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Phytochemical Composition and Antioxidant Activity of *Maerua angolensis* used for Cancer Treatment in Funakaye Local Government, Gombe State, Nigeria

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ABSTRACT

Maerua angolensis leaves and stembark have been used traditionally for the treatment of various diseases including cancer. Despite its widespread use and numerous reported beneficial uses, there is a few research on its antioxidant potential. The present study was therefore conducted to evaluate the qualitative and quantitative phytochemical constituents and antioxidant activity of the methanol leaves and stembark extracts. Phytochemical screening was carried out using standard methods and procedures as described by Evans for the identification of phytochemicals. The antioxidant properties of methanol extracts were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP). The phytochemical screening of *M. angolensis* leaves revealed the high concentration of steroids, saponins, coumarin, terpenoid, phenols, flavonoids, cardiac glycosides, tannins and appreciable amount of alkaloids while steroids, terpenoid, phenols, cardiac glycosides and tannins were found at high concentration with appreciable amount of saponins and alkaloids in the stem bark but coumarin and flavonoid were not detected. The activity of the extract against the free radicals was concentration dependent. The result revealed a linear correlation between the extract and its polyphenolic contents.The reducing ability of the extract also occurred in a dose dependent manner. The study provides evidence that the leaves and stembark of *M. angolensis* contain essential constituents which may serve as natural antioxidants. Therefore, it could be concluded that the plant contains phytochemicals needed in the treatment and management of various diseases.

Keywords: *Maerua angolensis*, Phytochemicals, Antioxidant, Medicinal plant

INTRODUCTION

Maerua angolensis is a deciduous tree belonging to the Family Capparaceae. The genus name “Maerua” means “drooping” in reference to the drooping foliage (Venter & Venter, 2015).As reported by Dharani, (2019) its common English name is “bead bean”.*Maerua angolensis* is a shrub or small to medium-sized and rounded tree which can grow up to 10 metres high and is widely distributed in continental tropical Africa (Dharani, 2019). The plant materials have been used for numerous ethno medicinal uses across the region. The bark on young stems is purplish to yellowish in colour with light grey corky lenticels, and smooth and grey to rough and dark grey, peeling off in small flakes on older stems (Venter & Venter, 2015).

The leaves locally known as ‘Leggel bale’ in Fulfulde Language are used to treat diabetes in parts of northern Nigeria; and the root and stem bark used as an aphrodisiac, and to cure diarrhoea and epilepsy in Tanzania (Maroyi *et al*., 2020). Scientific evaluation of the plant revealed that the extracts have been reported to show antimicrobial activity against some clinical isolates (Yusuf *et al*., 2017). Traditionally, *Maerua angolensis* leaves have long been

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used in the management of various diseases (Sepasal, 2012). Medicinally, the leaves are used either alone or in combination with other drug plants, to treat a range of human illnesses including stomach aches, asthenia, anorexia, headaches, and rheumatism (Yusuf *et al*., 2017). *Maerua angolensis* extract hinders vincristine–induced peripheral neuropathy in mice signifying that it may exert an analgesic effect in cancer patients with neuropathic pain and an antinociceptive effect in abdominal constrictions (Iliya *et al*., 2016). The stem bark extract of *Maerua angolensis* also protects the generation of free radicals and oxidative products of pentylenetetrazole induced oxidative stress and seizures in rats (Charles *et al*., 2018). *Maerua angolensis* is a potential source of antibiotics with a wide range of activity as demonstrated by the antimicrobial activity of the leaf extract against pathogenic microorganisms (Amina *et al*., 2017).

Plants have been used for therapeutic purposes since the time memorial and their importance and vitality have been appreciated by the human populace (Fateemah *et al*., 2018).Natural bioactive compounds from plants perform specific biological activities and modify different physiological functions to improve health of human being (Niaz *et al*., 2020).Several studies have shown that phytochemical compounds of plants have potential use as drugs, and the pharmacological activity of these constituents serves as the basis for their use in modern medicine based on scientific evidence (Ahn, 2017). Plant phytochemicals are potent antioxidants against reactive oxygen species and have numerous health benefits (Narzary *et al*., 2016).

Therefore, the medicinal values of plants depend on the chemical constituents in them that produce certain physiological actions like cell protective effects. Phytochemical study includes the process of extraction, screening and identification of the active substances of medicinal importance in plants. Such bioactive substances include cardiac glycosides phlobatannin alkaloids, saponin, carotenoids, flavonoids, antioxidants, tannin, and phenols (Mollakhalili *et al*., 2017). There are almost 35,000 species of plants that are presently used in herbal remedies across the world, out of which only 20% have been screened for their phytochemicals. With these large yet to be explored herbs, the future of medicinal plants seems to be very bright with the remarkable discovery of new and novel therapeutic products (Srivastava *et al*., 2019).

Unfortunately, little or no attention has been paid especially with the plant species in Sub-Saharan Africa in the literature to examine its phytochemical constituents and antioxidant activity despite its widespread use for treating cancer patients. Hence, this study was aimed at evaluating the phytochemical constituents and antioxidant activity of the leaves and stembark extracts of this important plant with high medicinal value called *Maerua angolensis.*

MATERIALS AND METHODS

Description of the Study Area

Funakaye is one of the eleven (11) Local Government Areas of Gombe State, Nigeria. Its headquarters is Bajoga. It is bounded in the east by the Gongola River and Lake Dadin Kowa, beyond which lie Yobe State and Borno State.It got its name from Fula language widely spoken in the area. It is largely made of Fulani tribe as well as other ones.It has an area of 1,415 km^2. It is located at 10°51′N 11°26′E / 10.85°N 11.433°E (Figure 1). It consists of the following communities: Ashaka, Bage, Wawa, Ribadu, Bajoga, Tong, Jinlayi, Tilde and Wuro bapparu. The major ethnic groups include Tera, Fulani, Kanuri, and Bolewa. Major crops include

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Cotton, Cassava, Sorghum, Groundnuts & Tomatoes.

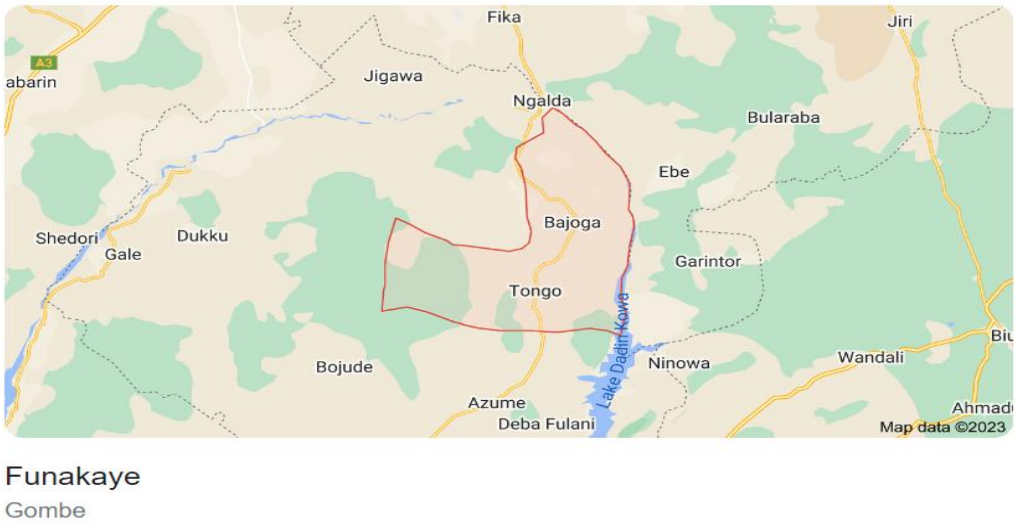

Figure 1: Map of the Study Area.

Sample Collection and Preparation

The sample collection and preparation were carried out according to the method of (Ilondu and Enwa, 2013). The Fresh leaves and stem bark of *Maerua angolensis* were carefully harvested in the early morning. The leaves and stem bark were washed with distilled water, sliced using a stainless-steel knife, and air dried under a sheath over a period of two weeks. The dried samples were milled into powder by pounding manually with a clean mortar; it was then sieved to obtain a fine powder and was stored in a sterile bottle in a cool dry place until required for use.

Sterilization of Glass Wares and Working Bench

The glass wares used were accurately washed with detergent and sterilized in an autoclave at 121°C for 15 minutes. The work was conducted under aseptic condition. The working bench was disinfected with 70% methanol.

Preparation of the Methanol extract

The sample was extracted using the method described by (Sadafbibi *et al*., 2015). The grounded leaves and stem bark of *M. angolensis* obtained above were weighed 100 gm and soaked in 1000 mL of methanol (95%) in a conical flask and were covered with cotton wool and aluminum foil to avoid contaminations. The conical flasks containing the extracts were agitated and allowed to stand at room temperature for 48 hours after which they were filtered using a muslin cloth. The filtrates were then evaporated to dryness in an oven at 37°C to yield crude extracts. The crude extracts obtained were preserved in sterile containers at room temperature until further use.

Qualitative Phytochemical Screening

The identification of chemical classes present in the extracts of *M. angolensis* leaves and stem bark were based on the observation of colour change or formation of precipitate after the addition of specific reagents. The major secondary metabolites classes such as steroids, flavonoids, alkaloids, phenols, Saponins, coumarins, tannin, terpenoids and cardiac glycosides were screened according to the standard phytochemical methods described by Evans, (2009) and Udayaprakash *et al*. (2013).

***Test for Flavonoid*:** 1 mL of 10% sodium hydroxide wad added to 3 mL of the extract.

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If anintense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids compounds.

***Tests for Tannins*:** 2 drops of 5% ferric chloride solution were added to 3 mL of the extract and coloured produced was noted. Condensed tannins usually give dark green colour while hydrolyzed tannins give blue-black colour.

***Test for Saponins*:** Saponins were detected using the froth test. 5 mL of the extract was added to 5 mL of sterile distilled water in a test tube. The test tube was then shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Presence of honeycomb froth indicates positive result for Saponins.

***Test for Alkaloids*:** 2 mL of the extract was stirred with 2 mL of 10% hydrochloric acid in a test tube.1 mL was treated with few drops of Wagner's reagent and a second 1 mL portion was treated similarly with Mayer's reagent. The samples were then observed for the presence of turbidity or yellow Precipitate which gives preliminary evidence for the presence of alkaloids.

***Test for Steroids*:** 0.5 mL of the extract was dissolved in 2 mL of chloroform. 2 mL of sulphuric acid was carefully added to the mixture to form lower layer. A reddish brown colour at the inter face indicate the presence of a steroid ring.

***Test for Phenols*:** In 1ml of the extract, 2ml of 5% aqueous ferric chloride was added. Formation of blue colour indicates the presence of phenols.

***Test for Cardiac Glycosides*:** 2 mL of 3.5% ferric chloride solution was added to 3 mL of the extract and was allowed to stand for a minute. Then 1 mL of concentrated sulphuric acid was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at their interface indicates the presence of cardiac glycoside.

***Test for Coumarins*:** To the extract, NaOH (10%) and chloroform were added at equal proportions. A yellow coloured solution indicated the presence of Coumarins.

***Test for Terpenoids*:** To 5 ml of the extract, 2 ml of chloroform and 3 ml of concentrated sulphuric acid were added. The presence of terpenoids was indicated by a reddish brown interface.

Quantitative Phytochemical Screening

***Total phenolic content (TPC)*:** According to Folin-Ciocalteu method, 100 µl of FolinCiocalteu reagent and 500 µl of distilled water were added to 100 µl of the extract and incubated for 6 min at room temperature. The volume was made to 3 ml using water, after adding 1.25 ml of 7% sodium carbonate. The absorbance was recorded at 760 nm, after incubation for 90 min and the result was expressed as mg Tannic acid equivalents (TAE) per gram dry weight (DW) of the plant material (Udayaprakash *et al.*, 2015).

***Total flavonoid content (TFC)*:** The solvent in 200 µl of the extract was evaporated and 5 ml of aluminium chloride (0.1 M) was added to the residue. After incubation for 40 min, the absorbance was measured at 415 nm. The TFC was expressed with reference to quercetin equivalents (QE) per gram dry weight (DW) of the plant material (Udayaprakash *et al*., 2015).

***Total tannin content (TTC)*:** According to Tambe and Bhambar, (2014) sodium bicarbonate (1 ml), Folin-Ciocalteau reagent (500 µl), water (8.4 ml) and the extract (100 µl) were mixed and incubated for 30 min. The results are expressed as mg Tannic acid equivalents (TAE) per gram dry weight (DW) of the plant material.

***Total alkaloid content (TAlC)*:** According to John *et al*., (2014) one ml of 2 N HCl was added to the extract and filtered. phosphate buffer (5 ml) and Boromocresol green (5 ml)were added to the filtrate. To this, chloroform was added in successive

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volumes (1–4 ml) in a separating funnel and mixed vigorously. The chloroform layer was then separated and the volume made up to 10 ml. The absorbance was measured at 470 nm and expressed as caffeine equivalents (CE) per gram dry weight (DW) of the plant material.

***Total Saponin Content (TSC)*:** The extracts were dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600c for 10min, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

***Total Steroids Content (TSC)*:** 1ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Varying concentrations of *M. angolensis* extracts (100, 200, 300, 400, & 500 µg/ml) were made up to 1 ml. To this, 1 ml of 0.01 mM DPPH was added and incubated in the dark for 30 min, after which the absorbance was measured at 517 nm (Udayaprakash *et al*., 2014).

Ferric reducing antioxidant power (FRAP) assay

2.5 ml of 0.2 M phosphate buffer (pH 7) and 2.5 ml of 1% potassium ferricyanide were added to the different concentrations of the extract (100, 200, 300, 400, & 500 µg/ml) and maintained at 50 C for 30 min. Additional, 10% trichloroacetic acid (2.5 ml) was added and centrifuged for 10 min at 6500 rpm. To the supernatant, 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride were added and the absorbance was measured at 700 nm (Udayaprakash *et al*., 2015).

The percent of inhibition exhibited by different concentrations of the extract in both DPPH and FRAP was calculated as:

$$\text{Percent inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) of three replicates. Statistical analysis was done using analysis of variance (ANOVA). Significant levels were tested at $P < 0.05$.

RESULTS

Qualitative Phytochemical Screening

The results of the qualitative phytochemical screening of *M. angolensis* leaves revealed the high concentration of steroids, saponins, coumarin, terpenoid, phenols, flavonoids, cardiac glycosides, tannins and appreciable amount of alkaloids while steroids, terpenoid, phenols, cardiac glycosides and tannins were found at high concentration with appreciable amount of saponins and alkaloids in the stem bark but coumarin and flavonoid were not detected as shown in Table 1.

Table 1: Qualitative Phytochemical Screening of Leaves and Stembark of *Maerua angolensis*

Phytoconstituents	*Leaves*	*Stembark*
Alkaloids	+	+
Saponins	++	+
Flavonoids	+++	-
Coumarins	+++	-
Steroids	++	+++
Terpenoids	++	+++
Tannins	+++	++
Phenols	+++	++
Cardiac glycosides	++	++

Key:

+++	Abundantly Present
++	Moderately Present
+	Present
-	Absent

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Quantitative Phytochemical Screening

Maerua angolensis leaves and Stembark were subjected to quantitative screening by standard methods and procedures. The methanol extract of the leaves and stembark of the plant were analysed for alkaloids, flavonoids, phenols, steroids, saponins and tannin.

The results obtained from the quantitative analysis of the methanol extract showed the presence of phytochemicals from highest to least extent. The table 2 result clearly indicated that the highest amount of alkaloids (122.19 µg/mg extract) is reported in the leaves and least amount of 112.98 µg/mg extract was observed in the stembark. The amount of flavonoid is only reported in the leaves with 124.98 µg/mg while it is absent in the stembark. The highest amount of phenols (201.75 µg/mg extract) is reported in the stembark and least amount of 182.56 µg/mg extract was observed in the leaves. When the methanol extract was quantitatively determined for the steroids, the leaves showed the highest amount of 83.83 µg/mg dry weight and the least value is observed in the stembark (52.65 µg/mg). The highest concentration of saponin (371.28 µg/mg) was determined in the stembark while the least was observed in the leaves (337.24 µg/mg). Finally, the highest amount of tannin (316.67 µg/mg extract) was reported in the stembark and least amount of 169.23 µg/mg extract was observed in the stembark as shown in table 2 below.

Table 2: Quantitative Phytochemical Analysis of Leaves and Stembark of *Maerua angolensis*

Parameters	**Quantitative Composition**	
	Leaves	***Stembark***
Alkaloids (mg/g Caffiene eqv.)	122.19 ± 0.05	112.98 ± 0.06
Total Phenolics (mg/g Tannic acid eqv.)	182.56 ± 0.82	201.75 ± 0.41
Saponins (mg/g Diosgenin eqv.)	337.24 ± 2.26	371.28 ± 0.75
Tannins (mg/g Tannic acid eqv.)	169.23 ± 3.63	316.67 ± 6.35
Steroids (mg/g Cholesterol eqv.)	83.83 ± 0.63	52.65 ± 0.63
Flavonoids (mg/g Quercetin eqv.)	124.98 ± 0.17	-

Values are expressed as mean ± Standard Error Mean (SEM) of replicate determinations

Ferric Reducing antioxidant power (FRAP)

The reducing ability of the extract occurred in a dose dependent manner. This was obvious in the transformation of ferric ions to its ferrous form at 700 nm as shown in Figure 2.

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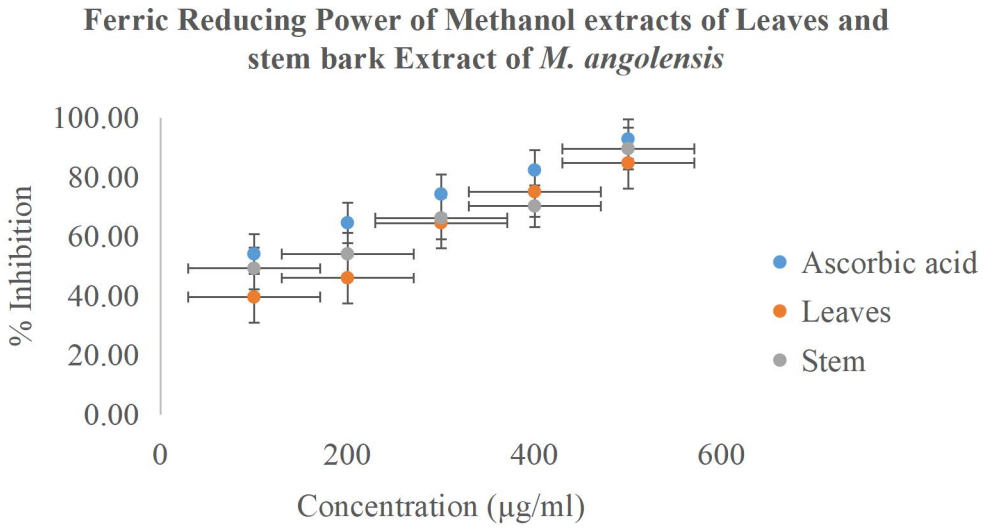

Figure 2: Ferric reducing ability of methanol extract of *M. angolensis* leaves and stembark.

DPPH radical scavenging assay

The percentage inhibition produced by the leaves and stembark extract was significantly different (P <0 .05) from that of ascorbic acid, the standard antioxidant. The extract showed concentration-dependent inhibition as presented in the figure 3 below.

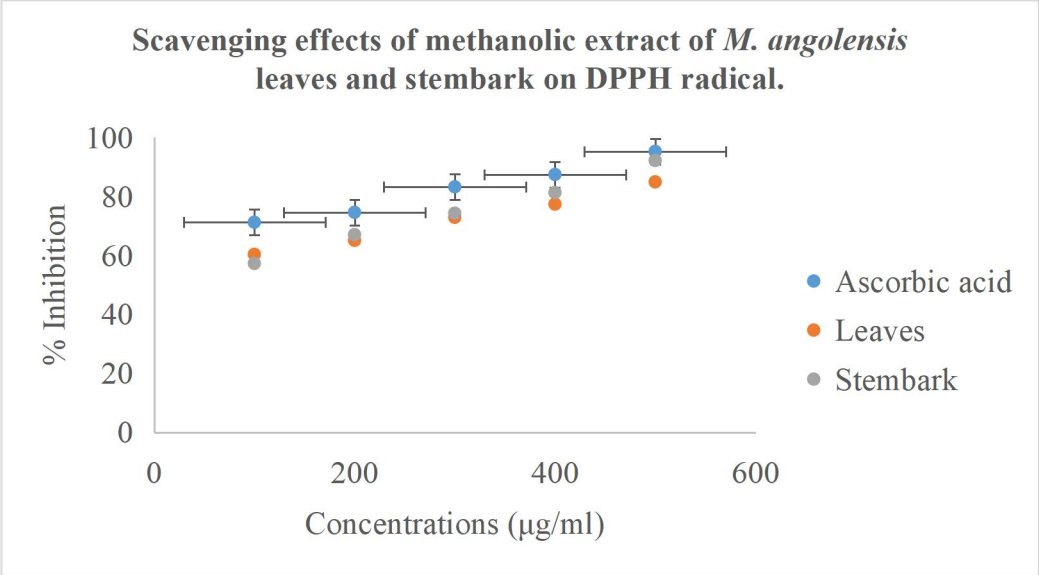

Figure 3: Scavenging activity of methanol extract of *M. angolensis* leaves and stembark on DPPH radical.

DISCUSSION

The phytochemical screening of *Maerua angolensis* methanol leaf extracts revealed the presence of rich secondary metabolites like flavonoid, cardiac glycosides, phenols, coumarin, tannin, terpenoid, saponin, steroid and alkaloids which justified the use of the plant by traditional medical practitioners (TMPs) for the treatment of various diseases. This agrees with previous studies reported by Amina *et al.* (2017). Although, in addition, the present study revealed the presence of cardiac glycosides, phenols, tannin, terpenoid, saponin, steroid and

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alkaloids but flavonoid and coumarin were absent in the stem bark methanolic extract of *Maerua angolensis* (Magaji *et al*., 2009). Tannins are reported to possess antioxidant and anti-microbial potencies (Majid, 2013). Flavonoids are significantly recognized for their antioxidant, anti-carcinogenic, antimicrobial and anti-tumour properties (Okwu, 2001). Polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit oxidative mechanisms that lead to degenerative diseases (Lee *et al*., 2014). They are therefore necessary for preventing haemolytic diseases such as anaemia (Upadhyay and Dixit, 2015). Terpenoids are naturally occurring hydrocarbons produced by a wide variety of plants and animals. Important therapeutic uses of terpenoids include antioxidant, antimicrobial, antifungal, antiviral, anti-inflammatory, antiparasitic, hyperglycemic, immunomodulatory and skin permeation enhancer (Priyarka and Pathik, 2013). Saponins have been reported to have beneficial effects on blood cholesterol levels (Oyewole and Akingbala, 2011). Saponin are amphipathic glycosides, they possess detergent properties that act as surfactants (Bissinger *et al*., 2014).Alkaloids, which are present in both the leaves and the stembark of the plant, are among the most important bioactive substances in phytomedicine with highly diverse group of compounds widely distributed in the plant kingdom (Lu j. *et al*., 2012).many alkaloids compounds have been successfully developed into anticancer drugs.

The quantitative analysis was conducted for Alkaloids, total Phenols, tannin saponin, steroid and Flavonoids which are the targeted phytochemicals in this study. The results pattern showed higher quantity except for flavonoid which is absent in stembark. Alkaloid, which is one of the most important secondary metabolites in phytomedicine, is found in abundance in both the leaves and the stembark. Majority of the antiproliferative drugs in clinical trials are considering the characteristics of alkaloid derivatives in their studies (Mondal *et al*., 2019). Saponins are widely utilized in veterinary vaccines because their character as an adjuvant and helps in the upgrading of immune response. Many of them are useful in intracellular histo-chemistry staining allowing antibody access to intracellular protein molecules. Phenolics are, perhaps, the most widely studied metabolites in chemopreventive studies. They can be characterised into simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids (Watson *et al*., (2013). This is the reason for their total quantification in phytochemical studies (Watson *et al*., (2013). They play a crucial role in plant reproduction and act as soldiers that protect plants against predators, parasites and pathogens (Acharya *et al*., 2010). Their antioxidant capacity and ability to prevent some diseases have been documented especially in cancer research (Mollakhalili *et al*., 2017).The results obtained from this study are usually in line with the studies connected with other researchers in the field of phytomedicine (Elumalai *et al*., 2011).

The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its potential antioxidant activity (Abbasi *et al*., 2013). The reductive ability of the extract occurred in a dose dependent manner. This can be attributed to the electron donating ability of the polyphenols present in the extract.

The methanolic extract of *M. angolensis* inhibited DPPH, signifying its antioxidant activity. The DPPH test provides information on the reactivity of compounds with a stable free radical DPPH that gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger the absorption decreases and the DPPH solution is decolorized as the colour

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changes from deep violet to light yellow. The degree of fall in absorbance is reflective of the antioxidant potency of the extract (Barreira *et al*., 2008).

CONCLUSION

The plant-based bio-active compounds have the effective dosage response with minimal side effects, when compared to the synthetic compounds. The study conducted on *M. angolensis* leaves and stembark revealed the presence of phytochemicals. The presence of this secondary plant product (phytochemicals) is responsible for their healing effects. It further reflects a hope for the development of many novel therapeutic agents from the plant which in future may serve to produced synthetically improved therapeutic drugs. The data obtained from this study implies that the methanol extract of *M. angolensis* leaves and stembark is rich in antioxidants. It is reasonable to speculate that the chemical constituents are responsible for the potent antioxidant properties exhibited by the plant. However, the mechanism of action of these compounds should be revealed.

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