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Antimicrobial, *In-vitro* Free Radical Scavenging, Antioxidant Properties of Leaf, Bark and Root Extracts from *Khaya senegalensis*

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Abstract

Khaya senegalensis is a very important medicinal plant in the tropics and has been utilized for treatment purposes which centred around protozoan as well as bacterial diseases. The phytochemical analysis, antioxidant, free radical scavenging activities and antimicrobial properties of *K. senegalensis* were investigated using various modern and modified techniques. The plant has been shown to contain secondary metabolites such as alkaloids, saponin, tannins, cardiac glycosides, steroids and flavonoids which are thought to give the plant its medicinal values. The bark and root extracts of *K. senegalensis* demonstrated hydroxyl (with EC_{50} values of 278.23, 401.34) and DPPH scavenging activities (with EC_{50} values of 95.76, 107.43) and lipid peroxidation inhibition properties with EC_{50} values of 132.12, 157.65 respectively which are by far higher than the EC_{50} ((50% effective concentration)) values of ascorbic acid of 223.55, 76.11 and 86.22 respectively. DPPH (2,2-diphenyl-1-picrylhydrazyl) is radical and a trap ("scavenger") for other radicals. The zones of inhibition created around test organisms (both bacterial and fungal isolates) are reasonably comparable with standard antibiotics used as control. In most cases there was no significant difference (at $p<0.05$) between the standard and antibiotics and the extracts with zones of inhibition ranging from 12.2±0.02-22.5±0.01 for the extracts and 16.2±1.02-27.0±0.04 for standard antibiotics used in these experiments. The pharmaceutical world should take a very close and deep look at this tropical tree (*Khaya senegalensis*) once again with the aim of harnessing its enormous potentials as antimicrobial as well as antioxidant.

Key words: *Khaya senegalensis*, antimicrobials, antioxidant, free radical, 2,2-diphenyl-1-picrylhydrazyl

INTRODUCTION

Higher plants as well as shrubs are believed to contain substances of high medicinal values. Secondary metabolites from plants have been harnessed for curative purposes by man which we believe, dates to antiquity. Modern research works have revealed that plants contain physiologically active principles which have antimicrobial activities against disease causing organisms. For instance, Racowski *et al.* (2016) studied the antifungal activities of Oregano, Laurel and Rosemary leaves and their essential oils against *Acremonium* sp. The antimicrobial activity of *Peganum harmala L.* extract was carried out by Abdel Moneim *et al.* (2016) while Sokamte *et al.* (2016) determined the *in-vitro* activity of *Syzygium aromaticum* against food spoilage fungi.

Plants such as *Azadirachta indica*, *Euphorbia hirta*, *Euphorbia heterophylla*, *Phylanthus niruri, Prunus amygdalus, Ziziphus spinachristi, Ziziphus mauritiana, Momordica charantia, Moringa oleifera* and numerous other plants, have been evaluated for either their antibacterial, antifungal or in-vitro free radical scavenging, antioxidant activities [Deka *et al*., 2013; Abalaka *et al.,* (2011); Abalaka *et al.,* (2012); Abdel Moneim *et al*., (2008),

Abdel Moneim *et al*., (2011); Danh *et al*., (2013)]. Plants that have antioxidant as well as free radical scavenging properties are likely to be more useful in ethnophamacology especially if they also posses antimicrobial properties. We have also studied, at some point, the antimicrobial activities of the plant *Khaya senegalensis* [Abalaka *et al* (2011); Abalaka and Sani (2009)].

According to world conservation monitoring centre (1998), *Khaya senegalensis* also known as African Mahogany, Benin Mahogany, Dry Zone Mahogany, Senegal Mahogany, is native to Benin; Burkina Faso; Cameroon; Central African Republic; Chad; Côte d'Ivoire; Gabon; Gambia; Ghana; Guinea; Guinea-Bissau; Mali; Niger; Nigeria; Senegal; Sierra Leone; South Sudan; Sudan; Togo; Uganda. It is a mahogany species, widespread in high-rainfall savannah woodland. African mahogany is a medium-sized tree which can grow up to 15–30 m in height and 1 m in diameter. The bark is dark grey to grey-brown while the heartwood is brown with a pink-red pigment made up of coarse interlocking grains. The tree is characterized by leaves arranged in a spiral formation clustered at the end of branches. The white flowers are sweet-scented; the fruit changes from grey to black when ripening (World Conservation Monitoring Centre, 1998).

Generally, plants parts are often used for either therapeutic purposes or traditional cleansing. Plants parts used by the locals include leaf, root, stem bark, flower, fruit, seed, pod. In the present study we utilized the leaf, stem bark and root of *Khaya senegalensis* with the aim of knowing and revealing the antimicrobials, in-vitro free radical scavenging, antioxidant and bioeffects of each part.

Materials and Methods

Collection of plant materials

Plant materials were collected from Bida. Bida, Niger State is on latitude 9006 N and longitude 6001E on the Nupe sand stone formation. Its geographical coordinates are 9° 5' 0" North, 6° 1' 0" East. It is located 19kms North of River Kaduna, along Mokwa Bida road and 86 kms South East of Minna, the Niger State Capital. The town is situated in a valley and uses stream tributary of Gbako River. The plant parts (leaf, stem bark and root) were collected with the help of the locals and was identified and authenticated by a Botanist and Taxonomist.

***Khaya senegalensis* growing in North Central Nigeria**

Extraction of plant materials

Adopting the method of Mann *et al*., (2008), 100 g of the ground samples (leaf, stem bark and root) were separately soaked in 500 ml of ethanol and allowed to stand for about 72 hours for extraction. After 72 hours, it was then filtered using No.1 Whatman filter paper. The filtered extract in solution were sterilized by passing through Millipore filter and then evaporated to dryness and kept for further use.

Phytochemical screening of plant parts for secondary metabolites

Plant parts were screened for secondary metabolites such as alkaloids, tannins, saponins, cardiac glycosides, flavonoids etc using the methods of Trease and Evans (1989) as adopted by Abalaka *et al.* (2011).

a.) Alkaloids- 1 ml of 1% HCl was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, cooled and filtered. About 2 drops of Mayer's reagent was added to1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.

b.) Tannins- 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract. A dirty white precipitate showed the presence of tannins.

c.) Glycosides- 10 ml of 50% H_2SO_4 was added to 1 ml of the extract and the mixture heated in boiling water for about 15 min. 10ml of Fehling's solution was then added and the mixture was boiled. A brick-red precipitate confirmed the presence of glycosides.

d.) Saponins- Frothing test: 2 ml of the extract was vigorously shaken in the test tube for 2 min. No frothing was observed.

e.) Flavonoids- 1 ml of 10% NaOH was added to 3 ml of the extract. There was no yellow colouration which was indication of the absence of flavonoids.

f. Steroids- Salkowski test: 5 drops of concentrated H_2SO_4 was added to 1 ml of the extract in a test tube. Red colouration was observed which is indication of the presence of steroids.

g. Phlobatanins- 1 ml of the extract was added to 1% HCl. No red precipitate was observed which means negative result.

h. Triterpenes- 1 ml of the extract was added to 5 drops of Acetic anhydride and a drop of concentrated H_2SO_4 added. The mixture was then steamed for 1 h and neutralized with NaOH followed by the addition of chloroform. Absence of blue-green colour indicates the absence of triterpenes.

In *vitro free* radical scavenging and antioxidant capabilities of plant parts

The *in vitro* free radical scavenging and antioxidant capabilities of plant parts were determined strictly using the methods of Chaminda *et al*., 2001, Braca *et al.* (2001), Luximon-Ramma *et al*. (2002), Gupta *et al.* (2000), and Raju *et al.* (2005), as quoted by Abalaka *et al*. (2011). These were conducted as outlined below:

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the interactions between deoxyribose and test extracts for hydroxyl radicals which were obtained by Fenton's reaction. The damage on deoxyribose due to the free radicals was determined calorimetrically by measuring the thiobarbituric acid reactive substances (TBARS) at optical density of 532 nm (Chaminda *et al*., 2001). Percentage of inhibition was also calculated and recorded.

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Braca *et al.* (2001). An aliquot of 3ml of 0.004% DPPH alcohol solution and 0.1ml of plant extract at various concentrations were mixed and incubated at 37°C for 30 min at an absorbance of 517 nm in the spectrophotometer. The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control using the formula of Luximon-Ramma *et al.* (2002) as indicated below:

Percentage of inhibition = $[(A_o - A_1) / A_o] \times 100$

Where A_o = Absorbance of the control

A_1 = Absorbance of the plant extract/ standard

Lipid peroxidation inhibition activity

The inhibition of lipid peroxidation was performed in line with the method described by Gupta *et al.* (2000). Determination of the extent of lipid peroxidation was carried out using rat liver homogenate as the source of polyunsaturated fatty acids. The absorbance was measured at 532 nm. Percentage of inhibition was calculated using the formula of Raju *et al.* (2005).

***In vitro* antimicrobial activity study of plant parts**

Test organisms and their standardization

Clinical isolates of *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Candida albicans* and environmental isolate of *Aspergillus niger* were used for this experiment. The test organisms were standardized prior to use by adopting the method described by Beka *et al.* (2013). In this test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard was used. This was prepared by adding a 0.5ml of 0.048 mol.L $BaCl_2$ (1.175% w/v $BaCl_2$.$2H_2O$) to 99.5 ml of 0.18 mol/L $H2SO_4$ (1% v/v) with constant stirring to maintain a suspension. McFarland regent was standardized by measuring absorbance at 625 nm and obtained at standard absorbance of 0.091.

Susceptibility test

Antibacterial Assay

The antibacterial assay was performed using the Kirby-Bauer Method against the three bacteria- *Staphylococcus aureus, Salmonella typhi, Escherichia coli.* In this analysis, sterile paper discs made by using 6mm paper perforator were soaked in varying concentrations of the ethanol extracts of the plant parts for about 60 minutes until the abstracts were well absorbed by the discs. The discs were aseptically inoculated onto the plates with the test organisms. After 24 hours of incubation, zones of inhibition were observed and recorded. Streptomycin and chloramphenicol were used as control.

Antifungal Assay

Antifungal assay was carried out using 2ml of prepared ethanol extracts of the plant parts at varying test concentrations against the fungi *Aspergillus niger* and *Candida albicans*. The plant extracts were aseptically poured on a sterile standard plate containing 20ml of Potato Dextrose Agar (PDA) and thoroughly mixed. The PDA had been previously sterilized in an autoclave. Plates were inoculated with a 10mm fungal disk and were incubated at room temperature (25°C). Fungal growth was monitored and growth measured using calibrated Vernier caliper at every 24 hours intervals for 7 days. Nystatin was used as control.

Statistical analysis

Descriptive statistics for antimicrobial screening of the extracts and minimum inhibitory concentrations were presented in line graphs and bar charts. The tests were carried out in triplicate and the mean values of the triplicate tests were recorded. All the values were expressed as mean ± standard error of mean (x ± SEM). One way ANOVA was used to compare mean MICs of ethanol extracts from different plant parts the test isolates ($P < 0.05$). SPSS statistics version 20.0 was used for all analyses.

RESULTS

Phytochemical screening of plant parts for secondary metabolites

Table 1 comparison of secondary metabolites in extracts of plant parts

	Ethanol extracts		
Metabolite	Leaf	Stem bark	Root
Triterpenes	-	-	-
Flavonoids	+	-	+
Saponins	+	+	+
Tannins	-	+	+
Alkaloids	+	+	+
Phlobatannins	-	-	-
Glycosides	+	+	+
Steroids	-	+	+

Key:
+=Present
-= Absent

The leaf extract contains the least number of secondary metabolites followed by the stem bark of *K. senegalensis*. The root extract has shown to have the highest number of metabolites.

Hydroxyl radical scavenging activity

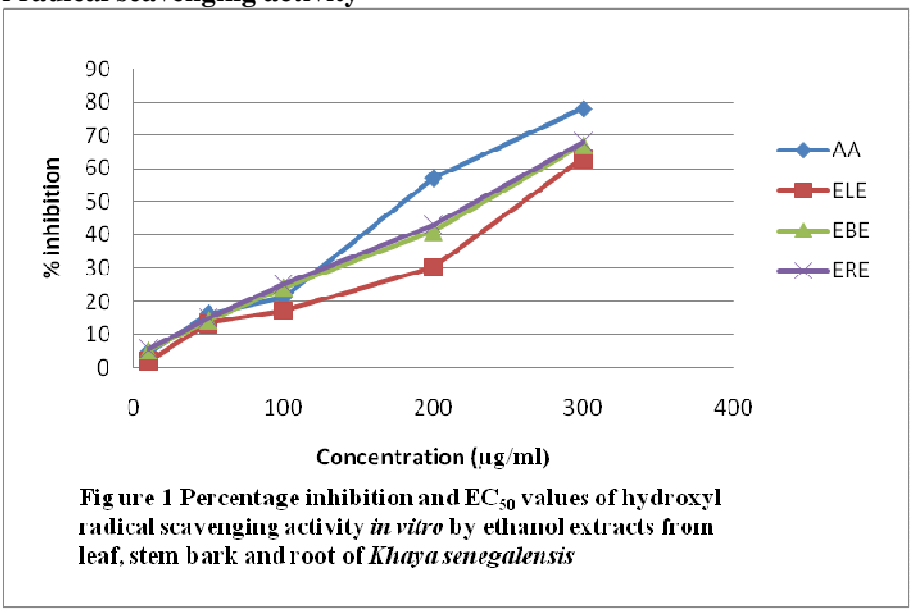

Figure 1 Percentage inhibition and EC_{50} values of hydroxyl radical scavenging activity *in vitro* by ethanol extracts from leaf, stem bark and root of *Khaya senegalensis*

Key:
AA – Ascorbic acid, ELE-Ethanol leaf extract of K. senegalensis, EBE- Ethanol stem bark extract of *K. senegalensis,* ERE- Ethanol root extract of *K. senegalensia*,

The results from figure 1 suggest that ascorbic acid (AA) has the highest percentage inhibition and EC_{50} values of hydroxyl radical scavenging activity *compared to* leaf, stem bark and root extracts of *K. senegalensis*.

DPPH radical scavenging activity

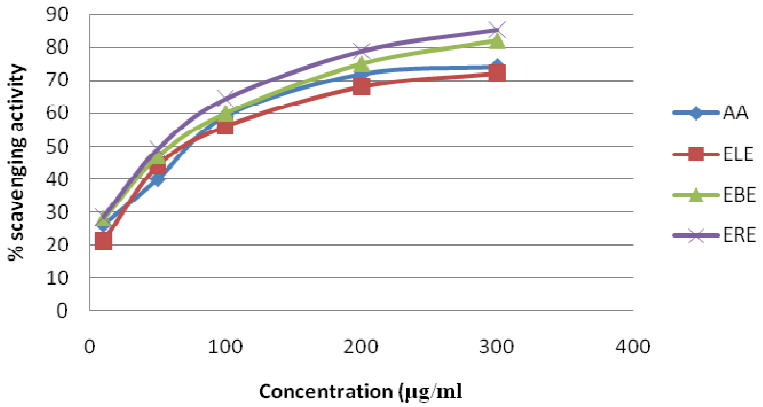

Figure 2 Percentage inhibition and EC_{50} values of DPPH radical scavenging activity *in vitro* by ethanol extracts from leaf, stem bark and root of *Khaya senegalensis*

Key:
AA – Ascorbic acid, ELE-Ethanol leaf extract of K. senegalensis, EBE- Ethanol stem bark extract of *K. senegalensis,* ERE- Ethanol root extract of *K. senegalensia*,

From figure 2 above it is clear that the stem bark and root extracts of the plant has the highest percentage inhibition and EC_{50} values of DPPH scavenging activity *compared to* ascorbic acid (AA) and leaf extract.

Lipid peroxidation inhibition activity

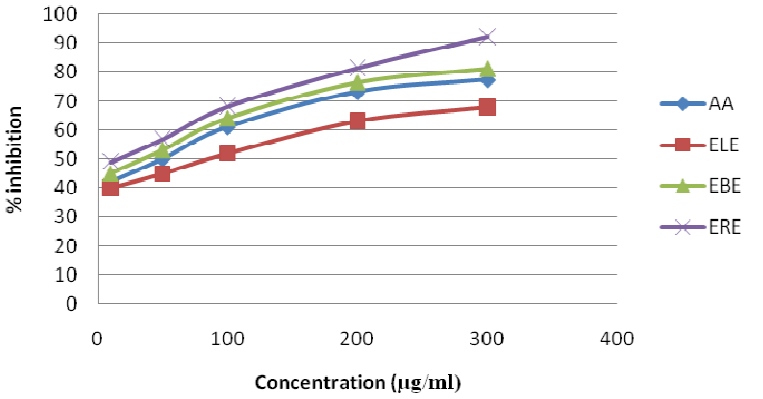

Figure 3 Percentage inhibition and EC_{50} values of Lipid peroxidation inhibition activity *in vitro* by ethanol extracts from leaf, stem bark and root of *Khaya senegalensis*

Key:
AA – Ascorbic acid, ELE-Ethanol leaf extract of K. senegalensis, EBE- Ethanol stem bark extract of *K. senegalensis,* ERE- Ethanol root extract of *K. senegalensia*,

The results above show root extract of *K. senegalensis* has the highest peroxide inhibition value followed by the stem bark, ascorbic acid and lastly the leaf extract

Antibacterial Assay

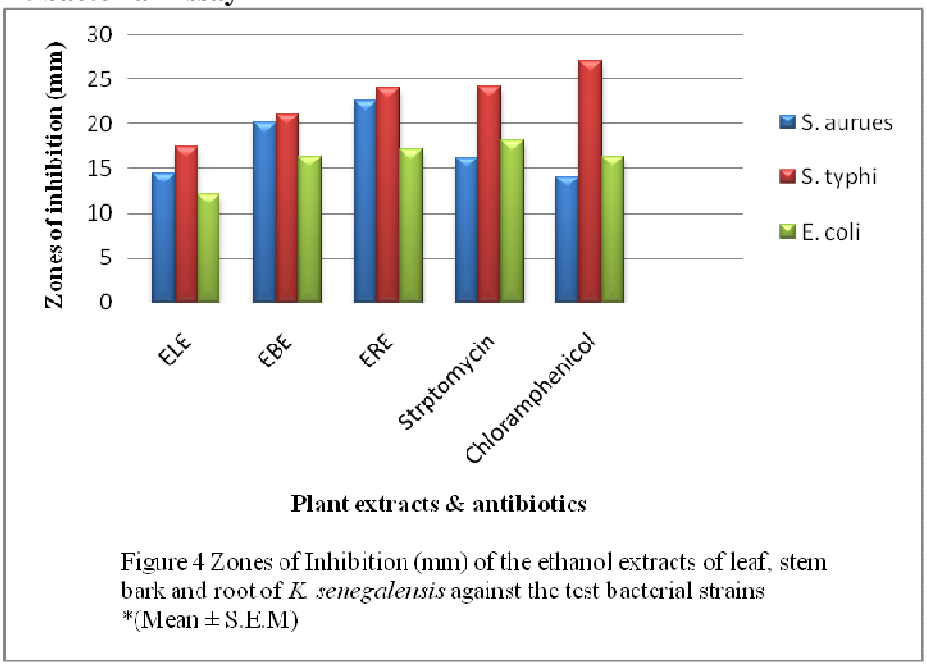

Figure 4 Zones of Inhibition (mm) of the ethanol extracts of leaf, stem bark and root of *K. senegalensis* against the test bacterial strains *(Mean ± S.E.M)

Key: ELE-Ethanol leaf extract, EBE- Ethanol stem bark extract, and ERE- Ethanol root extract of *K. senegalensia.*

Although the bacterial isolates were susceptible to the extracts at varying test concentrations, the root exhibited strongest antibacterial activity followed by the bark while the leaf had the least extract activity against the test organisms. Results are mean of triplicate trials ($p<0.05$)

Antifungal Assay

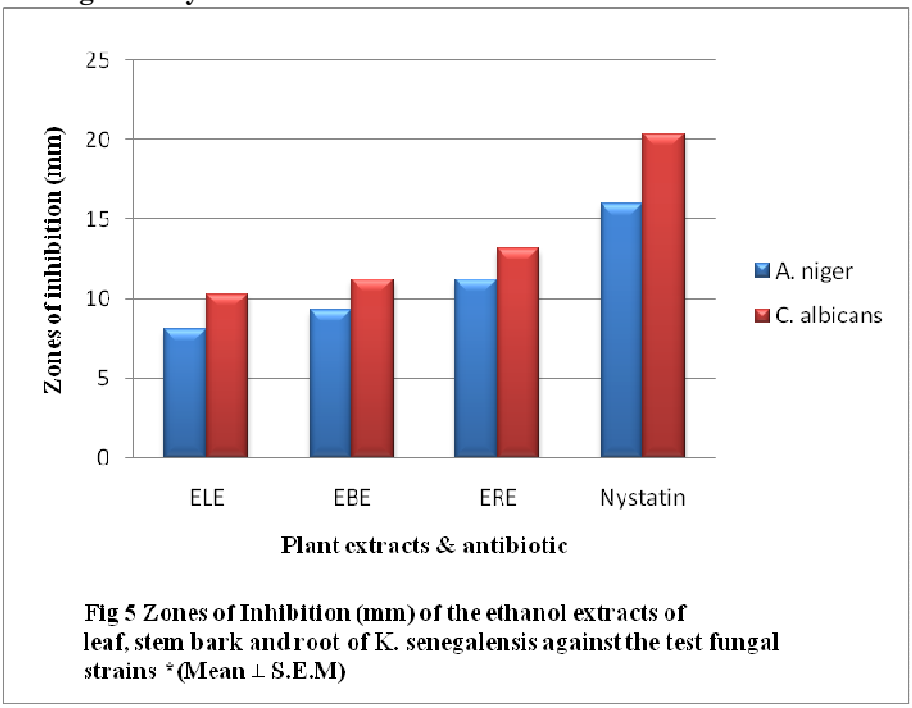

Fig 5 Zones of Inhibition (mm) of the ethanol extracts of leaf, stem bark and root of K. senegalensis against the test fungal strains *(Mean ± S.E.M)

Candida albicans is shown to be highly susceptible to the root as well as the stem bark extracts but it is mildly susceptible to the leaf extract. *Aspergillus niger* exhibited mild susceptibility towards the root and stem bark extracts but resistant to the leaf extract. Results are mean of triplicate trials ($p<0.05$)

DISCUSSION

The results in table 1 revealed that the root of the plant under investigation has more secondary metabolites than the bark while the leaf has the least concentration of metabolites. According to Deka *et al.* (2013) the methanol extract of *Azadirachta indica* posses more secondary metabolites compared with the petroleum ether extract of the plant and this accounted for the higher activity of the methanol extract compared to the petroleum ether extract.

Antioxidant capabilities and free radical scavenging properties are essential in using plant materials for curative purposes. The extracts from the three parts of the plant have exhibited antioxidant capabilities in comparison with the standard ascorbic acid (AA). Abalaka *et al.* (2011) reported the antioxidant and free radical scavenging activities of two medicinal plants, *Ziziphus mauritiana* and *Ziziphus spinachristi* and concluded that the plants have the potential of reducing oxidative stress in the body. Reporting further Abalaka *et al.* (2011) stated that a single hydroxyl radical can result in the formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely disrupt its function and lead to cell death. Figures 1-3 indicated that the plant has higher antioxidant as well as free radical scavenging potential compared to ascorbic acid.

Interestingly, the plant extracts have antimicrobial activities against the test organisms used in these experiments, *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Aspergillus niger* and *Candida albicans*. Figures 4 and 5 contain results of the antimicrobial activities of extracts from plant parts. In figure 4, it is clear that the root extract has more activity compared to the bark extract, while the leaf extract has the least activity against the bacterial isolates. The root extract was also found to exert more antifungal effects on the fungal isolates used in this experiment. Here in Nigeria, the locals who use this plant for treatments usually concentrate on the stem bark. One can see almost all the bark of this plant peeled off by those using it for medicinal purposes. The reason for this may be largely due to the fact that it is easier to get the bark and leaf than the root of the plant. However, from the foregoing, the root seems to be the most valuable. Also the bark of perennial trees is known to house one of the conducting tissues in the plant called phloem vessels. The phloem vessels conduct of transfer prepared polysaccharides and other metabolites from the leaf to other parts of a plant. Stem bark of plants also store metabolites as part of waste materials from plants. It is therefore expected that bark of medicinal plants should have more antimicrobial activity compared to other parts. The high antimicrobial activities of *Khaya senegalensis* could be as a result of both accumulation of metabolites as waste as well as and mineral elements absorbed from the soil.

The antimicrobial activities of medicinal plants have been closely associated with the secondary metabolites obtained from these plants. These metabolites have been shown to have physiological activity against known pathogens. Concentration of these metabolites however, may vary within parts of plant. That explains why the use of herbs for curative purposes is usually confined to particular parts of a plant. For example, leaves of certain plants are used rather than the stem or the stems are used rather than the root or all of the parts are used.

Enormous efforts are being devoted to research on natural remedies against pathogenic microorganisms mainly due to the development of resistance by microbial pathogens to both synthetic and naturally occurring antibiotics. Raja Naika *et al.* (2015); Faten *et al.* (2014); Shoib and Shahid (2015) all showed antioxidant as well as antimicrobial activities of plants materials against notable pathogens. It is important to note that these efforts are really yielding tangible results. As we continue our search for novel substances to use against the ever increasing resistant strains of pathogens, sooner than later we will get formidable active principles of plant origin that will be most useful against them.

CONCLUSION

Khaya senegalensis contains metabolites such as alkaloids, saponin, tannins, cardiac glycosides, steroids and flavonoids. These metabolites are concentrated more in the roots than the bark and leaf. Whereas the extracts have free radical scavenging and antioxidant potentials, the root showed more of such potentials compared to other parts of the plant. The antimicrobial activity of the plant is also concentrated on the root followed by the stem while the leaf has less activity against the pathogens. Concentration of metabolites in the root, more free radical scavenging and antioxidant activity of the root and the antimicrobial activities of the root clearly revealed that the root should be used for medicinal purposes more than the other parts of the plant.

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