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Original Article

PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF THE LEAVES OF *LANNEA KERSTINGII* ENGL *& K. KRAUSE* (ANACADIACEAE)

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Abstract :
The phytochemical and antimicrobial activity of the petroleum ether and crude methanol extracts, chloroform and ethyl acetate fractions of the leaves of *Lannea kerstingii* were investigated. Phytochemical screening revealed the presence of steroids and triterpenes in the petroleum ether extract, steroid, triterpene, flavonoids and tannins in both crude methanol extract and chloroform fraction while the ethyl acetate fraction contained only flavonoids and tannins. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 17.00 to 21.03, 20.10 to 25.24, 25.32 to 34.02 and 22.28 to 27.20 mm for petroleum ether extract, methanol extract, chloroform and ethyl acetate fractions respectively. The minimum inhibitory concentration was between 5 and 10mg/ml, 5mg/ml for the petroleum ether and methanol extract respectively, and between 2.5 and 5 mg/ml, 5mg/ml for the acetate fractions. The minimum bactericidal concentration for all the extracts was 40mg/ml respectively except for chloroform fraction which ranged from 20 to 40mg/ml. The minimum fungicidal concentration for all the extracts was found to be 40mg/ml respectively. This result indicates the broad spectrum antimicrobial potential of *L. Kerstingii* and justifies the use of this plant in traditional medicine.

Keywords : antimicrobial, phytochemical, barteri, *lannea kerstingii*

Introduction :

Humans have practiced the use of plants for the cure of diseases for centuries[1] and these plants are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of disease and ailments.[2] The search for eternal health and longevity and for remedies against pain and discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal products, minerals etc. and the development of a variety of therapeutic agents.[3] This evolving practice is recorded in both folklore and books of early practitioners.

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Despite decades of dramatic progress in their treatment and prevention, infectious diseases remain a major cause of death and debility and are responsible for worsening the living conditions of many millions of people around the world. Infections frequently challenge the physician's diagnostic skill and must be considered in the differential diagnoses of syndromes affecting every organ system.[4]

There is continuous increase in the number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics. Examples include methicillin-resistant staphylococci, pneumococci resistant to penicillin and macrolides, vancomycin-resistant Enterococci as well as multi-drug resistant gram-negative organisms.[5] There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross-infection and search for newer and safer antibiotics.[6]

Lannea kerstingii is a tree with a height of 12m and 40cm in diameter, with a wide-spreading and relatively dense crown. The bark is smooth to slightly fissured, fissures spiral around the trunk (spiral grain), pale grey with pinkish,

white-striped slash.[7] L. kerstingii Engl. and K. Krause (Anacardiaceae) is widely utilized in traditional medicine by various cultures worldwide; a decoction of the back is used to treat swellings,[8] infusion of the back, leaves and bud is used for flatulence, the fruit is used against rickets and scurvy. The plant has been reported for the treatment of diarrhoea,[9] gastritis, rheumatic, sterility, intestinal helminthiasis.[10]

Materials and Methods :

Plant material collection and extraction

The plant was collected in May, 2011 at Zaria, Kaduna State, Nigeria. It was then taken to the Herbarium of the Department of Biological Science, Ahmadu Bello University, Zaria for identification. It was identified by comparison with a herbarium specimen (voucher specimen 1832). After identification, the leaves were removed and dried under shade. The size was reduced using mortar and pestle, filtered for homogeneity and kept away from light until further use.

The leaves (100g) was extracted exhaustively using sequential solvent extraction. It was extracted with petroleum-ether followed by methanol using maceration method with intermittent shaking and solvents changed every 1 hour. The maceration process was then repeated several times for exhaustive extraction. The extracts were dried under reduced pressure. The dried methanolic extract (20g) was then dissolved in distilled water and partition using chloroform, and ethylacetate.

Phytochemical Screening

Basic phytochemical screening to detect the presence or absence of plant chemical constituents such as alkaloids, tannins, saponins, anthraquinones, flavonoids, cardiac glycoside, anthraquinones, steroids and triterpenoe were carried out using standard procedures[11,12] on the petroleum ether extract, crude methanol extract, chloroform and ethylacetate fractions of the leaves, of L. kerstingii.

Test Organisms

Reference strains and clinical isolates: Staphylococcus aureus NCTC6571, Streptococci faecalis, Bacillus subtilis, Corynobacterium ulcerane, Methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli NCTC10418, Klebsiella pneumoniae ATCC 10031, Salmonella typhi ATCC 9184, Shigella dysenteriae, Pseudomonas aeruginosa NCTC6750, and fungi Candida albicans, Candida tropicalis and Aspergillus flavus. were obtained from the Department of Phamaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. All the micro-organisms (clinical isolates) were checked for purity and maintained in slants of agar.

Cultivation and Standardization of Test Organism

A loop full of each of the test organisms were taken from the agar slant and sub cultured into test tubes containing 20 ml of sterile nutrient agar (for bacteria) and sabouraud dextrose agar medium (for fungi). The test tubes were then incubated for 24 hours at 37°C for two days (for bacteria) and 27°C for 2-7 days (for fungi). The growth culture was standardized using sterile normal saline to obtain a density of 10^6 cfu/ml for bacteria. A sporulated test fungal spores was harvested with 0.05%Tween80 in sterile Normal saline and standardized to 10^6 spores/ml.

Preparation of Culture Media

The prescribed quantities of the dehydrated bacteriological culture media was weighed and hydrated with distilled water according to the manufacturers specification. Where necessary, gentle heat was applied to aid dissolution and the resultant suspensions were dispensed into clean bottles and sterilized at 121°C for 15 minutes in an Adelphi bench autoclave.

Antimicrobial Profile (susceptibility test)

The antibacterial screening was carried out using agar diffusion method.[13] The extract was weighed and dissolved in DMSO to obtain the initial concentrations (40mg/ml) of the different extracts. Overnight culture of the various bacteria in blood agar and the fungi in sabouraud dextrose agar slant media were sterilized to produce inoculums size of 10^6cfu/ml. The medium was seeded with 0.1 ml of standard inoculums of the micro-

Keywords : antimicrobial, phytochemical, barteri, *Iannea kerstingii* - Njinga N.S.

organism (Mc-Forland 0.5). The inoculums were sprayed evenly by the use of sterile swab over the surface of the medium, the seeded plates were allowed to dry at 37°C and 27°C for the bacteria and fungi respectively for 30 mins inside incubator. A standard cork borer of diameter 6mm was used to cut a well at the centre of each seeded medium used and 0.1ml of the solution of the extracts was then introduced into each hole on the surface of the medium of each bacteria. In one medium, 0.1ml of DMSO was introduced to serve as negative control and in another, Spafloxacin and Fluconazole (10 µg ml) to serve as positive control for the bacteria and fungi respectively. The medium was incubated at 37°C for 24 hours (for bacteria) and 27°C for 2-7 days (for fungi) after which the plates were observed for zones of inhibition. The zones of inhibition were measured with a transparent ruler and the result recorded.

Determination of Minimum Inhibitory Concentration

This was done using broth dilution method.[14] In this method, 10ml nutrient broth (prepared according to manufacturers specifications) was dispensed into test tubes and sterilized at 121°C for 10 minutes and allowed to cool. Mc-Forland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was inoculated with each of the test micro-organisms and incubated at 37°C for 6 hours to make a turbid suspension of the micro-organisms. After incubation, dilution of the micro-organism in DMSO was done until the turbidity ($1.5x10^6$cfu/ml) matched that of the Mc-Forland scale by visual comparison. Two fold serial dilution of the extract in the broth was done to obtain the following concentrations; 20mg/ml, 10mg/ml, 2.5mg/ml, 1.25mg/ml and 0.625mg/ml. From the suspension of the micro-organism in DMSO, 0.1ml was inoculated into the different concentrations of the extract in the nutrient broth. The broths were incubated at 37°C for 24hrs (for bacteria) and 27°C for 2-7 days (for the fungi) after which the test tubes were observed for turbidity. The lowest concentration of the extract in the broth which shows no turbidity represents the MIC. The results after 24 hour were recorded.

Determination of Minimum Bactericidal Concentration (MBC)

Blood agar was prepared according to manufacturer's instruction, sterilized at 121°C for 15 minutes. It was poured into sterile petri-dishes. The plates were allowed to cool and solidify. The contents of the MIC test tubes in the serial dilution were then sub-cultured on to the prepared plates and the plates were then incubated at 37°C for 24 hours (for bacteria) and 27°C for 2-7 days (for the fungi) after which the plates were observed for growth. The plate without growth represents the minimum bactericidal concentration. After 24 hours the results were recorded.[14,15]

Results :

The leaves of L. kerstingii contained more methanol soluble phytochemicals (32.44%) followed by petroleum ether (30.2%). The yield of the chloroform and ethyl acetate fractions were found to be 10.05 and 8.5% respectively (table 1).

The crude methanolic extract and chloroform fraction were found to contain flavonoids, tannins, steroids and triterpenes. The ethyl acetate fraction of the leaves of L. kerstingii was found to contain only flavonoids and tannins. While the petroleum ether extract contains only steroids and triterpenes (table 2).

The petroleum ether, the crude methanol extract and all the other two fractions were inactive against C. ulcerane, P. aeruginosa, C. albicans and A. flavus. The crude methanol and the petroleum ether extract were also inactive against S. typhi. All the extracts were active against S. aureus, S. faecalis, B. subtilis, MRSA, E. coli, K. pneumonia, S. dysentariae and the fungi C. tropicalis. The chloroform fraction and the ethyl acetate fraction were also active against S. typhi (Table 3). The zone of inhibition of the crude methanol extract ranged from 20.10mm (E. coli) to 25.12mm (S. dysentariae), that of petroleum ether ranged from 17.00mm (E. coli and C. tropicalis) to 21.03mm (S. aureus). The chloroform fraction showed high activity when compared to the ethyl acetate fraction with zone of

inhibition ranging from 25.32mm (S. typhi) to 34.02mm (B. subtilis). While that of ethyl acetate ranged from 22.28mm (S. typhi) to 27.20mm (B. subtilis, K. pneumonia and S. dysentariae) (table 3).

The MIC of the crude methanol and ethyl acetate fractions were 5mg/ml respectively. That of the chloroform extract was found to be 2.5mg/ml for S. faecalis, B. subtilis, K. pneumonia, S. typhi and S. dysentariae and 5mg/ml for S. aureus, MRSA, E. coli and C. tropicalis respectively while the MIC of the petroleum ether extract was 5mg/ml for S. aureus, B. subtilis and K. pneumonia and 10mg/ml for S. faecalis, MRSA, E. coli, S. dysentariae and C. tropicalis respectively as shown in table 4.

The MBC of the petroleum ether and the crude methanol extracts were above 40mg/ml for all the organisms except for B. subtilis whose MBC was 40mg/ml for the methanol extract. The chloroform extract showed MBC/MFC for all tested organism except for B. subtilis whose MBC was 20mg/ml. The ethyl acetate showed MBC of 40mg/ml for S. aureus, B. subtilis, E. coli and K. pneumonia and MFC of above 40mg/ml for C. tropicalis (table 5)..

Table 1 : % yield of the different extracts obtained from the extraction of the leaves of Lannea kerstingii.

Fraction	Colour	Weight	% yield
Petroleum ether extract	Dark green	30.02g	30.2%
Crude methanol extract	Dark green	32.44g	32.44%
Chloroform fraction	Light green	2.01g	10.05%
Ethyl acetate fractopm	Light green	1.74g	8.5%

Table 2 : phytochemical constituents present in the different extracts of the leaves of Lannea kerstingii

Constituents	Petroleum ether extract	Crude methanol extract	Chloroform fraction	Ethyl acetate fraction
Anthraquinones	-	-	-	-
Flavonoids	-	+	+	+
Tannins	-	+	+	+
Alkaloids	-	-	-	-
Coumarins	-	-	-	-
Saponins	-	-	-	-
Steroids and triterpenes	+	+	+	-
Cardiac Glycoside	-	-	-	-

Key: - = not present, + = present

Table 3 : Zone of inhibition of crude methanol extract, chloroform and ethyl acetate fraction of the leaves of L. kerstingii.

TEST ORGANISM	ZONE OF INHIBITION (mm)					
	Petroluem ether fraction	Crude CH_3OH extract	$CHCl_3$ fraction	Ethyl acetate fraction	Sparflo-zin	Flucona -zole
S. aureus NCTC6571	21.03	20.10	27.30	25.10	27.00	
S. faecalis	19.10	21.23	30.10	24.10	32.02	
B. subtilis	20.10	25.24	34.02	27.20	47.00	
C. ulcerane	0.00	0.00	0.00	0.00	32.11	
Methicillin-resistant S. aureus (MRSA)	19.02	22.06	28.41	24.21	37.03	
E. coli NCTC10418	17.00	20.10	27.24	26.22	37.10	
K. pneumoniae ATCC 10031	20.42	24.10	31.34	27.20	47.02	
S. typhi ATCC 9184	0.00	0.00	25.32	22.28	32.23	
S. dysentariae	19.30	25.12	32.16	27.20	39.33	
P. aeruginosa NCTC6750	0.00	0.00	0.00	0.00	29.22	
C. albicans	0.00	0.00	0.00	0.00		32.00
C. tropicalis	17.00	22.03	27.00	24.10		29.21
A. flavus	0.00	0.00	0.00	0.00		34.01

Table 4 : Minimum Inhibitory Concentration of petroleum ether and crude methanol extract, chloroform and ethyl acetate fractions of the leaves of L. kerstingii against Test Organisms

TEST ORGANISM	MIC(mg/ml)			
	Petroleum ether extract	Crude methanol extract	Chloroform fraction	Ethyl acetate fraction
S. aureus NCTC6571	5	5	5	5
S. faecalis	10	5	2.5	5
B. subtilis	5	5	2.5	5
MRSA	10	5	5	5
E. coli NCTC10418	10	5	5	5
K. pneumoniae ATCC 10031	5	5	2.5	5
S. typhi ATCC 9184			2.5	5
S. dysentariae	10	5	2.5	5
C. tropicalis	10	5	5	5

Table 5 : Minimum Bactericidal / Fungicidal Concentration of petroleum ether and crude methanol extracts, chloroform and ethyl acetate fractions of the leaves of L. kerstingii against Test Organism

TEST ORGANISM	MBC/MFC (mg/ml)			
	Petroleum ether extract	Crude methanol	Chloroform fraction	Ethyl acetate fraction
Staphylococcus aureus NCTC6571	40	40	40	40
Streptococci faecalis	40	40	40	40
Bacillus subtilis	40	40	20	40
MRSA	40	40	40	40
Escherichia coli NCTC10418	40	40	40	40
Klebsiella pneumoniae ATCC 10031	40	40	40	40
Salmonella typhi ATCC 9184			40	40
Shegella dysentariae	40	40	40	40
C. tropicalis	40	40	40	40

Discussion :

Methanol was found to be a better extraction solvent than petroleum ether (table 1) which is with conformity with literature.[16] Flavonoids have been shown to have anti-viral and antimicrobial activities.[17,18] Tannins may exhibit antibiotic activity by complexing extracellular enzymes produced by the pathogens or by interference with the metabolism of the pathogen itself.[19] Thus the antimicrobial activity of the leaves may be due to the presence of flavonoids and tannins (table 2).

The methanol and ethanol extract of the leaves of L. kerstingii have been reported to be active against S. aureus, E. coli, P. vulgaris, S. typhi, S. lactis, Shigella sp..[20] This is in line with the current study in which the methanol extract show activity against S. aureus, E. coli and S. dysentariae but inactive against S. typhi (table 3). This difference may be due to age, physiological variations, environmental conditions, geographic variations, genetic factors and evolutional differences of the plant[21] or the presence of an antagonist in the extract.

Among the extracts, the petroleum ether fraction is the least active. Its activity is solely due to the presence of steroids and triterpenes present in the fraction (table 2). The chloroform fraction is the most active and its activity is be due to the presence of low molecular weight flavonoids, tannins, steroids and triterpenes (table 2). The results of this study corresponds with several investigation that flowering plants are potential source of antimicrobial substances.[22,23]

The low MIC of these extracts (table 3) especially the chloroform fraction showed the extract's activity against both Gram positive and Gram negative bacteria which are associated with different type of infections including urinary tract infections (S. aureus), and typhoid fever (S. typhi). S. aureus is also responsible for a wide variety of

diseases, including pneumonia, skin and soft tissue infections, and diabetic foot infections.[24] Similarly, P. aeruginosa is a common pathogen associated with burn wound infections, keratitis, and respiratory tract infections.[25] The extract also showed activity against E. coli (MIC 5mg/ml) which is the commonest cause of urinary tract infection and accounts for approximately 90% of first urinary tract infection in young women.[15] This indicates the usefulness of this plant in the treatment of urinary tract infection, respiratory tract infections, diabetic foot infections due to its activity against the organisms causing these infections. This result gives scientific base and credence for the claims of the therapeutic capabilities and folkloric usage of the leaves of *Lannea kerstingii* for the treatment of various ailments.

Conclusion :

The leaves of *Lannea kerstingii* contains phytochemicals which possess antimicrobial activity. This study therefore supports the traditional use of *Lannea kerstingii* for the treatment of various infectious diseases in Nigeria and different regions of the world, and may serve as a good source of novel antibiotics.

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