania somnifera	Leaves	Antioxidant	Fatima *et al.* (2017)
	Fruit	Antioxidant	
Datura innoxia	Leaves	Antioxidant	Fatima *et al.* (2017)
	Fruit	Antioxidant	
Kigelia africana	Leaves	Antioxidant	Fatima *et al.* (2017)
	Fruit		
Schinus molle	Leaves	Antioxidant	Phiri *et al.* (2021)
Vernonia amygdalina	Roots	Antimalarial	Abosi and Raseroka (2016)

Harpagophytum procumbens is one of the internationally recognized and utilized medicinal plants from Botswana. Scientific studies showed that *Harpagophytum procumbens* has anti-diabetic, anti-arthritic, anti-malarial, and anti-epileptic properties (Mahomed and Ojewole, 2004; Georgiev *et al.*, 2010; Akhtar and Haqqi, 2012; Brendler, 2021). *Harpagophytum procumbens* roots have also been proven to contain phytocompounds including harpagoside and iridoid glycoside phytochemicals effective against osteoarthritis, rheumatoid arthritis, heart diseases, and kidney inflammation (Stewart and Cole, 2005; Mncwangi *et al.*, 2012). *Hoodia gordonii*, a succulent plant locally used by San Bushmen in Botswana as an appetite suppressant during famine or prolonged hunting periods, is now marketed worldwide as a nutraceutical product for weight loss or diet control. Oxypregnane steroidal glycoside was found to be the

active phytochemical causing suppressed appetite after eating the plant (Mahomed and Ojewole, 2004; Brendler, 2021).

In a study by Owolabi *et al*., (2007), where they assessed the antibacterial and antifungal activity of ethanol crude extract of *K. africana* stembark, at a 20 mg/ml concentration, the extract was active against *S. aureus* and *Candida albicans* while *Escherichia coli* and *Pseudomonas aeruginosa* were not susceptible to the extract. In contrast to this study, Grace *et al.* (2002) demonstrated antimicrobial activity of *K. africana* stem-bark ethanol extract against *E. coli* and other microorganisms including *Bacillus subtilis*, *Klebsiella pneumoniae*, and *S. aureus* at a concentration of 2.5 mg/ml. The fruit, roots, and leaves also possess antimicrobial, antidiarrhoeal, antidiabetic, and anticancer properties (Akah, 1996; Khan *et al*., 2012; *Agyare et al.*, 2013; Arkhipov *et al.*, 2014). Different phytochemicals were identified from the different parts of *K. africana.* Quinones and iridoids such as lapachol, leonuride (ajugol), dehydro α-lapachone, verminoside, jioglutolide, 2-acetylfuro-1,4-naphthoquinone, kigelinol, kigelinone, pinnatal, norviburtinal, and tecomaquinone-I were identified in the leaves, bark, stem, roots, and fruits of the plant (Nabatanzi *et al.*, 2020). In other studies, phenolic compounds, coumarins, sterols, terpenes, terpenoids, and fatty acids were also identified in different plants parts of *K. africana* and were shown to contribute to its medicinal properties (Moronkola *et al.*, 2018; Grace *et al.*, 2002; Olatunji and Atolani, 2009; Sidjui *et al.*, 2015). Essential oils from the leaves and flowers were found to constitute phytochemicals with antioxidant, antimalarial, and antimicrobial properties. The phytochemicals include myristic acid, hexadecanoic acid, linalool, nonanal, limonene, pinene, β-phellandrene, myristaldehyde, lapachol, and eicostrienoic acid (Asekun *et al.*, 2007; Zofou *et al.*, 2013).

Chipinga *et al*. (2018) demonstrated the antimicrobial activity of *P. angolensis* dichloromethane bark extract against *Streptococcus agalactiae* and *Candida krusei*. Samie *et al*. (2009). Whereas Abubakar and Majinda (2016) demonstrated antimicrobial activity against *E. coli*, *B. cereus*, *S. aureus*, and *Enterococcus fecalis* with MIC of <500 µg/ml for the methanol, ethanol, and acetone crude extracts of *P. angolensis* bark. In the same studies, the chloroform and n-hexane bark extracts showed a weak activity against the same strains. Studies by Samie *et al*. (2009), Abubakar and Majinda (2016), and Santos *et al*. (2020) also demonstrated the presence of phytochemicals such as neophytadiene, tetradecanoic acid, eseroline, pregn-5-en-3-ol, 9-

octadecenamide, n-hexadecanoic acid, (-)-epicatechin, epicatechin-3-O-galate, epicatechin (4b–8)- epicatechin (B2), 7-dehydrodiosgenin, lupeol, a hexamer of epicatechin octadecanoic acid, caffeic acid, tetratriacontane, stigmasta-3,5-dien-7-one, friedelan-3-one, eicosamethylcyclodecasiloxane, and 1-octacosanol in the GC-MS, NMR and HPLC analysis of the n-hexane and chloroform stem-bark extract. Different studies demonstrated the anti-inflammatory, antimicrobial, anticancer, analgesic, antipyretic, antihypertensive, and larvicidal properties of some of these phytochemicals. Lupeol and friedelan-3-one have anti-inflammatory, antibacterial, and antifungal activity while 7-dehydrodiosgenin, 1-octacosanol, and stigmasta-3,5-dien-7-one have antioxidant, anticancer and antidiabetic properties (Karabay-Yavasoglu *et al.,* 2007; Saleem, 2009; Antonisamy *et al.*, 2011; Balogun *et al.*, 2013).

2.4. Current prospects for the use of medicinal plants

Currently, herbal medicine is regarded as part of complementary and alternative medicine and contribute to issues of self-medication (WHO, 2019). Although the use of herbal medicine rapidly declined during the 19th century, when synthetic pharmaceutical products were made available, people continued to use herbals due to perceptions that plant-based pharmaceuticals are safer than synthetic drugs (Mosihuzzaman, 2012; Pan *et al.*, 2014; Gunjan *et al.*, 2015). According to WHO (2009), 80% of the world's population in developing countries still use herbal medicine as a source of primary healthcare. This is approximately 5.6 billion people, and they are mainly situated in rural communities in parts of Asia, Central and South America, and Africa (Tchacondo *et al.*, 2011). Most people use herbal medicine as it is inexpensive and easily accessible and has a promising efficacy. The increasing demand for medicinal plants worldwide and a global market worth more than US$100 billion per annum is due to its recognized effects on different health conditions (Zhang *et al.*, 2015; Binns *et al.*, 2018). The estimated global value of herbal medicine was US$60 000 million in 2002. This value increased to US$ 83 billion between the years 2003 and 2019 and it has been estimated to reach US$ 550 billion by 2030 (Gunjan *et al.*, 2015). The total annual income for herbal medicine in China increased from US$ 14 billion to US$ 62.9 billion in the years 2005 to 2011, making China a major exporter of medicinal plants (Patwardhan *et al.*, 2005; Li *et al.*, 2015). Sales of herbal medicine include whole herbs, teas, and more processed products like essential oils, ointments, salves,

rubs, capsules, and tablets that contain a crushed or powdered form of a raw plant or its dried extract.

In the European and Southeast Asia region, herbal medicine now forms an integral part of healthcare and has been incorporated into the overall healthcare services (Xu and Yang, 2009; WHO, 2013; WHO, 2019). In China, national policies and regulatory frameworks have been set concerning medicinal plants used in TMS. Traditional herbal medicine as part of TMS in China, was one of the prominent strategies to control and treat the 2019 severe acute respiratory syndrome (SARS-CoV-2) (Xu and Yang, 2009; Luo *et al.*, 2020; Yang *et al.*, 2020). The European Parliament and Council released the European Directive 2004/24/EC with regulations and guidelines on the use of medicinal plants. The directive states that medicinal plants and their products must have a recognized level of safety and efficacy before being released and sold in the market (Wachtel and Benzie, 2011). On the other hand, in Africa, the government of Ghana's decision to prioritize herbal medicine in the country's healthcare system, included herbal medicine units in 17 piloted public hospitals. This led to the establishment of the 2005 policy on the practice of integrated medicine in the country (Appiah *et al.*, 2018). These policies helped in increasing investments in the evaluation and improvement of medicinal plant use and creating awareness of the importance of herbal medicine on a global level and as a recognized commodity with high efficacy among different communities. In 2012, the government of Botswana through the Ministry of Health also established a policy for medicine, vaccines, and health encompassing technologies whose initiatives were to promote evidence-based utilization of medicinal plants and alternative medicines through collaborative partnership between the formal healthcare system and traditional healers (Danley, 2006; MoH, 2011). Ministries of Environment, Wildlife and Tourism, Trade and Industry and Infrastructure, and Science and Technology were incorporated into this policy to facilitate commercialization of medicinal plants.

CHAPTER 3: MATERIALS AND METHODS

3.1. Plant material sampling and preparation

Plant materials (leaves, bark, roots, and fruits) were sampled from northwest Botswana. *K. africana* plant materials were collected near Tsodilo Hills at coordinates 18°48'26.10S, 21°43'32.10°E while *P. angolensis* was collected in Shakawe at coordinates 18°16'48.72°S, 21°46'50.28E. Plant materials from at least five plants of each species were collected. The plant materials from each part (leaves, bark, roots, and fruits) were washed with tap water and dried at room temperature (25 °C) for 72 hrs, and ground to a fine homogenous powder using a Planetary Ball Mill Pulverisette 5 (Fritsch, Idar-Oberstein, Germany) and/or a laboratory blender (Waring, Stamford, USA).

3.2. Crude plant extract preparation

Forty grams from each grounded plant material (leaves, bark, roots, and fruits) was used for the preparation of crude plant extracts. Crude plant extracts were prepared by macerating the plant material in a solvent for 72 hrs at room temperature (25 °C) in the dark. The solvents used were distilled water, methanol, ethyl acetate, and hexane. After maceration, the plant material and solvent mixtures were filtered using a Whatman filter paper 3 (Cytiva, Buckinghamshire, United Kingdom). The filtrate was concentrated using a rotary evaporator (Heidolph, Schwabach, Germany) and completely dried under the fume hood for 30 mins. Each aqueous extract filtrate was centrifuged at 20,000 × *g*, for 10 mins four times, removing the pelleted plant material each time. This was performed to clarify the crude extract before concentrating it. The stock of each crude plant extract was prepared to a final concentration of 1 g/mL by dissolving 5 g of dry crude extract in 5 mL of ≥99.9 % dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Saint-Louis, USA). Crude extracts from each plant part were prepared at least 3 separate times. Each time, at least 3 replicates per tissue type were prepared.

3.3. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Quantities of phenolic compounds and flavonoids within the leaf, bark, root, and fruit crude plant extracts were determined using the assays detailed in subsection 3.3.1. and 3.3.2.

3.3.1. Total Phenolic Content (TPC)

Total Phenolic Content (TPC) in each crude extract was determined using the Folin-Ciocalteu (F-C) colorimetric assay, where by 200 µL of prepared 1 mg/mL crude plant extract dilution, 200 µL of 10 % F-C reagent phenol (Sigma-Aldrich, Saint-Louis, USA), and 2 mL of distilled water were mixed in an assay tube, vortexed, and incubated at room temperature for 8 mins. After incubation, 600 µL of 20 % (w/v) sodium carbonate solution was added to stop the reaction. The assay tube was then incubated for 2 hrs in the dark and absorbance was measured at 760 nm against a ≥99.9 % methanol blank. Methanol (≥ 99.9 %) was used to prepare gallic acid or crude plant extract stock dilutions. Methanol was also used as a negative control. The gallic acid calibration curve was generated using serial dilutions of gallic acid in equivalence of 400, 200, 100, 50, 25, 12.5 and 0 µg/mL. The TPC of each plant crude extract was determined using the linear equation based on a gallic acid calibration curve and the TPC of the extracts was expressed as µg gallic acid equivalents (GAE) per mg dry extract (µg GAE/mg DE).

3.3.2. Total Flavonoid Content (TFC)

The total amount of flavonoids was determined using the aluminum chloride ($AlCl_3$) colorimetric assay. Serial dilutions of quercetin (standard) in equivalence of 400, 200, 100, 50, 25, 12.5 and 0 µg/mL were used to make a TFC calibration curve. One milliliter of 1 mg/mL crude plant extract or quercetin solution was mixed with 200 µL of 10 % aluminum chloride, 200 µL of 1M potassium acetate, and 600 µL of distilled water. The mixture was kept at room temperature for 30 mins. Absorbance was then measured at 420 nm using the SPECTRONIC 200 Spectrophotometer (Thermo-Fisher Scientific, Winsford, United Kingdom). Methanol (≥ 99.9 %) was used to prepare crude plant extract or quercetin dilutions. Methanol was also used as a negative control. The TFC of each crude plant extract was expressed as quercetin equivalents (QE) using the linear equation based on the generated quercetin TFC calibration curve.

3.4. Antioxidant activity assays

The antioxidant activity of each crude plant extract was determined using the 2,2 diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, Ferric Reducing Antioxidant Power (FRAP),

and Phosphomolybdenum inhibition total antioxidant capacity (TAC) assays. Estimations were made against the standard antioxidant compound, gallic acid.

3.4.1. DPPH free radical scavenging assay

The free radical scavenging capacity of each crude plant extract was determined using DPPH and a microplate-based method as described by Brand-Williams *et al*., (1995). In this assay, the purple color of DPPH in solution (absorbs light at λmax=517 nm) changes to a yellowish-colored product (diphenyl-picryl hydrazine) when the antioxidant is added in a concentration-dependent manner. In a 96 well microplate, 150 µL of 0.1M methanolic DPPH (SigmaAldrich, Saint-Louis, USA) solution was added to 50 µL of serially diluted concentrations of the crude plant extract or gallic acid (standard) in equivalence of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125 and 0 mg/mL. Methanol (≥ 99.9 %) was used to prepare gallic acid or crude plant extract stock dilutions. Methanol was also used was used as a negative control. The plate was incubated at room temperature in the dark for 30 mins. After incubation, the absorbance was measured at 514 nm using a microplate reader (Thermo-Fisher Scientific, Winsford, United Kingdom). The percentage scavenging capacity of the crude extract at 1 mg/mL was determined using the following formula:

$$\%\ DPPH\ scavernging\ capacity\ = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the negative control and A_{sample} is the absorbance of the plant extracts or standard. The IC_{50} (µg/mL) values for the DPPH free radical scavenging capacity of each crude plant extract were also determined as described in section 3.7. below.

3.4.2. Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was performed on each plant's crude extract according to the Modified Ferricyanide method (with incubation) described by Berker *et al*. (2007) using the microwell-based method. This assay is based on the direct reduction of Fe^{3+} to Fe^{2+} under acidic conditions (pH 3.6). The reduction of Fe^{3+} to Fe^{+2} in presence of 2,4,6-trypyridyl-s-triazine results in increased absorbance from the formation of a tripyridyl triazine complex (absorbs light at 593 nm). To evaluate FRAP percentage activity, 40 µL of 1 mg/mL plant crude extract or gallic

acid (standard) solution was mixed with 100 µL of 1N hydrochloric acid, 20 µL of 1 % sodium dodecyl sulphate (SDS), and 30 µL of potassium ferrocyanide in a well of a 96-well microplate. The plate was then incubated at 50 °C for 20 mins, and 20 µL of ferric chloride was added before reading the absorbance at 750 nm in a microplate reader. The same procedure was followed for serially diluted concentrations of gallic acid in equivalence of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.007812 and 0 mg/mL. A calibration curve was prepared using gallic acid values. Methanol (≥ 99.9 %) was used to prepare gallic acid or crude plant extract stock dilutions. Methanol was also used was used as a negative control. FRAP values for crude extracts were determined using a calibration curve linear equation and expressed as µg gallic acid equivalent per gram of dry extract (µg GAE/g DE). Percentage FRAP activity at 1 mg/mL of each crude plant extract was evaluated using the following formula:

$$\%\ FRAP\ activtity\ = \frac{A_{sample} - A_{control}}{A_{sample}} \times 100$$

where $A_{control}$ is the absorbance of the negative control and A_{sample} is the absorbance of the plant extracts or standard.

3.4.3. Phosphomolybdenum inhibition Total Antioxidant Capacity (TAC) assay

The total antioxidant capacity (TAC) through Phosphomolybdenum inhibition was performed for each crude extract according to Ahmed *et al*., (2014). In glass assay tubes, a volume of 0.1 mL of 1 mg/mL solution of each crude plant extract was added to 1 mL of reagent solution (4 mM ammonium molybdate, 0.6M sulphuric acid, and 28 mM sodium phosphate) and incubated for 90 mins at 95 °C in a water bath. After incubation, the mixture was allowed to cool under room temperature to 25 °C, and the absorbance of the mixture was measured at 695 nm using SPECTRONIC 200 Spectrophotometer (Thermo-Fisher Scientific, Winford, United Kingdom). Methanol (≥ 99.9 %) was used as a negative control. The same procedure was followed for serially diluted concentrations of gallic acid in equivalence of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.007812 and 0 mg/mL. A calibration curve was prepared using gallic acid values. The Phosphomolybdenum inhibition TAC activity of each 1 mg/mL plant crude extract solution was determined and expressed in µg gallic acid equivalent per mg (µg GAE /mg DE) using the linear equation from a gallic acid calibration curve.

3.5. Pearson's correlation of antioxidant activity, TFC, and TPC

The correlation analysis between the TPC, TFC, and antioxidant activity of all *K. africana* and *P. angolensis* was performed using GraphPad Prism 9 software. Multiple linear regression variable analyses of Pearson's correlation were used to generate a two-dimensional correlation heat map.

3.6. Antimicrobial activity assays

For the determination of antimicrobial activity of each crude plant extract, the Kirby Bauer Disc diffusion and serial microdilution (developed by Eloff, 1999) methods were used. The bacterial strains used were KWIK STIK *E. coli* ATCC® 25922, *S. aureus* ATCC® 29123, *P. aeruginosa* ATCC® 10145 (Microbiologics Saint-Cloud, USA), and *B. subtilis* (Carolina Biological Supply Company, Burlington, USA). The yeast strain used was the KWIK STIK *C. albicans* ATCC® 10231 (Microbiologics Saint-Cloud, USA). Mueller Hinton Agar/Broth (MHA/MHB) (Sigma Aldrich, Burlington, USA) media was used for bacterial cultures while Yeast Extract Peptone Dextrose (YPD) (Sigma Aldrich, Burlington, USA) media was used for yeast cultures.

3.6.1. Bacterial and yeast culture preparation

KWIK STICK bacterial and yeast strain swab pouches stored at 8 ºC were used and allowed to equilibrate to room temperature for four hours. Each bacterial and yeast strain swab sample was inoculated onto respective agar media plates. A sterile inoculation loop was then used to streak the inoculated sample and facilitate colony isolation. The inoculated plates were incubated for 18 hrs at 37 °C for bacterial cultures and 30 ºC for yeast cultures. Overnight cultures were prepared by picking pure colonies from the prepared culture agar plates by suspending each pure colony in sterile broth. The inoculated broth was incubated overnight (12 hrs) at 37 ^{o} C and and30 ^{o} C for bacterial cultures and yeast cultures respectively. The overnight cultures were used to prepare inoculums used for the antimicrobial assays. Inoculums were prepared by diluting the overnight cultures to a 0.5 McFarland turbidity (1.5×10^{8} CFU/mL) using sterile broth media. The turbidity of the suspension was adjusted using the McFarland Standard kit (Biomerieux, Marcy-l'Etoile, France) and the SPECTRONIC 200 Spectrophotometer (Thermo-Fisher Scientific, Winsford, UK).

3.6.2. Kirby Bauer Disc diffusion method - Minimum Inhibitory Concentration (MIC) determination

The Kirby Bauer Disc diffusion method was performed to investigate the susceptibility of each target microbial strain to each crude plant extract. The method involves placing antimicrobial agent (plant extract or antibiotic) impregnated paper discs on an inoculated solidified agar surface (the lawn of bacteria or yeast), then incubating the plate overnight (12 hrs) and measuring the zones of inhibition around the discs. Discs were prepared by puncturing Whatman filter paper 3 to make a disc of 6 mm diameter and soaking the discs in different concentrations of filter-sterilized crude plant extracts. The assay concentrations for crude plant extracts were 40 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml. The discs were soaked for 30 mins in crude extract dilutions and allowed to dry before placing them on inoculated agar plates. The inoculated agar plates were prepared by spread plating 100 µl of prepared 0.5 McFarland bacterial or yeast suspension. After placing the discs, the plates were then incubated overnight at 37 ºC for bacterial cultures and 30 ºC for yeast cultures. Gentamicin (10 µg) (Oxoid, Basingstoke, United Kingdom) and Amphotericin B (10 µg) (Gibco, Massachusetts, USA) discs were used as positive controls while distilled water, methanol, ethyl acetate, hexane, and DMSO impregnated discs were used as negative controls. Each crude plant extract concentration was tested in triplicates. Susceptibility and MIC values were determined by measuring the zone of inhibition. MIC is described as the lowest concentration of the crude extract inhibiting the growth of the microorganisms.

3.6.3. Serial microdilution method – Half-maximal inhibitory concentration (IC_{50}) determination

This assay was done according to the microplate method to determine the minimal inhibitory concentration of crude plant extracts for bacteria described by Eloff, (1999) with modification. Instead of the 25 – 100 µL volume range of 3 hours old bacterial or yeast suspensions used by Eloff (1999), a constant volume of 100 µL of prepared 0.5 McFarland bacterial yeast suspensions was used for each crude plant extract concentration. Half-maximal inhibitory concentration (IC_{50}) was defined as a concentration of crude plant extract required for 50 % inhibition of microorganisms. Crude extracts, gentamicin, or amphotericin B were prepared through a series of two-fold dilutions using sterile water to achieve concentrations of 20, 10, 5,

2.5, 1.25, 0.625 0.313, 0.156, and 0 mg/mL. In each test microplate (containing crude plant extract and test microorganism), in a test well, 100 µL of crude extract dilution was mixed with a constant volume of 100 µL of 0.5 McFarland suspensions. The plates were then incubated overnight (12 hrs) at 37 ℃ and 30 ℃ for bacteria and yeast respectively. A cell viability test was performed by dissolving 40 µL of 0.2 mg/ml p-iodonitrotetrazolium (INT) salt (Sigma-Aldrich, Saint-Louis, USA) in water to each test well and each test microplates was further incubated for 30 to 45 mins. The development of color from clear to reddish-purple was observed. After incubation, the absorbance was measured at 505 nm using a microplate reader. The same procedure was followed for Gentamicin and amphotericin B dilutions as positive controls against bacterial and yeast species respectively and distilled water, methanol, ethyl acetate, hexane, and DMSO-distilled water solution were used as negative controls.

3.7. *In-silico* assessment of human Epidermal Growth Factor Receptor (EGFR) protein inhibition and anticancer properties of *K. africana* and *P. angolensis* phytochemicals

This assessment included literature survey for already identified bioactive compounds from *K. africana* and *P. angolensis*, retrieving (Spatial Data Files) SDF files of the identified compounds, and EGFR protein, bioactive compound ligand and protein preparation, site-specific molecular docking, and ADMET analysis.

3.7.1. Literature survey for bioactive compounds

Natural bioactive compounds isolated from *K. africana* and *P. angolensis* were found through a literature survey using ScienceDirect, Web of Science, PubMed, and Google Scholar databases. The keywords bioactive compounds, phytochemicals, *K. africana*, and or *P. angolensis* were used for the search. Food and Drug Administration (FDA) approved EGFR inhibitors were also through literature survey and used as positive controls.

3.7.2. Ligand and protein preparation

Three-dimensional models of the identified bioactive compounds and FDA-approved EGFR inhibitors were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Kim *et al.*, 2016) and ZINC15 (http://zinc15.docking.org/) (Sterling and Irwin, 2015) online databases as SDF. Open Babel was used to converting 2D compounds to 3D compound SDF files and Protein Data

Bank, Partial Charge (Q), and Atom Type (T) (PDBQT) files. Bioactive compound and FDA-approved inhibitors PDBQT files were used for docking. A three-dimensional sequence structure model of the human Epidermal Growth Factor Receptor (EGFR) protein (PDB ID: 2J6M) was also retrieved as an SDF file from the RCBS protein data bank (https://www.rcsb.org/) (Kouranov *et al.*, 2006). The protein files were prepared by removing water molecules and heteroatoms using PyMOL Molecular Graphics (System Version 2.0 Schrodinger, LLC) (Schrödinger, 2015). Protein-ligand binding sites (target for protein inhibition) of the prepared protein were predicted using the PrankWeb (P2Rank) web-based front-end website (http://prankweb.cz/) (Jendele *et al.*, 2019).

3.7.3. Site-specific molecular docking

Protein-ligand docking was performed using AutoDock 4.2.6 and AutoDock Vina in PyRX 0.8 virtual screening tool (Dallakyan and Olson, 2015). All prepared compounds and proteins were loaded into AutoDock as PDQT files. The P2Rank predicted protein-ligand binding sites for pocket 1 (Table 1) was used to label amino acid residues of the active site and specify the vina search space. For docking simulation and conformational search, the Lamarckian Genetic Algorithm (LGA) was selected. Moreover, the number of evaluations was set to 10 while other parameters were kept at default.

3.7.4. Drug-likeness analysis, Absorption, Distribution, Metabolism, and Excretion, (ADME), and Toxicity predictions

Drug-likeness analysis and ADME/Toxicity predictions were performed for the top 10 bioactive compounds (bioactive compounds with high binding affinity to EGFR protein) using theSwissADME online tool by the Swiss Institute of Bioinformatics (http://www.swissadme.ch/) and PreADMET online tool (https://preadmet.webservice.bmdrc.org). ADME predictions were performed using the SwissADME tool. The predicted pharmacokinetic properties were gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeant, P-glycoprotein substrate (P-gy substrate), cytochrome P450 (CYP) 1A2 inhibitor, CYP 2C19 inhibitor, CYP 2C9 inhibitor, CYP 2D6 inhibitor, a CYP3A4 inhibitor, bioavailability score, and Log Kp (skin permeation). Drug-likeness properties were assessed in using the Lipinski (Pfizer), Ghose, Muegge, Veber, and Egan filters in SwissADME. Bioactive compound toxicity likeliness was predicted using

PreADMET. Predictions were followed by the Ames test (which predicts the mutagenicity of compounds) and the National Toxicology Program (NTP), which predicted the in-vivo carcinogenicity of the compounds in mice and rats for 2 years.

3.7.5. Visualization of binding position and interaction between EGFR protein and bioactive compounds

The 3-D crystal structure image of the EGFR protein (PDB ID:2J6M) and ligand with promising human EGFR inhibitor properties (according to the binding affinity, drug-likeness, and ADME and Toxicity analysis) were examined and visualized using PyMOL, and Discovery Studio Visualiser (Systèmes BIOVIA, 2020).

3.8. Statistical analysis

All experiments were performed in triplicate, and data analysis was performed using GraphPad Prism 9 software for windows (California, USA). The IC_{50} values for DPPH of the crude plant extracts were determined through Fit spine/ LOWNESS XY analyses. The mean values of the samples were compared using repeated measure one-way analysis of variance (ANOVA). Means were considered significant if $p<0.05$ and they were ranked compared to the control using Tukey's Honest Significant Difference assay. The correlation analysis results of antioxidant activity, with TPC, and TFC were expressed as Pearson's correlation coefficients in a heatmap. The results were presented as mean ± standard error (SE) values.

CHAPTER 4: RESULTS

In this study, the total phenolic contents, total flavonoid contents, antioxidant, antimicrobial, and anticancer properties of *K. africana* and *P. angolensis* were assessed. The anticancer drug potential activities of *K. africana* and *P. angolensis* phytochemicals were also assessed.

4.1. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

All crude extracts of *K. africana* and *P. angolensis* showed the presence of both phenolic and flavonoid compounds. The R^2 values for the gallic acid and quercetin calibration curves used to determine phenolic and flavonoid contents in the different extracts were 0.9924 (**Figure 4**) and 0.9900 (**Figure 5**) respectively.

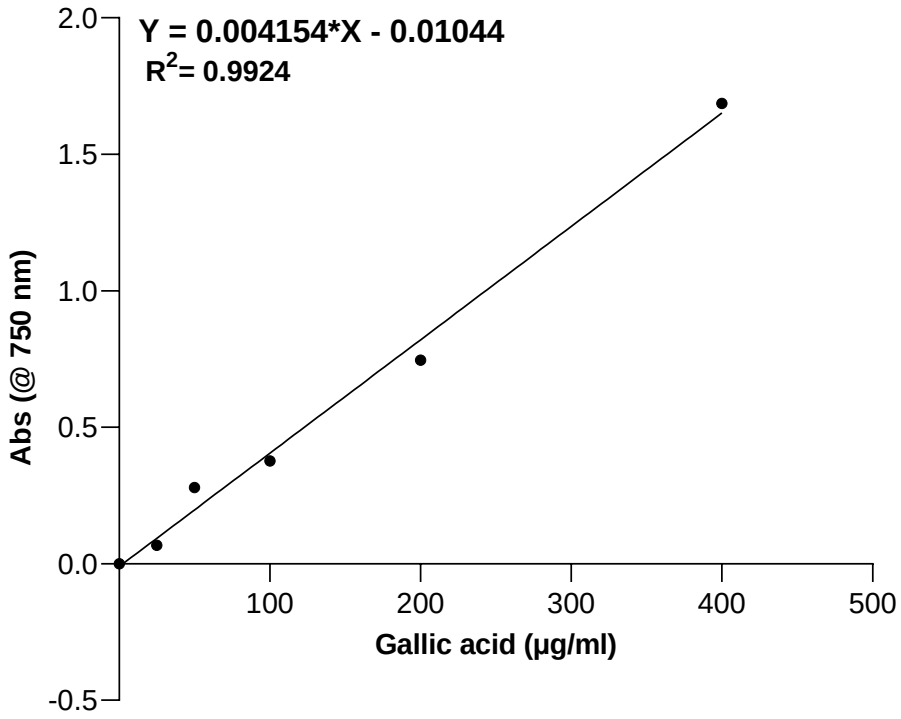


Figure 4: Gallic acid calibration curve used to determine TPC in crude extracts.

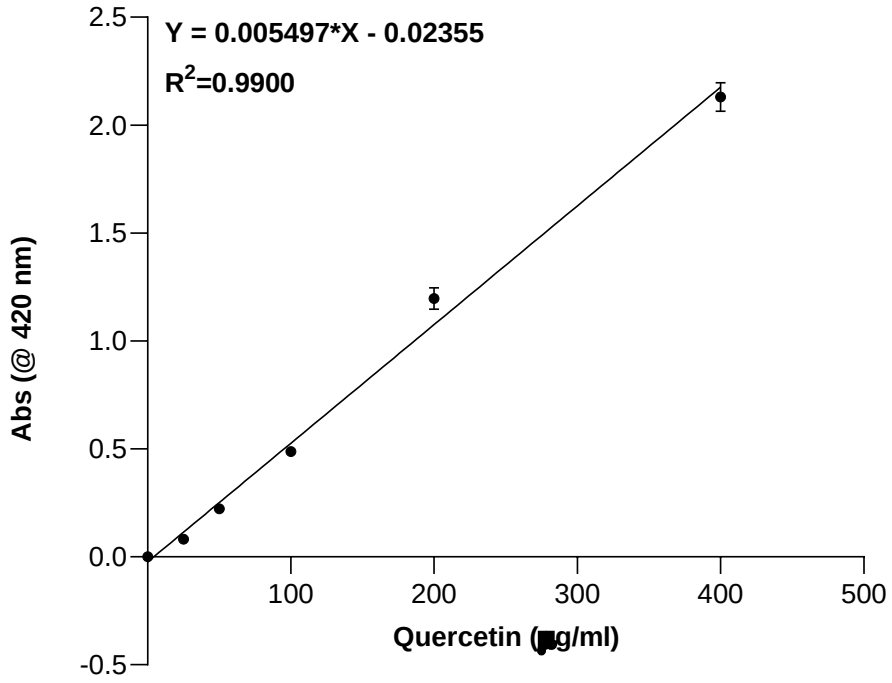


Figure 5: Quercetin calibration curve used to determine TFC in crude extracts.

4.1.1. Total Phenolic Content (TPC)

Total Phenolic Contents of all *K. africana* and *P. angolensis* crude extracts ranged from 13.3 ± 0.2 to 556.2 ± 3.7 µg/mg DE. *Pterocarpus angolensis* crude plant extracts (**Figure 7**) showed significantly higher amounts of phenolic compounds than *K. africana* crude extracts (**Figure 6**). *Pterocarpus angolensis* bark aqueous extract showed the highest TPC (556.2 ± 3.7 µg GAE/mg DE).

The TPC of *K. africana* extracts ranged from 13.3 ± 0.2 to 236.5 ± 6.1 µg GAE/mg DE (**Figure 6**). The leaves ethyl acetate extract showed the highest TPC of 236.5 ± 6.1 µg GAE/mg DE followed by leaves hexane extract at 201.7 ± 2.0 µg GAE/mg DE, leaves methanol extract at 164.3 ± 2.6 µg GAE/mg DE and leaves aqueous extract at 100.5 ± 0.2 µg GAE/mg DE. Solvents with lower polarity (ethyl acetate and hexane) extracted higher amounts of phenolic compounds from the leaves than solvents with high polarity (water and methanol). From the bark extracts, ethyl acetate and methanol extracts showed the highest TPC of 225.8 ± 2.4 and 222.3 ± 2.5 µg GAE/mg DE respectively while the aqueous extract had a TPC of 175.6 ± 0.6 µg GAE/mg DE. The bark hexane extract showed the lowest TPC (34.6 ± 1.5 µg GAE/mg DE). Similarly, in all *K. africana* root extracts, the methanol and ethyl acetate extract showed the highest TPC of 195.1 ± 1.3 and 190.7 ± 2 µg/mg DE respectively while the aqueous and hexane extract showed the lowest TPC of 137.6 ± 2.3 and 38.5 µg GAE/mg DE respectively. There was no significant difference between *K. africana* bark methanol (222.3 ± 2.5 µg GAE/mg DE), bark ethyl acetate (225.8 ± 2.4 µg GAE/mg DE), roots methanol (195.1 ± 1.3 µg GAE/mg DE) and roots acetate (190.7 ± 2 µg GAE/mg DE) extracts. The fruit hexane extract showed the lowest TPC (13.4 ± 0.2 µg GAE/mg DE). Amongst all *K. africana* fruit extracts, the highest observed TPC was 59.2 ± 3.2 µg GAE/mg DE exhibited by the fruit aqueous extract.

From *P. angolensis* leaves extracts, the ethyl acetate extract showed the highest TPC (346.8 ± 2.4 µg GAE/mg DE), then leaves methanol extract (322.7 ± 1.4 µg GAE/mg DE) (**Figure 7**). There was no significant difference between the TPC of the leaves aqueous (140.9 ± 4.1 µg GAE/mg DE) and leaves hexane (147.0 ± 1.1 µg GAE/mg DE). Water (aqueous) extracted the highest amount of phenolic compounds from the bark (556.2 ± 3.8 µg GAE/mg DE) followed by methanol (244.9 ±10.0 µg GAE/mg DE). The ethyl acetate and hexane bark extracts showed the lowest TPC of 70.4 ± 2.1 and 40.0 ± 1.0 µg GAE/mg DE respectively. Highly polar solvents,

water, and methanol extracted higher amounts of phenolic compounds from the bark than the least polar solvents ethyl acetate and hexane. Amongst the *P. angolensis* root extracts, the ethyl acetate extract exhibited the highest TPC of 285.8 ± 1.7 µg GAE/mg DE followed by the aqueous extract at 182.7 ± 0.7 µg GAE /mg DE. There was no significant difference between the root methanol (108.6 ± 1.4 µg/mg DE) and root hexane (101.9 ± 2.7 µg/mg DE) extracts.

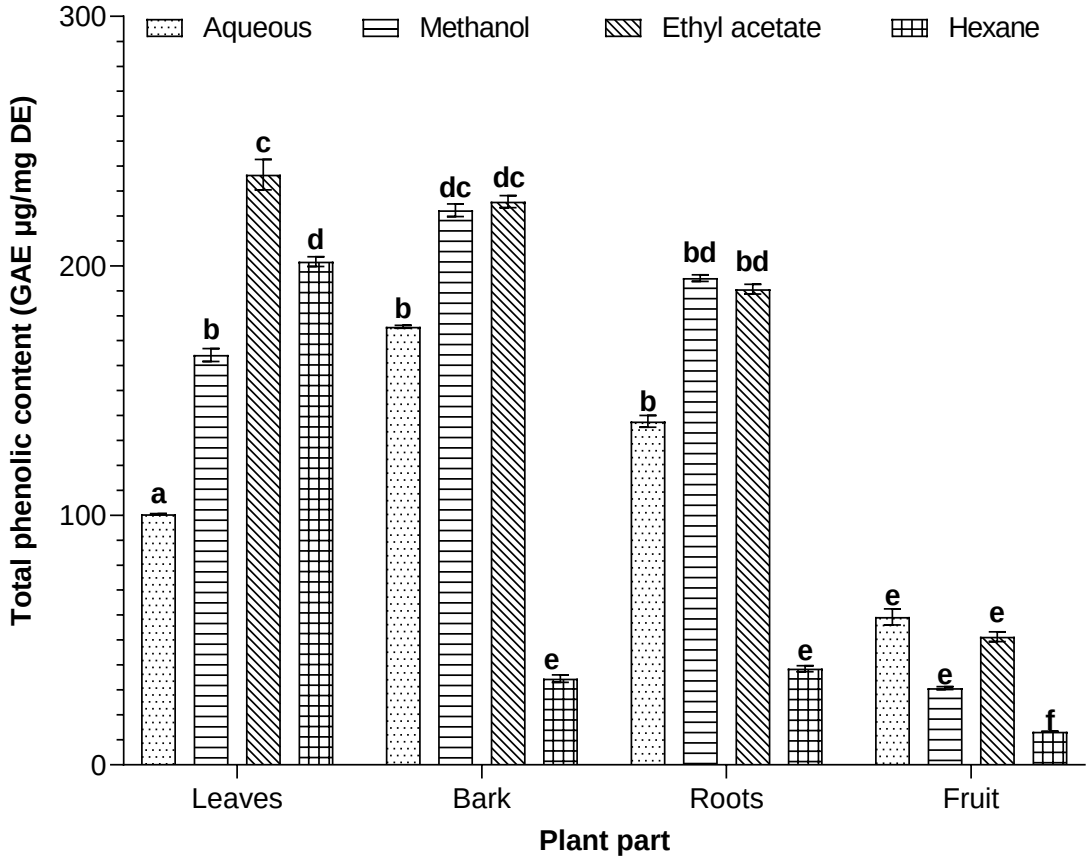


Figure 6: Total phenolic contents in different parts of *K. africana*. The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

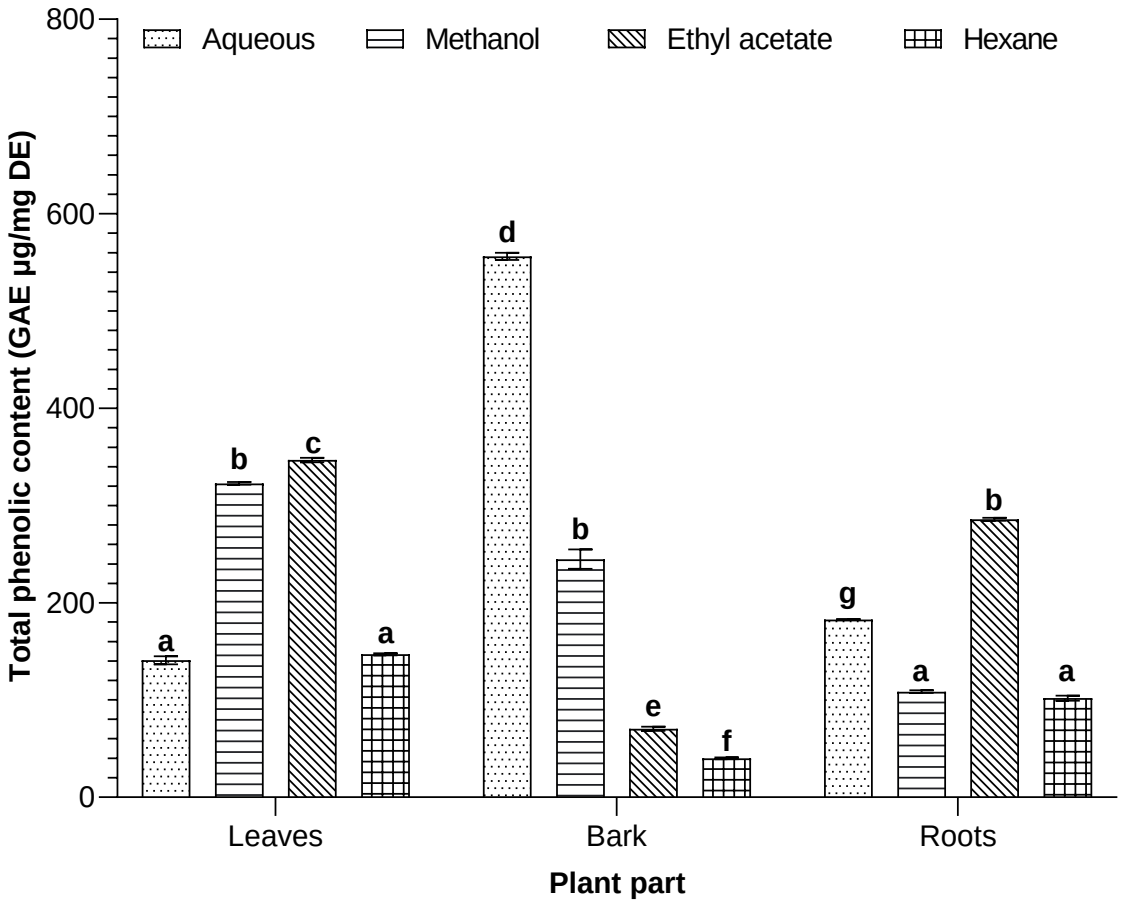


Figure 7: Total phenolic contents in different parts of *P. angolensi*s. The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

4.1.2. Total Flavonoid Content (TFC)

For Total Flavonoid Content of *K. africana* and *P. angolensis*, the crude plant extracts ranged from 12.7 ± 0.02 to 445.1 ± 1.6 µg QE/mg DE. *Pterocarpus angolensis* crude plant extracts (**Figure 9**) showed significantly higher amounts of flavonoid compounds than *K. africana* crude extracts (**Figure 8**). *Kigelia africana* leaves ethyl acetate extract exhibited a significantly higher amount of flavonoids (445.1 ± 1.6 µg QE/mg DE).

The TFC of the *K. africana* extracts (**Figure 8**) ranged from 4.1 ± 0.0 to 336.6 ± 1.6 µg QE/mg DE. From the leaves, the ethyl acetate extract had the highest amount of flavonoid compounds (445.1 ± 1.6 µg QE/mg DE). This was followed by the methanol extract (173.0 ± 0.2 µg QE/mg

DE), aqueous extract (52.4 ± 0.3 µg QE/mg DE) and hexane extract (16.2 ± 0.0 µg QE/mg DE) respectively. All *K. africana* leaves extracts were significantly different. Among *K. africana* bark extracts, the highest observed TFC was 85.8 ± 0.4 µg QE/mg DE exhibited by the ethyl acetate extract followed by 54.8 ± 1.5 µg QE/mg DE exhibited by the methanol extract. From the root, ethyl acetate extract showed the highest TFC (86.3 ± 1.3 µg QE/mg DE) followed by methanol extract (60.9 ± 1.0 µg QE/mg DE). There was no significant difference between the bark and root extracts. The highest recorded TFC among fruit extracts was 103.2 ± 0.3 µg QE/mg DE exhibited by fruit aqueous extract followed by 41.8 ± 1.1 µg QE/mg DE exhibited by fruit ethyl acetate extract. In all *K. africana* plant parts, ethyl acetate was a significantly better extraction solvent, followed by methanol and distilled water respectively.

The observed TFC of *P. angolensis* extracts is shown in **Figure 9**. From the leaves, the methanol extract had the highest TFC (75.8 ± 1.5 µg QE/mg DE) followed by the ethyl acetate extract (62.5 ± 1.8 µg QE/mg DE) and aqueous extract (60.1 ± 1.8 µg QE/mg DE). There was no significant difference between the TFC of leaves aqueous, methanol, and ethyl acetate extracts. Among the *P. angolensis* bark extracts, the highest observed TFC was 226.2 ± 2.8 µg QE/mg DE exhibited by the methanol extract followed by 157.3 ± 1.2 µg QE/mg DE exhibited aqueous extract. The highest observed TFC from the root extracts was 131.4 ±0.8 µg QE/mg DE exhibited by the aqueous extract followed by 129.3 ± 0.7 µg QE/mg DE and 112.9 ± 1.9 µg/mg DE exhibited by the methanol and ethyl acetate extract respectively. The was no significant difference between the aqueous, methanol, and ethyl acetate root extracts.

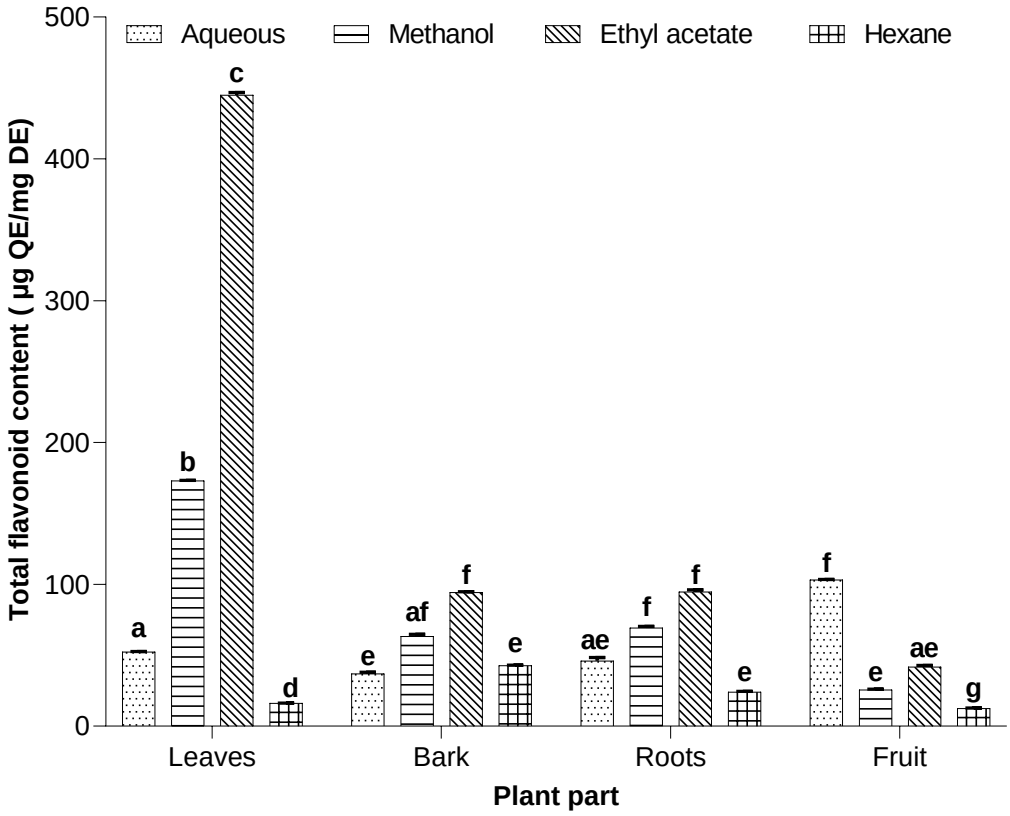


Figure 8: Total flavonoid contents in different parts of *K. africana*. The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

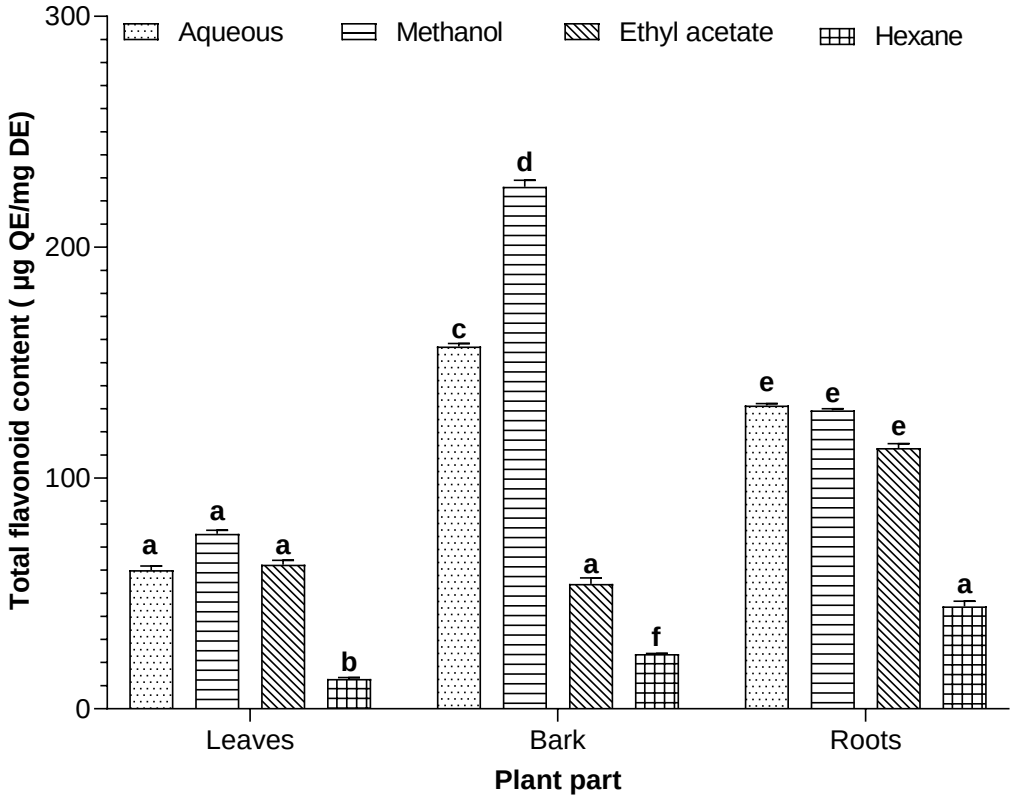


Figure 9: Total flavonoid contents in different parts of *P. angolensis*. The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

4.2. Antioxidant activity

The antioxidant activity of *K. africana* and *P. angolensis* crude extracts was determined using DPPH free radical scavenging, FRAP, and Phosphomolybdenum inhibition total antioxidant capacity (TAC) assays.

4.2.1. DPPH free radical scavenging activity

Percentage DPPH scavenging activity of *K. africana* and *P. angolensis* crude extracts are shown in **Figures 10** and **Figure 11** respectively, while the DPPH scavenging IC_{50} values are presented in **Table 3**.

The percentage of DPPH scavenging activity of *K. africana* crude extracts ranges from 89.1 ± 0.1 % to 32.8 ± 1.5 % (**Figure 10**). From the leaves, the methanol extract showed the highest

DPPH scavenging activity of 85.5 ± 0.0 % and IC_{50} of 49.0 ± 1.8 µg/ml followed by the aqueous extract activity of 76.2 ± 0.3 % and IC_{50} of 131.9 µg/ml. The bark extracts ethyl acetate and methanol showed the highest DPPH scavenging activity of 88.2 ± 0.1 % and 88.0 ± 0.0 % and IC_{50} of 36.7 ± 0.5 µg/ml and 32.8 ± 0.1 µg/ml respectively. Similarly, in all *K. africana* root extracts, the ethyl acetate and methanol extracts also exhibited the highest DPPH scavenging activity of 89.1 ± 0.1% and 87.9 ± 0.1 % and IC_{50} values of 54.7 ± 0.4 and 44.1 ± 0.1 µg/ml (**Table 3**) respectively. There was no significant difference between the % DPPH scavenging activity of *K. africana* bark methanol (88.0 ± 0.0 %), bark ethyl acetate (88.2 ± 0.1 %), roots methanol (87.9 ± 0.1 %) and roots ethyl acetate (89.1 ± 0.1 %) extracts. Amongst all *K. africana* fruit extracts, the lowest observed DPPH scavenging activity was 42.5 ± 0.2 % fruit aqueous extract, and IC_{50} were greater than 1 000 µg/ml (**Table 3**). Methanol and ethyl acetate were the best solvents to extract *K. africana* bioactive compounds with high DPPH scavenging activity.

The percentage of DPPH scavenging activity of *P. angolensis* extracts ranged from 87.2 ± 0.0 % to 42.2 ± 0.4 % (**Figure 11**). From *P. angolensis* leaves extracts, the methanol extract showed the highest percentage of DPPH scavenging activity (80.5 ± 0.1 %), with an IC_{50} of 61.3 ± 1.9 µg/mL. This was followed by leaves ethyl acetate extract with a percentage activity of 75.4 ± 0.6 and IC_{50} of 57.7 ± 2.4 µg/mL. There was no significant difference between the % DPPH scavenging activity and IC_{50} value of the leaves methanol (80.5 ± 0.1 %; 61.3 ± 1.9 µg/mL) and leaves ethyl acetate (75.4 ± 0.6 %; 57.7 ± 2.4 µg/mL) extracts. From the bark, the highest observed % DPPH scavenging activity was 87.2 ±0.0 % and 85.8 ± 0.6 % exhibited by the methanol and aqueous extracts respectively. The IC_{50} values of the methanol and aqueous extracts were 3.5 ± 1.6 and 6.9 ±0.7 µg/ml respectively and they were comparable with the gallic acid (positive control) IC_{50} value at 2.1 ± 0.1 µg/ml (**Table 3**). The methanol root extracts, exhibited the highest % DPPH scavenging activity of 85.4 ± 0.5 % with an IC_{50} value of 25.7 ± 0.3 µg/ml. This was followed by the ethyl acetate extract with a % activity of 73.9 ± 0.9 % and an IC_{50} value of 50.1 ± 0.1 µg/ml. In all *P. angolensis* extracts, methanol was the best solvent for the extraction of bioactive compounds with high DPPH scavenging activity (**Table 3**).

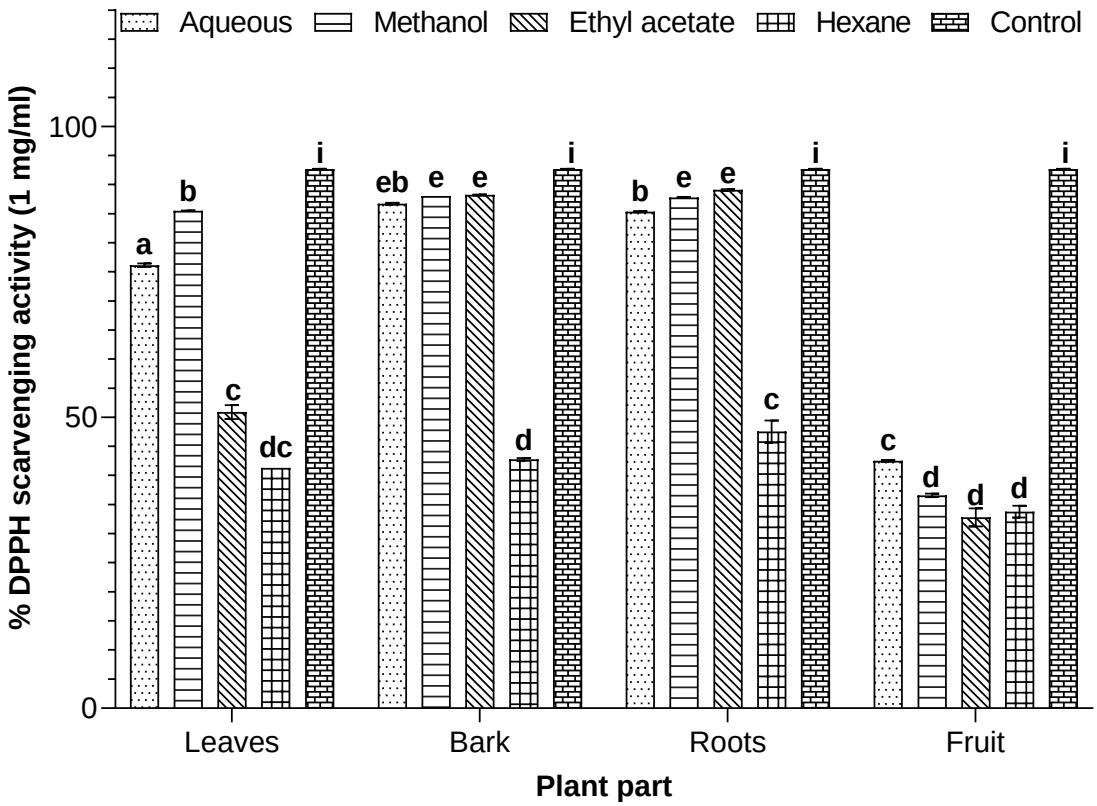


Figure 10: Percentage of DPPH scavenging activity of aqueous, methanol, ethyl acetate, and hexane crude extracts from different parts of *K. africana*. The different letters show (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

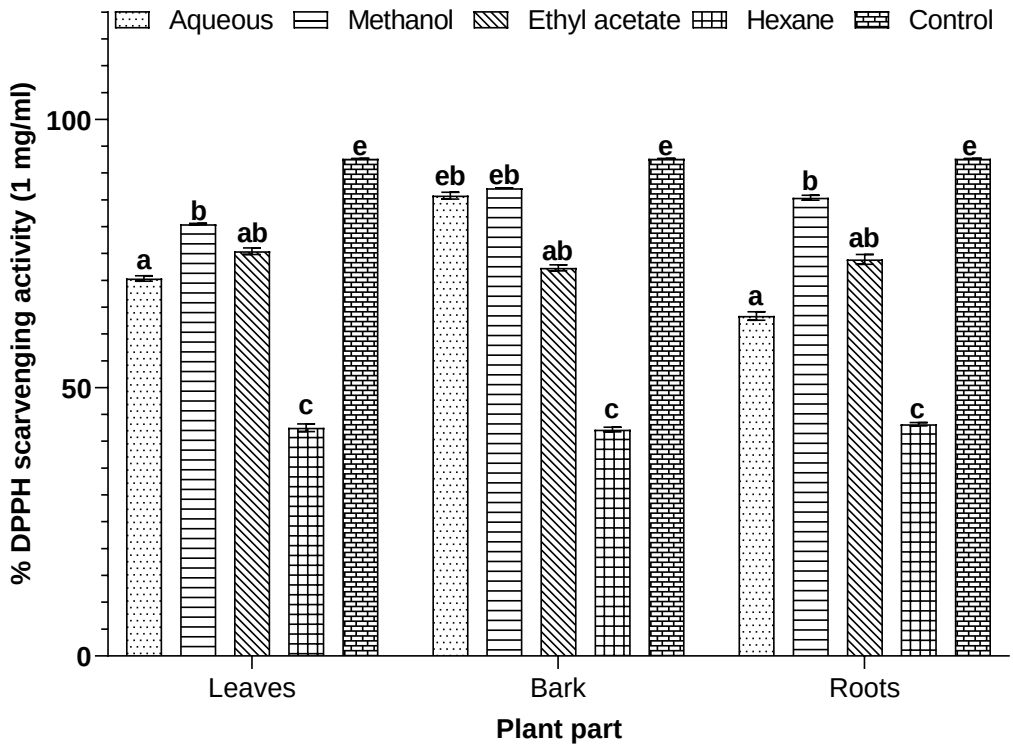


Figure 11: Percentage of DPPH scavenging activity of aqueous, methanol, ethyl acetate, and hexane crude extracts from different parts of *P. angolensis.* The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

Table 3: DPPH free radical scavenging activity IC_{50} values of aqueous, methanol, ethyl acetate, and hexane crude extracts from different parts of *P. angolensis* and *K. africana.*

Plant part	**Solvent**	**DPPH IC_{50} (µg/mL)**	
		K. africana	***P. angolensis***
Leaves	Aqueous	131.9 ± 12.9^{a}	93.7 ± 5.6^{a}
	Methanol	49.0 ± 1.8^{a}	61.3 ± 1.9^{a}
	Ethyl acetate	961.6 ± 28.1^{a}	57.7 ± 2.4^{a}
	Hexane	> 1 000	>1 000
Bark	Aqueous	61.8 ± 0.4^{a}	6.9 ± 0.7^{eb}
	Methanol	32.8 ± 0.1^{a}	3.5 ± 1.6^{eb}

	Ethyl acetate	36.7 ± 0.5[a]	54.2 ± 1.5[a]
	Hexane	> 1 000	>1 000
Roots	Aqueous	76.6 ± 5.6[a]	56.0 ± 1.0[a]
	Methanol	44.1 ± 0.1[a]	25.7 ± 0.3[c]
	Ethyl acetate	54.7 ± 0.4[a]	50.1 ± 0.5[a]
	Hexane	> 1 000	> 1 000
Fruit	Aqueous	629.9 ± 4.9[c]	-
	Methanol	841.2 ± 6.2[c]	-
	Ethyl acetate	891.5 ± 11.0[c]	-
	Hexane	>1 000	-
Control	Gallic acid	2 ± 0.08[e]	2 ± 0.08[e]

Values are expressed as mean ± SE. The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different.

4.2.2. Ferric Reducing Antioxidant Power (FRAP) activity

FRAP values for *K. africana* and *P. angolensis* crude extracts were determined based on the calibration curve linear equation (R^2 = 0.9768; **Figure 12**) and expressed as µg gallic acid equivalent (GAE) per gram dry extract (µg GAE/g DE). *Pterocarpus angolensis* bark crude extracts exhibited significantly higher FRAP activity than *P. angolensis* leaves and roots crude extracts and all *K. africana* crude extracts.

The FRAP activity of the *K. africana* extracts ranged from 2.5 ± 0.9 to 500.6 ± 2.3 µg GAE/mg DE (**Table 4**) while the % FRAP activity of the extracts ranged from 73.8 ± 0.6 to 97.4 ± 0.0 % (**Figure 13**). From the leaves, the methanol extract had the highest FRAP activity of FRAP of 231.6 ± 0.8 µg GAE/mg DE with a % FRAP activity of 97.4 ± 0.0 %. This was followed by the ethyl acetate extract at a FRAP activity equivalent to FRAP of 151.7 ± 1.5 µg GAE/mg DE and a % FRAP activity of 93.1 ± 0.0 %. All *K. africana* leaves extracts were significantly different. Among *K. africana* bark extracts, the highest observed % FRAP activity was 97.4 ± 0.0 % exhibited by the methanol and ethyl acetate extract. The FRAP activity of the methanol and ethyl acetate extracts were 500.6 ± 2.3 and 495.9 ± 1.6 µg GAE/mg DE respectively. From the root extracts, methanol extract showed the highest FRAP activity of 475.9 ± 10.4 µg GAE/mg

DE and a % FRAP activity of 97.3 ±0.1 %. This was followed by ethyl acetate extract with a FRAP activity equivalent to FRAP activity of 440.5 ± 4.1 µg GAE/mg DE and a % FRAP of 97.1 ± 0.0 %. There was no significant difference between the bark and root extracts. The highest recorded FRAP activity among fruit extracts was 31.3 ± 2.3 µg GAE/mg DE and showed a % FRAP activity of 84.0 ± 0.4 %. This was exhibited by fruit aqueous extract. In all *K. africana* plant parts, methanol and ethyl acetate extracted the highest amounts of compounds with FRAP properties.

The FRAP activity of *P. angolensis* extracts ranged from 5.0 ± 2.4 to 1042.2 ± 76.9 µg GAE/mg DE (**Table 4**) while the % FRAP activity of the extracts from 52.0 ± 0.4 to 98.7 ± 0.1 % (**Figure 14**). From the leaves, the ethyl acetate extract exhibited the highest FRAP activity (152.1 ± 15.2 µg GAE/mg DE) and % FRAP activity (93.0 ± 0.5 %). This was followed by leaves methanol extract at a FRAP activity equivalence of 135.9 ± 12.3 µg GAE/mg DE with % FRAP activity of 92.5 ± 0.5 %. From the bark, the highest FRAP activity was exhibited by the methanol extract and it was equivalent to 1042.2 ± 76.9 µg GAE/mg DE with a % FRAP activity of 98.7 ± 0.1 %. This was followed by the bark aqueous extract with a FRAP activity equivalence of 1028.9 ± 102.5 µg GAE/mg DE and a % FRAP activity of 98.6 ± 0.1 %. The aqueous root extract showed the highest FRAP activity of 459.4 ± 6.7 µg GAE/mg DE and % FRAP activity of 97.2 ± 0.0 % followed by the methanol extract with FRAP activity of 422.9 ± 8.4 µg GAE/mg DE and % FRAP activity of 97.0 ± 0.1 %. There was no significant difference between the % FRAP activity of the leaves methanol (92.5 ± 0.5 %) and ethyl acetate (93.0 ± 0.5 %) extracts, bark aqueous (98.6 ± 0.1 %) and methanol (98.7 ± 0.1 %) extracts, root aqueous (97.2 ±0.04) methanol (97.0 ± 0.1 %) and ethyl acetate (96.6 ± 0.1 %) extracts, and gallic acid (98.6 ± 0.1 %) (positive control).

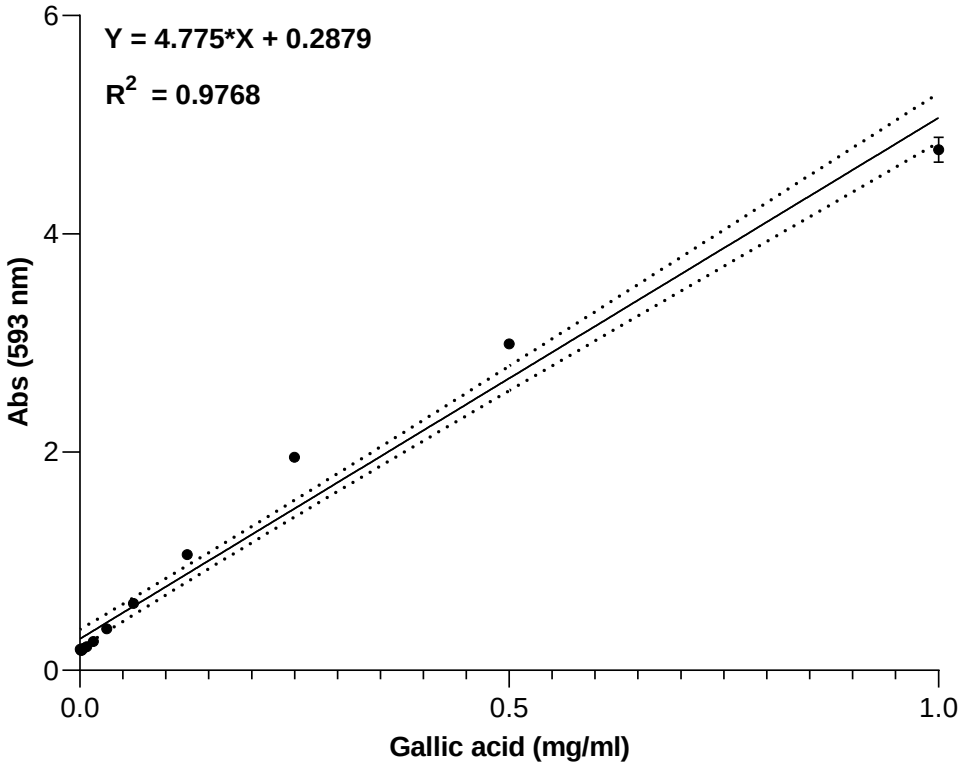


Figure 12: Gallic acid calibration curve used to determine crude extract FRAP antioxidant activity in µg gallic acid equivalent /mg DE (µg GAE/mg DE).

Table 4: *K. africana* and *P. angolensis* plant extract FRAP activity in gallic acid equivalent (GAE) µg/mg DE.

Plant part	**Solvent**	***K. africana***	***P. angolensis***
		µg GAE/mg DE	**µg GAE/mg DE**
Leaves	Aqueous	74.8 ± 1.8^{a}	69.9 ± 1.4^{a}
	Methanol	231.6 ± 0.7^{b}	135.9 ± 12.3^{ab}
	Ethyl acetate	151.7 ± 1.5^{c}	152.1 ± 15.2^{ab}
	Hexane	12.6 ± 0.9^{d}	30.1 ± 0.2^{c}
Bark	Aqueous	215.4 ± 7.4^{b}	1029.9 ± 102.5^{d}
	Methanol	500.6 ± 2.3^{e}	1042.2 ± 76.9^{bd}
	Ethyl acetate	495.9 ± 1.6^{e}	39.9 ± 0.2
	Hexane	27.2 ± 1.2^{d}	12.9 ± 1.5
Roots	Aqueous	208.9 ± 4.6^{b}	459.4 ± 6.7
	Methanol	475.9 ± 10.4^{e}	422.9 ± 8.4
	Ethyl acetate	440.5 ± 4.1^{e}	370.3 ± 17.8^{ab}
	Hexane	3.2 ± 1.4^{d}	5.0 ± 2.4
Fruit	Aqueous	31.3 ± 2.3^{g}	-
	Methanol	4.8 ± 1.2^{d}	-
	Ethyl acetate	4.6 ± 1.2^{d}	-
	Hexane	2.5 ± 0.9^{d}	-

Values are expressed as mean ± SE. The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different.

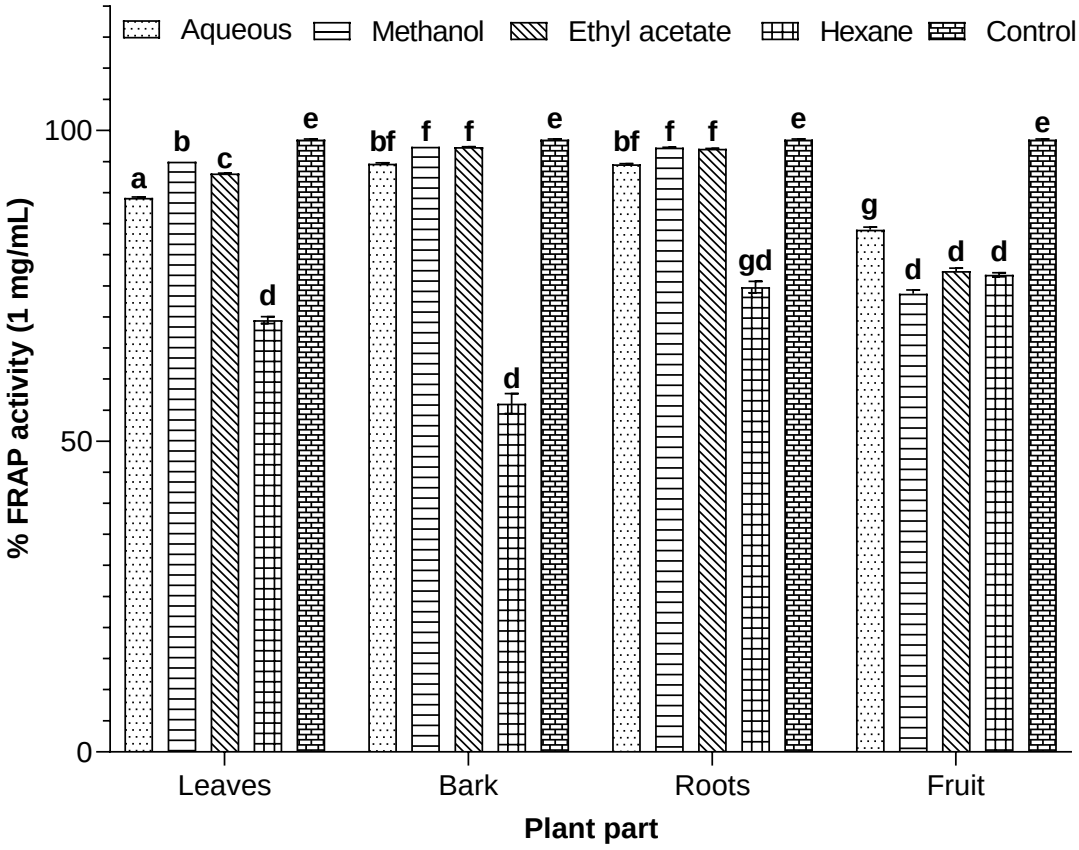


Figure 13: Percentage FRAP activity of aqueous, methanol, ethyl acetate, and hexane crude extracts from different parts of *K. africana.* The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

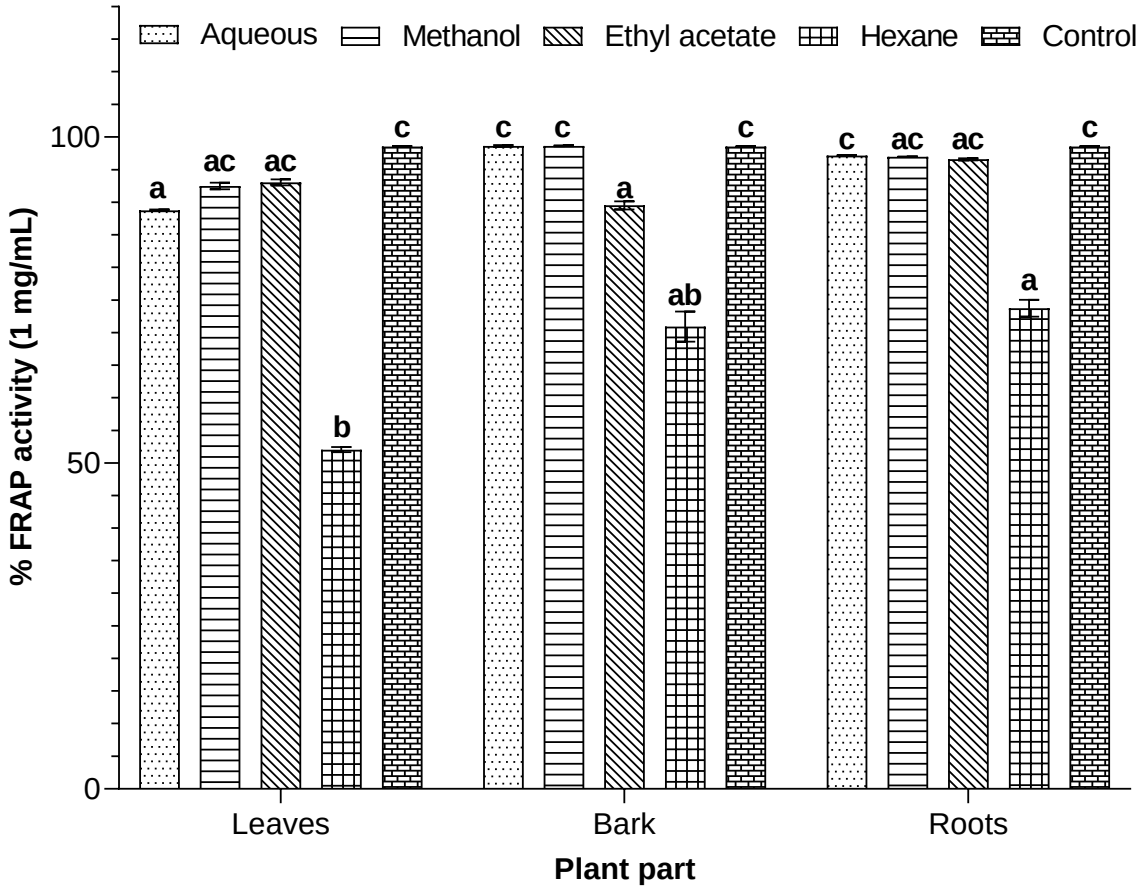


Figure 14: Percentage FRAP activity of aqueous, methanol, ethyl acetate, and hexane crude extracts from different parts of *P. angolensis.* The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

4.2.3. Phosphomolybdenum inhibition Total Antioxidant Capacity (TAC) activity

Phosphomolybdenum inhibition TAC values for *K. africana* and *P. angolensis* crude extracts were determined based on the calibration curve linear equation (R^2 = 0.9768; **Figure 15**) and expressed as µg gallic acid equivalent (GAE) per gram dry extract (µg GAE/g DE).

The phosphomolybdenum inhibition TAC (µg GAE/mg DE) values of *K. africana* are presented in **Figure 16**. The highest Phosphomolybdenum inhibition TAC among all *K. africana* extracts was 1551.1 ± 82.2 µg GAE/mg DE from bark methanol extract. The leaves showed the highest Phosphomolybdenum inhibition TAC activity of 1411.6 ± 58.4 µg GAE/mg DE followed by

1230.2 ± 53.6 µg GAE/mg DE exhibited by ethyl acetate and methanol extracts respectively. The *K. africana* bark extracts which showed the highest Phosphomolybdenum inhibition TAC activity was from methanol extract (1551.1 ± 82.3 µg GAE/mg DE) followed by the ethyl acetate extract (1518.6 ± 71.6 µg GAE/mg DE). Similarly, for the root extracts the methanol extract also exhibited the highest Phosphomolybdenum inhibition TAC activity of 1402.3 ± 81.1 µg GAE/mg DE followed by the ethyl acetate extract at 1082.7 ± 29.5 µg GAE/mg DE. The was no significant difference between the bark and root methanol extracts and bark ethyl acetate extract. From the fruit extracts, the methanol extract exhibited the highest Phosphomolybdenum inhibition TAC activity of 658.0 ± 27.1 µg GAE/mg DE.

The Phosphomolybdenum inhibition TAC (µg GAE/mg DE) values of *P. angolensis* extracts ranged from 246.3 ± 4.4 to 2564.7 ± 30.5 µg GAE/mg DE (**Figure 17**). The methanol leaves extract exhibited the highest Phosphomolybdenum inhibition TAC (1118.5 ± 0.5 µg GAE/mg DE) followed by the ethyl acetate extract (1044.6 ± 8.9 µg GAE/mg DE) and). There was no significant difference between the two extracts. Among the bark extracts, the highest Phosphomolybdenum inhibition TAC was shown by the aqueous extract (2564.7 ± 30.5 µg GAE/mg DE) followed by the methanol extract (2206.5 ± 8.0 µg GAE/mg DE). In all root extracts, the highest Phosphomolybdenum inhibition TAC was shown by the aqueous extract (1811.1 ± 12.3 µg GAE/mg DE) followed by the ethyl acetate extract (1387.9 ± 52.4 µg GAE/mg DE).

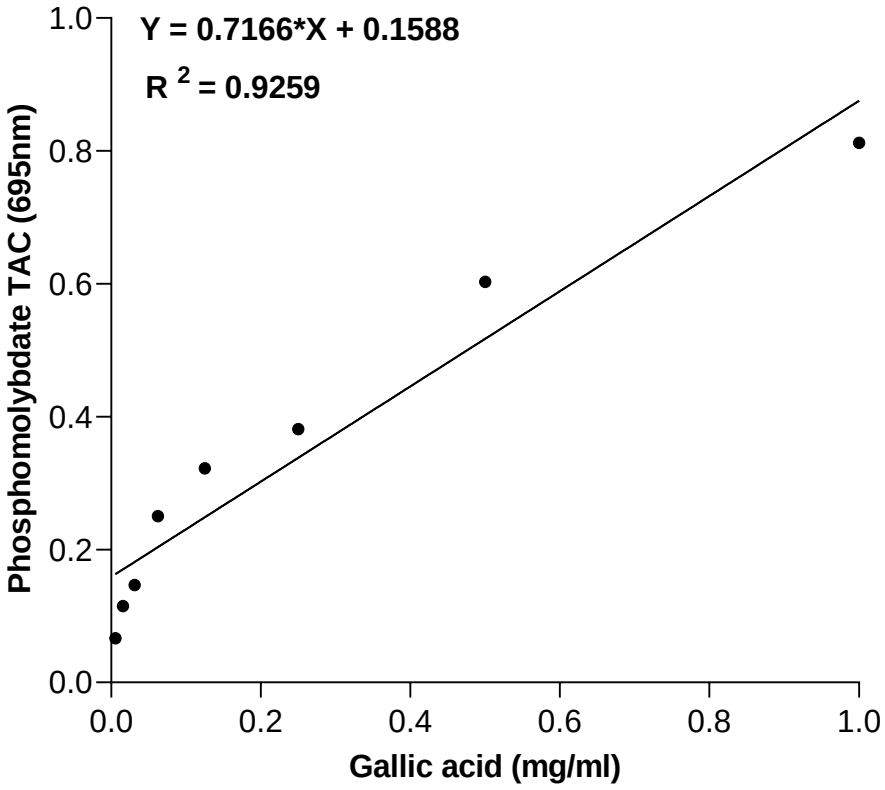


Figure 15: Gallic acid calibration curve for crude extract phosphomolybdenum inhibition TAC in µg gallic acid equivalent (GAE) (µg GAE/mg DE).

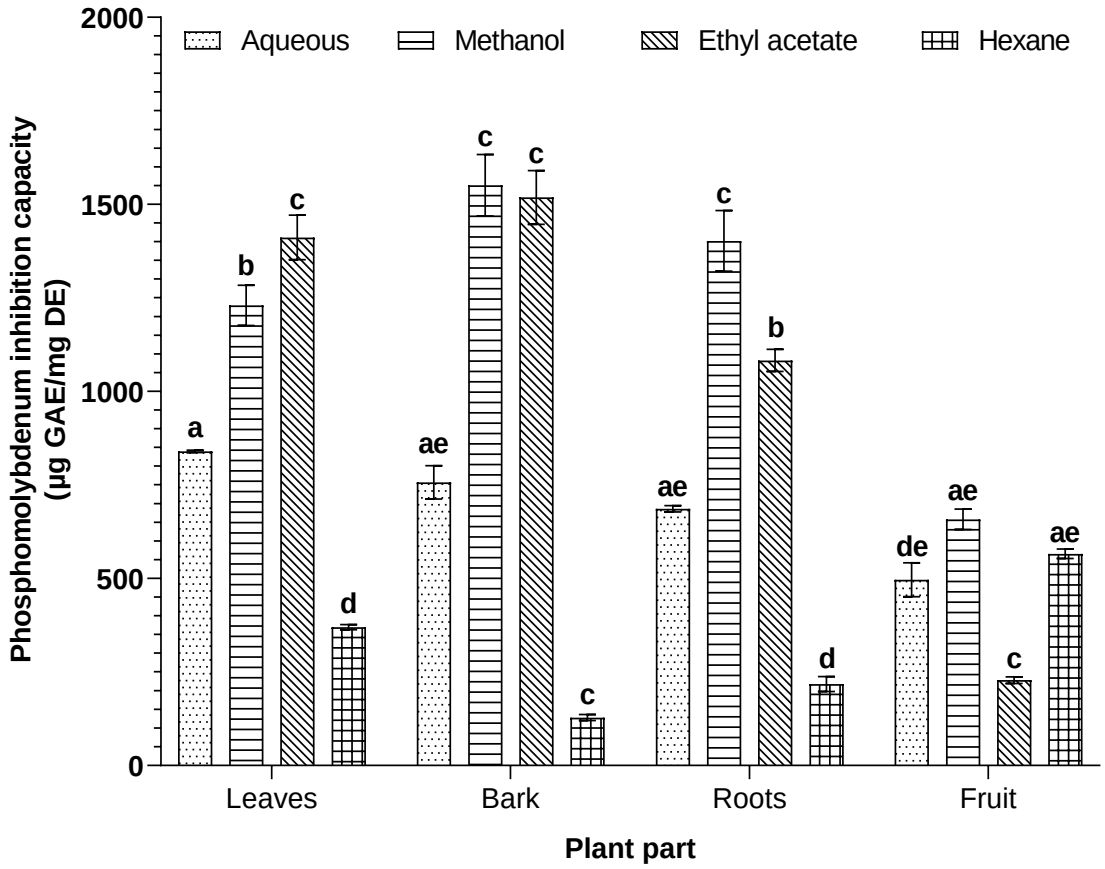


Figure 16: Phosphomolybdenum inhibition capacity (µg GAE/mg DE) of aqueous, methanol, ethyl acetate, and hexane extracts of *K. africana.* The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

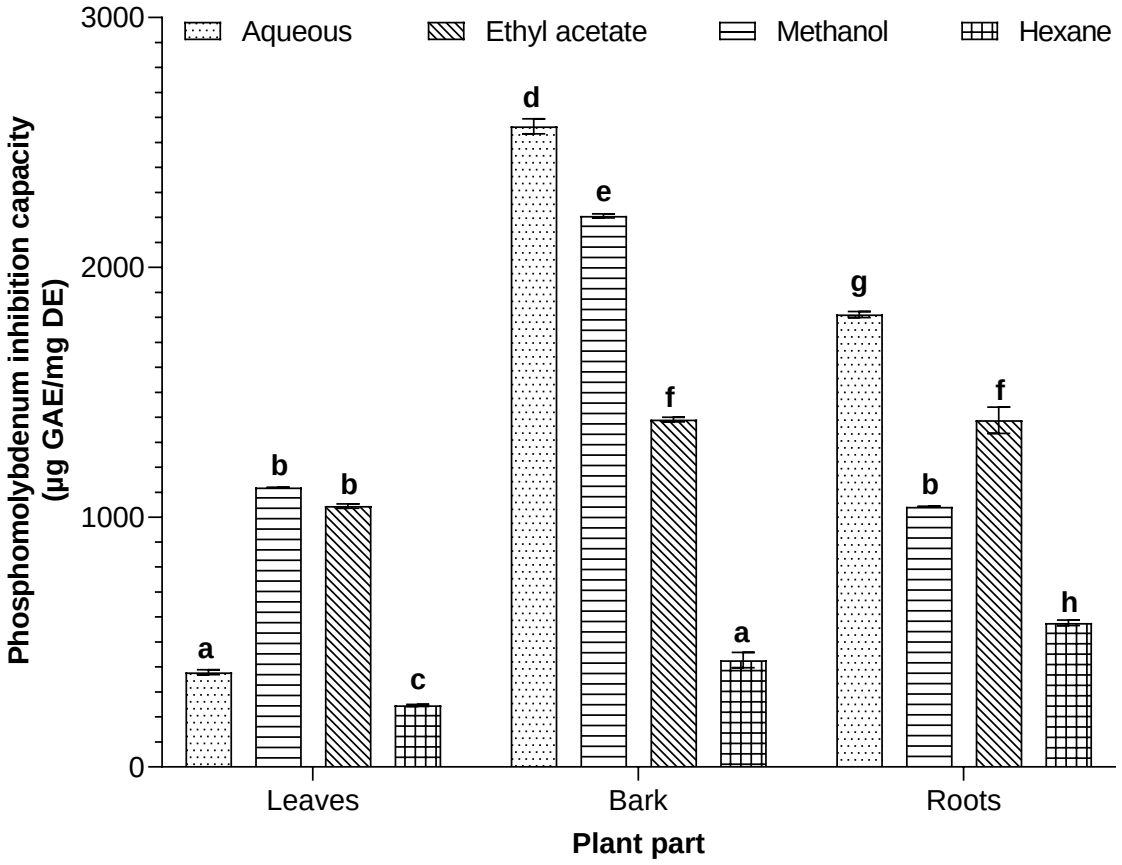
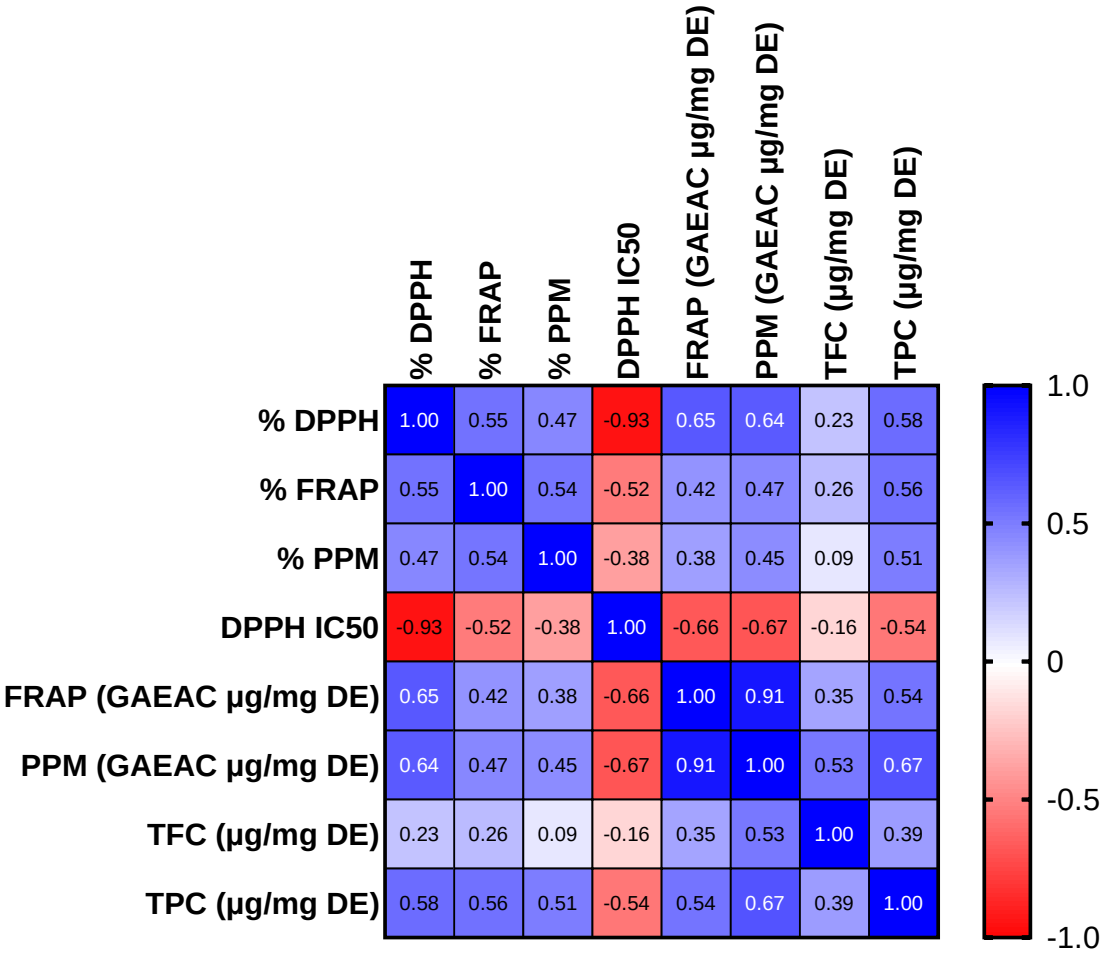


Figure 17: Phosphomolybdenum inhibition capacity (µg GAE/mg DE) of aqueous, methanol, ethyl acetate, and hexane extracts of *P. angolensis*. The different letters show the significant differences (P-value < 0.05) between sample means. Means with the same letters are not significantly different. Bars represent SE (n≥3).

4.3. Pearson's correlation of antioxidant activity, TFC, and TPC

Correlation analysis between antioxidant activity, TFC, and TPC of *K. africana* and *P. angolensis* crude extracts was performed and presented as a heatmap in Figure 14. There was a positive correlation between antioxidant activity and TPC with a correlation coefficient of r^2=0.58, r^2=*0.56*, and r^2=*0.51* for % DPPH scavenging activity, % FRAP activity, and Phosphomolybdenum inhibition TAC activity respectively.



Key:

% DPPH – Percentage DPPH scavenging capacity per mg/mL

% FRAP – Percentage FRAP activity per mg/mL

% PPM – Percentage Phosphomolybdenum inhibition at 1 mg/mL

µg GAE/g DE – Gallic acid equivalent antioxidant capacity µg/mg DE

TFC µg QE/mg DW – Total flavonoid content µg GAE/mg DE

TPC µg GAE/mg DE – Total phenol content µg GAE/mg DE

Figure 18: Heatmap analysis of Pearson's correlation between the % DPPH scavenging activity, % FRAP activity, phosphomolybdenum TAC activity, TFC, and TPC of *P. angolensis* and *K. africana* extracts. The values and color intensity represent the degree of association between the samples with a P value > 0.05.

4.4. Antimicrobial activity

The antimicrobial activity results of *K. africana and P. angolensis* crude plant extracts against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa,* and *C. albicans* using Kirby Bauer's disc diffusion assay are shown in **Table 5**. *Kigelia africana* crude extracts were active against both bacterial and yeast strains while *P. angolensis* extracts were active against gram positive strains only.

The different *K. africana* crude extracts showed antimicrobial activity against all strains (bacterial and yeast). From the leaves, the aqueous extract was only active against *P. aeruginosa* with a MIC of 20 mg/mL. The observed zone at this concentration was 7.3 ± 0.7 mm. The leaves methanol extract was active against *B. subtilis*, *E. coli*, and *C. albicans* with MIC of 20 mg/mL, 20 mg/mL, and 40 mg/mL respectively. The leaves' ethyl acetate extract was active against *B. subtilis*, *S. aureus*, and *E. coli.* The MIC of extract against the strains was 20 mg/mL for *B. subtilis* and *E. coli* and 10 mg/mL for *S. aureus*. The leaves hexane extract was active against *B. subtilis*, *S. aureus*, and *P. aeruginosa* with MIC of 40 mg/mL, 40mg/mL, and 20 mg/mL.

Among the *K. africana* bark extracts, the aqueous extract was active against *P. aeruginosa* with a MIC of 40 mg/mL and a 6.7 ± 0.3 mm zone of inhibition. The methanol and ethyl acetate extracts were active against all bacterial strains. The MIC of the methanol extract was 20 mg/mL for *B. subtilis* and 40 mg/mL for *S. aureus*, *P. aeruginosa,* and *E. coli* with zones of inhibitions of 6.7 ± 0.3 mm, 16.3 ± 0.7 mm, 7.7 ± 0.3 mm, and 7.7 ± 0.3 mm for *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli* respectively. The MIC of ethyl acetate extract was 20 mg/mL for B. subtilis and P. aeruginosa and 40 mg/mL for S. aureus and E. coli. The MICs of each strain exhibited zones of inhibitions of 13.7 ± 0.3 mm, 16.3 ± 0.3 mm, 6.7 ± 0.3 mm, and 7.7 ± 0.3 mm for *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli* respectively. The hexane bark extract was active against *B. subtilis*, *S. aureus*, and *P. aeruginosa* with MIC of 40 mg/mL, 20 mg/mL, and 40 mg/mL against each strain respectively.

The aqueous root extract was active against *P. aeruginosa* and *E. coli* only with a MIC of 10 mg/mL and 40 mg/mL respectively, and zone of inhibition of 7.0 ± 0.0 and 7.7 ± 0.3 mm for each MIC respectively. The methanol extract was active against *B. subtilis*, *P. aeruginosa*, *E. coli*, and *C. albicans* with zones of inhibitions of 8.7 ± 0.3 mm, 8.7 ± 0.3 mm, 9.3 ± 0.7 mm, 7.0 ± 0.0 mm for their MIC which was 20 mg/mL for all the strains. *Kigelia africana* ethyl

acetate root extracts showed antimicrobial activity against all tested bacterial strains. The extract showed the highest antimicrobial activity against *B. subtilis* with a MIC of 2.5 mg/mL. The extract showed zone of inhibition of 19.7 ± 0.3 mm against the strain at 40 mg/mL. It also showed a zone of inhibition of 16.0 ± 0.0 mm, 9.7 ± 0.3 mm and 9.0 ± 0.0 mm, and 10.0 ± 0.0 mm against *S. aureus*, *P. aeruginosa*, *E. coli,* and *C. albicans* respectively. The hexane root extract was active against *B. subtilis*, *S. aureus*, and *C. albicans* with the MIC of 20 mg/mL for all the strains. The MIC of 20 mg/mL showed a zone of inhibition of 7.7 ± 0.3 mm, 8.0 ± 0.0 mm, and 7.0 ± 0.0 mm against *B. subtilis*, *S. aureus*, and *C. albicans* respectively.

Kigelia africana aqueous fruit extract was active against *S. aureus* only with MIC and zone of inhibition of 40 mg/mL and 9.0 ± 0.0 mm respectively. Fruit methanol and hexane extracts were active against *B. subtilis* only with MIC of 20 mg/mL and 10 mg/mL respectively. The zones of inhibition of MIC were 9.0 ± 0.0 for 20 mg/mL methanol extract and 8.7 ± 0.3 mm for the 10 mg/mL hexane extract. Fruit ethyl acetate extract was active against *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *C. albicans*. The MICs for each strain were 10 mg/mL for *P. aeruginosa* (6.7 ± 0.3 mm zone of inhibition), 20 mg/mL for *B. subtilis* (9.7 ± 0.3 mm zone of inhibition), and *C. albicans* (7.0 ± 0.0 mm zone of inhibition) and 40 mg/mL for *S. aureus* (6.7±0.3 mm zone of inhibition).

Amongst all *P. angolensis* crude extracts, antimicrobial activity was observed for the methanol bark extract only. The extract was only active against gram-positive strains with MIC of 20 mg/mL and 10 mg/mL for *B. subtilis* and *S. aureus* respectively (**Table 5**). It had zones of inhibition of 7.7 ± 0.3 mm for both *B. subtilis* at 20 mg/mL concentration and *S. aureus* at 10 mg/mL concentration. The establishment of MIC and IC_{50} values using the microplate method was not generative because plant extract pigments disturbed the absorbance of INT salt metabolization activity by live cell readings from the microplate reader.

Table 5: Antimicrobial activity of *K. africana* and *P. angolensis* aqueous, methanol, ethyl acetate, and hexane extracts of the leaves, bark, root, and fruit extracts at different concentrations (mg/mL).

Plant species	Plant part	Solvent	Conc. (mg/ml)	Zone of inhibition (mm)				
				Gram-positive		Gram-negative		Yeast
				B. subtilis	*S. aureus*	P. *aeruginosa*	*E. coli*	*C. albicans*
K. africana	**Leaves**	**Water**	40	-	-	9.0 ± 0.0	-	-
			20	-	-	7.3 ± 0.7	-	-
			10	-	-	-	-	-
		Methanol	40	9.7±0.3	-	-	9.7±0.3	6.7± 0.3
			20	7.7±0.3	-	-	6.7±0.3	-
			10	-	-	-	-	-
		Ethyl acetate	40	8.7 ± 0.3	12.3±0.7	-	9.7±0.3	-
			20	6.7 ± 0.3	10.7 ± 0.3	-	7.7±0.3	-
			10	-	7.7±0.3	-	-	-
		Hexane	40	8.7±0.3	7.7±0.3	7.7 ± 0.3	-	-
			20	-	-	7.0 ± 0.0	-	-
			10	-	-	-	-	-
	Bark	**Water**	40	-		6.7 ± 0.3	-	-
			20	-		-	-	-
			10	-		-	-	-
		Methanol	40	10.3±0.7	16.3 ± 0.7	7.7 ± 0.3	7.7±0.3	-
			20	6.7±0.3		-	-	-
			10	-		-	-	-
		Ethyl acetate	40	21.3±0.7	16.3±0.3	7.7±0.3	7.7±0.3	-
			20	13.7±0.3	-	6.7 ± 0.3	-	-
			10	-	-	-	-	-
		Hexane	40	9.7 ± 0.3	8.0± 0.0	7.7 ± 0.3	-	-
			20	9.7 ± 0.3	-	-	-	-
			10	-	-	-	-	-
	Roots	**Water**	40	-	-	9.7 ± 0.3	7.7±0.3	-
			20	-	-	7.7 ± 0.3	-	-
			10	-	-	7.0 ± 0.0	-	-
		Methanol	40	8.7 ± 0.3	-	10.7 ± 0.3	9.7±0.3	12.0± 0.0
			20	8.7 ± 0.3	-	8.7 ± 0.3	9.3±0.7	7.0± 0.0
			10	-	-	-	-	-
		Ethyl acetate	40	19.7±0.3	16.0± 0.0	9.7 ± 0.3	9.0±0.0	10.0± 0.0
			20	19.3±0.7	10.0 ± 0.0	8 ± 1	7 ± 1	7 ± 1
			10	19.7±0.3	8.0± 0.0	-	-	-
			5	11.7±0.3	-	-	-	-
			2.5	8.7 ± 0.3	-	-	-	-
			1.25	-	-	-	-	-
		Hexane	40	8.3 ± 0.7	12.0± 0.0	-	-	8.7 ± 0.3
			20	7.7 ± 0.3	8.0± 0.0	-	-	7.0± 0.0
			10	-		-	-	-

	Fruit	**Water**	40	-	9.0± 0.0	-	-	-
			40	-	-	-	-	-
			20	-	-	-	-	-
		Methanol	40	8.7 ± 0.3		-	-	-
			20	9.0 ± 0.0		-	-	-
			10	-		-	-	-
		Ethyl acetate	40	10.0±0.0	6.7 ± 0.3	9.0 ± 0.0	-	8.3± 0.7
			20	9.7 ± 0.3	-	8.3 ± 0.7	-	7.0 ± 0.0
			10	-	-	6.7 ± 0.3	-	-
		Hexane	40	12.3±0.7	-	-	-	-
			20	9.7 ± 0.3	-	-	-	-
			10	8.7 ± 0.3	-	-	-	-
P. angolensis	**Leaves**	**Water**	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
		Methanol	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
		Ethyl acetate	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
		Hexane	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
	Bark	**Water**	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
		Methanol	40	9.7 ± 0.3	9.7 ± 0.3	-	-	-
			20	7.7 ± 0.3	9.0 ± 0.0	-	-	-
			10	-	7.7 ± 0.3	-	-	-
		Ethyl acetate	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
		Hexane	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
	Roots	**Water**	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-

		Methanol	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
		Ethyl acetate	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
		Hexane	40	-	-	-	-	-
			20	-	-	-	-	-
			10	-	-	-	-	-
Distilled water	**(–) control**		99.9 %	-	-	-	-	-
Methanol	**(–) control**		99.9 %	-	-	-	-	-
Ethyl acetate	**(–) control**		99.9 %	-	-	-	-	-
Hexane	**(–) control**		99.9 %	-	-	-	-	-
DMSO	**(–) control**		99.9 %	-	-	-	-	-
Gentamicin**Gen**	**(+) control**	-	10µg	28.7±0.3	19.7 ± 0.3	15.7 ± 0.3	19.7 ± 0.3	-
Amp B	**(+) control**	-	-	-	-	-	-	19.7 ± 0.7

Results are presented average (mean) zone of inhibition ± SE.
Key:
- – no activity
Conc. – Concentration
Gen – Gentamicin (positive control against bacterial strains)
Amp B – Amphotericin B (positive control against yeast strain)
(–) – negative
(+) – positive

4.5. *In-silico* assessment of human Epidermal Growth Factor Receptor (EGFR) protein inhibition anticancer properties of *K. africana* and *P. angolensis* phytochemicals.

4.5.1. Literature survey for bioactive compounds

A total of 213 natural bioactive compounds from *K. africana* and *P. angolensis* were found through a literature survey (**Table 6**). The phytochemicals belong to different classification groups including alkaloids, phenolic compounds, naphthoquinones, coumarins, sterols, esters, alkanes, quinones, terpenes, terpenoids, iridoids, and unsaturated fatty acids. Gefitinib and

AEE_788, two Food and Drug Administration (FDA) approved human Epidermal Growth Factor Receptor (EGFR) protein inhibitors were also retrieved and used as positive controls.

Table 6: Phytochemicals identified and isolated from *K. africana and P. angolensis*.

Plant species	Plant part	Bioactive compound	References
K. africana	**Leaves**	Luteolin, 6-hydroxy luteolin, quercetin, 7-hydroxyeucommic acid, 7-hydroxy eucommiol, jioglutolide, 1-Dehydroxy-3,4-dihydroaucubigenin, (9Z,12Z)-Methyl octadeca-9,12-dienoate, 1-iodohexadecane, tritriacontane, n-hentriacontane, 11-(2,2-dimethylpropyl) heneicosane, luteolin 7-O-glucoside, 2,6,10-trimethyldodecane,1-iododecane, Norviburtinal, Nonacosane, Ajugol, 4,4-dimethylundecane, 6-demethylkigelin, phytol, sonovoburtinal, hentriacontane, isovitexin, des-p-hydroxy benzoyl kisasagenol B, 6-Trans-caffeoyl, quercetin-glycoside, luteolin-glycoside, 3-hydro-4,8-phytene, and vernolic acid,	Olubunmi and Gabriel (2010); Zofou *et al*. (2011); Sidjui *et al*. (2014); Bello *et al.* (2016); Nabatanzi *et al.* (2020)
	Flower	Methyl-12-methyltetradecanoate, and palmitic acid	Atawodi and Olowoniyi (2015); Nabatanzi *et al*. (2020)
	Twigs	7-hydroxy eucommiol, caffeic acid, catalpol, specioside, minecoside, caffeic acid, rehmaglutin C, 4-hydroxy cinnamic acid, shoreaphenol, verminoside, ajugol, and 7-hydroxy viteoid II	Khan *et al*. (2012)

	Bark	Minecoside, 2-(4-hydroxyphenyl) ethyl ester, 6-methoxymellein, kigelin, 3-demethyl-kigelin, ferulic acid, kigelinone, balaphonin, 6-demethylkigelin β-sitosterol, kigeliol, 1,3-dimethylkigelin, caffeic acid, stigmasterol, and vernolic acid	Khan *et al*. (2012); Atawodi and Olowoniyi (2015); Bello *et al.* (2016); Nabatanzi *et al.* (2020)
	Stem	Minecoside, oleanolic acid, specioside, verminoside, balanophonin, tecomaquinone I, isokigelin, kojic acid, 2-(1-hydroxyethyl) naphtho [2,3-b] furan-4,9-dione, 2-acetylnaphtho[2,3-b] furan-4,9-quinone, kigelinone, 6-demethylkigelin, vernolic acid, ferulic acid, 1,3-dimethylkigelin, lapachol, pomolic acid, 2β,3β,19α-Trihydroxy-urs-12-en-28-oic acid, 6-methoxymellein, stigmasterol, caffeic acid nonacosanoic acid, β-sitosterol, 2-(4-hydroxyphenyl) ethyl ester and atranorin	Khan *et al*. (2012); Atawodi and Olowoniyi (2015); Nabatanzi *et al.* (2020)
	Wood	Luteolin, lapachol, stigmasterol, kigeliol, kigelin, 6-hydroxy luteolin, β-sitosterol, vernolic acid, and rehmaglutin C	Sidjui *et al*. (2014); Moronkola *et al*. (2018);
	Root	6-methoxymellein, stigmasterol, lapachol, kiglin, specioside, 3-demethyl-kigelin, kigelinone, isopinnatal, β-Sitosterol, γ-sitosterol, stigmasterol, dehydro-alpha-lapachol, p-coumaric acid, norviburtinal, pinnatal, ferulic acid, kigelinone, caffeic acid, luteolin, and 6-hydroxy-luteolin	Atawodi and Olowoniyi (2015); Bello *et al.* (2016); Nabatanzi *et al.* (2020)

	Fruit	Verminoside, β-Sitosterol, isoschaftoside, physostigmine, fluazifop, specioside, dexamethasone, 6-demethylkigelin, γ-sitosterol, caffeic acid, sulfisomidine, desmethylmirtazapine, lapachol 6-p-coumaroyl-sucrose, cycluron, diclomezine, buphedrone, N, N-dimethylcathinone, gemfibrozil, phenmetrazine, nicotinamide, yohimbine, 19-Noretiocholanolone, Clobazam, leucomalachite green, trimetazidine, fenoxanil, doxapram, digoxigenin Diflucortolone pivalate, Zidovadine, (9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid 2, (4-hydroxyphenyl)ethyl ester, N, N Dimethylaniline, Butylamine, N-Ethylbuphedrone, ferulic acid, Pentedrone, Phendimetrazine, 1-Tridecene, 2,3-Dihydrobenzofuran, stigmasterol, 2-Methoxy-4-vinylphenol, 2 Benzofurancarboxaldehyde, Linoleic acid ethyl ester, (Z)-Docos-13-enamide (Erucamide), Bis(2-ethylhexyl) phthalate, octadecanamide, and octadecanoic acid	Shama *et al.* (2013); Atawodi and Olowoniyi (2015); Bello *et al.* (2016); Costa *et al*. (2017); Nabatanzi *et al.* (2020); Fagbohun *et al*. (2021)
P. angolensis	**Leaves**	Gallic acid, ellagic acid, chlorogenic acid, vanillic acid, ferulic acid, p-coumaric acid, rosmarinic acid, caffeic acid, hexadecanamide, neophytadiene, eseroline, 7,9-Di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione, Pregn-5-en-3-ol, taxifolin, syringic acid, rutin,	Santos *et al*. (2020)

		eicosamethylcyclodecasiloxane, 9-octadecenamide, hexadecanoic acid, and lup-20(29)-en-3β-ol	
	Stembark	Tetratriacontane, Stigmasta-3,5-dien-7-one, Lupeol, Octadecanoic acid, Friedelin, Hexadecanoic acid methyl ester, Tetradecanoic acid, 1-Octacosanol, 7-Dehydrodiosgenin, Phthalate, Hexamer epicatechin, Epicatechin-3-O-galate, (-)-epicatechin, Epicatechin, and (4β-8)-epicatechin	Samie *et al*. (2009); Abubakar and Majinda (2016)
	Wood	Acetyloleanolic acid, (-)-pterocarpin, (αS)-4-O-methylangolensin, Prunetin, (-)-Homopterocapin, Angolensin, Muningin, Liquiritigenin, Isoliquiritigenin, *α*,2′-Dihydroxy-4,4′-di-methoxychalcone Minecoside, Prunetin, (αR,l" S,4" S,4"αR,8"αR)-4-O-T-cadinylangolensin, and (αR,1" R,4" S,4"αR,8″αR)-4-O-α cadinylangolensin	King and Jurd (1952); Bezuidenhoudt *et al*. (1980); Abouelela *et al*. (2019)
FDA-approved EGFR inhibitors	**Positive controls**	Gefitinib, and AEE788	Yun *et al.* (2007); Yoshikawa *et al.* (2013); Solassol *et al.* (2019)

4.5.2. Ligand and protein preparation

The predicted binding sites (pockets) for the EGFR protein (PDB ID: 2J6M) generated using PrankWeb (P2Rank) are shown in **Figure 18** and **Table 7**. Two binding pockets were predicted, and pocket 1 binding site showed a score of 21.71 and the highest binding probability of 0.855. The active site (pocket 1) is within chain A of the protein and the predicted binding amino acid

residues acids in this pocket are Leu718, Gly719, Phe723, Val726, Ala743, Lys745, Glu762, Met766, Cys775, Leu788, Thr790, Gln791, Leu792, Met793, Pro794, Gly796, Cys797, Asp800, Arg841, Asn842, Leu844, Thr854 and Asp855.

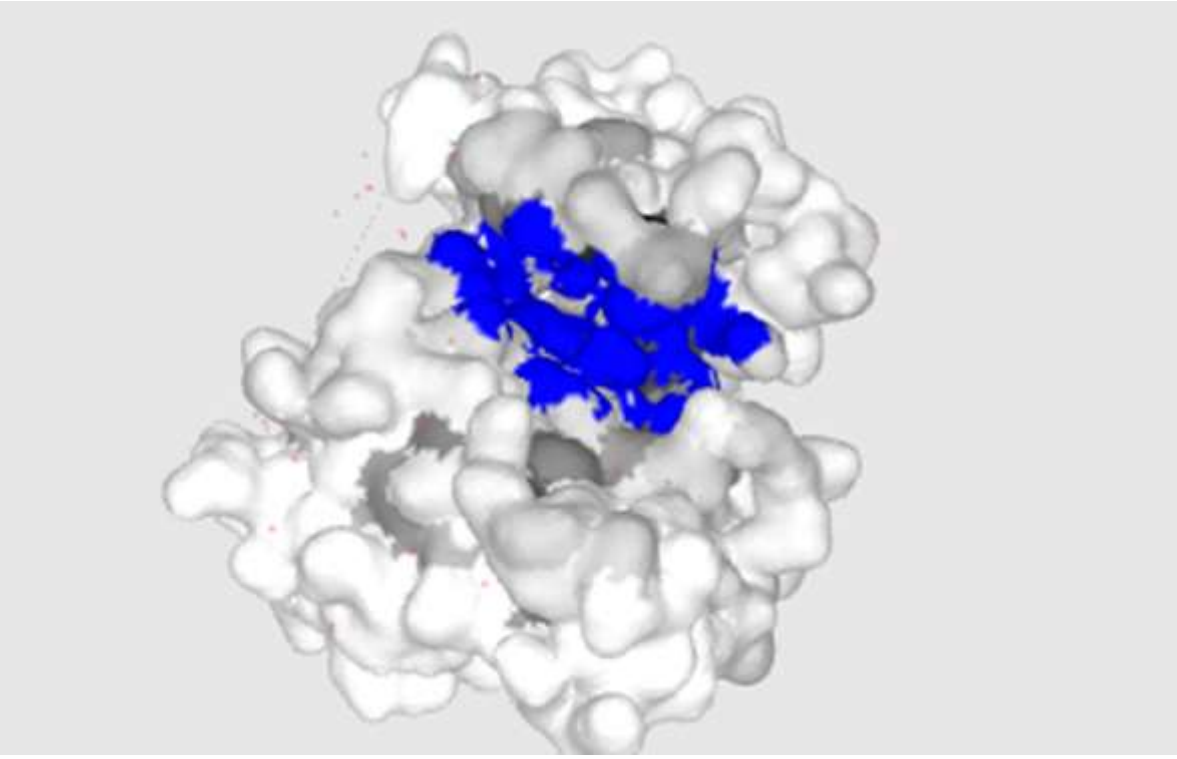


Figure 18: PRANKWeb (P2Rank) predicted the active site of the EGFR protein PDB ID: 2J6M. The active site (pocket 1) is represented by the blue colour.

Table 7: EGFR (PDB ID: 2j6m) P2Rank predicted active site amino acid residues and vina search space X, Y, and Z coordinates.

Predicted Binding Pockets	**Rank**	**Score**	**Probability**	**center_x**	**center_y**	**center_z**	**Amino acids residue ids**
Pocket 1	1	21.71	0.855	-50.507	-2.7526	-25.557	Leu718, Gly719, Gly721, Phe723, Val726, Ala743, Lys745, Glu758, Ile759, Glu762, Met766, Cys775,

							Leu788, Thr790, Leu792, Met793, Pro794, Gly796, Cys797, Asp800, Asp837, Arg841, Asn842, Leu844, Thr854, Asp855, Gly857, Leu858, Lys875
Pocket 2	2	2.19	0.145	-48.609	-7.2721	-30.536	Phe723, Lys745, Glu758, Glu762, Asp837, Arg841, Asn842, Asp855, Gly857, Leu858

4.5.3. Site-specific molecular docking

Of the 213 identified bioactive, the top 10 EGFR inhibitors according to their molecular docking binding affinity energy to the EGFR protein were 7- Dehydrodiosgenin, Yohimbine, Lupeol, 3 beta-O-acetyloleanolic acid, Cynaroside, Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3beta), Digoxigenin, 19-Noretiocholanolone, Diflucortolone pivalate, and Dexamethasone and their binding affinity ranged from -9.6 to -8 kcal/mol (**Table 8**; **Figure 19**). Spirosta-5,7-dien-3-ol (7-Dehydrodiosgenin), a bioactive compound isolated from *P. angolensis* exhibited the highest binding affinity of –9.6 kcal/mol followed by Yohimbine (*K. africana*) at –9.2 kcal/mol and Lupeol (*P. angolensis*) at –9.2 kcal/mol. The interactions between the compounds and the protein were predicted to be through Carbon hydrogen-bond, Conventional hydrogen-bond, van der Waals, Halogen (Fluorine) bond, Alkyl bond, Pi-Alkyl bond, and Pi-sigma bonds (**Table 8**).

Table 8: Molecular docking results of *K. africana* and *P. angolensis* bioactive compounds against EGFR protein.

Plant species	**Ligand (Bioactive compound)**	**Binding affinity (kcal/mol)**	**Contact receptor amino acid residues**	**Types of interactions (bonds)**
K. africana	Yohimbine	-9.2	Asp855, Phe723, Gly796, Glu762, Lys745, Thr854, Met766, Val726, Thr790, Ala743, Met793, Leu844, Leu792, Leu718, Pro794	Conventional hydrogen-bond van der Waals Pi-sigma bond Alkyl bond Pi-Alkyl bond
K. africana	Cynaroside	-8.7	Leu718, Gly719, Val726, Ala743, Lys745, Glu762, Met766, Leu788, Thr790, Leu792, Met793, Gly796, Cys797, Asp800, Asn842, Leu844, Thr854, Asp855	Conventional hydrogen-bond Carbon hydrogen-bond van der Waals Pi-Alkyl bond
K. africana	Digoxigenin	-8.7	Leu718, Phe723, Val726, Ala743, Lys745, Leu792, Gly796, Cys797, Arg841, Asn842, Leu844, Thr854, Asp855,	Carbon hydrogen-bond van der Waals Alkyl bond
K. africana	19-Noretiocholanolone	-8.6	Leu718, Phe723, Val726, Ala743, Lys745, Glu762, Met766, Thr790 Met793, Gly796, Cys797, Leu844, Thr854, Asp844, Phe856	van der Waals Conventional hydrogen-bond Alkyl bond

K. africana	Diflucortolone pivalate	-8.5	Leu718, Gly719, Phe723, Val726, Ala743, Lys 745, Glu762, Met766, Leu788, Gly796, Cys797, Leu844, Thr854, Asp855, Phe856	van der Waals Conventional hydrogen-bond Halogen (Fluorine)
K. africana	Dexamethasone	-8.3	Leu718, Phe723, Val726, Ala743, Lys745, Glu762, Met766, Thr790, Met793, Gly796, Cys797, Leu844, Thr854, Asp855	Carbon hydrogen-bond Conventional hydrogen-bond van der Waals
P. angolensis	Spirosta-5,7-dien-3-ol or 7-Dehydrodiosgenin	-9.6	Arg841, Gly796, Gly719, Thr854, Thr790, Leu788, Leu844, Val726, Asp855, Ala743, Glu762, Lys745, Leu777, Met766	Carbon hydrogen-bond Conventional hydrogen bond Van der Waals
P. angolensis	Lupeol	-9.2	Leu718, Gly719, Gly721, Phe723, Val726, Ala743, Lys745, Thr790, Leu792, Leu844, Thr854, Asp 855	Alkyl bond van der Waals
P. angolensis	3 beta-O-acetyloleanolic acid	-8.9	Leu718, Gly719, Phe723, Val726, Ala743, Lys745, Glu762, Met766, Cys775, Leu788, Thr790, Cys797, Leu799, Asp800, Arg841, Leu844, Thr854, Asp855	Carbon hydrogen-bond Conventional hydrogen-bond van der Waals

P. angolensis	Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3beta)-	-8.3	Leu718, Val726, Ala743, Lys745, Glu762, Met766, Thr790, Met793, Gly796, Cys797, Leu844, Thr854, Asp855	Alkyl bond van der Waals
Control	Gefitinib	-8	Leu718, Gly719, Val722, Phe723, Ala743, Ile744, Lys745, Glu762, Met766, Leu788, Thr790, Cys797, Asp800, Arg841, Leu844, Thr854, Asp855,	Pi-sigma bond Pi-alkyl bond Alkyl bond Van der Waals Carbon hydrogen-bond
Control	AEE_788	-9.2	Gln791, Leu777, Cys775, Asp855, Thr854, Glu762, Met766, Leu788, Thr790, Lys745, Ile744, Phe795, Val726, Ala743, Gly796, Glu804, Asn842, Asp837, Phe723, Arg841, Asp800, Pro794, Leu718, Leu844, Leu792, Met793	Pi-Pi stacked Pi-alkyl bond Carbon hydrogen-bond Van der Waals

Table shows the bioactive compound and their binding affinity to the EGFR protein (PDB ID: 2J6M). The table also shows the contact interacting amino acid residues of each compound.

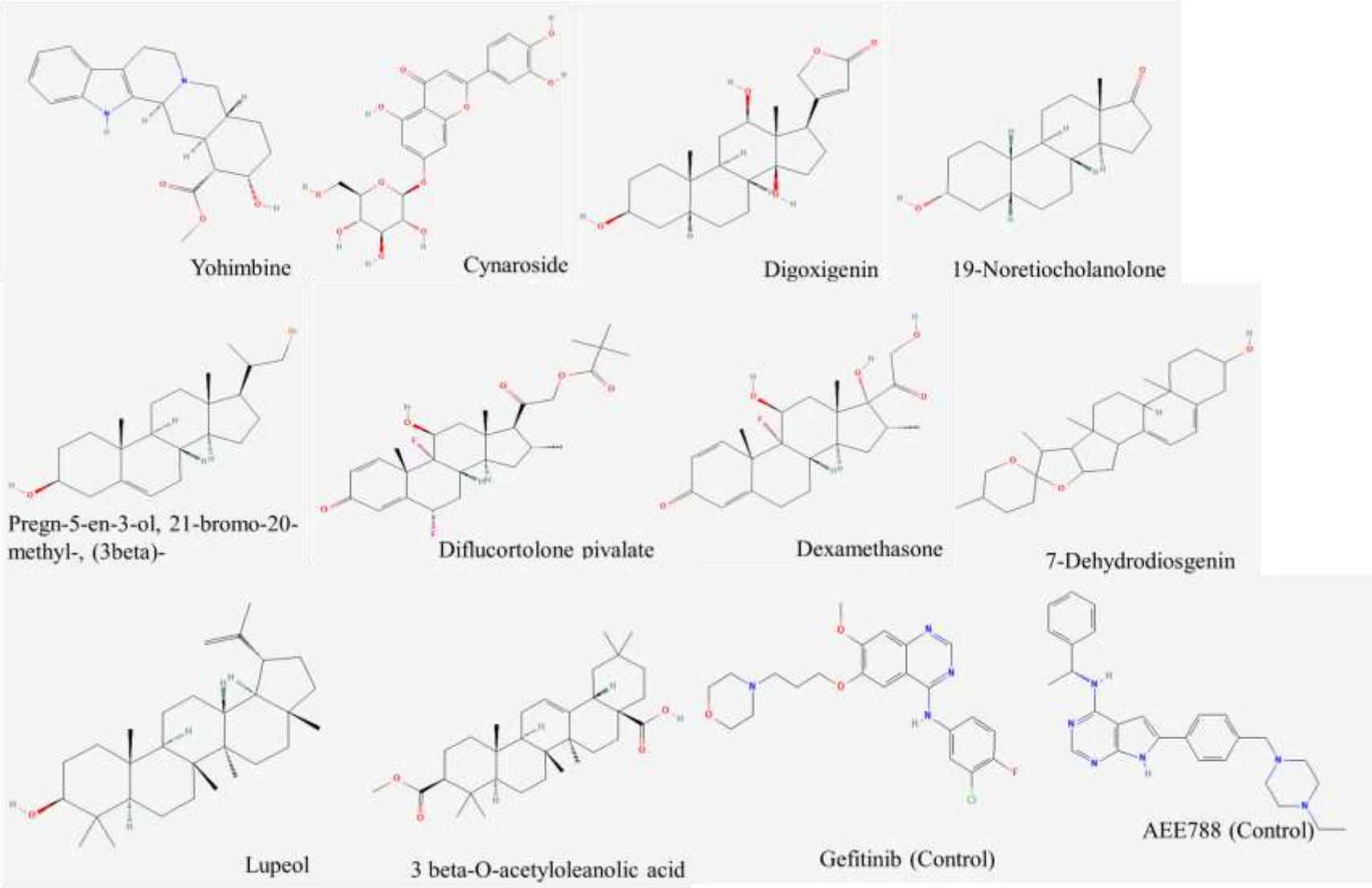


Figure 19: Top 10 *K. africana* and *P. angolensis* phytochemicals (bioactive compounds) according to their binding affinity to the EGFR protein (PDB ID: 2J6M) through molecular docking.

4.5.4. Drug-likeness analysis, Absorption, Distribution, Metabolism, and Excretion, (ADME), and Toxicity predictions

The drug-likeness, ADME, and Toxicity predictions of the top 10 ligands are shown in **Tables 9**, **10**, and **11**. For the drug-likeness properties of the top 10 ligands (**Table 10**), the bioactive compounds Yohimbine, Digoxigenin, 19-Noretiocholanolone, Diflucortolone pivalate and Dexamethasone showed no violations against all Lipinski (Pfizer), Ghose, Muegge, Veber, and Egan rules for drug-likeness. Although 7-Dehydrodiosgenin exhibited a high binding affinity to the EGFR protein it violated the Lipinski (Pfizer), Ghose and Muegge rules. It was also positive as a possible carcinogen according to the Mouse Carcinogenicity test (**Table 10**). Yohimbine and Digoxigenin bioactive compounds were predicted as non-mutagen according to Ames's mutagenic test. They were also negative as possible carcinogens for the rodent carcinogenicity tests. According to the ADME predictions from SwissADME (**Table 11**), Yohime has a high GI absorption, and it is BBB permeant and a P-gp substrate. The compound is also a non-inhibitor of all CYP enzyme variants.

Table 9: Drug-likeness properties of *K. africana* and *P. angolensis* bioactive compounds.

Plant species	Bioactive compounds	Lipnski (Pfizer)	Ghose	Veber	Egan	Muegge
K. africana	Yohimbine	YES	YES	YES	YES	YES
K. africana	Cynaroside	NO; 2 VIOLATIONS: NORO>10 NHOROH>5	YES	NO; 1 VIOLATIONTPSA>140	NO; 1 VIOLATION: TPSA>131.6	NO; 3 VIOLATIONS: TPSA>150 H-ACC>10 H-DON>5
K. africana	Digoxigenin	YES	YES	YES	YES	YES

K. africana	19-Noretiocholanolone	YES	YES	YES	YES	YES
K. africana	Diflucortolone pivalate	YES	YES	YES	YES	YES
K. africana	Dexamethasone	YES	YES	YES	YES	YES
P. angolensis	7-Dehydrodiosgenin	NO; 1 VIOLATION: MLOGP >4.15	NO; 2 VIOLATIONS: WLOGP>5.6 ATOMS >70	YES	YES	NO: 1 VIOLATION: XLOG3>5
P. angolensis	Lupeol	NO; 1 VIOLATION: MLOG >4.15	NO; 2 VIOLATIONS: WLOP >5.6 MR>130	YES	NO; 1 VIOLATIONWLOGP>5.88	NO; 2 VIOLATIONS XLOGP3 >5 HETEROATOMS <2
P. angolensis	3 beta-O-acetyloleanolic acid	NO; 1 VIOLATION: MLOGP >4.15	NO; 4 VIOLATIONS: MW>480 WLOGP>5.6 MR>130 #ATOMS>70	YES	NO; 1 VIOLATION: LOGP>5.88	NO; 1 VIOLATION XLOGP3 >5

P. angolensis	Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3)-	NO: 1 VIOLATION: MLOGP >4.15	NO; 1 VIOLATION WLOGP>5.6	YES	NO; 1 VIOLATION WLOGP >5.88	NO; 2 VIOLATIONS: XLOGP3 >5 HETEROATOMS <2
Control	Gefitinib	YES	YES	YES	YES	YES
Control	AEE_788	YES	NO MR>130	YES	YES	YES

NO means the bioactive compound violated one or more of the stated rules within the used drug-likeness properties analysis while a YES means the bioactive compound violated none of the stated rules.

Table 10: Toxicity predictions of *K. africana* and *P. angolensis* bioactive compounds according to Ames's mutagenic and Rodent Carcinogenicity test in PreADMET.

Plant species	**Ligand (Bioactive compound)**	**Ames's mutagenic test**	**Rodent carcinogenicity**	
			Carcinogenicity _Mouse	**Carcinogenicity _Rat**
K. africana	Yohimbine	Non-Mutagen	Negative	Negative
K. africana	Cynaroside	Mutagen	Positive	Negative
K. africana	Digoxigenin	Non-mutagen	Negative	Negative
K. africana	19-Noretiocholanolone	Mutagen	Negative	Negative
K. africana	Diflucortolone pivalate	Non-mutagen	Positive	Positive

K. africana	Dexamethasone	Non-mutagen	Positive	Positive
P. angolensis	7-Dehydrodiosgenin	Non-mutagen	Positive	Negative
P. angolensis	Lupeol	Mutagen	Negative	Positive
P. angolensis	3 beta-O-acetyloleanolic acid	Non-mutagen	Positive	Positive
P. angolensis	Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3beta)-	Non-mutagen	Positive	Positive
Control	Gefitinib	Mutagen	Positive	Positive
Control	AEE_788	Mutagen	Positive	Positive

Table 11: ADME properties predictions of *K. africana* and *P. angolensis* bioactive compounds in comparison to two FDA-approved EGFR inhibitors AEE_788 and Gefitinib.

Ligand (Bioactive compound)	GI absorption	BBB permeant	P-gp substrate	CYP1A2 Inhibitor	CPY2C19 Inhibitor	CYP2C9 inhibitor	CYP3A4 inhibitor	Log *K*p skin permiation (cm/s)	Bio-availability score
7-Dehydrodiosgenin	HIGH	YES	NO	NO	NO	NO	NO	-4.80	0.55
Yohimbine	HIGH	YES	YES	NO	NO	NO	NO	-6.37	0.55
Lupeol	LOW	NO	NO	NO	NO	NO	NO	-1.90	0.55
3 beta-O-acetyloleanolic acid	LOW	NO	NO	NO	NO	YES	NO	-3.56	0.85
Cynaroside	LOW	NO	YES	NO	NO	NO	NO	-8.00	0.17
Digoxigenin	HIGH	NO	YES	NO	NO	NO	NO	-7.90	0.55
19-Noretiocholanolone	HIGH	YES	NO	NO	NO	NO	NO	-5.65	0.55
Diflucortolone pivalate	HIGH	NO	YES	NO	NO	NO	YES	-6.21	0.55
AEE_788	HIGH	YES	YES	NO	NO	NO	NO	-6.50	0.55
Dexamethasone	HIGH	NO	YES	NO	NO	NO	NO	-7.32	0.55

Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3)-	HIGH	NO	NO	NO	NO	YES	NO	-4.08	0.55
Gefitinib	HIGH	YES	YES	NO	YES	NO	YES	-6.67	0.55

ADME predictions are based on the SwissADME predictive models for physicochemical properties, pharmacokinetics, drug-likeness, and medicinal chemistry.

3.6.5. Visualization of binding position and interaction between EGFR protein and bioactive compounds

The three-dimensional crystal structure of binding poses of Yohimbine in the predicted pocket 1 of EGFR protein is shown in **Figure 20** while the binding interaction are shown **Figure 21**. Yohimbine interacted with amino acids Phe723, Gly796, Asp855, Glu762, Lys745, Thr854, Met766, Val726, Thr790, Ala743, Met793, Leu844, Leu792, Leu718 and Pro794. The interaction between Yohimbine and EGFR were through van der Waal, Conventional Hydrogen bonds, Pi-sigma, Alkyl and Pi-Alkyl bonds.

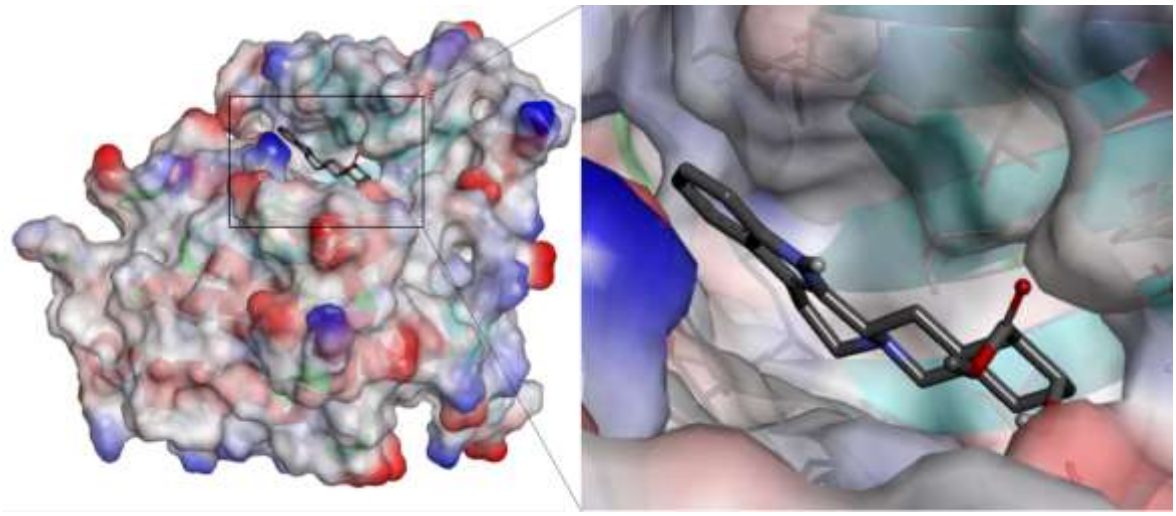


Figure 20: Crystal structure of EGFR (2J6M) bound, Yohimbine (*K. africana* bioactive compound).

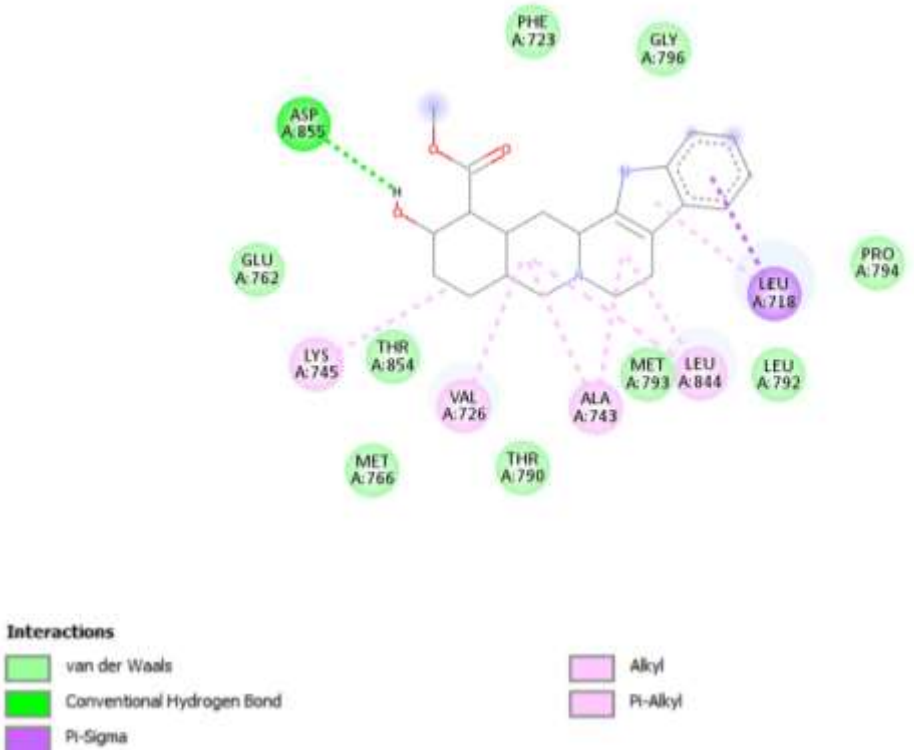


Figure 21: Yohimbine interaction types with EGFR protein amino acid residues.

CHAPTER 5: DISCUSSION

5.1. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

5.1.1. Total Phenolic Content (TPC)

Our study demonstrated that the different plant parts of *K. africana* and *P. angolensis* contain phenolic compounds and the TPC of the plants varied according to the plant species, plant part and the solvent used for extraction. For example, bark and leaves of *K. africana* contained significantly (P value ≤ 0.05) higher amounts of phenolic compounds than the *K africana* roots. While *K. africana* fruit contained the least amount of phenolic compounds compared to other plant parts. Similarly, *P. angolensis* bark extracts contained significantly higher phenolic compounds than *P. angolensis* leaves and roots. These results supported Santos *et al*. (2020) and Mulaiudzi *et al*. (2011) who reported higher phenolic compounds extraction from bark than leaves of *P. angolensis*. According to Santos *et al*. (2020), methanolic extract of *P. angolensis* bark exhibited higher TPC (258.40 ± 5.81 mg ascorbic acid equivalent [AAE]/mg) than methanolic extract of *P. angolensis* leaves (262.00 ± 1.70 mg AAE/mg). Whereas Mulaudzi *et al.* (2011) through a protocol of 50 % aqueous methanolic extraction recorded a higher TPC from bark than the leaves (i.e., 12.07 ± 0.09 mg GAE/g for *P. angolensis* bark compared to 8.85 ± 0.10 mg GAE/g for *P. angolensis* leaves).

The higher levels of TPC observed in the plant's aerial parts (i.e., bark and leaves) compared to the roots may be attributed to the plant's physiological processes. For example, the leaves and bark are subjected to various stresses such as plant tissue wounding (e.g., due to animal, and human interaction), cold and hot temperatures, and light irradiation (Luna-Guevara *et al*., 2019; De la Rosa *et al*., 2019). These stresses ultimately trigger the plant's defence system which is characterised by increased production of protective phenolic compounds such as flavonoids, tannins, coumarins, lignans, and phenolic acids.

Conversely, since the roots are underground, they are not subjected to stress such as light irradiation. However, the roots are subjected to stresses such as salinity, heavy metals, drought, and plant pathogenic soil microbes (Lin et al., 2016; Luna-Guevara et al., 2019), which cause a rise in the production of phenolic compounds in the roots. As a result, the differential

distribution patterns of these TPCs are most likely determined by which parts of the plant experienced the most stress.

Further, the phenolic contents of the same parts of *K. africana* and *P. angolensis* varied significantly even when using the same solvent for extraction. For example, a significantly higher TPC in *P. angolensis* leaves methanolic extract (322.7 ± 1.4 µg GAE/mg DE) compared to *K. africana* leaves methanolic extract (164.3 ± 2.6 µg GAE/mg DE) was observed. Moreover, the methanol extract of *P. angolensis* bark exhibited higher TPC (244.8 ± 10.0 µg GAE/mg DE) compared to methanol extract of *K. africana* bark (222.3 ± 2.5 µg GAE/mg DE). Similar observations were shown by Mulaudzi *et al.* (2011). Mulaudzi *et al.* (2011) showed that *P. angolensis* bark (12.07 ± 0.09 mg GAE/g) contained significantly higher TPC than *Acacia digitata* (2.20 ± 0.14 mg GAE/g DE), and *Bolusanthus specious* (8.69 ± 0.56 mg GAE/g DE) bark.

Furthermore, Santos *et al*. (2020) demonstrated that methanolic extract of *Julbernardia paniculate* bark contains significantly higher TPC (410.93 ± 16.72 mg AAE mg/g) compared to methanolic extract of *P. angolensis* bark (258.40 ± 5.81 mg AAE/mg). In a study by Johnson and Ayoola (2015), the methanolic extracts of *K. africana* leaves exhibited significantly higher TPC (46.24 mg GAE/g plant material (PM)) than *Alafia barteri* leaves (43.82 mg GAE/g PM), and *Anthocleista djalonesis* leaves (33.42 mg GAE/g PM).

Factors such the plant's genetic makeup, environmental conditions, seasonal changes, and geographical and environmental location differences have a significant impact on a plant's phytochemical composition (Hu, 2012; Pelkonen *et al*., 2014; Lin *et al.*, 2016; De la Rosa *et al.*, 2019; Samaniego *et al*., 2020). Thus, these factors may have contributed to the varying TPC in the two plant species. This may explain why it is also critical to state a plant's location/origin when reporting medicinal values of plants, as environmental conditions vary with geographic complexity and may influence the plant's composition of active compounds.

Regarding the effect solvents used on the TPC of the plants, we observed that in both *K. africana*, and *P. angolensis* the moderately and highly polar solvents, distilled water (aqueous), methanol, and ethyl acetate extracted more phenolic compounds than low polarity solvent

hexane. This supports what was observed by Swarnalatha *et al*. (2019) and Fagbohun *et al.* (2020). Swarnalatha *et al*. (2019) performed a phytochemical screening of *K. africana* bark extracts using water, methanol, ethyl acetate and hexane as solvents. Hexane extracted an insignificant amount of phenolic compounds while water (38.19 mg catechol equivalent (CE) /g DE), methanol (17.22 mg CE/g DE), and ethyl acetate extracts (13.89 mg CE/g DE) extracted significantly higher phenolic compounds. Similarly, Fagbohun *et al.* (2020) recorded TPC of 62.51 ± 2.86 mg AAE/g DE from *K. africana* fruit while using ethanol as an extraction solvent. This was a high TPC compared to the TPC of *K. africana* fruit aqueous (57.53 ± 3.68 µg GAE/g DE), methanol (30.39 ± 0.40 µg GAE/g DE) and ethyl acetate (52.54 ± 2.24 µg GAE/g DE) extracts observed in this study. Ethanol has a higher polarity than methanol and ethyl acetate and this might have contributed to it extracting more phenolic compounds from *K. africana* fruit

The significantly higher TPC recorded for polar solvents may be due to the sugars or unsubstituted hydroxyl (−OH) groups on the chemical structure of phenolic compounds. This chemical property makes them polar and hence more soluble in polar solvents (Ajila *et al*., 2011; Akomeng and Adusei, 2021). Therefore, polar solvents such as methanol, acetone, distilled water, and ethyl acetate will dissolve and extract higher amounts of phenolic compounds and flavonoids than non-polar solvents such as hexane and dichloromethane (Bello *et al*., 2016; Swanalatha *et al*., 2019; Fagbohun *et al*., 2020).

5.1.2. Total Flavonoid Content (TFC)

Crude extracts of various parts of *K. africana* and *P. angolensis* contained flavonoid compounds. The TFC of the various plant parts differed, and the solvent used also had an effect on the amount of flavonoids in the extracts. *Kigelia africana* leaves extracts exhibited significantly higher TFC than *K. africana* bark, root, and fruit extracts. The *P. angolensis* bark extracts exhibited significantly higher TFC than the leaves, and root extracts. Similarly, Mulaudzi *et al.* (2011) also recorded a higher TFC of 8.34 ± 0.13 µg catechin equivalent (CAE)/ g DE for bark extract compared to 0.29 ± 0.00 µg CAE/g DE exhibited by the leaves. As a subgroup of phenolic compounds (Atawodi and Olowoniyi, 2015; Fagbohun *et al*., 2020; Santos *et al*., 2020), increased production flavonoids in plants is also in response to experienced abiotic and biotic stresses (Luna-Guevara *et al*., 2019; De la Rosa *et al*., 2019). Thus, plant parts

experiencing higher or frequent stresses will potentially produce more flavonoid compounds. This consequently contributes to a varying TFC being observed in the different plant parts of the same plant.

In consistence to our study, Apalowo *et al*. (2019) observed higher TFC in *K. africana* leaves ethyl acetate extract (4.63 ± 0.19 mg QE/g) than the water (0.81 ± 0.07 mg QE/g), ethanol (0.67 ± 0.27 mg QE/g), and butanol (0.51 ± 0.34 mg QE/g) extracts. In this study, ethyl acetate also extracted higher amounts flavonoids in the bark and roots of *K. africana* than other solvents. The moderate polarity of ethyl acetate (i.e., polarity index of 4.4) (Pattanaik and Mandalia, 2011; Ghosh *et al*., 2016) could have potentially enabled it to dissolve both polar and non-polar flavonoids and thus higher flavonoid extraction potential compared to highly polar (i.e., water, and methanol) and low polarity (i.e., hexane) solvents. Flavonoids such as aurones, chalcones, flavones, isoflavones, and flavanones are non-polar classes of flavonoids and dissolve better in non-polar solvents such as chloroform, hexane, and dichloromethane. While anthocyanin and anthocyanidin flavonoids have sugars or unsubstituted hydroxyl (−OH) groups which makes them polar compounds and hence their solubility in polar solvents (Ajila *et al*., 2011; Rodríguez De Luna *et al*., 2020; Akomeng and Adusei, 2021). Both polar and non-polar flavonoids in *K. africana* and *P. angolensis* can potentially be dissolved in ethyl acetate leading to a high TFC extraction yield.

5.2. Antioxidant activity

Oxidative stress has been implicated as one of the leading causes of human diseases such as hypertension, atherosclerosis, cardiovascular diseases, ischemia injury, diabetes mellitus, neurodegenerative diseases, and cancer (Zhang *et al*., 2015; Salehi *et al*., 2020). Plants are a vast reservoir for antioxidant compounds. Evaluation of the antioxidant properties of medicinal plants is important for the discovery and development of compounds that are useful in the pharmaceutical, cosmetic, and food industries (Škrovánková *et al.*, 2012; Tabart *et al.*, 2014). Our study demonstrated the presence of both primary and secondary antioxidants in *K. africana* and *P. angolensis*. The different plant parts and solvents used for extraction varied in antioxidant activity and capacity. Results show that crude extracts from highly polar solvents (distilled water and methanol) and medium polar (ethyl acetate) have high DPPH radical scavenging, FRAP, and Phosphomolybdenum inhibition TAC activity for the different plant parts of *K.*

africana and *P. angolensis* while hexane (low polarity) extracts showed the least activity. The bark extracts showed the highest antioxidant activity.

5.2.1. DPPH free radical scavenging activity

All *K. africana* and *P. angolensis* crude extracts demonstrated DPPH free radical scavenging properties. *Pterocarpus angolensis* bark extracts showed significantly higher DPPH free radical scavenging activity than *P. angolensis* leaves, and roots. Correspondingly, a study by Santos *et al*. (2020) demonstrated that methanolic extracts of *P. angolensis* bark have higher DPPH free scavenging antioxidant (i.e., DPPH scavenging IC_{50} value of 7.11 ± 1.19 mg/L) compared to methanolic extracts of *P. angolensis* leaves (i.e., DPPH scavenging IC_{50} value of 8.67 ± 1.22 mg/L). Methanol and water are polar solvents with high effectiveness to dissolve polyphenols (Tabart *et al.*, 2014; Burton-Freeman *et al*., 2016; Rodríguez De Luna *et al*., 2020). Therefore, the use of methanol and water might have encouraged higher extraction yields of polyphenols in *P. angolensis* and the present polyphenols in these extracts might have high DPPH free radical scavenging activity.

Generally, crude extracts from polar solvents (water, methanol, and ethyl acetate) exhibited greater DPPH free radical scavenging activity compared to low polarity solvent (hexane) in both *K. africana* and *P. angolensis.* This could be due to the fact that phenolics are frequently extracted in higher concentrations in more polar solvents (Burton-Freeman *et al*., 2016; Rodríguez De Luna *et al*., 2020; Akomeng and Adusei, 2021). It has also been widely established that plant extracts' free radical scavenging activity is primarily due to phenolic compounds (Ajila *et al*., 2011; Burton-Freeman *et al*., 2016; Rodríguez De Luna *et al*., 2020).

A percentage DPPH free radical scavenging activity of less than 50 % and IC_{50} values of greater than 1 000 µg/mL were observed for all *K. africana* fruit extracts. These findings were in difference to what was observed by Fatima *et al.* (2017) and Fagbohun *et al*. (2019). Fatima *et al.* (2017) recorded a % DPPH free radical scavenging activity of 62.66 % while Fagbohun *et al*. (2019) recorded an IC_{50} value of 150 ± 3 µg/mL for ethanol extract *K. africana* fruit. Thus, ethanol might be a better potential solvent compared to water, methanol, ethyl acetate, and hexane for extracting DPPH scavenging compounds from *K. africana* fruit. Further, ethanol is highly polar, and it can dissolve and extract polyphenols, alkaloids, flavonoids, glycosides,

tannins, terpenoids, and terpenoids (Tiwari *et al*., 2011; Azmir *et al*., 2013). This property might have influenced the higher DPPH free radical scavenging activity observed by Fatima *et al.* (2017) and Fagbohun *et al*. (2019).

The DPPH free radical scavenging activity of *K. africana* and *P. angolensis* bark and roots were not significantly different, and their DPPH free radical scavenging activity was greater than that of the leaves and fruit. In support to our study, Hussain *et al.* (2016) showed that the *K. africana* bark ethanol extract has higher % DPPH free radical scavenging activity (i.e., 67.33 %) compared to *K. africana* leaves and fruit ethanol extracts (% DPPH free radical scavenging activity of 59.66 % and 62.66 % for leaves and fruit respectively). In contrast to our study, Agyare *et al*. (2013) demonstrated a DPPH free scavenging IC_{50} of 13.7 µg/mL for *K. africana* stem-bark methanol extract, while we recorded an IC_{50} of 33 ± 1 µg/mL for bark (without stem) methanol extract. The stem might have contributed to higher antioxidant activity and lower IC_{50} recorded by Agyare *et al*. (2013). Antioxidant activity of a plant part is influenced by the present bioactive compounds of the plant part. And the bioactive compounds present are influenced by the plant's physiological processes (Luna-Guevara *et al*., 2019; De la Rosa *et al*., 2019).

Santos *et al*. (2020) recorded DPPH free radical scavenging antioxidant IC_{50} value of 8.67 ± 1.22 µg/mL and 7.11 ± 1.19 µg/mL for methanolic extracts of *P. angolensis* leaves and bark respectively. While in this study IC_{50} values of 61.3 ± 1.9 µg/mL and 3.5 ± 1.6 µg/mL were recorded for methanolic extracts of *P. angolensis* leaves and bark respectively. Compared Santos *et al*. (2020), we demonstrated higher activity for the bark than the leaves while they demonstrated higher activity for the leaves than the bark. The observed differences in antioxidant activity in this study compared Santos *et al*. (2020) while using the same plant parts and solvent may be accounted for by the different extraction methods used.

Santos *et al. (*2020) used ultrasound sonication extraction. Ultrasound sonication extraction utilises acoustic cavitation mechanism to extract bioactive compounds in plants. It has been considered an efficient and superior extraction method since it ensures maximum extraction and it is cost and time efficient (Azmin *et al.*, 2016; Dhanani *et al.*, 2017; Zhang *et al.*, 2018). The extraction method may have influenced the difference in DPPH free radical scavenging

antioxidant activity. However, the plant's physiological progressions and geographical location should also be considered.

5.2.3. Ferric Reducing antioxidant Power (FRAP) activity

Pterocarpus angolensis bark extracts exhibited higher FRAP activity than *P. angolensis* leaves and roots and all *K. africana* extracts. *Pterocarpus angolensis* bark aqueous and methanol extract showed the highest FRAP activity of (i.e., 98.6 ± 0.3 % and 1029 ± 177.6 µg GAE/mg DE for aqueous and 98.7 ± 0.15 % and 1042.2 ± 133.2 µg GAE/mg DE for methanol). The highest recorded percentage of FRAP activity for *K. africana* extracts was 97.4 ± 0.01 % and 495.9 ± 2.8 µg GAE/mg DE exhibited by *K. africana* bark methanol extract. As mentioned before, the levels of bioactive compounds observed in different plant parts may be attributed to the plant's physiological processes (Luna-Guevara *et al*., 2019; De la Rosa *et al*., 2019). The plant's bioactive compound composition affects its antioxidant capacity. Thus, the plant's physiological processes have an overall effect on its antioxidant activity.

Oseni and Williams, (2018) observed a higher FRAP for the *K. africana* fruit methanol extract prepared using maceration extraction (24 hrs). They recorded a FRAP activity of 102 µg AAE/mg DE which was higher compared to the recorded 4.8 µg GAE/mg DE FRAP activity of *K. africana* fruit methanol extract in this study. Maceration extraction was used in both studies, however, the extraction duration differed. Extraction in this study was done in 72 hrs while Oseni and Williams, (2018) extracted in 24 hrs. We extracted for longer hours but recorded a lower FRAP activity. Thus, the maceration extraction duration might not have an effect the FRAP activity of the extracts. Further, the quantity and FRAP strength of extracted bioactive compounds from *K. africana* fruit may have differed due to the difference in location or origin.

5.2.3. Phosphomolybdenum inhibition Total Antioxidant Capacity (TAC) activity

The organic solvents methanol and ethyl acetate extracted antioxidant compounds with significantly higher Phosphomolybdenum inhibition TAC *in K. africana* leaves, bark, and roots. Methanol and ethyl acetate also extracted significantly higher Phosphomolybdenum inhibiting compounds in *P. angolensis* leaves. Further, even though among *P. angolensis* bark extracts the aqueous exhibited the highest Phosphomolybdenum inhibition TAC (i.e., 2564.7 ± 52.8 µg

GAE/mg DE), methanol and ethyl acetate extracts also exhibited high Phosphomolybdenum inhibition TAC (i.e., 2206.5 ± 14 µg GAE/mg DE and 2001.9 ± 32.2 µg GAE/mg DE for methanol and ethyl acetate respectively). High extraction yield of Phosphomolybdenum inhibiting compounds from *K. africana* fruit was while using organic solvents was also observed by Fagbohun *et al*., (2020). Fagbohun *et al*., (2020) recorded a Phosphomolybdenum inhibition TAC of 175.20 ± 7.09, 79.27 ± 2.94, 52.11 ± 1.49, and 44.95 ± 3.35 mg AAE/ g extract in *K. africana* fruit ethyl acetate, hexane, butanol, and butanol extracts respectively. These were higher compared to 15.03 ± 1.63 mg AAE/ g extract recorded for *K. africana* fruit aqueous extract.

The observed high extraction yield of Phosphomolybdenum inhibiting compounds in these organic solvents might be contributed to their polarity and ability to dissolve both polar and non-polar natural organic compounds. Organic solvents are effective at extracting methylated hydrophobic bioactive compounds than water (Min *et al*., 2015; Akomeng and Adusei, 2021). The recorded antioxidant activity does not necessarily indicate high extraction yield (i.e., more active compounds) but it also measures the potency/activity of available bioactive compounds (Min *et al*., 2015; Akomeng and Adusei, 2021). Thus, these organic compounds might have extracted higher amount of both polar and methylated bioactive compounds with highly active phosphomolybdenum inhibiting compounds.

Water, an inorganic solvent used in this study, extracted higher amounts or highly active phosphomolybdenum inhibiting compounds in *K. africana* fruit and *P. angolensis* bark, and roots. Phosphomolybdenum inhibiting compounds in these plant parts might be mostly polar (i.e., with high affinity to polar solvents). Water is considered a universal solvent and it has high polarity index. High yield of antioxidants while using water as a solvent is advantageous as water is also regarded as the greenest solvent (Chemat *et al*., 2012; Castro-Puyana *et al*., 2017). The title "greenest solvent" is given because water has a lower health toxicity, and environment impacts compared to organic solvents. Using water in industrial level extractions of natural compounds is also inexpensive in terms of production, transportation, and disposal (Chemat *et al*., 2012; Castro-Puyana *et al*., 2017).

The Phosphomolybdenum inhibition (TAC) assay is based on the ability of a compound or extracts to reduce a Phosphomolybdenum ion from a Mo^{6+} to Mo^{5+} phosphate complex that

absorbs light at 695 nm under acid conditions (Prieto *et al.*, 1999). The assay follows the electron and hydrogen transfer antioxidant mechanism and evaluates estimated quantities of both water-soluble and fat-soluble antioxidants such phenolics, carotenoids, and vitamins (i.e., ascorbic acid and alpha-tocopherol) (Bibi-Sadeer *et al.*, 2020). Thus, this assay has primarily elucidated presence of both primary and secondary antioxidants in *K. africana* and *P. angolensis*. The primary and secondary antioxidants acted as electron donors and reduced Phosphomolybdenum ions.

5.3. Pearson's correlation of Antioxidant activity, TPC, and TFC

A moderately positive correlation between the antioxidant capacity and TPC was observed for *K. africana* and *P. angolensis* crude extracts. The Pearson's correlation coefficient (r) between TPC and % DPPH scavenging activity, % FRAP activity and % Phosphomolybdenum inhibition was 0.58, 0.56 and 0.51 (p-value < 0.002) respectively. A very low positive Pearson's correlation coefficient (r) was observed for TFC and % DPPH scavenging activity (of 0.23), % FRAP activity (0.26) and % Phosphomolybdenum inhibition (0.09) (p values < 0.09). Therefore, flavonoid compounds contributed less to the antioxidant properties of the plant's extracts. Other phenolic compounds such as lignans, phenolic acids, and coumarins may have also played part in the antioxidant evaluations of the extracts. The hydroxyl groups in phenolic compounds are good hydrogen donors, therefore, play a role in the antioxidant properties of different medicinal plants (Škrovánková *et al*., 2012; Zhang *et al*., 2015). This property of phenolic compounds in *K. africana* and *P. angolensis* extracts might have potentially contributed to their antioxidant property observed in this study and other studies (i.e., Fatima *et al.* (2017), Oseni and Williams (2018), Fagbohun *et al*. 2019, and Santos *et al.* (2020)).

Our study demonstrated that the level of phenolic and flavonoid compounds and the antioxidant activity potential of *K. africana* and *P. angolensis* was dependent on the plant species examined, the solvent used. The results may have been also potential influenced by the plant's environmental location, experienced stresses, and maturity. Phenolic compounds including flavonoids, coumarins, phenolic acids, tannins, stilbenes, curcuminoids, and quinones have been implicated to play an important role in the prevention and treatment of different diseases including cancer, diabetes, cardio-vascular diseases, rheumatic diseases, and microbial infections (Ajila *et al*., 2011; Forni *et al*., 2019; Alabi *et al*., 2020). Therefore, phenolic

compounds produced by *K. africana* and *P. angolensis* may be contributing to their therapeutic effects when used traditionally to treat diseases including cancer, rheumatic diseases, and microbial infections.

5.4. Antimicrobial activity

This study demonstrated that the *K. africana* and *P. angolensis* plant parts contain antimicrobial compounds. Different plant parts demonstrated varying antimicrobial activity. *Kigelia africana* root extracts exhibited higher and broad-spectrum (antibacterial and anti-yeast) antimicrobial activity. The *K. africana* leaves and fruit extracts also exhibited broad spectrum antimicrobial activity while *K. africana* bark extracts were only active against tested bacterial strains (*B. subtilis, S. aureus, E. coli*, and *P. aeruginosa*). The broad-spectrum activity of *K. africana* was also demonstrated by Grace *et* al. (2002) and Agyare *et al.* (2013). Grace *et al*. (2002) showed that *K. africana* fruit extracts have antibacterial activity against strains *P. vulgaris,* and *K. pneumoniae* and antifungal activity against strains *A. niger,* and *A. flavus*. While Agyare *et al.* (2013) also demonstrated the broad-spectrum antimicrobial activity of *K. afri*cana leaves against *S. aureus, B. subtilis, E. coli, P. aeruginosa,* and *C. albicans.*

Ethyl acetate extract of *K. africana* roots contained compounds with high and broad-spectrum antimicrobial properties than aqueous, methanol, and hexane *K. africana* root extracts. At 40 mg/mL concentration, the extract was active against *B. subtilis, S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* with zone of inhibitions of 19.7 ± 0.3 mm, 16.0 ± 0.0 mm, 9.7 ± 0.3 mm, 9.0 ± 0.0 mm, and 10.0 ± 0.0 mm against the strains respectively. In contrast, in a study by Hassan *et al*. (2015), the aqueous extract of *K. africana* roots showed grater antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* than the ethyl acetate extract. At 60 mg/mL concentration of *K. africana* root aqueous extract, Hassan *et al*. (2015) recorded a zone of inhibition of 13.00 ± 0.47mm, 10.27 ± 0.49 mm, and 10.20 ± 0.40 mm against *S. aureus*, *E. coli* and *P. aeruginosa* respectively while the ethyl acetate root extract exhibited lower zone of inhibition of 6.00 ± 0.00 mm against *S. aureus*, *E. coli* and *P. aeruginosa*.

Grace *et al*. (2002) and Agyare *et al.* (2013) showed that the *K. africana* extracts used in their studies contained phytochemicals such as phenolics, alkaloids, glycosides, terpenes, terpenoids, tannins, and reducing sugars. These different phytochemicals have been shown to have different

antimicrobial mechanisms including destabilization of the cell membrane, interference with microbial metabolic processes, gene expression, or signal transduction pathway modulation (Patra, 2012; Omojate *et al.*, 2014; Barbieri *et al.*, 2017). Thus, these phytochemicals could have contributed to the antimicrobial potency of *K. africana* extracts.

In all *P. angolensis* extracts, only the bark methanol extract showed antimicrobial activity. The extract was active against gram-positive species *B. subtilis* and *S. aureus*. In contrast to this study, Chipinga *et al*, (2018) demonstrated anti-yeast activity of *P. angolensis* leaves, bark and root extracts against *C. krusei* with MIC values ranging from 0.166 g/mL to 0.0417 g/mL. In support to our study, the bark methanol extract (166 mg/ml) was also active against gram-positive species (*S. aureus* and *S. agalactiae*) only while gram-negative specie (*E. coli*) was resistant to the extract. Further, at a concentration of 166 mg/ml, *P. angolensis* bark aqueous extract was also active against *C. krusei, S. aureus*, and *S. agalactiae* while the *P. angolensis* root aqueous extract was active against *S. agalactiae* and *C. krusei* (Chipinga *et al*., 2018). At a concentration of 166 mg/ml, *P. angolensis* leaves and roots methanol extracts were active against *S. agalactiae, S. aureus*, and *C. krusei*. In our study, *P. angolensis* leaves and roots did not show antimicrobial activity. Thus, these findings show that the solvent used for extraction, and the concentration of the extract influences the antimicrobial activity of different plants and plant parts.

Regarding the tested strains, in contrast to gram-positive (*B. subtilis* and *S. aureus*), the gram-negative strains *P. aeruginosa*, and *E. coli* were resistant to the methanol extract of *P. angolensis* bark. On the other hand, gram-positive strain *B. subtilis* was more susceptible to the extracts than other microbial strains. The resistance of gram-negative bacteria (i.e., *S. agalactiae* and *E. faecalis*) to *P. angolensis* extracts was also observed in studies by Abubakar and Majinda, (2016) and McGaw *et al*. (2007). The observed resistance could be due to the low crude extract concentrations used in the studies. It may also be attributed to gram-negative bacteria's outer membrane which makes them less sensitive to antimicrobial compounds than gram-positive bacteria (Garka, 2018; Nazzaro *et al*., 2019). Gram-negative bacteria are also capable of altering their outer membrane proteins (porins), and hydrophobicity through DNA mutations leading to a developed resistance to antimicrobial compounds (Miller, 2016; Exner *et al*., 2017).

Candida albicans was resistant to most *K. africana* and all *P. angolensis* extracts. However, Chipinga *et al*. (2018) showed that *C. krusei*, a yeast strain in the same genus as *C. albicans*, was sensitive to methanol extract of *P. angolensis* leaves, bark, roots, and fruit and resistant to the aqueous extracts only. The higher extract concentration of ≤166 mg/mL used by Chipinga *et al*. (2018) compared to the ≤ 40 mg/mL used in this study might have contributed to the observed anti-yeast observed by Chipinga *et al*. (2018).

Further, our study also demonstrated that the antimicrobial activity of crude extracts is concentration dependent. The zone of inhibition decreased with decreasing crude extract concentration. Correspondingly, studies by Epidi *et al.* (2019) and Izah *et al.* (2018) also observed decreasing activity and zone of inhibition with decreasing crude extract concentration. A trend noted, which showed a higher concentration of the crude extract was required compared to conventional antibiotics (Gentamicin and Amphotericin B) for optimal antimicrobial activity. This could be for the reason that conventional antibiotics are more purified and used at a established optimal concentration than a mixture of unknown and non-tested/optimized compounds. Crude extracts from more polar solvents had greater antimicrobial activity. Different aspects such as the microbe's metabolic characteristics, extraction method, and solvents, and time of plant sampling may also contribute to the susceptibility pattern of microorganisms to medicinal plants (Vashist and Jindal, 2012; Yeo *et al.*, 2014) and thus the observed varrying susceptibility among tested strains in this study.

5.5. *In-silico* assessment of human Epidermal Growth Factor Receptor (EGFR) protein inhibition and anticancer properties of *K. africana* and *P. angolensis* phytochemicals

The EGFR is also known Erythroblastosis oncogene B1 (ErbB1), is a transmembrane tyrosine kinase receptor that activates several downstream intracellular signaling pathways, including the Ras mitogen-activated protein kinase (Ras/Raf/MAPK) and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) (JAK-STAT). Ras/Raf/MAPK and JAK-STAT play an essential role in the regulation of cellular processes such as cell proliferation, differentiation, and apoptosis (Morgillo *et al*., 2016). Damage or mutations in the EGFR genes have been linked to overexpression of the protein in a variety of cancers, including non-small cell lung cancers, colorectal cancers, breast cancers, and glioblastomas. EGFR protein

overexpression promotes cancer cell multiplication, differentiation, survival, and migration (Arteaga and Engelman, 2014; Chanprapaph *et al*., 2014; Sigismund *et al*., 2014). As a result, the EGFR protein is an intriguing alternative target for cancer treatment.

Gefitinib, afatinib, erlotinib, and osimertinib are examples of Food and Drug Administration (FDA) and clinically approved EGFR protein inhibitors used in cancer treatment (Yun *et al*., 2007; Yoshikawa *et al*., 2013; Solassol *et al*., 2019). These chemicals have been linked to a variety of side effects such as generalized pruritus, paronychia, conjunctivitis, alopecia, skin fissures, and hypertrichosis (Chanprapaph *et al*., 2014; Huang *et al*., 2020). Drug resistance is also develops in cancer patients with a mutant EGFR protein in colorectal, lung, and neck cancers within 3-24 months of treatment (Chong and Jänne, 2013; Morgillo *et al*., 2016). Drug resistance is also known to develop in cancer patients with a mutant EGFR protein in colorectal, lung, and neck cancers within 3-24 months of treatment (Chong and Jänne, 2013; Morgillo *et al*., 2016). This necessitates the development of novel mutant EGFR protein inhibitors or combination cancer treatment strategies. We conducted an *in-silico* prediction assessment (molecular docking, ADMET, and drug-likeness computational modelling) of potential competitive inhibition (binding to the active site) of wild type EGFR protein and anticancer drug potentials from bioactive compounds derived from *K. africana* and *P. angolensis*.

5.5.1. Literature survey for bioactive compounds

Plant bioactive compounds (phytochemicals) contribute to the therapeutic or medicinal effects of plants in the human or animal body (Arendt and Zannini, 2013; Diep *et al.*, 2015). Plants produce these compounds as a defense mechanism against abiotic and biotic environmental stressors. Phytochemicals from various plants have been identified, isolated, and scientifically evaluated for their medicinal properties (anticancer/antitumor, antimicrobial, antidiabetic, antioxidant, and so on. Some of these phytochemicals are now being used in clinical trials to treat diseases (Tam *et al.*, 2015; Wang *et al.*, 2015; Li *et al.*, 2021). A total of 213 phytochemicals from *K. africana* and *P. angolensis* were identified in the literature survey. The identified compounds are classified as iridoids, quinones, phenolic compounds, naphthoquinones, coumarins, alkaloids, steroids, sterols, fatty acids, nitrogen-containing, sulfur-containing compounds, terpenes, and terpenoid (King and Jurd, 1952; Bezuidenhoudt *et al*., 1980; Samie *et al*., 2009; Olubunmi and Gabriel, 2010; Zofou *et al*., 2011; Khan *et al*., 2012;

Sidjui *et al*., 2014; Atawodi and Olowoniyi, 2015; Abubakar and Majinda, 2016; Bello *et al*., 2016; Abouelela *et al*., 2019; Nabatanzi *et al*., 2020; Santos *et al*., 2020).

Fagbohun *et al*. (2021) demonstrated the *in silico* (molecular docking) competitive inhibitory activity of compounds identified from the ethyl acetate and butanol fractions of *K. africana* fruit extract against DNA topoisomerase-IIα enzyme (PDB ID: 1QZR). From the study, the better performing compounds according to their binding affinity energies (ranged from −7.5 to −8.6kcal/mol) to the enzyme were zidovudine, fenoxanil, yohimbine, digoxigenin, sulfisomidine, fluazifop, physostigmine, diflucortolone pivalate, dexamethasone, clobazam, diclomezine, desmethylmirtazapine, and 19-noretiocholanolone. Fluazifop showed the highest binding affinity energy (-8.6 kcal/mol) to the enzyme, and it interacted with amino acids in the active site through hydrogen bonds. N-hexadecanoic acid is compound that was identified in both *K. africana* and *P. angolensis* (Abubakar and Majinda, 2016; Ravi and Krishnan, 2017). In a study by Ravi and Krishnan (2017), N-hexadecanoic acid was identified and isolated from *K. africana* leaves chloroform extract. The compound showed anticancer activity against colon cancer cell (HCT 116) with an IC_{50} of 0.8 µg/mL and a binding affinity energy of -6.7 kcal/mol to DNA topoisomerase 1 (PDB ID: 1TL8). DNA topoisomerase enzymes are important for the maintenance of the topological (supercoiling nature) features of DNA during the process of gene expression, DNA replication, DNA recombination and chromosome segregation (Bjornsti and Kaufmann, 2019). Different clinical cancer drugs targeting the DNA topoisomerases include Doxorubicin, Irinotecan, MM398, and Mitoxantrone and they induce competitive inhibition of the enzyme leading to reduced cancer cell proliferation and apoptosis (Bjornsti and Kaufmann, 2019; Skok *et al*., 2019). The naphthoquinone compound Lapachol was isolated from *K. africana* root extracts and had anticancer properties against cervical cancer cell line (HeLa cells) with a IC_{50} of 19.04 ± 0.7 µg/mL (Atolani *et al*., 2021).

Lupeol is triterpenoid (phytosterol) isolated from hexane extract of *P. angolensis* stembark (Abubakar and Majinda, 2016). In a study by Saleem *et al*. (2004), Lupeol was found to have antitumor-promoting effects in a 12-O-tetradecanoyl-phorbol-13-acetate (TPA) induced skin tumorigenesis mouse model. The antitumor-promoting activity was shown to be through inhibition of (TPA) induced activation of the Phosphoinositide 3-Kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) and nuclear factor-*k*B (NF-*k*B)

transcription factor pathways. The PI3K/Akt/mTOR and NF-*k*B are targeted in cancer treatments because they implicated in tumor angiogenesis and invasiveness, cancer cell evasion of apoptosis and the development of drug resistance in cancer patients (Saleem *et al*., 2004; Karin, 2006; Becht *et al*., 2016). Lupeol was also shown to induce apoptosis in human acute myeloid leukaemia cells (HL-60) and human pancreatic tumor cells (AsPC-1 and PANC-1) (Hirotakakatsuzaki and Komiya, 2004; Murtaza *et al*., 2009).

5.5.2. Ligand and protein preparation

The EGFR protein PDB ID: 2J6M is a wild type of tyrosine kinase transmembrane receptor. The PrankWeb predicted two pockets (active sites) on the protein. Pocket 1 had score of 21.71 and highest probability (0.855). The predicted amino acid residues within these pockets were, Lys745, Glu762, Met766, Cys775, Leu788, Thr790, Leu792, Met793, Pro794, Gly796, Cys797, Glu758, Ile759, Arg841, Leu718, Gly719, Gly721, Phe723, Val726, Asp800, Asp837, Ala743Asn842, Leu844, Thr854, Asp855, Gly857, Leu858, and Lys875. These amino acid residues lie within the tyrosine kinase domain of different wild and mutant types of human EGFR protein defined by Stamos *et al*. (2002), Yun *et al*. 2007 and Jura *et al*. (2009). The tyrosine kinase domain is the intracellular domain of the receptor protein and encompasses the activation loop (A-loop), catalytic loop and phosphate-binding loop (P-loop). The wild type of tyrosine kinase domain of the EGFR is in an auto-inhibited state. It is activated when a ligand binds to the extracellular domain of the EGFR protein. In the activated state of the EGFR protein, the tyrosine kinase domain is responsible for the onward transmission of intracellular signals that are important in cell survival, cell differentiation, cell division, autophagy, and others. Common oncogenic mutations within the EGFR protein result in mutant EGFR that mimics the ligand-bond (active) state of the EGFR and a continually activated tyrosine domain. Oncogenic mutations within the tyrosine kinase domain are common in patients with human non-small cell lung cancer (NSCLC) (Arteaga and Engelman, 2014; Chanprapaph *et al.*, 2014; Sigismund *et al.*, 2018).

5.5.3. Site specific molecular docking

A site-specific binding of an inhibitor to a protein is desired to ensure maximum inhibition (Roy *et al*., 2015). Furthermore, a higher compound binding affinity energy to the EGFR protein ensures a persistent inhibition leading to reduced EGFR protein signaling and downstream effects (Arcon *et al.*, 2017; Pantsar and Poso, 2018). In site specific molecular docking, we

performed site specific protein-ligand docking using PrankWeb predicted active site coordinates, and the results were presented as binding affinity energies between the protein molecule and the bioactive compound (ligand), and their interaction position. From the 213 identified *K. africana* and *P. angolensis* phytochemicals, the top 10 bioactive compounds according to their binding affinity to the EGFR protein were 7- Dehydrodiosgenin, Yohimbine, Lupeol, 3 beta-O-acetyloleanolic acid, Cynaroside, Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3beta), Digoxigenin, 19-Noretiocholanolone, Diflucortolone pivalate, and Dexamethasone. The binding affinities of the top 10 compounds ranged from -9.6 to -8 kcal/mol. The *P. angolensis* bioactive compound 7-Dehydrodiosgenin had the highest predicted binding affinity energy of -9.6 kcal/mol than all identified compounds including the FDA approved EGFR protein inhibitors Gefitinib (-8 kcal/mol) and AEE788(-9.2 kcal/mol). Spirosta-5,7-dien-3-ol (7-Dehydrodiosgenin) is a diosgenin derivative isolated from the stembark of *P. angolensis*. It was shown to have antimicrobial, antioxidant, and cytotoxic properties (Abubakar and Majinda, 2016). In a study by Raju *et al*. (2021), 7-Dehydrodiosgenin was also identified from ethanolic extract *Lagerstroemi a speciosa* (L.) Pers. seeds and it showed potential anticancer properties through inhibition of breast cancer promoting proteins Progesterone receptor, Estrogen receptor alpha, and Ribosome Modulation Factor VP1593. In the study 7-Dehydrodiosgenin exhibited binding affinity energy of -8.9 kcal/mol to the Progesterone receptor (PDB ID: 4OAR) and Estrogen receptor alpha (PDB IB: 3ERT) and -10.0 kcal/mol to the Ribosome Modulation Factor VP1593 protein (PDB ID: 2JRM).

Yohimbine (*K. africana*) and Lupeol (*P. angolensis*) also showed high binding affinity energy (-9.2 kcal/mol). Lupeol showed anticancer properties against myeloid leukaemia, and pancreatic cancer cell lines and TPA induced skin tumorigenesis (Hirotakakatsuzaki and Komiya, 2004; Saleem *et al*., 2004; Murtaza et al., 2009). Yohimbine is an indole alkaloid with aphrodisiac, hepatotoxic, and anticancer properties (De Smet, 1997; Roy, 2017; Jabir *et al*., 2022). It was shown to have anticancer activity against multidrug-resistant human KB carcinoma cell line (KB-ChR-8-5) with an IC_{50} of 44 µM (Jabir *et al*., 2022).

5.5.4. Drug-likeness analysis, Absorption, Distribution, Metabolism, and Excretion, (ADME), and Toxicity predictions

7-Dehydrodiosgenin presented good ADME, and druglike-ness properties and its binding affinity was also higher than that of AEE_788 and Gefitinib was thus its better potential

inhibitor. It passed Lipinski (Pfizer)'s rule of five filters which states that an oral drug with good GI absorption and permeation has (i) presence of less than 10 hydrogen bond acceptors (HBAs), (ii) presence of less than 5 hydrogen bond donors (HBD's), (iii) calculated octanol/water partition coefficient (logP) < 5, and (iv) molecular weight (MW) < 500 (Roy *et al*., 2015; Daina *et al*., 2017). However, 7-Dehydrodiogesin failed the required toxicity requirement, predicted as a possible carcinogen in the mouse carcinogenicity test (Table 8). According to the Ames test, a predicted mutagen means the compound can induce reversion to the growth of different strains of bacterium Salmonella typhimurium in a histidine-free medium. The strains have mutations in genes involved in histidine synthesis (so that they require histidine for growth) (Ames *et al*., 1972). For the Rodent Carcinogenicity test, the prediction is based on the carcinogenicity tests (*in vivo*) in mice and rats in a 2-year experimental period as demonstrated by the NTP National Toxicology Program (NTP) (Yamashita *et al*., 2000).

Bioactive compounds like lupeol, 3β-O-acetylolealenolic acid, cynaroside, 19-Noretiocholanolone, diflucortone pivalate, dexamethasone, and digoxigenin also showed high affinity ranging from -9.2 to 8.0 kcal/mol. Although these molecules showed high affinity to the EGFR protein, they failed the ADMET and drug-likeness pharmacokinetic profiling prediction. They predicted to be possible mutagens and carcinogens. This shows that medicinal plant consumption is not always safe thus the safety of medicinal plants and proper dosage amounts should be established to avoid the possible occurrence of bad side effects from medicinal plants consumption.

5.5.5. Visualization of binding position and interaction between EGFR protein and bioactive compounds

The binding predictions show that the compound was interacting with amino acid residues within the active site pocket. Interactions were through van der Waals, hydrogen bond, and hydrophobic non-covalent binding. The compound also showed a binding affinity of -7.6 kcal/mol to topoisomerase IIα protein in a study by Fagbohun *et al*. (2021) and thus shows a possibility of multi-target potential in anticancer activity. The compound was isolated from ethyl acetate fruit extract of *K. africana* (Fagbohun *et al*., 2021). Yohimbine passed the required predictive ADMET, and drug-likeness pharmacokinetic profiling was performed in this study and the study by Fagbohun *et al*. (2021). The compound was a non-inhibitor of cytochrome P450 (CYP) enzyme or isoenzyme. CPY enzymes are essential for drug elimination through

biotransformation in the human body. Toxic or unwanted adverse effects of drugs have been associated with CPY enzyme or isoenzymes inhibition property. CPY inhibition leads to lower elimination and accumulation of the drug or its metabolites (Hay *et al*., 2014; Kirchmair *et al*., 2015; Daina *et al*., 2017).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

In the assessment of the overall medicinal properties of different plants, it is important to examine and distinguish the most potential part by assessing different plant parts (e.g., leaves, bark, roots, and fruit) of that plant. It is also important to design an effective extraction method to ensure maximum extraction of the bioactive compounds in the plant materials. We assessed the TPC and TFC and antioxidant, antimicrobial, and anticancer properties of traditional medicinal plants *K. africana* and *P. angolensis*. The study demonstrated that *K. africana* and *P. angolensis* have promising medicinal properties.

The different parts of both plants demonstrated the presence of phenolic and flavonoid compounds. The highest content of phenolic compounds were in *P. angolensis* crude plant extracts compared to *K. africana* extracts. *Pterocarpus angolensis* bark contained a significantly higher content of phenolic compounds. Amongst all *K. africana* plant parts, the leaves contained higher amounts of phenolic compounds than the bark, roots, and fruit. The highest content of flavonoid compounds were in *K. africana* leaves. The amount of phenolic and flavonoid compounds in *K. africana* and *P. angolensis* crude plant extracts was mostly affected by the solvent used for extraction. Polar solvents water, methanol, and ethyl acetate extracted higher amounts of phenolic and flavonoid compounds in *K. africana* and *P. angolensis* plant parts than non-polar solvent hexane. The present phenolic compounds in the plants are a possible explanation for their effectiveness when used in the traditional treatment of diseases.

The use of more than one assay in determining the antioxidant capacity of crude plant extracts is important in elucidating the antioxidant mechanisms of the bioactive compounds in the extracts. The bark and root extracts of both *K. africana* and *P. angolensis* showed the highest antioxidant activity than the leaves and fruit extracts. Polar solvents distilled water (aqueous), methanol, and ethyl acetate extracted the highest amount of compounds with antioxidant properties. The antioxidant activity of plant compounds was through sharing of hydrogen and electrons in redox reaction and possibly through metal chelation as an antioxidant was demonstrated through DPPH free radical scavenging activity, FRAP antioxidant activity, and Phosphomolybdenum inhibition TAC. The crude extracts contained both primary and secondary antioxidants. There was a positive correlation between the TPC, TFC, and antioxidant activity of the extracts thus, the phenolic compounds (including flavonoid compounds) in the crude plant extracts were responsible for the antioxidant properties of plants.

Further analysis of the antioxidant activity of both plants is recommended. Further analysis should include the identification and purification of *K. africana* and *P. angolensis* phytochemicals and the assessment of their antioxidant activity using both *in-vitro* and *in-vivo* analysis.

Kigelia africana and *P. angolensis* crude extracts were active against bacterial and yeast strains. *Kigelia africana* crude extracts demonstrated greater antimicrobial than *P. angolensis* crude extracts. The *K. africana* crude extracts showed antifungal and antibacterial properties. *Kigelia africana* roots crude extracts showed greater antimicrobial activity potential and the extracts were active against all tested microbial strains. Ethyl acetate extracted bioactive compounds with significantly higher and broad-spectrum antimicrobial activity. Methanol extracted bioactive compounds with antimicrobial activity against gram-positive bacteria only from *P. angolensis* bark. Methanol and ethyl acetate extracted higher amount of compounds with potential antimicrobial properties from both plants. Therefore, *K. africana* and *P. angolensis* can potentially be used as an alternative treatment in the prevention of microbial infections. High antimicrobial active in crude extract can potentially be achieved by using a mixed solution of different solvents during extraction therefore it is recommended in future studies. Further analysis of the antimicrobial activity of *K. africana* and *P. angolensis* pure phytochemicals should be also conducted.

The phytochemicals 7- Dehydrodiosgenin, Yohimbine, Lupeol, 3 beta-O-acetyloleanolic acid, Cynaroside, Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3beta), Digoxigenin, 19-Noretiocholanolone, Diflucortolone pivalate, and Dexamethasone showed the higher binding affinity energies to the EGFR. The compounds showed potential competitive inhibition properties as they interacted with the predicted amino acid residues of the EGFR protein's active site. Bioactive compounds like lupeol, 3β-O-acetylolealenolic acid, cynaroside, 19-Noretiocholanolone, diflucortone pivalate, dexamethasone, and digoxigenin also showed high binding affinity energy to the EGFR protein (ranging from -9.2 to 8.0 kcal/mol). However, although these molecules showed high affinity to the EGFR protein, they failed the ADMET and drug-likeness pharmacokinetic profiling prediction. They predicted to be possible mutagens and carcinogens. This shows that medicinal plant consumption is not always safe thus the safety of medicinal plants and proper dosage amounts should be established to avoid the possible occurrence of bad side effects from medicinal plants consumption. Yohimbine, a phytochemical

derived from *K. africa*na, was predicted to have anticancer drug potential due to its high binding affinity energy to the active site of EGFR. It also demonstrated good ADMET and drug-likeness profiling properties.

CHAPTER 7: REFERENCES

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CHAPTER 8: APPENDICES

Appendices 8.1: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Appendix 1: TPC calibration curve absorbance readings at 750nm.

Gallic acid solution (Dissolved in methanol) (µg/ml)	Abs 1	Abs 2	Abs 3	Av Abs	SE (±)
0	0	0	0	0	0
25	0.068	0.064	0.068	0.066666667	0.001885618
50	0.266	0.279	0.271	0.272	0.005354126
100	0.376	0.371	0.378	0.375	0.00294392
200	0.631	0.647	0.644	0.640666667	0.006944222
400	1.64	1.69	1.64	1.656666667	0.023570226

Appendix 2: TPC plant crude extract absorbance reading at 750nm.

Plant species	Plant part	Solvent	Abs 1	Abs 2	Abs 3	GAE (µg/mg DE)	SE (±)
K. africana	Leaves	Aqueous	0.406	0.409	0.406	100.571	0.368
		Methanol	0.693	0.665	0.658	162.6	1.685
		Ethyl acetate	1.02	0.934	0.962	232.653	4.742
		Hexane	0.83	0.812	0.84	201.465	3.375
	Bark	Aqueous	0.724	0.717	0.716	175.198	0.368
		Methanol	0.928	0.893	0.918	221.098	3.185
		Ethyl acetate	0.937	0.907	0.938	224.976	3.791
		Hexane	0.141	0.138	0.121	33.996	2.114
	Roots	Aqueous	0.562	0.544	0.578	137.591	4.093
		Methanol	0.791	0.81	0.799	195.821	1.464
		Ethyl acetate	0.779	0.797	0.769	190.899	3.375
		Hexane	0.141	0.149	0.159	39.238	1.346
	Fruit	Aqueous	0.257	0.239	0.211	57.534	3.682
		Methanol	0.122	0.114	0.116	30.385	0.404
		Ethyl acetate	0.19	0.218	0.201	52.425	2.237
		Hexane	0.0444	0.0463	0.0441	13.373	0.267
P. angolensis	Leaves	Aqueous	0.59	0.541	0.593	140.854	7.028
		Methanol	1.32	1.33	1.34	322.687	2.407
		Ethyl acetate	1.42	1.42	1.45	346.760	4.170
		Hexane	0.609	0.599	0.593	147.033	1.946
	Bark	Aqueous	2.33	2.29	2.28	556.196	6.369
		Methanol	1.09	0.967	0.963	244.850	17.380
		Ethyl acetate	0.275	0.299	0.272	70.400	3.563
		Hexane	0.164	0.153	0.151	40.067	1.685
	Roots	Aqueous	0.753	0.743	0.75	182.741	1.235
		Methanol	0.433	0.437	0.452	108.596	2.411
		Ethyl acetate	1.19	1.17	1.17	285.774	2.780
		Hexane	0.391	0.425	0.423	101.935	4.593

Appendix 3: Tukey's multiple comparisons test of TPC of *Kigelia africana* crude extracts.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Leaves:Aqueous vs. Leaves:Methanol	-63.79	-93.77 to -33.82	Yes	*	0.0128
Leaves:Aqueous vs. Leaves:Ethyl acetate	-136.0	-206.7 to -65.32	Yes	*	0.0140

Leaves:Aqueous vs. Leaves:Hexane	-101.2	-125.9 to -76.43	Yes	***	0.0009
Leaves:Aqueous vs. Bark:Aqueous	-75.11	-83.39 to -66.83	Yes	****	<0.0001
Leaves:Aqueous vs. Bark:Methanol	-121.8	-152.6 to -90.99	Yes	**	0.0012
Leaves:Aqueous vs. Bark:Ethyl acetate	-125.3	-155.5 to -94.99	Yes	***	0.0008
Leaves:Aqueous vs. Bark:Hexane	65.88	49.82 to 81.94	Yes	***	0.0008
Leaves:Aqueous vs. Roots:Aqueous	-37.15	-66.19 to -8.118	Yes	*	0.0309
Leaves:Aqueous vs. Roots:Methanol	-94.61	-107.1 to -82.09	Yes	****	<0.0001
Leaves:Aqueous vs. Roots:Ethyl acetate	-90.19	-109.9 to -70.51	Yes	***	0.0002
Leaves:Aqueous vs. Roots:Hexane	61.95	47.41 to 76.49	Yes	***	0.0005
Leaves:Aqueous vs. Fruit:Aqueous	41.25	5.212 to 77.28	Yes	*	0.0387
Leaves:Aqueous vs. Fruit:Methanol	69.73	61.11 to 78.35	Yes	****	<0.0001
Leaves:Aqueous vs. Fruit:Ethyl acetate	49.11	29.50 to 68.72	Yes	*	0.0107
Leaves:Aqueous vs. Fruit:Hexane	87.16	86.27 to 88.05	Yes	****	<0.0001
Leaves:Methanol vs. Leaves:Ethyl acetate	-72.22	-117.9 to -26.52	Yes	*	0.0191
Leaves:Methanol vs. Leaves:Hexane	-37.39	-74.37 to -0.4209	Yes	*	0.0489
Leaves:Methanol vs. Bark:Aqueous	-11.31	-33.50 to 10.87	No	ns	0.1765
Leaves:Methanol vs. Bark:Methanol	-58.02	-84.34 to -31.69	Yes	*	0.0125
Leaves:Methanol vs. Bark:Ethyl acetate	-61.47	-94.93 to -28.00	Yes	*	0.0147
Leaves:Methanol vs. Bark:Hexane	129.7	110.0 to 149.4	Yes	****	<0.0001
Leaves:Methanol vs. Roots:Aqueous	26.64	-15.64 to 68.93	No	ns	0.1202
Leaves:Methanol vs. Roots:Methanol	-30.81	-71.59 to 9.959	No	ns	0.0854
Leaves:Methanol vs. Roots:Ethyl acetate	-26.40	-62.43 to 9.634	No	ns	0.0906
Leaves:Methanol vs. Roots:Hexane	125.7	83.40 to 168.1	Yes	**	0.0070
Leaves:Methanol vs. Fruit:Aqueous	105.0	88.60 to 121.5	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Methanol	133.5	110.3 to 156.8	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Ethyl acetate	112.9	66.17 to 159.6	Yes	*	0.0113
Leaves:Methanol vs. Fruit:Hexane	151.0	121.5 to 180.4	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Leaves:Hexane	34.83	-26.60 to 96.26	No	ns	0.1459
Leaves:Ethyl acetate vs. Bark:Aqueous	60.91	-1.625 to 123.4	No	ns	0.0526
Leaves:Ethyl acetate vs. Bark:Methanol	14.20	-30.58 to 58.98	No	ns	0.3867
Leaves:Ethyl acetate vs. Bark:Ethyl acetate	10.75	-41.25 to 62.75	No	ns	0.6638
Leaves:Ethyl acetate vs. Bark:Hexane	201.9	136.9 to 266.9	Yes	**	0.0059
Leaves:Ethyl acetate vs. Roots:Aqueous	98.86	34.55 to 163.2	Yes	*	0.0203
Leaves:Ethyl acetate vs. Roots:Methanol	41.41	-41.64 to 124.5	No	ns	0.1838
Leaves:Ethyl acetate vs. Roots:Ethyl acetate	45.82	-35.63 to 127.3	No	ns	0.1480
Leaves:Ethyl acetate vs. Roots:Hexane	198.0	119.9 to 276.0	Yes	*	0.0105
Leaves:Ethyl acetate vs. Fruit:Aqueous	177.3	120.5 to 234.1	Yes	**	0.0058

Leaves:Ethyl acetate vs. Fruit:Methanol	205.7	143.6 to 267.9	Yes	**	0.0043
Leaves:Ethyl acetate vs. Fruit:Ethyl acetate	185.1	95.28 to 275.0	Yes	*	0.0132
Leaves:Ethyl acetate vs. Fruit:Hexane	223.2	153.3 to 293.1	Yes	**	0.0052
Leaves:Hexane vs. Bark:Aqueous	26.08	3.172 to 48.99	Yes	*	0.0391
Leaves:Hexane vs. Bark:Methanol	-20.62	-37.50 to -3.746	Yes	*	0.0340
Leaves:Hexane vs. Bark:Ethyl acetate	-24.07	-33.84 to -14.30	Yes	*	0.0110
Leaves:Hexane vs. Bark:Hexane	167.1	131.2 to 202.9	Yes	***	0.0002
Leaves:Hexane vs. Roots:Aqueous	64.03	58.61 to 69.46	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Methanol	6.580	-27.78 to 40.94	No	ns	0.7143
Leaves:Hexane vs. Roots:Ethyl acetate	10.99	-33.41 to 55.40	No	ns	0.5424
Leaves:Hexane vs. Roots:Hexane	163.1	142.3 to 184.0	Yes	****	<0.0001
Leaves:Hexane vs. Fruit:Aqueous	142.4	91.84 to 193.0	Yes	**	0.0083
Leaves:Hexane vs. Fruit:Methanol	170.9	150.4 to 191.4	Yes	****	<0.0001
Leaves:Hexane vs. Fruit:Ethyl acetate	150.3	109.2 to 191.4	Yes	**	0.0023
Leaves:Hexane vs. Fruit:Hexane	188.3	164.3 to 212.4	Yes	****	<0.0001
Bark:Aqueous vs. Bark:Methanol	-46.70	-71.14 to -22.26	Yes	*	0.0141
Bark:Aqueous vs. Bark:Ethyl acetate	-50.15	-75.97 to -24.33	Yes	*	0.0138
Bark:Aqueous vs. Bark:Hexane	141.0	128.0 to 154.0	Yes	****	<0.0001
Bark:Aqueous vs. Roots:Aqueous	37.96	9.950 to 65.96	Yes	*	0.0272
Bark:Aqueous vs. Roots:Methanol	-19.50	-40.02 to 1.022	No	ns	0.0551
Bark:Aqueous vs. Roots:Ethyl acetate	-15.09	-38.63 to 8.454	No	ns	0.1165
Bark:Aqueous vs. Roots:Hexane	137.1	116.6 to 157.5	Yes	****	<0.0001
Bark:Aqueous vs. Fruit:Aqueous	116.4	85.76 to 146.9	Yes	**	0.0017
Bark:Aqueous vs. Fruit:Methanol	144.8	142.5 to 147.2	Yes	****	<0.0001
Bark:Aqueous vs. Fruit:Ethyl acetate	124.2	96.80 to 151.6	Yes	***	0.0003
Bark:Aqueous vs. Fruit:Hexane	162.3	154.7 to 169.8	Yes	****	<0.0001
Bark:Methanol vs. Bark:Ethyl acetate	-3.450	-12.07 to 5.167	No	ns	0.2679
Bark:Methanol vs. Bark:Hexane	187.7	153.4 to 222.0	Yes	****	<0.0001
Bark:Methanol vs. Roots:Aqueous	84.66	64.00 to 105.3	Yes	***	0.0008
Bark:Methanol vs. Roots:Methanol	27.20	-15.82 to 70.23	No	ns	0.1194
Bark:Methanol vs. Roots:Ethyl acetate	31.62	-16.26 to 79.50	No	ns	0.1102
Bark:Methanol vs. Roots:Hexane	183.8	149.6 to 217.9	Yes	****	<0.0001
Bark:Methanol vs. Fruit:Aqueous	163.1	120.7 to 205.4	Yes	**	0.0015
Bark:Methanol vs. Fruit:Methanol	191.5	168.7 to 214.3	Yes	****	<0.0001
Bark:Methanol vs. Fruit:Ethyl acetate	170.9	120.7 to 221.1	Yes	**	0.0037
Bark:Methanol vs. Fruit:Hexane	209.0	179.0 to 238.9	Yes	****	<0.0001
Bark:Ethyl acetate vs. Bark:Hexane	191.1	153.5 to 228.8	Yes	****	<0.0001
Bark:Ethyl acetate vs. Roots:Aqueous	88.11	75.69 to 100.5	Yes	****	<0.0001
Bark:Ethyl acetate vs. Roots:Methanol	30.65	-10.81 to 72.12	No	ns	0.0891
Bark:Ethyl acetate vs. Roots:Ethyl acetate	35.07	-14.02 to 84.16	No	ns	0.0951
Bark:Ethyl acetate vs. Roots:Hexane	187.2	157.4 to 217.0	Yes	****	<0.0001
Bark:Ethyl acetate vs. Fruit:Aqueous	166.5	117.7 to 215.3	Yes	**	0.0037
Bark:Ethyl acetate vs. Fruit:Methanol	195.0	171.3 to 218.7	Yes	****	<0.0001
Bark:Ethyl acetate vs. Fruit:Ethyl acetate	174.4	125.9 to 222.9	Yes	**	0.0026
Bark:Ethyl acetate vs. Fruit:Hexane	212.4	183.0 to 241.8	Yes	****	<0.0001
Bark:Hexane vs. Roots:Aqueous	-103.0	-144.0 to -62.02	Yes	*	0.0107
Bark:Hexane vs. Roots:Methanol	-160.5	-183.6 to -137.4	Yes	****	<0.0001
Bark:Hexane vs. Roots:Ethyl acetate	-156.1	-172.5 to -139.6	Yes	****	<0.0001
Bark:Hexane vs. Roots:Hexane	-3.932	-34.53 to 26.66	No	ns	0.9174

Bark:Hexane vs. Fruit:Aqueous	-24.63	-45.06 to -4.213	Yes	*	0.0349
Bark:Hexane vs. Fruit:Methanol	3.852	-11.56 to 19.26	No	ns	0.5360
Bark:Hexane vs. Fruit:Ethyl acetate	-16.77	-44.78 to 11.23	No	ns	0.1320
Bark:Hexane vs. Fruit:Hexane	21.28	5.233 to 37.33	Yes	*	0.0286
Roots:Aqueous vs. Roots:Methanol	-57.45	-95.02 to -19.89	Yes	*	0.0205
Roots:Aqueous vs. Roots:Ethyl acetate	-53.04	-101.8 to -4.325	Yes	*	0.0426
Roots:Aqueous vs. Roots:Hexane	99.10	76.46 to 121.7	Yes	***	0.0004
Roots:Aqueous vs. Fruit:Aqueous	78.40	22.39 to 134.4	Yes	*	0.0253
Roots:Aqueous vs. Fruit:Methanol	106.9	81.27 to 132.5	Yes	***	0.0007
Roots:Aqueous vs. Fruit:Ethyl acetate	86.26	42.27 to 130.3	Yes	*	0.0137
Roots:Aqueous vs. Fruit:Hexane	124.3	95.95 to 152.7	Yes	***	0.0004
Roots:Methanol vs. Roots:Ethyl acetate	4.413	-11.42 to 20.24	No	ns	0.4648
Roots:Methanol vs. Roots:Hexane	156.6	140.1 to 173.0	Yes	****	<0.0001
Roots:Methanol vs. Fruit:Aqueous	135.9	92.65 to 179.1	Yes	**	0.0056
Roots:Methanol vs. Fruit:Methanol	164.3	143.2 to 185.5	Yes	****	<0.0001
Roots:Methanol vs. Fruit:Ethyl acetate	143.7	136.5 to 150.9	Yes	****	<0.0001
Roots:Methanol vs. Fruit:Hexane	181.8	168.4 to 195.1	Yes	****	<0.0001
Roots:Ethyl acetate vs. Roots:Hexane	152.1	121.3 to 183.0	Yes	****	<0.0001
Roots:Ethyl acetate vs. Fruit:Aqueous	131.4	98.92 to 164.0	Yes	***	0.0010
Roots:Ethyl acetate vs. Fruit:Methanol	159.9	134.4 to 185.4	Yes	****	<0.0001
Roots:Ethyl acetate vs. Fruit:Ethyl acetate	139.3	122.9 to 155.7	Yes	****	<0.0001
Roots:Ethyl acetate vs. Fruit:Hexane	177.4	157.0 to 197.7	Yes	****	<0.0001
Roots:Hexane vs. Fruit:Aqueous	-20.70	-71.07 to 29.66	No	ns	0.2564
Roots:Hexane vs. Fruit:Methanol	7.784	-11.34 to 26.90	No	ns	0.2606
Roots:Hexane vs. Fruit:Ethyl acetate	-12.84	-34.76 to 9.086	No	ns	0.1375
Roots:Hexane vs. Fruit:Hexane	25.21	10.63 to 39.79	Yes	*	0.0160
Fruit:Aqueous vs. Fruit:Methanol	28.49	-4.086 to 61.06	No	ns	0.0646
Fruit:Aqueous vs. Fruit:Ethyl acetate	7.864	-39.45 to 55.18	No	ns	0.8003
Fruit:Aqueous vs. Fruit:Hexane	45.92	10.06 to 81.77	Yes	*	0.0308
Fruit:Methanol vs. Fruit:Ethyl acetate	-20.62	-48.80 to 7.557	No	ns	0.0908
Fruit:Methanol vs. Fruit:Hexane	17.43	9.654 to 25.20	Yes	*	0.0123
Fruit:Ethyl acetate vs. Fruit:Hexane	38.05	17.57 to 58.53	Yes	*	0.0145

Appendix 4: Tukey's multiple comparisons test of TPC of *Pterocarpus angolensis* crude extracts.

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**
Leaves:Aqueous vs. Leaves:Methanol	-181.8	-225.9 to -137.8	Yes	**	0.0010
Leaves:Aqueous vs. Leaves:Ethyl acetate	-205.9	-241.5 to -170.3	Yes	****	<0.0001
Leaves:Aqueous vs. Leaves:Hexane	-6.179	-49.07 to 36.71	No	ns	0.8622
Leaves:Aqueous vs. Bark:Aqueous	-415.3	-463.9 to -366.7	Yes	****	<0.0001
Leaves:Aqueous vs. Bark:Methanol	-104.0	-198.6 to -9.414	Yes	*	0.0417
Leaves:Aqueous vs. Bark:Ethyl acetate	70.45	6.661 to 134.2	Yes	*	0.0413
Leaves:Aqueous vs. Bark:Hexane	100.8	60.55 to 141.0	Yes	*	0.0103
Leaves:Aqueous vs. Roots:Aqueous	-41.89	-77.33 to -6.447	Yes	*	0.0362
Leaves:Aqueous vs. Roots:Methanol	32.26	-7.173 to 71.69	No	ns	0.0735
Leaves:Aqueous vs. Roots:Ethyl acetate	-144.9	-182.7 to -107.1	Yes	**	0.0018
Leaves:Aqueous vs. Roots:Hexane	38.92	-22.18 to 100.0	No	ns	0.1177

Leaves:Methanol vs. Leaves:Ethyl acetate	-24.07	-38.58 to -9.568	Yes	*	0.0177
Leaves:Methanol vs. Leaves:Hexane	175.7	149.5 to 201.8	Yes	****	<0.0001
Leaves:Methanol vs. Bark:Aqueous	-233.5	-285.8 to -181.2	Yes	***	0.0005
Leaves:Methanol vs. Bark:Methanol	77.84	-39.84 to 195.5	No	ns	0.1097
Leaves:Methanol vs. Bark:Ethyl acetate	252.3	225.2 to 279.4	Yes	****	<0.0001
Leaves:Methanol vs. Bark:Hexane	282.6	258.4 to 306.8	Yes	****	<0.0001
Leaves:Methanol vs. Roots:Aqueous	139.9	121.8 to 158.1	Yes	****	<0.0001
Leaves:Methanol vs. Roots:Methanol	214.1	209.4 to 218.8	Yes	****	<0.0001
Leaves:Methanol vs. Roots:Ethyl acetate	36.91	6.718 to 67.11	Yes	*	0.0338
Leaves:Methanol vs. Roots:Hexane	220.8	203.3 to 238.2	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Leaves:Hexane	199.7	164.6 to 234.8	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Bark:Aqueous	-209.4	-267.5 to -151.4	Yes	**	0.0028
Leaves:Ethyl acetate vs. Bark:Methanol	101.9	-17.90 to 221.7	No	ns	0.0682
Leaves:Ethyl acetate vs. Bark:Ethyl acetate	276.4	234.9 to 317.9	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Bark:Hexane	306.7	274.3 to 339.1	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Aqueous	164.0	139.5 to 188.6	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Methanol	238.2	226.9 to 249.4	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Ethyl acetate	60.99	24.48 to 97.49	Yes	*	0.0175
Leaves:Ethyl acetate vs. Roots:Hexane	244.8	217.2 to 272.5	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Aqueous	-409.2	-436.1 to -382.2	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Methanol	-97.82	-191.6 to -4.014	Yes	*	0.0461
Leaves:Hexane vs. Bark:Ethyl acetate	76.63	51.75 to 101.5	Yes	**	0.0061
Leaves:Hexane vs. Bark:Hexane	107.0	103.9 to 110.0	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Aqueous	-35.71	-46.60 to -24.82	Yes	**	0.0047
Leaves:Hexane vs. Roots:Methanol	38.44	12.88 to 63.99	Yes	*	0.0218
Leaves:Hexane vs. Roots:Ethyl acetate	-138.7	-146.0 to -131.4	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Hexane	45.10	6.466 to 83.73	Yes	*	0.0371
Bark:Aqueous vs. Bark:Methanol	311.3	244.2 to 378.5	Yes	***	0.0003
Bark:Aqueous vs. Bark:Ethyl acetate	485.8	437.7 to 533.9	Yes	****	<0.0001
Bark:Aqueous vs. Bark:Hexane	516.1	487.9 to 544.4	Yes	****	<0.0001
Bark:Aqueous vs. Roots:Aqueous	373.5	338.9 to 408.0	Yes	****	<0.0001
Bark:Aqueous vs. Roots:Methanol	447.6	396.9 to 498.3	Yes	****	<0.0001
Bark:Aqueous vs. Roots:Ethyl acetate	270.4	248.3 to 292.6	Yes	****	<0.0001
Bark:Aqueous vs. Roots:Hexane	454.3	388.7 to 519.8	Yes	****	<0.0001
Bark:Methanol vs. Bark:Ethyl acetate	174.5	59.76 to 289.1	Yes	*	0.0212
Bark:Methanol vs. Bark:Hexane	204.8	110.1 to 299.4	Yes	*	0.0123
Bark:Methanol vs. Roots:Aqueous	62.11	-37.45 to 161.7	No	ns	0.1223
Bark:Methanol vs. Roots:Methanol	136.3	21.12 to 251.4	Yes	*	0.0361
Bark:Methanol vs. Roots:Ethyl acetate	-40.92	-128.9 to 47.05	No	ns	0.2070
Bark:Methanol vs. Roots:Hexane	142.9	10.59 to 275.2	Yes	*	0.0431
Bark:Ethyl acetate vs. Bark:Hexane	30.33	4.183 to 56.48	Yes	*	0.0375
Bark:Ethyl acetate vs. Roots:Aqueous	-112.3	-140.8 to -83.87	Yes	**	0.0015

Bark:Ethyl acetate vs. Roots:Methanol	-38.20	-68.67 to -7.725	Yes	*	0.0321
Bark:Ethyl acetate vs. Roots:Ethyl acetate	-215.4	-247.6 to -183.2	Yes	****	<0.0001
Bark:Ethyl acetate vs. Roots:Hexane	-31.54	-57.68 to -5.387	Yes	*	0.0348
Bark:Hexane vs. Roots:Aqueous	-142.7	-150.7 to -134.7	Yes	****	<0.0001
Bark:Hexane vs. Roots:Methanol	-68.53	-91.75 to -45.31	Yes	**	0.0070
Bark:Hexane vs. Roots:Ethyl acetate	-245.7	-252.6 to -238.9	Yes	****	<0.0001
Bark:Hexane vs. Roots:Hexane	-61.87	-99.55 to -24.18	Yes	*	0.0181
Roots:Aqueous vs. Roots:Methanol	74.15	57.99 to 90.30	Yes	***	0.0003
Roots:Aqueous vs. Roots:Ethyl acetate	-103.0	-115.4 to -90.64	Yes	****	<0.0001
Roots:Aqueous vs. Roots:Hexane	80.81	47.09 to 114.5	Yes	*	0.0110
Roots:Methanol vs. Roots:Ethyl acetate	-177.2	-205.7 to -148.6	Yes	****	<0.0001
Roots:Methanol vs. Roots:Hexane	6.660	-15.16 to 28.48	No	ns	0.4061
Roots:Ethyl acetate vs. Roots:Hexane	183.8	139.4 to 228.2	Yes	***	0.0010

Appendix 5: TFC calibration curve quercetin absorbance readings at 420nm.

Quercetin solution (Dissolved in methanol) (µg/ml)	Abs 1	Abs 2	Abs 3	Average Abs	SE (±)
0	0.000	0.000	0.000	0.000	0.000
25	0.087	0.075	0.084	0.082	0.006244998
50	0.221	0.223	0.223	0.222333333	0.001154701
100	0.493	0.488	0.482	0.487666667	0.005507571
200	1.220	1.270	1.101	1.197	0.086815897
400	2.196	2.196	1.998	2.13	0.114315353

Appendix 6: TFC plant crude extract absorbance readings at 420nm and calculated quercetin equivalence (µg QE/mg DE) (mean ±SD)

Plant species	**Plant part**	**Solvent**	**Abs 1**	**Abs 2**	**Abs 3**	**QE (µg/mg DE)**	**SE (±)**
K. africana	Leaves	Aqueous	0.261	0.267	0.265	52.371	0.556
		Methanol	0.926	0.927	0.93	173.043	0.379
		Ethyl acetate	2.44	2.41	2.42	445.131	2.779
		Hexane	0.0651	0.0657	0.0658	16.206	0.069
	Bark	Aqueous	0.173	0.174	0.192	36.969	1.945
		Methanol	0.339	0.324	0.311	63.347	2.549
		Ethyl acetate	0.491	0.495	0.499	94.333	0.728
		Hexane	0.213	0.213	0.21	42.851	0.315
	Roots	Aqueous	0.255	0.218	0.218	46.186	3.886
		Methanol	0.348	0.362	0.365	69.471	1.651
		Ethyl acetate	0.491	0.49	0.512	94.818	2.260
		Hexane	0.111	0.105	0.111	24.113	0.630
	Fruit	Aqueous	0.542	0.547	0.542	103.187	0.525
		Methanol	0.122	0.114	0.116	25.629	0.757
		Ethyl acetate	0.218	0.198	0.203	41.820	1.893
		Hexane	0.0463	0.0464	0.0461	12.701	0.028
P. angolensis	Leaves	Aqueous	0.326	0.296	0.298	51.504	3.051
		Methanol	0.382	0.389	0.409	67.270	2.549
		Ethyl acetate	0.332	0.328	0.3	53.929	3.172
		Hexane	0.042	0.052	0.049	4.387	0.934
	Bark	Aqueous	0.85	0.842	0.827	148.466	2.124
		Methanol	1.25	1.209	1.2	217.594	4.848
		Ethyl acetate	0.272	0.299	0.25	45.501	4.465

		Hexane	0.11	0.106	0.105	15.181	0.481
	Roots	Aqueous	0.707	0.697	0.693	122.876	1.312
		Methanol	0.691	0.691	0.679	120.693	1.260
		Ethyl acetate	0.587	0.618	0.587	104.381	3.256
		Hexane	0.233	0.195	0.233	35.798	3.991

Appendix 7: Tukey's multiple comparisons test of TPC of *Kigelia africana* crude extracts.

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**
Leaves:Aqueous vs. Leaves:Methanol	-120.7	-124.1 to -117.3	Yes	****	<0.0001
Leaves:Aqueous vs. Leaves:Ethyl acetate	-392.8	-414.4 to -371.1	Yes	****	<0.0001
Leaves:Aqueous vs. Leaves:Hexane	36.17	32.95 to 39.38	Yes	****	<0.0001
Leaves:Aqueous vs. Bark:Aqueous	15.40	3.095 to 27.71	Yes	*	0.0324
Leaves:Aqueous vs. Bark:Methanol	-10.98	-30.20 to 8.249	No	ns	0.1441
Leaves:Aqueous vs. Bark:Ethyl acetate	-41.96	-45.57 to -38.35	Yes	****	<0.0001
Leaves:Aqueous vs. Bark:Hexane	9.520	5.044 to 14.00	Yes	*	0.0128
Leaves:Aqueous vs. Roots:Aqueous	6.185	-22.51 to 34.88	No	ns	0.6359
Leaves:Aqueous vs. Roots:Methanol	-17.10	-24.85 to -9.347	Yes	*	0.0125
Leaves:Aqueous vs. Roots:Ethyl acetate	-42.45	-57.04 to -27.85	Yes	**	0.0076
Leaves:Aqueous vs. Roots:Hexane	28.26	21.03 to 35.48	Yes	**	0.0013
Leaves:Aqueous vs. Fruit:Aqueous	-50.82	-53.28 to -48.35	Yes	****	<0.0001
Leaves:Aqueous vs. Fruit:Methanol	26.74	18.22 to 35.27	Yes	**	0.0057
Leaves:Aqueous vs. Fruit:Ethyl acetate	10.55	-5.357 to 26.46	No	ns	0.1092
Leaves:Aqueous vs. Fruit:Hexane	39.67	36.08 to 43.26	Yes	****	<0.0001
Leaves:Methanol vs. Leaves:Ethyl acetate	-272.1	-291.3 to -252.9	Yes	****	<0.0001
Leaves:Methanol vs. Leaves:Hexane	156.8	154.7 to 159.0	Yes	****	<0.0001
Leaves:Methanol vs. Bark:Aqueous	136.1	125.8 to 146.3	Yes	****	<0.0001
Leaves:Methanol vs. Bark:Methanol	109.7	90.78 to 128.6	Yes	****	<0.0001
Leaves:Methanol vs. Bark:Ethyl acetate	78.71	76.25 to 81.17	Yes	****	<0.0001
Leaves:Methanol vs. Bark:Hexane	130.2	125.7 to 134.7	Yes	****	<0.0001
Leaves:Methanol vs. Roots:Aqueous	126.9	99.83 to 153.9	Yes	***	0.0002
Leaves:Methanol vs. Roots:Methanol	103.6	94.70 to 112.4	Yes	****	<0.0001
Leaves:Methanol vs. Roots:Ethyl acetate	78.22	65.88 to 90.57	Yes	****	<0.0001
Leaves:Methanol vs. Roots:Hexane	148.9	144.8 to 153.1	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Aqueous	69.86	65.13 to 74.59	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Methanol	147.4	140.9 to 153.9	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Ethyl acetate	131.2	117.5 to 144.9	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Hexane	160.3	157.7 to 163.0	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Leaves:Hexane	428.9	410.5 to 447.4	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Bark:Aqueous	408.2	383.8 to 432.5	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Bark:Methanol	381.8	368.0 to 395.6	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Bark:Ethyl acetate	350.8	329.3 to 372.3	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Bark:Hexane	402.3	384.5 to 420.1	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Aqueous	398.9	388.8 to 409.0	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Methanol	375.7	347.7 to 403.6	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Ethyl acetate	350.3	325.4 to 375.2	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Hexane	421.0	405.8 to 436.2	Yes	****	<0.0001

Leaves:Ethyl acetate vs. Fruit:Aqueous	341.9	321.2 to 362.7	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Fruit:Methanol	419.5	406.3 to 432.7	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Fruit:Ethyl acetate	403.3	397.4 to 409.2	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Fruit:Hexane	432.4	414.3 to 450.5	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Aqueous	-20.76	-33.12 to -8.404	Yes	*	0.0169
Leaves:Hexane vs. Bark:Methanol	-47.14	-64.13 to -30.15	Yes	**	0.0087
Leaves:Hexane vs. Bark:Ethyl acetate	-78.13	-82.45 to -73.81	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Hexane	-26.64	-28.99 to -24.30	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Aqueous	-29.98	-55.68 to -4.277	Yes	*	0.0373
Leaves:Hexane vs. Roots:Methanol	-53.27	-63.55 to -42.98	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Ethyl acetate	-78.61	-93.05 to -64.18	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Hexane	-7.907	-12.19 to -3.621	Yes	*	0.0146
Leaves:Hexane vs. Fruit:Aqueous	-86.98	-90.25 to -83.71	Yes	****	<0.0001
Leaves:Hexane vs. Fruit:Methanol	-9.423	-14.77 to -4.081	Yes	*	0.0155
Leaves:Hexane vs. Fruit:Ethyl acetate	-25.61	-38.34 to -12.89	Yes	*	0.0134
Leaves:Hexane vs. Fruit:Hexane	3.505	2.972 to 4.038	Yes	****	<0.0001
Bark:Aqueous vs. Bark:Methanol	-26.38	-54.63 to 1.877	No	ns	0.0570
Bark:Aqueous vs. Bark:Ethyl acetate	-57.36	-66.08 to -48.65	Yes	****	<0.0001
Bark:Aqueous vs. Bark:Hexane	-5.882	-20.57 to 8.807	No	ns	0.2679
Bark:Aqueous vs. Roots:Aqueous	-9.217	-43.02 to 24.59	No	ns	0.4790
Bark:Aqueous vs. Roots:Methanol	-32.50	-42.13 to -22.87	Yes	**	0.0039
Bark:Aqueous vs. Roots:Ethyl acetate	-57.85	-60.21 to -55.48	Yes	****	<0.0001
Bark:Aqueous vs. Roots:Hexane	12.86	1.493 to 24.22	Yes	*	0.0396
Bark:Aqueous vs. Fruit:Aqueous	-66.22	-80.75 to -51.69	Yes	***	0.0002
Bark:Aqueous vs. Fruit:Methanol	11.34	-3.633 to 26.31	No	ns	0.0851
Bark:Aqueous vs. Fruit:Ethyl acetate	-4.851	-25.14 to 15.43	No	ns	0.5656
Bark:Aqueous vs. Fruit:Hexane	24.27	11.46 to 37.08	Yes	*	0.0142
Bark:Methanol vs. Bark:Ethyl acetate	-30.99	-52.28 to -9.692	Yes	*	0.0231
Bark:Methanol vs. Bark:Hexane	20.50	5.617 to 35.37	Yes	*	0.0262
Bark:Methanol vs. Roots:Aqueous	17.16	4.083 to 30.24	Yes	*	0.0293
Bark:Methanol vs. Roots:Methanol	-6.125	-33.10 to 20.85	No	ns	0.6007
Bark:Methanol vs. Roots:Ethyl acetate	-31.47	-61.31 to -1.628	Yes	*	0.0452
Bark:Methanol vs. Roots:Hexane	39.23	22.33 to 56.14	Yes	*	0.0119
Bark:Methanol vs. Fruit:Aqueous	-39.84	-56.89 to -22.79	Yes	*	0.0118
Bark:Methanol vs. Fruit:Methanol	37.72	24.43 to 51.01	Yes	**	0.0082
Bark:Methanol vs. Fruit:Ethyl acetate	21.53	10.54 to 32.51	Yes	*	0.0137
Bark:Methanol vs. Fruit:Hexane	50.65	34.19 to 67.10	Yes	**	0.0061
Bark:Ethyl acetate vs. Bark:Hexane	51.48	44.90 to 58.07	Yes	****	<0.0001
Bark:Ethyl acetate vs. Roots:Aqueous	48.15	18.70 to 77.60	Yes	*	0.0178
Bark:Ethyl acetate vs. Roots:Methanol	24.86	18.35 to 31.37	Yes	**	0.0016
Bark:Ethyl acetate vs. Roots:Ethyl acetate	-0.4851	-11.47 to 10.50	No	ns	>0.9999
Bark:Ethyl acetate vs. Roots:Hexane	70.22	63.96 to 76.48	Yes	****	<0.0001
Bark:Ethyl acetate vs. Fruit:Aqueous	-8.853	-14.69 to -3.021	Yes	*	0.0209
Bark:Ethyl acetate vs. Fruit:Methanol	68.70	59.75 to 77.66	Yes	****	<0.0001
Bark:Ethyl acetate vs. Fruit:Ethyl acetate	52.51	36.46 to 68.57	Yes	**	0.0046
Bark:Ethyl acetate vs. Fruit:Hexane	81.63	76.78 to 86.48	Yes	****	<0.0001
Bark:Hexane vs. Roots:Aqueous	-3.335	-27.64 to 20.96	No	ns	0.8931
Bark:Hexane vs. Roots:Methanol	-26.62	-38.76 to -14.49	Yes	*	0.0125
Bark:Hexane vs. Roots:Ethyl acetate	-51.97	-68.70 to -35.23	Yes	**	0.0059
Bark:Hexane vs. Roots:Hexane	18.74	13.32 to 24.16	Yes	**	0.0034
Bark:Hexane vs. Fruit:Aqueous	-60.34	-63.31 to -57.36	Yes	****	<0.0001

Bark:Hexane vs. Fruit:Methanol	17.22	12.44 to 22.00	Yes	**	0.0026
Bark:Hexane vs. Fruit:Ethyl acetate	1.031	-10.87 to 12.93	No	ns	0.9898
Bark:Hexane vs. Fruit:Hexane	30.15	28.27 to 32.03	Yes	****	<0.0001
Roots:Aqueous vs. Roots:Methanol	-23.29	-59.17 to 12.60	No	ns	0.1138
Roots:Aqueous vs. Roots:Ethyl acetate	-48.63	-83.25 to -14.01	Yes	*	0.0250
Roots:Aqueous vs. Roots:Hexane	22.07	-1.408 to 45.55	No	ns	0.0562
Roots:Aqueous vs. Fruit:Aqueous	-57.00	-84.13 to -29.87	Yes	*	0.0130
Roots:Aqueous vs. Fruit:Methanol	20.56	0.04222 to 41.07	Yes	*	0.0498
Roots:Aqueous vs. Fruit:Ethyl acetate	4.366	-9.270 to 18.00	No	ns	0.3814
Roots:Aqueous vs. Fruit:Hexane	33.48	8.259 to 58.71	Yes	*	0.0285
Roots:Methanol vs. Roots:Ethyl acetate	-25.35	-37.19 to -13.50	Yes	*	0.0128
Roots:Methanol vs. Roots:Hexane	45.36	32.60 to 58.11	Yes	**	0.0028
Roots:Methanol vs. Fruit:Aqueous	-33.72	-43.77 to -23.66	Yes	**	0.0041
Roots:Methanol vs. Fruit:Methanol	43.84	28.47 to 59.21	Yes	**	0.0080
Roots:Methanol vs. Fruit:Ethyl acetate	27.65	5.092 to 50.21	Yes	*	0.0338
Roots:Methanol vs. Fruit:Hexane	56.77	45.98 to 67.56	Yes	****	<0.0001
Roots:Ethyl acetate vs. Roots:Hexane	70.71	57.73 to 83.68	Yes	****	<0.0001
Roots:Ethyl acetate vs. Fruit:Aqueous	-8.368	-25.13 to 8.396	No	ns	0.1834
Roots:Ethyl acetate vs. Fruit:Methanol	69.19	52.62 to 85.76	Yes	***	0.0007
Roots:Ethyl acetate vs. Fruit:Ethyl acetate	53.00	31.70 to 74.29	Yes	*	0.0109
Roots:Ethyl acetate vs. Fruit:Hexane	82.12	67.26 to 96.98	Yes	****	<0.0001
Roots:Hexane vs. Fruit:Aqueous	-79.07	-86.58 to -71.56	Yes	****	<0.0001
Roots:Hexane vs. Fruit:Methanol	-1.516	-5.128 to 2.096	No	ns	0.2476
Roots:Hexane vs. Fruit:Ethyl acetate	-17.71	-27.62 to -7.790	Yes	*	0.0153
Roots:Hexane vs. Fruit:Hexane	11.41	7.178 to 15.65	Yes	**	0.0093
Fruit:Aqueous vs. Fruit:Methanol	77.56	69.86 to 85.25	Yes	****	<0.0001
Fruit:Aqueous vs. Fruit:Ethyl acetate	61.37	46.49 to 76.25	Yes	***	0.0008
Fruit:Aqueous vs. Fruit:Hexane	90.49	87.21 to 93.76	Yes	****	<0.0001
Fruit:Methanol vs. Fruit:Ethyl acetate	-16.19	-23.57 to -8.806	Yes	*	0.0125
Fruit:Methanol vs. Fruit:Hexane	12.93	7.993 to 17.86	Yes	**	0.0099
Fruit:Ethyl acetate vs. Fruit:Hexane	29.12	16.80 to 41.44	Yes	*	0.0116

Appendix 8: Tukey's multiple comparisons test of TPC of *Pterocarpus angolensis* crude extracts.

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**
Leaves:Aqueous vs. Leaves:Methanol	-15.77	-46.50 to 14.97	No	ns	0.1743
Leaves:Aqueous vs. Leaves:Ethyl acetate	-2.426	-20.28 to 15.43	No	ns	0.8869
Leaves:Aqueous vs. Leaves:Hexane	47.12	23.23 to 71.01	Yes	*	0.0136
Leaves:Aqueous vs. Bark:Aqueous	-96.96	-109.6 to -84.32	Yes	****	<0.0001
Leaves:Aqueous vs. Bark:Methanol	-166.1	-178.1 to -154.0	Yes	****	<0.0001
Leaves:Aqueous vs. Bark:Ethyl acetate	6.003	-28.33 to 40.33	No	ns	0.7610
Leaves:Aqueous vs. Bark:Hexane	36.32	20.73 to 51.91	Yes	*	0.0114
Leaves:Aqueous vs. Roots:Aqueous	-71.37	-82.62 to -60.12	Yes	****	<0.0001
Leaves:Aqueous vs. Roots:Methanol	-69.19	-85.64 to -52.74	Yes	***	0.0008
Leaves:Aqueous vs. Roots:Ethyl acetate	-52.88	-86.35 to -19.41	Yes	*	0.0196
Leaves:Aqueous vs. Roots:Hexane	15.71	-5.014 to 36.43	No	ns	0.0849
Leaves:Methanol vs. Leaves:Ethyl acetate	13.34	-21.05 to 47.73	No	ns	0.2808
Leaves:Methanol vs. Leaves:Hexane	62.88	49.18 to 76.59	Yes	***	0.0003

Leaves:Methanol vs. Bark:Aqueous	-81.20	-109.3 to -53.07	Yes	**	0.0075
Leaves:Methanol vs. Bark:Methanol	-150.3	-193.0 to -107.7	Yes	**	0.0032
Leaves:Methanol vs. Bark:Ethyl acetate	21.77	-17.14 to 60.68	No	ns	0.1492
Leaves:Methanol vs. Bark:Hexane	52.09	34.27 to 69.91	Yes	**	0.0072
Leaves:Methanol vs. Roots:Aqueous	-55.61	-78.19 to -33.03	Yes	*	0.0105
Leaves:Methanol vs. Roots:Methanol	-53.42	-76.21 to -30.63	Yes	*	0.0113
Leaves:Methanol vs. Roots:Ethyl acetate	-37.11	-65.08 to -9.144	Yes	*	0.0286
Leaves:Methanol vs. Roots:Hexane	31.47	6.645 to 56.30	Yes	*	0.0314
Leaves:Ethyl acetate vs. Leaves:Hexane	49.54	27.89 to 71.20	Yes	*	0.0116
Leaves:Ethyl acetate vs. Bark:Aqueous	-94.54	-101.8 to -87.24	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Bark:Methanol	-163.7	-183.9 to -143.4	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Bark:Ethyl acetate	8.429	-8.914 to 25.77	No	ns	0.1917
Leaves:Ethyl acetate vs. Bark:Hexane	38.75	21.66 to 55.83	Yes	*	0.0117
Leaves:Ethyl acetate vs. Roots:Aqueous	-68.95	-82.64 to -55.26	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Methanol	-66.76	-78.36 to -55.16	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Roots:Ethyl acetate	-50.45	-71.72 to -29.19	Yes	*	0.0111
Leaves:Ethyl acetate vs. Roots:Hexane	18.13	-18.05 to 54.31	No	ns	0.1817
Leaves:Hexane vs. Bark:Aqueous	-144.1	-160.6 to -127.5	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Methanol	-213.2	-247.5 to -178.9	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Ethyl acetate	-41.11	-66.61 to -15.62	Yes	*	0.0188
Leaves:Hexane vs. Bark:Hexane	-10.79	-19.09 to -2.494	Yes	*	0.0298
Leaves:Hexane vs. Roots:Aqueous	-118.5	-131.5 to -105.5	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Methanol	-116.3	-126.7 to -105.9	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Ethyl acetate	-99.99	-116.0 to -84.02	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Hexane	-31.41	-59.83 to -2.990	Yes	*	0.0412
Bark:Aqueous vs. Bark:Methanol	-69.13	-88.40 to -49.86	Yes	**	0.0029
Bark:Aqueous vs. Bark:Ethyl acetate	103.0	81.13 to 124.8	Yes	***	0.0002
Bark:Aqueous vs. Bark:Hexane	133.3	122.9 to 143.6	Yes	****	<0.0001
Bark:Aqueous vs. Roots:Aqueous	25.59	19.17 to 32.01	Yes	**	0.0014
Bark:Aqueous vs. Roots:Methanol	27.77	21.54 to 34.01	Yes	***	0.0005
Bark:Aqueous vs. Roots:Ethyl acetate	44.08	22.60 to 65.57	Yes	*	0.0130
Bark:Aqueous vs. Roots:Hexane	112.7	83.54 to 141.8	Yes	**	0.0017
Bark:Methanol vs. Bark:Ethyl acetate	172.1	134.6 to 209.6	Yes	***	0.0003
Bark:Methanol vs. Bark:Hexane	202.4	176.1 to 228.7	Yes	****	<0.0001
Bark:Methanol vs. Roots:Aqueous	94.72	73.34 to 116.1	Yes	***	0.0005
Bark:Methanol vs. Roots:Methanol	96.90	71.85 to 122.0	Yes	**	0.0017
Bark:Methanol vs. Roots:Ethyl acetate	113.2	72.77 to 153.7	Yes	**	0.0082
Bark:Methanol vs. Roots:Hexane	181.8	151.1 to 212.5	Yes	****	<0.0001
Bark:Ethyl acetate vs. Bark:Hexane	30.32	3.643 to 57.00	Yes	*	0.0391
Bark:Ethyl acetate vs. Roots:Aqueous	-77.38	-103.7 to -51.06	Yes	**	0.0071
Bark:Ethyl acetate vs. Roots:Methanol	-75.19	-96.17 to -54.21	Yes	**	0.0029
Bark:Ethyl acetate vs. Roots:Ethyl acetate	-58.88	-71.73 to -46.04	Yes	***	0.0003
Bark:Ethyl acetate vs. Roots:Hexane	9.702	-39.88 to 59.29	No	ns	0.6917
Bark:Hexane vs. Roots:Aqueous	-107.7	-112.7 to -102.7	Yes	****	<0.0001
Bark:Hexane vs. Roots:Methanol	-105.5	-111.6 to -99.41	Yes	****	<0.0001
Bark:Hexane vs. Roots:Ethyl acetate	-89.20	-109.9 to -68.45	Yes	***	0.0007
Bark:Hexane vs. Roots:Hexane	-20.62	-43.88 to 2.643	No	ns	0.0630

Roots:Aqueous vs. Roots:Methanol	2.183	-3.617 to 7.983	No	ns	0.2949
Roots:Aqueous vs. Roots:Ethyl acetate	18.49	-4.349 to 41.34	No	ns	0.0749
Roots:Aqueous vs. Roots:Hexane	87.08	63.64 to 110.5	Yes	**	0.0023
Roots:Methanol vs. Roots:Ethyl acetate	16.31	-0.8215 to 33.45	No	ns	0.0549
Roots:Methanol vs. Roots:Hexane	84.89	56.28 to 113.5	Yes	**	0.0069
Roots:Ethyl acetate vs. Roots:Hexane	68.58	24.92 to 112.2	Yes	*	0.0198

Appendices 8.2: Antioxidant activity

Appendix 9: Antioxidant activity of *K. africana* leaves, bark, roots, and fruit extracts. Values are expressed as mean ± SE.

Plant Species	**Plant part**	**Solvent**	**% DPPH scavenging capacity (1mg/mL)**	**% FRAP activity (1mg/mL)**	**FRAP IC_{50} (mg/mL)**	**% TAC inhibition**
K. africana	Leaves	Aqueous	76.2 ± 0.3	89.2 ± 0.253	0.144 ± 0.01	91.4 ± 0.06
		Methanol	85.5 ± 0.05	95.0 ± 0.023	0.048 ± 0.0004	94.0 ± 0.4
		Ethyl acetate	50.9 ± 1.2	93.1 ± 0.1	0.076 ± 0.005	94.2 ± 0.4
		Hexane	41.3 ± 0.0	70.0 ± 1.0	0.570 ± 0.04	85.4 ± 0.3
	Bark	Aqueous	86.7 ± 0.2	94.7±0.24	0.045 ± 0.003	90.7 ± 0.7
		Methanol	88.0 ± 0.0	97.4 ± 0.018	0.027 ± 0.0005	94.7 ± 0.4
		Ethyl acetate	88.2 ± 0.1	97.4 ± 0.01	0.030 ± 0.001	94.6 ± 0.4
		Hexane	42.8 ± 0.2	56.0 ± 2.8	0.912 ±0.03	77.3 ± 0.7
	Roots	Aqueous	85.4 ± 0.1	94.6 ± 0.16	0.045 ±0.004	90.1 ± 0.1
		Methanol	87.8 ± 0.1	97.3 ± 0.1	0.031 ±0.0008	94.2 ± 0.5
		Ethyl acetate	89.1 ± 0.1	97.1 ± 0.04	0.036 ± 0.001	93.0 ± 0.3
		Hexane	47.5 ± 1.9	74.8 ± 1.6	0.479 ±0.09	81.1 ± 1.2
	Fruit	Aqueous	42.5 ± 0.2	84.0 ± 0.7	0.148 ± 0.01	88.0 ± 1.2
		Methanol	36.6 ± 0.3	73.8 ± 1.0	0.431 ± 0.018	90.0 ± 0.5
		Ethyl acetate	32.8 ± 1.5	77.4 ± 0.7	0.333±0.007	82.0 ± 0.5
		Hexane	33.8 ± 1.0	76.8 ± 0.5	0.549 ± 0.167	89.0 ± 0.3
	Control	Gallic acid	92.7 ± 0.0	98.5 ± 0.06	0.002 ±0.00002	89.8 ± 0.06

Appendix 10: Two-way repeated measure ANOVA analysis summary for % DPPH scavenging activity of *K. africana* crude extract. Comparison of mean was performed using Tukey's multiple comparisons tests

Source of Variation	% of total variation	P value	P value summary	Significant?
Row Factor	31.30	<0.0001	****	Yes
Column Factor	49.36	<0.0001	****	Yes
Row Factor x Column Factor	19.18	0.0006	***	Yes
Subject x Row Factor	0.02478			
Subject x Column Factor	0.007844			
Subject	0.004601			

Appendix 11: Tukey's multiple comparisons test for % DPPH scavenging activity of *K. africana* crude extracts.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Leaves:Aqueous vs. Leaves:Methanol	-9.343	-12.95 to -5.737	Yes	*	0.0105
Leaves:Aqueous vs. Leaves:Ethyl acetate	25.26	7.673 to 42.84	Yes	*	0.0236
Leaves:Aqueous vs. Leaves:Hexane	34.84	31.33 to 38.35	Yes	****	<0.0001
Leaves:Aqueous vs. Leaves:Control	-16.50	-20.10 to -12.89	Yes	***	0.0002
Leaves:Aqueous vs. Bark:Aqueous	-10.56	-13.77 to -7.344	Yes	**	0.0045
Leaves:Aqueous vs. Bark:Methanol	-11.87	-15.39 to -8.361	Yes	**	0.0037
Leaves:Aqueous vs. Bark:Ethyl acetate	-12.07	-14.59 to -9.551	Yes	****	<0.0001
Leaves:Aqueous vs. Bark:Hexane	33.38	32.23 to 34.54	Yes	****	<0.0001
Leaves:Aqueous vs. Bark:Control	-16.50	-20.10 to -12.89	Yes	***	0.0002
Leaves:Aqueous vs. Roots:Aqueous	-9.197	-11.84 to -6.551	Yes	**	0.0031
Leaves:Aqueous vs. Roots:Methanol	-11.63	-15.42 to -7.843	Yes	**	0.0063
Leaves:Aqueous vs. Roots:Ethyl acetate	-12.94	-15.03 to -10.86	Yes	****	<0.0001
Leaves:Aqueous vs. Roots:Hexane	28.61	8.090 to 49.14	Yes	*	0.0254
Leaves:Aqueous vs. Roots:Control	-16.50	-20.10 to -12.89	Yes	***	0.0002
Leaves:Aqueous vs. Fruit:Aqueous	33.62	32.17 to 35.07	Yes	****	<0.0001
Leaves:Aqueous vs. Fruit:Methanol	39.56	33.87 to 45.24	Yes	****	<0.0001
Leaves:Aqueous vs. Fruit:Ethyl acetate	43.36	28.55 to 58.16	Yes	**	0.0076
Leaves:Aqueous vs. Fruit:Hexane	42.36	28.32 to 56.39	Yes	**	0.0067
Leaves:Aqueous vs. Fruit:Control	-16.50	-20.10 to -12.89	Yes	***	0.0002
Leaves:Methanol vs. Leaves:Ethyl acetate	34.60	20.60 to 48.60	Yes	*	0.0113
Leaves:Methanol vs. Leaves:Hexane	44.18	43.61 to 44.76	Yes	****	<0.0001
Leaves:Methanol vs. Leaves:Control	-7.153	-7.153 to -7.153	Yes	****	<0.0001
Leaves:Methanol vs. Bark:Aqueous	-1.217	-2.744 to 0.3113	No	ns	0.0774
Leaves:Methanol vs. Bark:Methanol	-2.530	-3.108 to -1.953	Yes	***	0.0003
Leaves:Methanol vs. Bark:Ethyl acetate	-2.725	-4.253 to -1.197	Yes	*	0.0150
Leaves:Methanol vs. Bark:Hexane	42.73	39.84 to 45.61	Yes	****	<0.0001
Leaves:Methanol vs. Bark:Control	-7.153	-7.153 to -7.153	Yes	****	<0.0001

Leaves:Methanol vs. Roots:Aqueous	0.1460	-0.8543 to 1.146	No	ns	0.8732
Leaves:Methanol vs. Roots:Methanol	-2.287	-2.865 to -1.710	Yes	***	0.0010
Leaves:Methanol vs. Roots:Ethyl acetate	-3.601	-5.129 to -2.073	Yes	*	0.0120
Leaves:Methanol vs. Roots:Hexane	37.96	14.89 to 61.03	Yes	*	0.0172
Leaves:Methanol vs. Roots:Control	-7.153	-7.153 to -7.153	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Aqueous	42.97	40.69 to 45.24	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Methanol	48.90	45.84 to 51.96	Yes	****	<0.0001
Leaves:Methanol vs. Fruit:Ethyl acetate	52.70	34.50 to 70.90	Yes	**	0.0079
Leaves:Methanol vs. Fruit:Hexane	51.70	40.11 to 63.29	Yes	***	0.0002
Leaves:Methanol vs. Fruit:Control	-7.153	-7.153 to -7.153	Yes	****	<0.0001
Leaves:Ethyl acetate vs. Leaves:Hexane	9.586	-4.642 to 23.81	No	ns	0.1062
Leaves:Ethyl acetate vs. Leaves:Control	-41.75	-55.76 to -27.75	Yes	**	0.0071
Leaves:Ethyl acetate vs. Bark:Aqueous	-35.82	-50.39 to -21.24	Yes	*	0.0114
Leaves:Ethyl acetate vs. Bark:Methanol	-37.13	-51.36 to -22.90	Yes	*	0.0103
Leaves:Ethyl acetate vs. Bark:Ethyl acetate	-37.32	-52.70 to -21.95	Yes	*	0.0116
Leaves:Ethyl acetate vs. Bark:Hexane	8.127	-8.740 to 24.99	No	ns	0.1957
Leaves:Ethyl acetate vs. Bark:Control	-41.75	-55.76 to -27.75	Yes	**	0.0071
Leaves:Ethyl acetate vs. Roots:Aqueous	-34.45	-49.39 to -19.52	Yes	*	0.0123
Leaves:Ethyl acetate vs. Roots:Methanol	-36.89	-50.69 to -23.09	Yes	**	0.0098
Leaves:Ethyl acetate vs. Roots:Ethyl acetate	-38.20	-53.71 to -22.69	Yes	*	0.0114
Leaves:Ethyl acetate vs. Roots:Hexane	3.358	-32.20 to 38.92	No	ns	0.9854
Leaves:Ethyl acetate vs. Roots:Control	-41.75	-55.76 to -27.75	Yes	**	0.0071
Leaves:Ethyl acetate vs. Fruit:Aqueous	8.367	-7.907 to 24.64	No	ns	0.1744
Leaves:Ethyl acetate vs. Fruit:Methanol	14.30	1.785 to 26.82	Yes	*	0.0391
Leaves:Ethyl acetate vs. Fruit:Ethyl acetate	18.10	-13.31 to 49.52	No	ns	0.1419
Leaves:Ethyl acetate vs. Fruit:Hexane	17.10	6.935 to 27.27	Yes	*	0.0165
Leaves:Ethyl acetate vs. Fruit:Control	-41.75	-55.76 to -27.75	Yes	**	0.0071
Leaves:Hexane vs. Leaves:Control	-51.34	-51.92 to -50.76	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Aqueous	-45.40	-47.40 to -43.40	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Methanol	-46.72		Yes	****	<0.0001
Leaves:Hexane vs. Bark:Ethyl acetate	-46.91	-48.06 to -45.75	Yes	****	<0.0001
Leaves:Hexane vs. Bark:Hexane	-1.460	-4.106 to 1.187	No	ns	0.1536

Leaves:Hexane vs. Bark:Control	-51.34	-51.92 to -50.76	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Aqueous	-44.04	-45.19 to -42.88	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Methanol	-46.47	-47.63 to -45.32	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Ethyl acetate	-47.79	-49.31 to -46.26	Yes	****	<0.0001
Leaves:Hexane vs. Roots:Hexane	-6.229	-28.82 to 16.37	No	ns	0.4748
Leaves:Hexane vs. Roots:Control	-51.34	-51.92 to -50.76	Yes	****	<0.0001
Leaves:Hexane vs. Fruit:Aqueous	-1.219	-3.313 to 0.8738	No	ns	0.1391
Leaves:Hexane vs. Fruit:Methanol	4.714	1.088 to 8.339	Yes	*	0.0300
Leaves:Hexane vs. Fruit:Ethyl acetate	8.514	-9.730 to 26.76	No	ns	0.2069
Leaves:Hexane vs. Fruit:Hexane	7.514	-4.608 to 19.64	No	ns	0.1240
Leaves:Hexane vs. Fruit:Control	-51.34	-51.92 to -50.76	Yes	****	<0.0001
Leaves:Control vs. Bark:Aqueous	5.937	4.409 to 7.465	Yes	**	0.0012
Leaves:Control vs. Bark:Methanol	4.623	4.045 to 5.200	Yes	****	<0.0001
Leaves:Control vs. Bark:Ethyl acetate	4.428	2.900 to 5.956	Yes	**	0.0078
Leaves:Control vs. Bark:Hexane	49.88	46.99 to 52.77	Yes	****	<0.0001
Leaves:Control vs. Bark:Control	0.000				
Leaves:Control vs. Roots:Aqueous	7.299	6.299 to 8.300	Yes	****	<0.0001
Leaves:Control vs. Roots:Methanol	4.866	4.289 to 5.444	Yes	****	<0.0001
Leaves:Control vs. Roots:Ethyl acetate	3.552	2.024 to 5.080	Yes	*	0.0122
Leaves:Control vs. Roots:Hexane	45.11	22.04 to 68.18	Yes	*	0.0137
Leaves:Control vs. Roots:Control	0.000				
Leaves:Control vs. Fruit:Aqueous	50.12	47.84 to 52.39	Yes	****	<0.0001
Leaves:Control vs. Fruit:Methanol	56.05	52.99 to 59.11	Yes	****	<0.0001
Leaves:Control vs. Fruit:Ethyl acetate	59.85	41.66 to 78.05	Yes	**	0.0044
Leaves:Control vs. Fruit:Hexane	58.85	47.27 to 70.44	Yes	****	<0.0001
Leaves:Control vs. Fruit:Control	0.000				
Bark:Aqueous vs. Bark:Methanol	-1.314	-3.314 to 0.6866	No	ns	0.1114
Bark:Aqueous vs. Bark:Ethyl acetate	-1.509	-3.818 to 0.8014	No	ns	0.1126
Bark:Aqueous vs. Bark:Hexane	43.94	40.94 to 46.94	Yes	****	<0.0001
Bark:Aqueous vs. Bark:Control	-5.937	-7.465 to -4.409	Yes	**	0.0012
Bark:Aqueous vs. Roots:Aqueous	1.363	0.2076 to 2.517	Yes	*	0.0368
Bark:Aqueous vs. Roots:Methanol	-1.071	-2.226 to 0.08442	No	ns	0.0577
Bark:Aqueous vs. Roots:Ethyl acetate	-2.384	-3.912 to -0.8566	Yes	*	0.0192
Bark:Aqueous vs. Roots:Hexane	39.17	15.67 to 62.68	Yes	*	0.0167
Bark:Aqueous vs. Roots:Control	-5.937	-7.465 to -4.409	Yes	**	0.0012
Bark:Aqueous vs. Fruit:Aqueous	44.18	41.81 to 46.56	Yes	****	<0.0001
Bark:Aqueous vs. Fruit:Methanol	50.12	47.63 to 52.60	Yes	****	<0.0001
Bark:Aqueous vs. Fruit:Ethyl acetate	53.92	36.76 to 71.07	Yes	**	0.0056
Bark:Aqueous vs. Fruit:Hexane	52.92	41.93 to 63.91	Yes	****	<0.0001
Bark:Aqueous vs. Fruit:Control	-5.937	-7.465 to -4.409	Yes	**	0.0012
Bark:Methanol vs. Bark:Ethyl acetate	-0.1946	-1.350 to 0.9603	No	ns	0.7987
Bark:Methanol vs. Bark:Hexane	45.26	42.61 to 47.90	Yes	****	<0.0001

Bark:Methanol vs. Bark:Control	-4.623	-5.200 to -4.045	Yes	****	<0.0001
Bark:Methanol vs. Roots:Aqueous	2.676	1.521 to 3.831	Yes	*	0.0122
Bark:Methanol vs. Roots:Methanol	0.2433	-0.9117 to 1.398	No	ns	0.6562
Bark:Methanol vs. Roots:Ethyl acetate	-1.071	-2.598 to 0.4574	No	ns	0.0987
Bark:Methanol vs. Roots:Hexane	40.49	17.89 to 63.08	Yes	*	0.0149
Bark:Methanol vs. Roots:Control	-4.623	-5.200 to -4.045	Yes	****	<0.0001
Bark:Methanol vs. Fruit:Aqueous	45.50	43.40 to 47.59	Yes	****	<0.0001
Bark:Methanol vs. Fruit:Methanol	51.43	47.80 to 55.05	Yes	****	<0.0001
Bark:Methanol vs. Fruit:Ethyl acetate	55.23	36.99 to 73.47	Yes	**	0.0067
Bark:Methanol vs. Fruit:Hexane	54.23	42.11 to 66.35	Yes	***	0.0002
Bark:Methanol vs. Fruit:Control	-4.623	-5.200 to -4.045	Yes	****	<0.0001
Bark:Ethyl acetate vs. Bark:Hexane	45.45	43.92 to 46.98	Yes	****	<0.0001
Bark:Ethyl acetate vs. Bark:Control	-4.428	-5.956 to -2.900	Yes	**	0.0078
Bark:Ethyl acetate vs. Roots:Aqueous	2.871	1.716 to 4.026	Yes	*	0.0112
Bark:Ethyl acetate vs. Roots:Methanol	0.4380	-1.563 to 2.438	No	ns	0.6299
Bark:Ethyl acetate vs. Roots:Ethyl acetate	-0.8759	-1.876 to 0.1244	No	ns	0.0644
Bark:Ethyl acetate vs. Roots:Hexane	40.68	19.13 to 62.23	Yes	*	0.0141
Bark:Ethyl acetate vs. Roots:Control	-4.428	-5.956 to -2.900	Yes	**	0.0078
Bark:Ethyl acetate vs. Fruit:Aqueous	45.69	44.62 to 46.76	Yes	****	<0.0001
Bark:Ethyl acetate vs. Fruit:Methanol	51.62	47.16 to 56.09	Yes	****	<0.0001
Bark:Ethyl acetate vs. Fruit:Ethyl acetate	55.42	38.11 to 72.74	Yes	**	0.0051
Bark:Ethyl acetate vs. Fruit:Hexane	54.42	41.38 to 67.47	Yes	***	0.0006
Bark:Ethyl acetate vs. Fruit:Control	-4.428	-5.956 to -2.900	Yes	**	0.0078
Bark:Hexane vs. Bark:Control	-49.88	-52.77 to -46.99	Yes	****	<0.0001
Bark:Hexane vs. Roots:Aqueous	-42.58	-44.66 to -40.50	Yes	****	<0.0001
Bark:Hexane vs. Roots:Methanol	-45.01	-48.23 to -41.80	Yes	****	<0.0001
Bark:Hexane vs. Roots:Ethyl acetate	-46.33	-47.85 to -44.80	Yes	****	<0.0001
Bark:Hexane vs. Roots:Hexane	-4.769	-25.28 to 15.74	No	ns	0.5886
Bark:Hexane vs. Roots:Control	-49.88	-52.77 to -46.99	Yes	****	<0.0001
Bark:Hexane vs. Fruit:Aqueous	0.2404	-0.4074 to 0.8882	No	ns	0.3044
Bark:Hexane vs. Fruit:Methanol	6.174	0.7200 to 11.63	Yes	*	0.0398
Bark:Hexane vs. Fruit:Ethyl acetate	9.974	-5.905 to 25.85	No	ns	0.1210
Bark:Hexane vs. Fruit:Hexane	8.974	-5.017 to 22.96	No	ns	0.1164
Bark:Hexane vs. Fruit:Control	-49.88	-52.77 to -46.99	Yes	****	<0.0001
Bark:Control vs. Roots:Aqueous	7.299	6.299 to 8.300	Yes	****	<0.0001
Bark:Control vs. Roots:Methanol	4.866	4.289 to 5.444	Yes	****	<0.0001
Bark:Control vs. Roots:Ethyl acetate	3.552	2.024 to 5.080	Yes	*	0.0122
Bark:Control vs. Roots:Hexane	45.11	22.04 to 68.18	Yes	*	0.0137
Bark:Control vs. Roots:Control	0.000				
Bark:Control vs. Fruit:Aqueous	50.12	47.84 to 52.39	Yes	****	<0.0001

Bark:Control vs. Fruit:Methanol	56.05	52.99 to 59.11	Yes	****	<0.0001
Bark:Control vs. Fruit:Ethyl acetate	59.85	41.66 to 78.05	Yes	**	0.0044
Bark:Control vs. Fruit:Hexane	58.85	47.27 to 70.44	Yes	****	<0.0001
Bark:Control vs. Fruit:Control	0.000				
Roots:Aqueous vs. Roots:Methanol	-2.433	-3.588 to -1.278	Yes	*	0.0131
Roots:Aqueous vs. Roots:Ethyl acetate	-3.747	-4.324 to -3.169	Yes	****	<0.0001
Roots:Aqueous vs. Roots:Hexane	37.81	15.29 to 60.33	Yes	*	0.0165
Roots:Aqueous vs. Roots:Control	-7.299	-8.300 to -6.299	Yes	****	<0.0001
Roots:Aqueous vs. Fruit:Aqueous	42.82	41.38 to 44.25	Yes	****	<0.0001
Roots:Aqueous vs. Fruit:Methanol	48.75	45.33 to 52.18	Yes	****	<0.0001
Roots:Aqueous vs. Fruit:Ethyl acetate	52.55	35.35 to 69.75	Yes	**	0.0064
Roots:Aqueous vs. Fruit:Hexane	51.55	39.55 to 63.56	Yes	***	0.0004
Roots:Aqueous vs. Fruit:Control	-7.299	-8.300 to -6.299	Yes	****	<0.0001
Roots:Methanol vs. Roots:Ethyl acetate	-1.314	-3.046 to 0.4186	No	ns	0.0849
Roots:Methanol vs. Roots:Hexane	40.24	16.69 to 63.79	Yes	*	0.0160
Roots:Methanol vs. Roots:Control	-4.866	-5.444 to -4.289	Yes	****	<0.0001
Roots:Methanol vs. Fruit:Aqueous	45.25	42.68 to 47.83	Yes	****	<0.0001
Roots:Methanol vs. Fruit:Methanol	51.19	48.68 to 53.69	Yes	****	<0.0001
Roots:Methanol vs. Fruit:Ethyl acetate	54.99	36.82 to 73.15	Yes	**	0.0067
Roots:Methanol vs. Fruit:Hexane	53.99	42.93 to 65.04	Yes	****	<0.0001
Roots:Methanol vs. Fruit:Control	-4.866	-5.444 to -4.289	Yes	****	<0.0001
Roots:Ethyl acetate vs. Roots:Hexane	41.56	19.54 to 63.57	Yes	*	0.0141
Roots:Ethyl acetate vs. Roots:Control	-3.552	-5.080 to -2.024	Yes	*	0.0122
Roots:Ethyl acetate vs. Fruit:Aqueous	46.57	45.69 to 47.45	Yes	****	<0.0001
Roots:Ethyl acetate vs. Fruit:Methanol	52.50	48.57 to 56.43	Yes	****	<0.0001
Roots:Ethyl acetate vs. Fruit:Ethyl acetate	56.30	39.58 to 73.02	Yes	**	0.0038
Roots:Ethyl acetate vs. Fruit:Hexane	55.30	42.80 to 67.79	Yes	***	0.0003
Roots:Ethyl acetate vs. Fruit:Control	-3.552	-5.080 to -2.024	Yes	*	0.0122
Roots:Hexane vs. Roots:Control	-45.11	-68.18 to -22.04	Yes	*	0.0137
Roots:Hexane vs. Fruit:Aqueous	5.009	-16.13 to 26.15	No	ns	0.5757
Roots:Hexane vs. Fruit:Methanol	10.94	-15.00 to 36.89	No	ns	0.2463
Roots:Hexane vs. Fruit:Ethyl acetate	14.74	-6.163 to 35.65	No	ns	0.0975
Roots:Hexane vs. Fruit:Hexane	13.74	-20.75 to 48.24	No	ns	0.2710
Roots:Hexane vs. Fruit:Control	-45.11	-68.18 to -22.04	Yes	*	0.0137
Roots:Control vs. Fruit:Aqueous	50.12	47.84 to 52.39	Yes	****	<0.0001
Roots:Control vs. Fruit:Methanol	56.05	52.99 to 59.11	Yes	****	<0.0001
Roots:Control vs. Fruit:Ethyl acetate	59.85	41.66 to 78.05	Yes	**	0.0044
Roots:Control vs. Fruit:Hexane	58.85	47.27 to 70.44	Yes	****	<0.0001
Roots:Control vs. Fruit:Control	0.000				
Fruit:Aqueous vs. Fruit:Methanol	5.933	1.121 to 10.75	Yes	*	0.0336

Fruit:Aqueous vs. Fruit:Ethyl acetate	9.733	-6.514 to 25.98	No	ns	0.1321
Fruit:Aqueous vs. Fruit:Hexane	8.733	-4.629 to 22.10	No	ns	0.1124
Fruit:Aqueous vs. Fruit:Control	-50.12	-52.39 to -47.84	Yes	****	<0.0001
Fruit:Methanol vs. Fruit:Ethyl acetate	3.800	-15.13 to 22.73	No	ns	0.6886
Fruit:Methanol vs. Fruit:Hexane	2.800	-5.785 to 11.39	No	ns	0.3726
Fruit:Methanol vs. Fruit:Control	-56.05	-59.11 to -52.99	Yes	****	<0.0001
Fruit:Ethyl acetate vs. Fruit:Hexane	-1.000	-25.52 to 23.52	No	ns	>0.9999
Fruit:Ethyl acetate vs. Fruit:Control	-59.85	-78.05 to -41.66	Yes	**	0.0044
Fruit:Hexane vs. Fruit:Control	-58.85	-70.44 to -47.27	Yes	****	<0.0001

Appendix 12: Antioxidant activity of *P. angolensis* leaves, bark, roots, and fruit extracts. Values are expressed as mean ± SE.

Plant Species	**Plant part**	**Solvent**	**% DPPH scavenging capacity (1mg/mL)**	**DPPH IC_{50} (mg/mL)**	**% FRAP activity (1mg/mL)**	**FRAP IC_{50} (mg/mL)**	**% TAC inhibition**
P. angolensis	Leaves	Aqueous	70.4 ± 0.5	0.094 ± 0.01	70.4 ± 0.9	0.084 ± 0.02	83.6 ± 0.4
		Methanol	80.5 ± 0.1	0.061 ± 0.003	80.5 ± 0.2	0.063 ± 0.007	92.6 ± 0.004
		Ethyl acetate	75.4 ± 0.6	0.058 ± 0.004	75.4 ± 1	0.064 ± 0.003	92.2 ± 0.05
		Hexane	42.5 ± 0.7	_	42.5 ± 1	0.908 ± 0.03	78.9 ± 0.3
	Bark	Aqueous	85.8 ± 0.6	0.007 ± 0.001	60.6 ± 1.7	0.013 ± 0.001	96.4 ± 0.07
		Methanol	87.2 ± 0.05	0.003 ± 0.003	88.1 ± 0.3	0.012 ± 0.05	96.0 ± 0.02
		Ethyl acetate	72.3 ± 0.6	0.106 ± 0.003	41.9 ± 0.6	_	55.4 ± 0.6
		Hexane	42.2 ± 0.4	0.105 ± 0.003	48.3 ± 3	0.405 ± 0.1	85.5 ± 0.2
	Roots	Aqueous	63.4 ± 0.8	0.056 ± 0.002	55.9 ± 1	0.034 ± 0.001	95.1 ± 0.9
		Methanol	85.4 ± 0.5	0.026 ± 0.001	84.0 ± 3	0.034 ± 0.003	92.5 ± 0.3
		Ethyl acetate	73.9 ± 0.9	0.050 ± 0.001	84.7 ± 2	0.035 ± 0.008	94.0 ± 0.2
		Hexane	43.2 ± 0.3	0.146 ± 0.04	43.9 ± 1	0.0429 ± 0.045	89.4 ± 0.3
	Control	Gallic acid	92.7 ± 0.0	0.002 ± 0.00008	98.5 ± 0.06	0.002 ±0.00002	89.8 ± 0.06

Appendix 13: Two-way repeated measure ANOVA analysis Phosphomolybdenum inhibition TAC (mg AAE/g) of *P. angolensis* plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests

Source of Variation	% of total variation	P-value	P-value summary	Significant?
Row Factor	38.98	<0.0001	****	Yes
Column Factor	39.31	<0.0001	****	Yes
Row Factor x Column Factor	21.58	<0.0001	****	Yes
Subject x Row Factor	0.005756			
Subject x Column Factor	0.02316			
Subject	0.01701			

Appendix 14: Two-way repeated measure ANOVA analysis of % PhosphomolybdenumTAC (mg AAE/g) of *K. africana* plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests

Source of Variation	% of total variation	P-value	P-value summary	Significant?
Row Factor	17.73	0.0019	**	Yes
Column Factor	52.45	0.0024	**	Yes
Row Factor x Column Factor	28.09	0.0023	**	Yes
Subject x Row Factor	0.2078			
Subject x Column Factor	0.7930			
Subject	0.2679			

Appendix 15: Two-way repeated measure ANOVA analysis of % FRAP activity of *P. angolensis* plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests

Source of Variation	% of total variation	P-value	P-value summary	Significant?
Row Factor	7.498	0.0028	**	Yes
Column Factor	46.18	0.0003	***	Yes
Row Factor x Column Factor	46.05	0.0004	***	Yes
Subject x Row Factor	0.04280			
Subject x Column Factor	0.05047			
Subject	0.03274			

Appendix 16: Two-way repeated measure ANOVA analysis of FRAP GAE for *P. angolensis* plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests

Source of Variation	% of total variation	P-value	P-value summary	Significant?
Row Factor	21.44	0.0049	**	Yes
Column Factor	39.57	0.0005	***	Yes

Row Factor x Column Factor	36.93	0.0021	**	Yes
Subject x Row Factor	0.3661			
Subject x Column Factor	0.4022			
Subject	0.2123			

Appendix 17: Two-way repeated measure ANOVA analysis of % FRAP activity for *K. africana* plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests

Source of Variation	% of total variation	P-value	P-value summary	Significant?
Row Factor	9.516	0.0006	***	Yes
Column Factor	65.89	0.0002	***	Yes
Row Factor x Column Factor	24.28	0.0005	***	Yes
Subject x Row Factor	0.04409			
Subject x Column Factor	0.03017			
Subject	0.04086			

Appendix 18: Two-way repeated measure ANOVA analysis of % FRAP activity for *K. africana* plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests

Source of Variation	% of total variation	P-value	P-value summary	Significant?
Row Factor	38.05	0.0001	***	Yes
Column Factor	40.53	<0.0001	****	Yes
Row Factor x Column Factor	21.35	<0.0001	****	Yes
Subject x Row Factor	0.01359			
Subject x Column Factor	0.01797			
Subject	0.01285			

Appendix 19: Two-way repeated measure ANOVA analysis of % DPPH scavenging activity for P. angolensis *plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests*

Source of Variation	% of total variation	P-value	P-value summary	Significant?
Row Factor	0.2492	0.0010	**	Yes
Column Factor	81.97	<0.0001	****	Yes
Row Factor x Column Factor	17.64	0.0004	***	Yes
Subject x Row Factor	0.006885			
Subject x Column Factor	0.07971			
Subject	0.0009680			

Appendix 20: Two-way repeated measure ANOVA analysis of % DPPH scavenging activity for K. africana *plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests*

Source of Variation	% of total variation	P-value	P-value summary	Significant ?
Row Factor	31.30	<0.0001	****	Yes
Column Factor	49.36	<0.0001	****	Yes
Row Factor x Column Factor	19.18	0.0006	***	Yes
Subject x Row Factor	0.02478			
Subject x Column Factor	0.007844			
Subject	0.004601			

Appendix 21: Two-way repeated measure ANOVA analysis for total flavonoid content for K. africana *plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests*

Source of Variation	**% of total variation**	**P-value**	**P-value summary**	**Significant?**
Row Factor	25.29	<0.0001	****	Yes
Column Factor	27.80	<0.0001	****	Yes
Row Factor x Column Factor	46.89	<0.0001	****	Yes
Subject x Row Factor	0.0005532			
Subject x Column Factor	0.001350			
Subject	0.001540			

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Appendix 22: Two-way repeated measure ANOVA analysis for total flavonoid content for P. angolensis *plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests*

Source of Variation	% of total variation	P-value	P-value summary	Significant?
Row Factor	20.99	0.0001	***	Yes
Column Factor	54.58	<0.0001	****	Yes
Row Factor x Column Factor	24.26	<0.0001	****	Yes
Subject x Row Factor	0.01413			
Subject x Column Factor	0.05803			
Subject	0.02540			

Appendix 23: Two-way repeated measure ANOVA analysis for total phenolic content for K. africana *plant extract. Comparison of mean was performed using Tukey's multiple comparisons tests*

Source of Variation	**% of total variation**	**P-value**	**P-value summary**	**Significant?**
Row Factor (Solvents)	47.63	<0.0001	****	Yes
Column Factor (Plant Part)	24.98	<0.0001	****	Yes
Row Factor x Column Factor	27.21	<0.0001	****	Yes
Subject x Row Factor	0.04487			
Subject x Column Factor	0.007023			
Subject	0.01984			

Appendix 24: Two-way repeated measure ANOVA analysis for total phenolic content for P. angolensis *plant extract. Comparison of mean was performed using Tukey's multiple comparisons test*

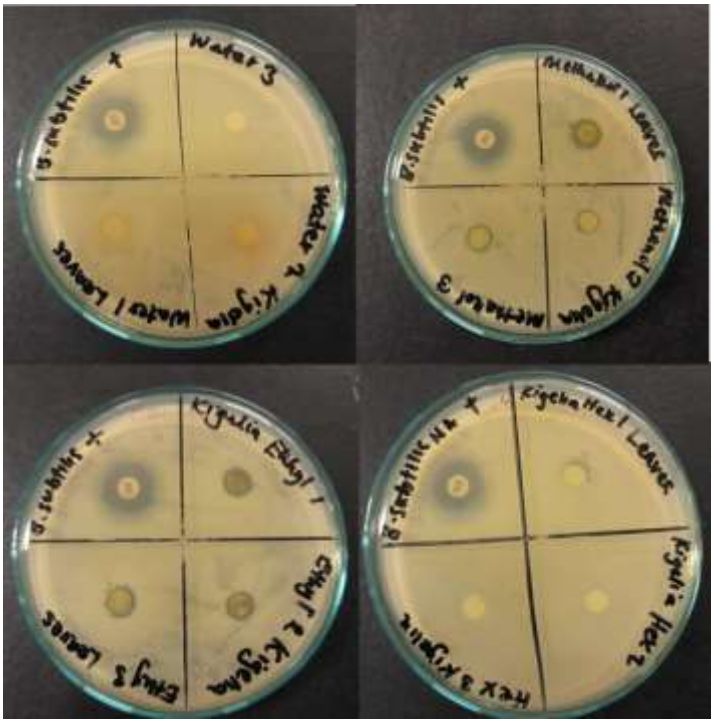
Source of Variation	**% of total variation**	**P-value**	**P-value summary**	**Significant?**
Row Factor (Solvents)	4.979	0.0007	***	Yes
Column Factor (Plant Part)	26.57	0.0008	***	Yes
Row Factor x Column Factor	68.13	0.0013	**	Yes
Subject x Row Factor	0.01168			
Subject x Column Factor	0.06200			
Subject	0.002890			

Appendix 25: Pearson's correlation p-value.

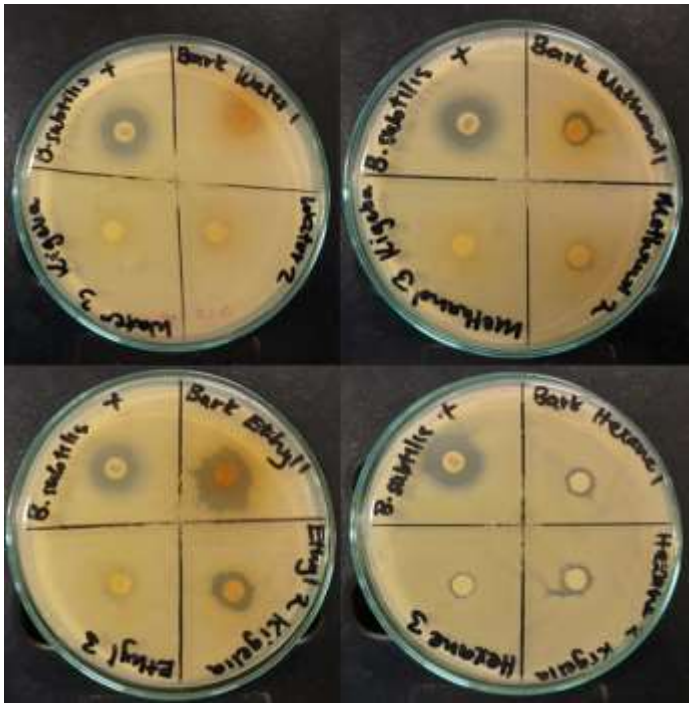
	% DPPH scavenging capacity	% FRAP activity	% Phosphomolybdenum activity	TFC	TPC
% DPPH scavenging capacity		0.0000033216758862526	0.000359281054911823	0.087801478557230 9	0.000370052758797338
% FRAP activity	0.00000332167588625269		0.0030807107832401	0.012825167309571 2	0.002845914218303 46
Phosphomolybdenum inhibition TAC (µg GAE/mg DE)	0.00035928105491182 3	0.0030807107832401		0.002762119377839 66	0.001620899130325 6

TFC (µg QE/mg DE)	0.087801478557 2309	0.01282516730 95712	0.00276211937 783966		0.01696789242 68164
TPC (µg GAE/mg DE)	0.000370052758 797338	0.00284591421 830346	0.00162089913 03256	0.0169678924 268164	

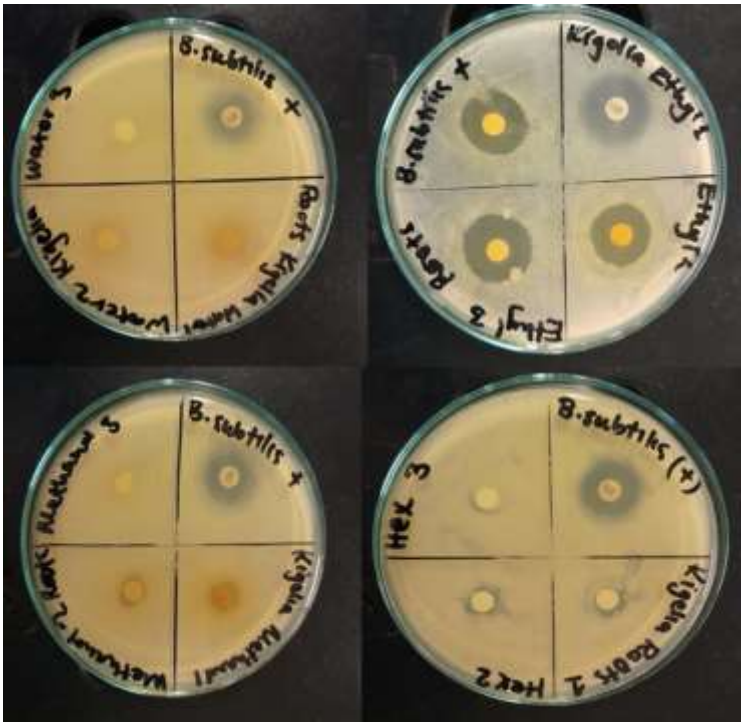
Appendices 8.3: Antimicrobial activity



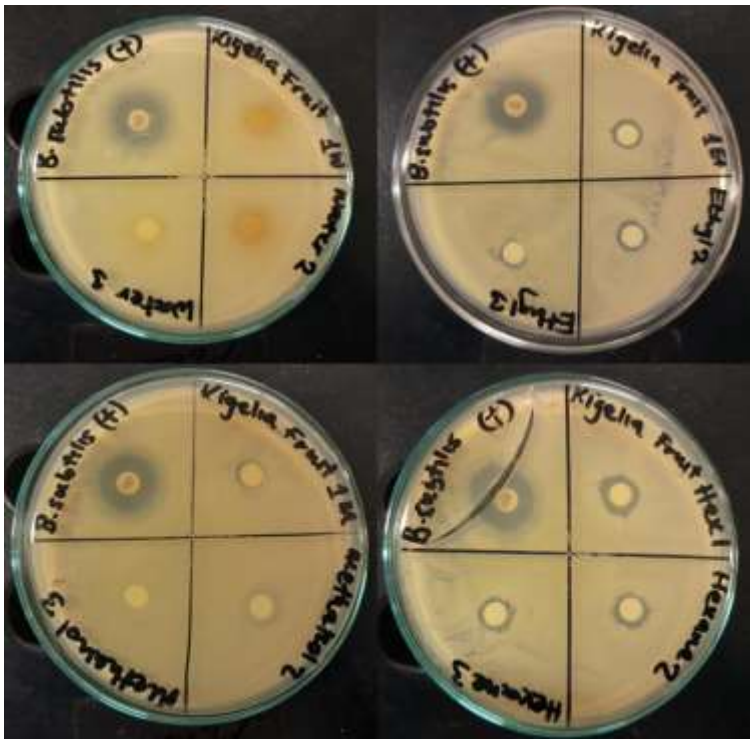
Appendix 26:Antimicrobial activity of *K. africana* leaves crude extracts against *B. subtilis*.



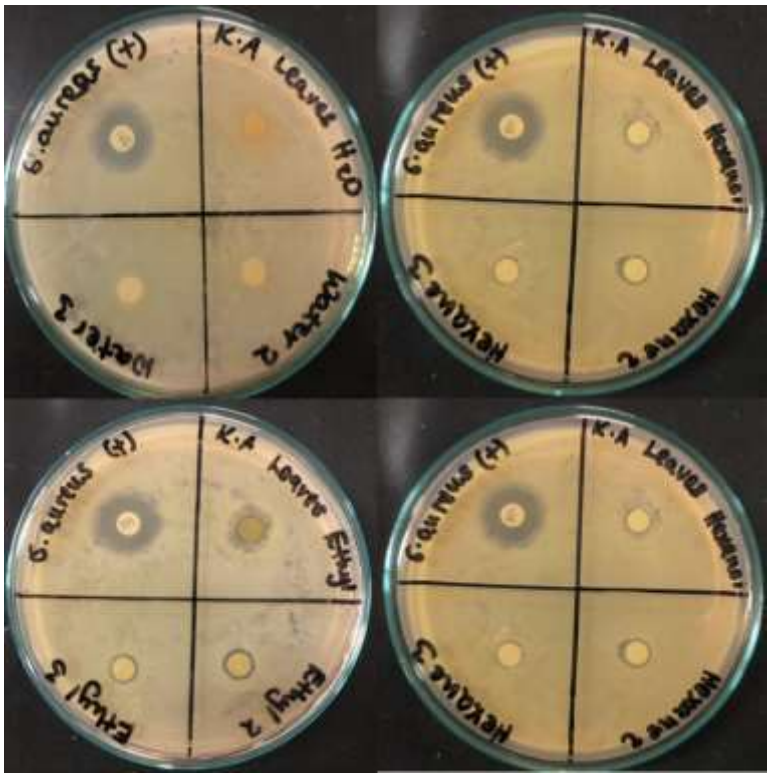
Appendix 27: Antimicrobial activity of *K. africana* bark crude extracts against *B. subtilis*.



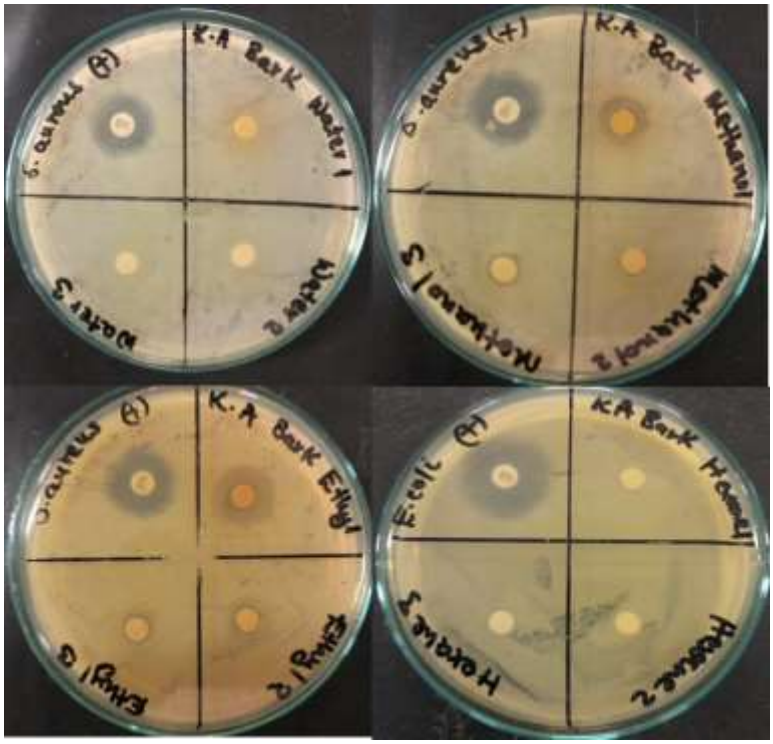
Appendix 28: Antimicrobial activity of *K. africana* roots crude extracts against *B. subtilis*.



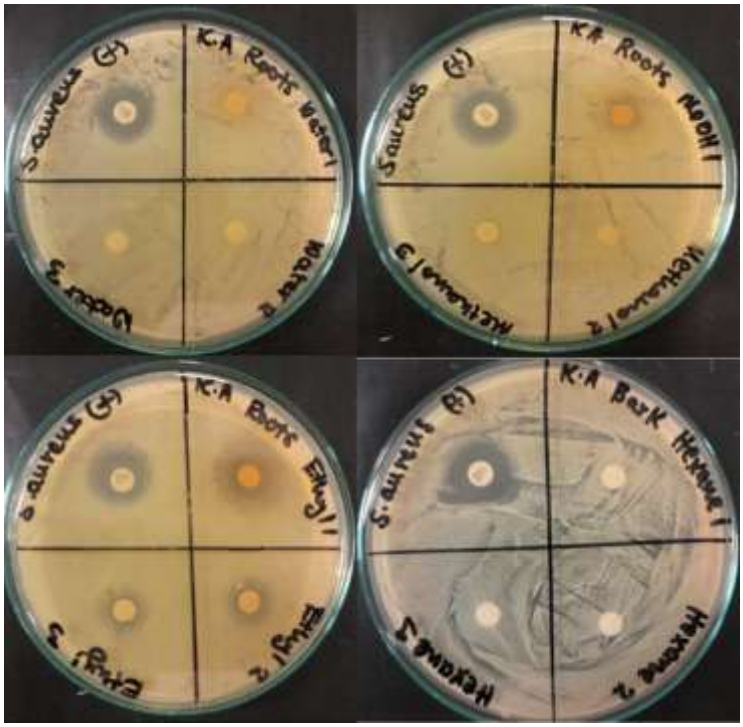
Appendix 29: Antimicrobial activity of *K. africana* fruit crude extracts against *B. subtilis*.



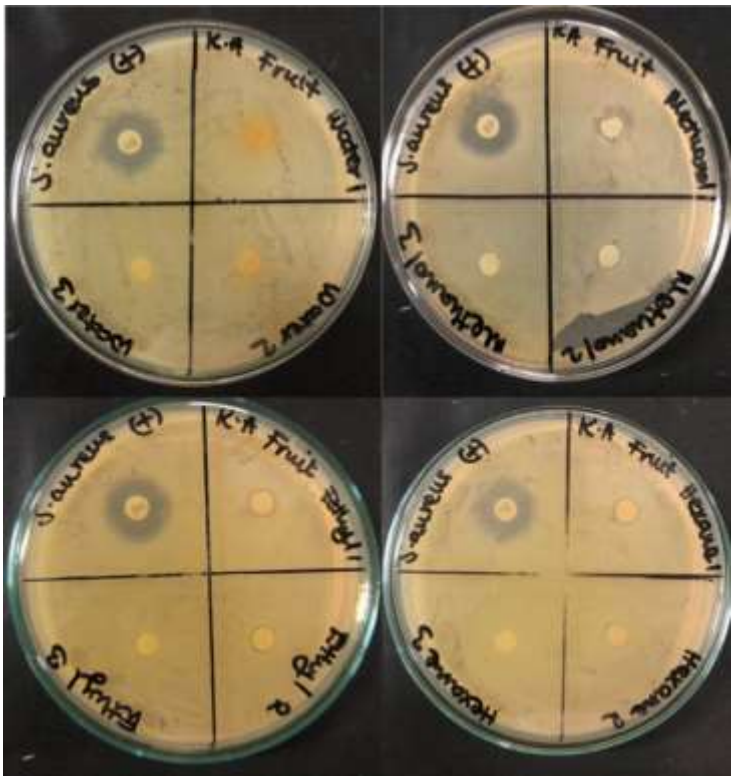
Appendix 30: Antimicrobial activity of *K. africana* leaves crude extracts against *S. aureus*.



Appendix 31: Antimicrobial activity of *K. africana* bark crude extracts against *S. aureus*.



Appendix 32: Antimicrobial activity of *K. africana* roots crude extracts against *S. aureus*.



Appendix 33: Antimicrobial activity of *K. africana* roots crude extracts against *S. aureus*.