Phytochemical, antioxidant and antimicrobial studies of partitioned fractions of Lannea kerstingii Engl. and K. Krause (Anarcadiaceae)

*Ngaitad S. Njinga[1], Susan M. David[2], Abiodun O. Shittu[3], Bilqis A. Lawal[4], Ronke H. Bello[2], Francis A.U. Attah[4], Sukurat O. Usman[4], Abdulrasheed A. Abdullahi[4], and Moji T. Bakare-Odunola[1]

[1]Department of Pharmaceutical and Medicinal Chemistry, University of Ilorin, Nigeria
[2]Department of Pharmaceutical Microbiology and Biotechnology, University of Ilorin, Ilorin, Nigeria
[3]Department of Pharmaceutics and Industrial Pharmacy, University of Ilorin, Ilorin, Nigeria
[4]Department of Pharmacognosy and Drug Development, University of Ilorin, Ilorin, Nigeria

Corresponding author: Ngaitad S. Njinga
Email: ngastanjin@yahoo.com; Phone: +234 7064933604

ABSTRACT

Background: Antimicrobial resistance is a serious threat to global public health and requires actions like the development of new antimicrobial with significant activities over existing drugs.

Objectives: This study aims at investigating the phytochemical, antioxidant and antimicrobial potentials of partitioned fractions of Lannea kerstingii.

Methods: A quantitative phytochemistry of chloroform, ethyl acetate, acetone and methanol fractions of L. kerstingii for total flavonoid, phenol and alkaloid were done using standard method; antioxidant and antimicrobial activity were determined using 1,1-Diphenyl-2-picrylhydrazyl and agar diffusion method respectively.

Results: The chloroform fraction contained only alkaloid (11%) and steroids while all the other fractions contained phenolic compounds in the range 0.43 to 0.67 mg/g garlic acid. The total flavonoid content ranged from 0.43 to 0.67 mg/g of quercetin. The total flavonoid in the ethyl acetate was significantly different from that of the methanol fraction but not with the acetone fraction. The acetone fraction showed highest antioxidant activity (60.4%) at 0.05 mg/mL though not as comparable to vitamin C. The ethyl acetate showed high antimicrobial activity as it was active against most of the organisms tested upon and zone of inhibition ranged from 13±0.02 to 29±0.1 mm. The acetone fraction was active only against T. mentagrophytes while the methanol fraction showed no activity.

Conclusion: The antioxidant and antimicrobial activities may be due to the presence of flavonoids, as well as the presence of tannins and terpenoids present in the different fractions. This makes the ethyl acetate fraction a good source of antioxidant and antimicrobial agent.

Keywords: Quantitative phytochemistry, Antimicrobial assay, Antioxidant assay, Lannea kerstingii

Études phytochimiques, antioxydantes et antimicrobiennes de fractions cloisonnées de Lannea kerstingii Engl. et K. Krause (Anarcadiaceae)

Ngaitad S. Njinga[1], Susan M. David[2], Abiodun O. Shittu[3], Bilqis A. Lawal[4], Ronke H. Bello[2], Francis A.U. Attah[4], Sukurat O. Usman[4], Abdulrasheed A. Abdullahi[4], and Moji T. Bakare-Odunola[1]

[1]Département de chimie pharmaceutique et médicinale, Université d'Ilorin, Nigéria
[2]Département de biotechnologie et microbiologie pharmaceutique, Université d'Ilorin, Ilorin, Nigéria
[3]Département de Pharmaceutique et de Pharmacie Industrielle, Université d'Ilorin, Ilorin, Nigéria
[4]Département de pharmacognosie et de développement de médicaments, Université d'Ilorin, Ilorin, Nigéria

Auteur correspondant : Ngaitad S. Njinga
E-mail : ngastanjin@yahoo.com ; Téléphone : +234 7064933604

RESUME

Contexte : La résistance aux antimicrobiens est une menace sérieuse pour la santé publique mondiale et nécessite des mesures comme la mise au point de nouveaux antimicrobiens avec des activités significatives sur les médicaments existants.

Objectifs : Cette étude vise à étudier les potentiels phyto-chimiques, antioxydants et antimicrobiens des fractions cloisonnées de Lannea kerstingii.

Méthodes : Une phyto-chimie quantitative de fractions de chloroforme, d'acétate d'éthylique, d'acétone et de méthanol de L. kerstingii pour le flavonoïde total, le phénol et l'alcaloïde ont été faites utilisant la méthode standard ; L'activité anti-oxydante et antimicrobienne a été déterminée à l'aide de la méthode de diffusion de 1,1-Diphényl-2-picrylhydrazyle et d'agar respectivement.

Résultats : La fraction chloroforme ne contenait que des alcaloïdes (11%) et les stéroïdes tandis que toutes les autres fractions contenaient des composés phénoliques dans la gamme 0,43 à 0,67 mg/g d'acide d'ail. La teneur totale en flavonoïdes variait de 0,43 à 0,67 mg/g de quercétine. Le flavonoïde total dans l'acétate d'éthyle était significativement différent de celui de la fraction de méthanol mais pas avec la fraction d'acétone. La fraction d'acétone a montré l'activité anti-oxydante la plus élevée (60.4%) à 0,05 mg/mL mais pas aussi comparable à la vitamine C. L'acétate d'éthyle a montré une activité antimicrobienne élevée car il était actif contre la plupart des organismes testés et la zone d'inhibition de 13±0,02 à 29±0,1 mm. La fraction d'acétone était active seulement contre T. mentagrophytes tandis que la fraction de méthanol n'a montré aucune activité.

Conclusion : Cette activité peut être due à la présence de flavonoïdes, ainsi qu'à la présence de tanins et de terpénoïdes présents dans les différentes fractions. Cela fait de la fraction d'acétate d'éthylique une bonne source d'antioxydants et d'agents antimicrobiens.

Mots-clés : phyto-chimie quantitative, test antimicrobien, test antioxydant, Lannea kerstingii

INTRODUCTION

From time immemorial, medicinal plants have been a rich source of antimicrobial agent.[1] In recent years, antimicrobial resistance to modern pharmaceuticals have become a major public health concern globally with over 70% of pathogens found in hospitals acquired resistance for at least one antibiotic.[2] Infectious diseases is associated with oxidative stress in a number of ways e.g. organ damage combined with altered metabolism, inflammation and other factors are responsible for the development of oxidative stress in infected patients.[3] Under aerobic conditions, antibiotics that are bactericidal with specific targets in bacterial cells stimulate production of harmful reactive oxygen species (ROS), which contribute to killing of the cells.[4,5]

Medicinal plants have been investigated for their antioxidant properties since natural antioxidants are very effective in preventing the destructive processes of oxidants.[6] Reactive oxygen species (ROS) and other oxidants play key roles in causing numerous disorders and diseases, thus making scientists to appreciate antioxidants for prevention and treatment of diseases and maintenance of human health.[7] Many of the biological responses such as the anti-carcinogenic, anti-aging, and anti-mutagenic responses originate from the inherent antioxidative mechanism in the human body.[8] Antioxidants stabilize or deactivate ROS and other oxidants, often before they attack targets in biological cells.[9] This has made the interest in naturally occurring antioxidants to increase considerably for use in food, cosmetic and pharmaceutical products.[10]

The tendency that medicines derived from plant products could be safer than their synthetic counterparts are being explored.[11,12] The discovery and development of new antioxidant and antimicrobial agents from natural resources may therefore offer an alternative to currently available products.

Lannea kerstingii Engl. and K. Krause (Anacardiaceae) is a widely used plant in traditional medicine. The bark of the plant has been reported to treat a lot of ailments like swellings and anaemia and malaria.[13,14] Previous studies showed that the plant contains tannins, flavonoids, alkaloids, steroids and triterpenes.[15,16] Phytochemicals isolated from the stem bark of this plant include ?-sitosterol-3-O-glucoside[17], catechin-3-O-rhamnoside[18] with both compounds having significant antioxidant and antimicrobial activity against Methicillin Resistant Staphylococcus aureus (MRSA), S. aureus, B. subtilis, E. coli, K. pneumoniae, S. typhi, S. dysentariae, C. albicans and C. tropicalis. This study is aimed at determining the quantitative Phytochemical, antioxidant, antimicrobial properties of partitioned fractions of L. kerstingii.

MATERIALS AND METHODS

Plant collection and identification

The plant L. kerstingii was collected in May, 2011 in Zaria, Kaduna State in the Northern part of Nigeria where it was identified by a botanist Mallam Umar Galla. A voucher specimen (1832) was deposited in the herbarium for future references. After identification, the stem bark was dried under shade for two weeks, after which the size was reduced using mortar and pestle, sieved for homogeneity and kept away from light until further use.

Extraction

The stem bark (30g) was extracted using maceration method with petroleum ether (3x300mL) at room temperature. The marc was sequentially extracted with chloroform (3 x 300mL), ethyl acetate (EtOAc) (3 x 300mL), acetone (3 x 300mL) and methanol (3 x 300mL) at room temperature. All extracts were concentrated under reduced pressure using rotary evaporator and their percentage yield calculated using the formula

$$\text{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical analysis

Qualitative phytochemical analysis of fractions of L. Kerstingii

The chloroform, ethyl acetate, acetone and methanol extracts were subjected to preliminary phytochemical screening to identify the chemical constituents using the standard procedures[19,20,21] with some modifications.

Determination of total phenolics

Total phenolic contents of the methanolic extracts were evaluated on each extract with Folin-Ciocalteu's phenol reagent.[22] To 2.0 mg/mL of the extract solution in methanol was mixed with 2.0 mL Folin-Ciocalteu reagent previously diluted with water (1:9 v/v). After 5 minutes, 1.6 mL of 7% Na_2CO_3 solution was added with mixing. The tubes were shaken for 5 seconds and allowed to stand for 30 min at 40°C in an oven for color development.

Absorbance was then measured at 765 nm using UV-Vis spectrophotometer. Samples of extract were evaluated at a final concentration of 0.01 mg/mL. Gallic acid at different concentrations of 0.01 to 0.07 mg/mL was used as standard. All tests were performed in triplicates.

Total phenolic content was expressed as mg/g Gallic acid equivalent using the following equation based on the calibration curve: y = 1.6232x, R2 = 0.6658, where y was the absorbance and x was the concentration.

Total flavonoids determination

Colorimetric aluminum chloride method was used for flavonoid determination.[22,23] A 3.0 mL solution of each extract dissolved in methanol was separately mixed with 3.0 mL of 2.0% aluminum chloride. After one hour at room temperature, the absorbance was measured at 420 nm. Extracts were evaluated at a final concentration of 0.1 mg/ml. Quercetin was used as standard. All tests were performed in triplicates. Total flavonoid content were calculated as quercetin equivalents (mg/g) using the following equation based on the calibration curve: y = 0.0.23x, R^2 = 0.9424, where y was the absorbance and x was the concentration.

Alkaloids determination

Three gram of the powdered sample was weighed into 150 mL of 20% acetic acid in ethanol and allowed for 4 h. The mixture was filtered and the filtrate concentrated using a water bath at 55°C to one-quarter of its original volume. Concentrated NH_4OH was added drop wise into the extract until precipitation was complete. The whole solution was allowed to settle and then filtered. The precipitate which is the crude alkaloid was washed with dilute NH4OH solution.[24] The crude alkaloid was weighed and calculated according to the equation:

$$\%\ \text{amount of alkaloid (mg/g)} = \frac{\text{weight of precipitate}}{\text{weight of sample}} \mathbf{X}\ 100$$

Antioxidant assay

Different concentrations (0.03 - 0.1 mg/ml) of each extract were separately added, to an equal volume of methanolic solution of 100 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH). The mixture was allowed to react at room temperature in the dark for 30 minutes. Vitamin C was used as standard control while a mixture without the extract was taken as blank. After 30 minutes, the absorbance (A) was measured at 518 nm.[25] Each test was carried out in duplicates converted into the percentage antioxidant activity using the following equation:

$$\%\ \text{scavenged} = \frac{\text{Absorbance (DPPH)} - \text{Absorbance (Extract)}}{\text{Absorbance (DPPH)}} \times 100$$

The EC50 values of each fractions were calculated by nonlinear regression using graph pad prism. Where the abscissa represented the concentration of fractionated extract and the ordinate represented the average percent of scavenging capacity from the triplicates.

Bacterial and fungal strains

The clinical isolates *Tricophyton mentagrophytes, Tricophyton rubrum, Shigella species, Salmonella species, Candida albicans, Pseudomonas aeruginosa, Staphyloccocus aureus, Citrobacter freundii, Citrobacter diversus, Escherichia coli, Enterobacter cloacae, Serratia marcescens, Yersinia enterocolitica, Pseudomonas aeruginosa, Salmonella arizonae, Klebsiella oxytoca, Klebsiella pneumoniae,* and two typed strains Escherichia Coli 25218 and Staphylococcus aureus ATCC 25913, were collected from the Department of Pharmaceutical microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Ilorin, Nigeria.

Antimicrobial assay

Overnight cultures of the test organisms were standardized to 0.5 McFarland turbidity, and 0.2 ml of each inoculum was spread on sterile Mueller Hinton agar and saboraud dextrose agar in petri plates with the excesses drained off. Plates were made to stand for about 20minutes for proper diffusion. A sterile cork borer was then used to make wells of 6 mm diameter on each of the plates containing cultures of the different test organisms and each base was sealed with molten agar. A stock concentration of each of the fractionated extract (50 µg/mL) in DMSO was prepared and 0.1 ml was introduced into the wells using sterile Pasteur pipettes. To serve as negative control, 0.1 ml of DMSO was introduced in

another well. Standard antibiotics ciprofloxacin (5 µg/ml) and an antifungal agent fluconazole (5 µg/ml) were included as positive control. The culture plates were allowed to stand for 30 min and were then incubated at 37°c for 24 h (bacteria) and 28°c for 48 h (fungi) respectively. After incubation, the diameter (mm) of the zones of inhibition were measured to determine the activity against the test organisms.[17,26] The zones of inhibition were measured in triplicates.

Statistical analysis

The data were analyzed using Graph Pad prism 6 (Graph Pad prism 6 software, Inc, USA), and the results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used in which differences of $P<0.05$ was taken as statistically significant.

RESULTS

Percentage yield of fractions of *L. kerstingii*

The percentage yield for the ethyl acetate fraction was 5.84 % w/w followed by methanol extract (1.74%). The chloroform fraction showed the lowest percentage extractive value (0.57 % w/w) as shown in table 1.

Table 1. Percentage extractive values of the different fractions of the stem bark of *L. kerstingii*

Extract	Color of fraction	Weight of fractions (g)	% yield
Chloroform	Green	0.17	0.57
Ethyl acetate	Brown	1.75	5.84
Acetone	Dark brown	0.27	0.89
Methanol	Dark brown	0.52	1.74

Qualitative phytochemistry

The chloroform fraction contained carbohydrates and steroids only. The upper part of the ethyl acetate contains only carbohydrates and flavonoids while the lower part contains steroids cardiac glycosides tannins and terpenoids. The acetone and methanol fractions both contains cardiac glycoside, flavonoids and tannins as shown in table 2.

Table 2. Phytochemical constituents present in sequential partitioned fractions of the stem-bark of *L. kerstingii*

Phytochemical constituent	Test	Fractions				
		$CHCl_3$	Ethyl acetate (upper part)	Ethyl acetate (lower part)	Acetone	CH_3OH
Carbohydrates	Molish test	–	+	+	+	–
Reducing sugars	Fehling's Test	–	–	–	–	–
Alkaloids	Wagners' Test	+	–	–	–	–
Cardiac glycosides	Keller-kilaniTest	–	–	+	+	+
Flavonoids	NaOH, Shinoda	–	+	–	+	+
Saponins	Frothing Test	–	–	–	+	–
Steroids	Acetic anhydride + Conc. H_2SO_4	+	–	–	–	–
Tannins	$Fecl_3$	–	–	+	+	+
Terpenoids	$CHCl_3 + H_2SO_4$	–	–	+	+	–

Key: + = positive, - = absent

Quantitative phytochemistry

Total phenols and flavonoid

The level of these phenolic compounds in the ethyl acetate, acetone and methanol fractions of the stem bark of L. kerstingii were considerable as shown in Table 3. The total phenolic content of the stem bark extract of *L. kerstingii* bark ranged from 0.43 to 0.67 mg/g garlic acid (Table 3). The total flavonoid content of ethyl acetate was higher than that of the other fractions while the methanol fraction showed the least. The upper ethyl acetate fraction contained more flavonoid than the lower part as shown in Table 3. Quantitative estimation indicated that the alkaloid content in the chloroform fraction of *L. kerstingii* was 11 % while the other fractions did not contain alkaloids (Table 3).

Table 3: Total phenolic, total flavonoid, and total alkaloid constituents identified in the different fractions of *L. kerstingii*

Fraction of extract	Phenol (mg/g garlic acid)	Flavonoid (mg/g quercetin)	Alkaloids (% w/w)
chloroform	–	–	11 ± 0.04
Ethyl acetate (lower part)	1.61 ± 0.01	0.43 ± 0.00	–
Ethyl acetate (upper part)	1.61 ± 0.02	0.67 ± 0.01	–
Acetone	1.40 ± 0.00	0.62 ± 0.00	–
EtOAc	1.66 ± 0.00	0.66 ± 0.00	–
Methanol	1.61 ± 0.01	0.48 ± 0.01	

Key: - = absent

Antioxidant activity

The scavenging activity was expressed as percentage inhibition of DPPH free radical (Figure 1). The results of the DPPH assay also showed that the upper ethyl acetate fraction has a stronger scavenging activity followed by the acetone fraction with the lower part of ethyl acetate having the lowest scavenging activity. As shown in Figure 2, the DPPH radical-scavenging activity of all the different fractions of *L. kerstingii* is significantly different than of vitamin C.

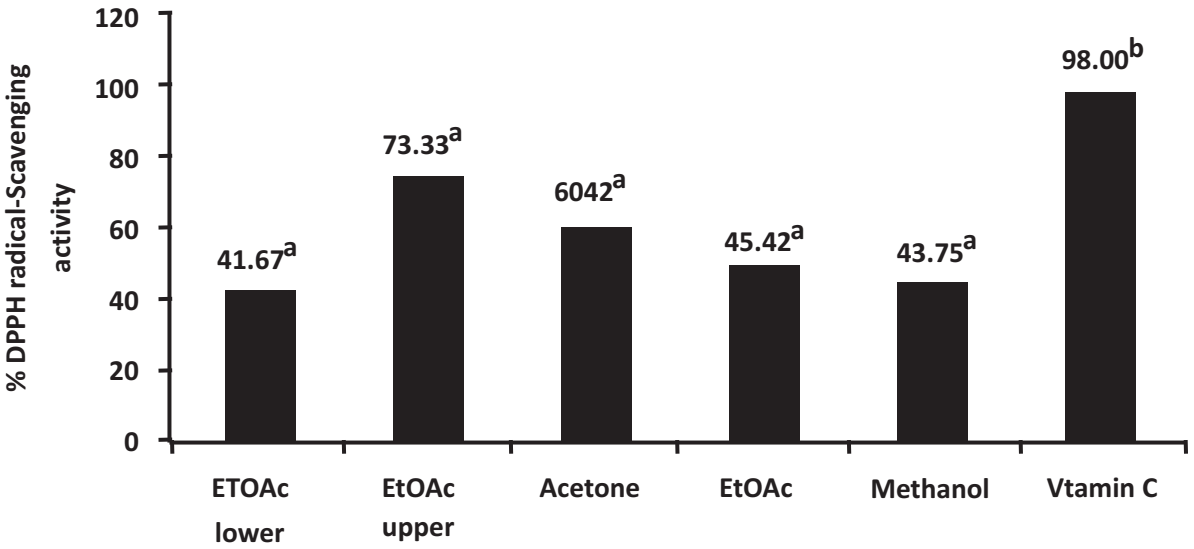

Fig. 1. Scavenging activity of L. kerstingii at concentration of 5.0 µg/ml (Values are expressed as mean ± SD (n=3) when compared to vitamin C. (Alphabets different form that of vitamin C are significant at P<0.05).

Antimicrobial

Figure 2 showed the antimicrobial activity of the partitioned fractions of *L. kerstingii.* The upper EtOAc fraction was the most active as it was active against most of the gram negative bacteria as well as the fungi tested upon. The lower EtOAc fraction was the second most active as it was active just against all the fungi and just two bacteria strain (Shigella sp and Salmonella sp) while the third most active was the acetone fraction which was active only against the fungus *T. mentagrophytes.* The methanol fraction showed no activity. Figure 6 showed the zone of the inhibition of the microorganism against standard antibiotics.

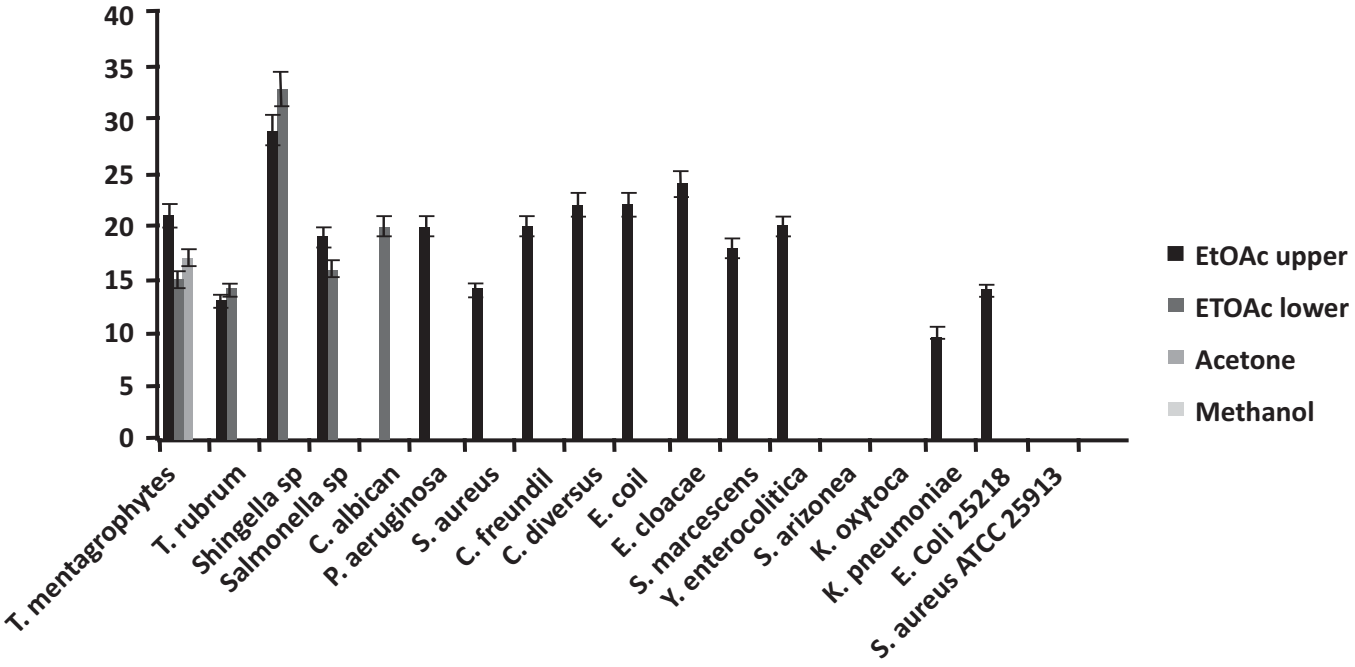

Fig. 2: Zones of inhibition of extracts of different fractions (50 µg/mL) of the stem bark of L. kerstingii against micro-organisms.

Table 4: Standard antibiotics ciprofloxacin and fluconazole against the isolates

Isolates	Zones of inhibition (mm)	
	Ciprofloxacin	fluconazole
Tricophyton mentagrophytes	–	18 ± 0.02
Tricophyton rubrum	–	18 ± 0.09
Shigella sp	19 ± 0.06	–
Salmonella sp	22 ± 0.12	–
Candida albican	-	28 ± 0.13
Pseudomonas aeruginosa	20 ± 0.01	–
Staphyloccus aureus	12 ± 0.10	–
Citrobacter freundii	12 ± 0.12	–
Citrobacter diversus	24 ± 0.11	–
Escherichia coli,	10 ± 0.08	–
Enterobacter cloacae	14 ± 0.05	–
Serratia marcescens	10 ± 0.01	–
Yersinia enterocolitica	16 ± 0.09	–
Salmonella arizonae	10 ± 0.13	–
Klebsiella oxytoca	10 ± 0.03	–
Klebsiella pneumoniae	14 ± 0.15	–
Escherichia Coli 25218	24 ± 0.02	–
Staphylococcus aureus ATCC 25913	10 ± 0.01	–

DISCUSSION

The results obtained in the present study revealed the presence of mostly ethyl acetate soluble phytochemicals as extractive values. This is in contrary to methanol fraction being the most extractive values in *L. coromandelica* reported by Kumar and Jain.[24] Kumar also reported high total flavonoid and phenolic content in the ethyl acetate fraction. The results also revealed high total phenolic and total flavonoid in the ethyl acetate fraction which is consistent that reported in literature[24], though there was no significant difference ($P < 0.05$) in total phenolic content amongst the different fractions. On the contrary, there was a significant difference ($P < 0.05$) in the total flavonoid present in the ethyl acetate and the methanol fractions but none between the extracts of the ethyl acetate and acetone fraction. The chloroform fraction contains mostly alkaloids which is in contrary with that reported by Diallo *et al*.[25] in which they reported the absence of alkaloid in *L kerstingii.*

Flavonoids have been shown to reduce free radicals by up-regulating, quenching and chelating radical intermediate compounds.[29] Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities.[4]

The electron donation ability of extracts can be measured by DPPH.[4] The scavenging of DPPH is through the addition of antioxidant or a radical species that decolourizes the DPPH solution and the degree of colour change is proportional to the potency of the antioxidants. Decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the test drug.[29]

In the present study among all the fractions tested, the acetone fraction showed highest antioxidant activity though not as significant as that of vitamin C. This is in contrary with literature in which it was reported high antioxidant activity on the ethyl acetate fraction[24]. The upper ethyl acetate fraction showed more antioxidant activity which was significantly higher than those of the other extracts. Polyphenols from plants act as antioxidants by the hydrogen-donating property of their hydroxyl groups.[30] The high presence of flavonoids in the upper ethyl acetate justifies its high antioxidant activity.

The inability of the different fractions to show activity as strong as that of vitamin C could be attributed to the fact that the synergistic effects or additive of polyphenols make the antioxidant activity of the extracts weaker than that of the isolated bioactive compounds.[31] The phenolic compounds present in all the extracts of the different fractions could be responsible for the observed % DPPH radical scavenging activity, since they can readily donate hydrogen atom to the radical.[32]

Studies on flavonoid and its derivatives have shown a wide range of antibacterial, anti-fungal, antiviral, anti-inflammatory, and anticancer activities.[33,34] The upper layer of the ethyl acetate was observed to possess the greatest antimicrobial activity as it was active against both gram positive (Staph. aureus) and Gram negative bacteria strains and also against fungal strains *(T. mentagrophytes, T. rubrum)* tested. The antimicrobial activity of the partitioned fractions was closely followed by the lower layer of the ethyl acetate fraction which was observed to be active against all the fungi. Saponins has been shown to be active antifungal agents[30] thus, justifying the activity of the acetone fraction against *T. mentagrophytes.* The methanol fraction was inactive though it contained flavonoid and tannins. The inactivity might be due to antagonistic relationship between the phytochimcals that are responsible of antimicrobial activity. The microbial activity of this plant is in agreement with that reported by Njinga *et al*.[17] who reported microbial activityes on the leaves and also in line with other reports members of the same species.[35]

The limitation of this study is the utilization of "one-dimensional" character of methods used to evaluate antioxidant activity. Also, the yield of the chloroform extract was too low and prevented further investigations.

CONCLUSION

This study clearly indicates that *L. kerstingii* contains flavonoids, saponins and alkaloids. Among all the fractions, the acetone and upper ethyl acetate fraction showed significant DPPH antioxidant activity. The ethyl acetate fractions showed wide spectrum antimicrobial activity as it was active against both gram positive and gram negative isolates tested and also against the fungal strains. This study thus provide justification for the therapeutic use of this plant in folkloric medicine.

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REFERENCES

1. Kane JH, Finlay AC and Sobin BA (1950). Antimicrobial agents from natural sources. Annals of the New York Academy of Sciences 53:226-228
2. Hashemi SH, Esna-Ashari F, Tavakoli S and Mamani M (2013). The prevalence of antibiotic resistance of Enterobacteriaceae strains isolated in community- and hospital-acquired infections in teaching hospitals of Hamadan, west of Iran. *Journal of Research in Health Sciences 13(1):75-80*
3. Tawadrous GA, Aziz AA, Amin DG, Eldemery A and Mostafa MAA (2012). RANTES, TNF-alpha, oxidative stress, and hematological abnormalities in hepatitis C virus infection. *Journal of Investigative Medicine 60(6):878-882*
4. Kohanski MA, Dwyer DJ, Hayete B, Lawrence C and Collins J (2007) A common mechanism of cellular death induced by bactericidal antibiotics, Cell 130:797-810.
5. Van Acker H and Coenye T (2017). The role of reactive oxygen species in antibiotic-mediated killing of bacteria, Trends Microbiol. 25:456-466.
6. Zengin G, Cakmak YS, Guler GO and Aktumsek A (2011). Antioxidant properties of methanolic extract and fatty acid composition of Centaurea urvillei DC. subsp. Hayekiana Wagenitz. Records of Natural Products 5:123-132.
7. Halliwell B and Gutteridge JMC (1981). Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS Letters 128:347-352.
8. Gulcin I (2012). Antioxidant activity of food constituents: an overview. Archives of Toxicology 86:345-391.
9. Nunes PX, Silva SF, Guedes RJ and Almeida S (2012). Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health.
10. Wannes WA, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G, Kchouk ME and Marzouk B (2010). Antioxidant activities of the essential oil and methanol extracts from myrtle (Myrtuscommunis var. italica L.) leaf, stem and flower. Food and Chemical Toxicology 48:1362-1370.
11. Verma S and Singh S (2008). Current and future status of herbal medicines. Veterinary world 1(11):347-350
12. Oluyemi KA, Okwuonu UC, Baxter DG and Oyesola TO (2007). Toxic effects of methanolic exract of Aspilia africana leaf on the estrous cycle and uterine tissues of Wistar rats. *International Journal of Morphology 25:609-614*
13. Doka IG and Yagi SM (2009). Ethnobotanical Survey of Medicinal Plants in West Kordofan (Western Sudan). Ethnobotanical Leaflets.13:1409-1416
14. Diallo A, Eklu-Gadegkeku K, Mobio T, Moukha S, Agbonon A, Aklikokou K, Creppy EE and Gbeassor M (2009). Protective effect of Moringa oleifera Lam. and Lannea kerstingii extracts against cadmium and ethanol-induced lipid peroxidation. *Journal of Pharmacology and Toxicology 4:160-166*
15. Koné WM, Soro D, Dro B, Yao K and Kamanzi K (2011). Chemical Composition, Antioxidant, Antimicrobial and Acetylcholinesterase Inhibitory Properties of Lannea Barteri (Anacardiaceae). *Australian Journal of Basic and Applied Sciences 5(10);1516-1523.*
16. Njinga NS, Sule MI, Patteh UU, Hassan HS, Usman MA and Haruna MS (2013). Phytochemical and Antidiarrhea Activity of the Methanolic Extract of the Stem Bark of Lannea kerstingii Engl. and K. Krause (Anacardiaceae). *Journal of Natural Product and Plant Resources 3(3):43-47.*
17. Njinga NS, Sule MI, Pateh UU, Hassan HS, Ahmad MM, Abdullahi ST, Danja BA and Bawa B (2014). Phytochemical and antimicrobial activity of the leaves of Lannea kerstingii Engl & K. Krause (anacadiaceae). *Nitte University Journal of Health Science 2014;4(4):4-9.*
18. Njinga NS, Sule MI, Pateh UU, Hassan HS, Abdullahi ST and Ache RN (2016). Isolation and Antimicrobial Activity of ?-Sitosterol-3-O-Glucoside from Lannea kerstingii Engl. & K. Krause (Anacardiacea). *Nitte University Journal of Health Science. 6(1):4-8.*
19. Harborne JB (1973). Phytochemical Methods London Chapman and Hall, Ltd.49-188.
20. Sofowora A (1993). Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd. Screening Plants for Bioactive Agents.134-156.
21. Trease GE and Evans WC (2002). Pharmacognosy. 15th edition. Saunders Publishers, London.
22. Adedapo A, Jimoh F, Koduru S, Masika J and Afolayan A (2009). Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of Buddlejasaligna. BMC Complementary and Alternative Medicine 9:9-21
23. Nabavi SM, Ebrahimzadeh M, Nabavi S, Hamidinia A and Bekhradnia AR (2008). Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica MEY. Pharmacology online 2008;2:560-567

24. Kumar T. and Jain V (2015). Appraisal of Total Phenol, Flavonoid Contents, and Antioxidant Potential of Folkloric Lannea coromandelica Using In Vitro and In Vivo Assays. Scientifica. Volume 2015 Article ID 203679
25. Diallo A, Eklu-Gadegbeku K, Agbonon A, Aklikokou K, Napo-Koura G, Creppy E and Gbeassor M (2017). Repeated-dose toxicological studies of hydroalcoholic extract of Lannea kerstingii Engl and K. krause (anacardiaceae) and identification of toxicity mechanisms. *International Journal of Pharmaceutical Sciences And Research. 6(2): 604-611*
26. Obadoni BO and Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta State of Nigeria. *Global Journal of Pure and Applied Sciences 8:203-208*
27. Ndhlala AR, Finnie JF and Van Staden J (2010). In vitro antioxidant properties, HIV-1 reverse transcriptase and acetylcholinesterase inhibitory effects of traditional herbal preparations sold in South Africa. Molecules. 15:6888-6904.
28. Lino A and Deogracios O (2006). The in-vitro antibacterial activity of Annona senegalensis, Securidaccalongi pendiculata and Steanotaenia araliaceae, Ugandan Medicinal plants. African Health Sciences 2006;6(1):31-35
29. Krishnaiah D, Sarbatly R and Nithyanandam RR (2011). A review of the antioxidant potential of medicinal plant species. Food and Bioproducts Processing 89:217-233.
30. Aberoumand A and Deokule SS (2008). Comparison of phenolic compounds of some edible plants of Iran and India. *Pakistan Journal of Nutrition 7:582-585.*
31. Hayouni EA, Abedrabba M, Bouix M and Hamdi M (2007). The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian Quercus coccifera L. and Juniperus phoenicea L. fruit extracts. Food Chemistry 105:1126-1134.
32. Tung YT, Wub HJ, Hsieh C, Ping-Sheng Chen PS and Chang ST (2009). Free radicalscavenging phytochemicals of hot water extracts of Acacia confusa leaves detected by an on-line screening method. Food Chemistry115:1019-1024.
33. Di Carlo G, Mascolo N, Izzo AA and Capasso F (1999). Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sciences 65:337-353.
34. Montoro P, Braca A, Pizza C and De Tommasi N (2005). Structure-antioxidant activity relationships of flavonoids isolated from different plant species. Food Chemistry 92:349-355.
35. Deji-Agboola AM (2010). Antimicrobial Activity of Lannea welwitschii Stem Bark Against Wound Pathogens. *Ethiopian Pharmaceutical Journal. 28:2 (2010).*