ANTIBACTERIAL STUDIES ON STEM-BARK OF *Lannea barteri* (OLIV.) ENGL. (ANACARDIACEA)

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I. Sabo[1], A. M. Zakariya[2], A. Ahmed[3] and Z. Abdulhamid[3]
[1]Department of Pharmacognosy & Drug Development, Gombe State University, Nigeria
[2]Department of Biological Sciences, Sule Lamido University, Jigawa State, Nigeria
[3]Department of Pharmacognosy & Drug Development, Ahmadu Bello University, Zaria, Nigeria
*Correspondent author: ibrahimsabo@gsu.edu.ng, successfulsabo@gmail.com

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Abstract: *In vitro* antibacterial activities of stem bark extract and fractions of *Lannea barteri* was investigated against four (4) reference bacterial strains including gram-positive and gram-negative bacteria. Powdered stem bark of *L. barteri* was extracted with methanol using cold maceration technique. A weight of 300 g of methanolic extract was obtained after extraction. Partitioning of the crude extract with hexane, ethyl acetate and n-butanol solvents gave three successive fractions, Hexane (0.1 g), ethyl acetate (4.46 g), n-butanol (4.83 g), respectively. Antibacterial activity of the extract and resultant fractions was performed by disk diffusion and broth microdilution with varying concentrations of 100, 50, 25 and 12.5 mg/ml. The crude extract, ethyl acetate and n-butanol fractions exhibited inhibitory action against the Gram-positive organisms at concentrations of 100, 50, 25 and 12.5 mg/ml with the exception of crude extract with no activity recorded against *B. subtilis* at concentration of 12 mg/ml, while hexane fraction exhibited inhibitory action against *E coli* at concentrations of 100 and 50 mg/ml with no activity recorded at 25 and 12.5 mg/ml for both gram-negative organisms tested, the crude extract possess activity on the gram positive only with highest zone of inhibition of 20 mm. The highest zone of inhibition recorded was 22 mm for the fractions and 40 mm for the standard drug used (ciprofloxacin). The results obtained from this research further justify the traditional claim on the wound healing activity of the stem-bark of *L. barteri*.

Keywords: Gram +ve, gram –ve, *L. barteri*, antibacterial, ciprofloxacin

Introduction

The increasing acceptance of traditional medicine as an alternative form of health care especially in Africa, Asia and some part of Europe and America have led researchers to investigate the anti-bacterial activities of medicinal plants (Njinga *et al*., 2013; Geslen *et al*., 2000; Ayman and marzen, 2014; Elumalai *et al*., 2011; Bashar *et al*., 2018). Species used in traditional medicines continue to be the most reliable sources for the discovery of useful compounds.

Antibiotic resistance has become a global concern (Westh *et al.*, 2004). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. Search for new antibacterial agents should be continued by screening many plant families. This work revealed the potential of *L. barteri* as sources of drugs. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile *et al.*, 2000). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon *et al.*, 2004); some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone *et al.*, 2004). In Nigeria, the prevalence of bacterial infection varies from location to locations, in Nasarawa, the prevalence has been reported to be up to 30% of the populations (Kolewale *et al.*, 2009) while in Jos the prevalence rate of 25.6% significant bacteriuria recorded by Nedolisa (1998) at the Jos University Teaching Hospital (JUTH), Nigeria and 22% by Ekweozor and Onyemenen (1996) in Ibadan. 38.6% rate was recorded by Akinyemi *et al*. (1997) in Lagos, Nigeria, and 35.5% rate recorded by Ebie *et al*. (2001) in Rukuba Military Cantoment, Jos, Plateau State Mbata (2007) recorded 77.9% among Prison inmates in Nigeria. The commonest bacteria found in infected wound are *Pseudomonas spp*, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and their prevalence in Nigeria tertiary hospitals (Unversity teaching hospitals) between1995-2001 were recorded as Pseudomonas spp. - 29.9%, *Staphylococcus aureus* (27.5 %). *Escherichia coli* (7 %) (Lateef *et al.*, 2003).

Lannea barteri (Oliv) Engl. belongs to the family Anacardiaceae. Members of the family are abundantly found in tropical and sub-tropical regions in Africa (Acharya, 2000). *Lannea barteri* is used traditionally for the treatment of typhoid, gastrointestinal disorders and wound healing. They are used as medicinal plants in the urban area of Chindwara town in India (Acharya, 2000). They are also used in Iran for curing infectious maladies. Members are also widely distributed in Nigeria. The bark is stomachic. A decoction is drunk as a treatment for gastric pains, diarrhoea, oedema, paralysis, epilepsy and madness (Kone *et al*., 2011).

When combined with the bark of ukuku (Yoruba, species) it is used as a vermifuge. The bark is used externally to treat ulcers, sores and leprosy. A root decoction is taken to cure hernia. The root is ground, wrapped in the leaves of an unknown species, and applied as a poultice on wounds. A leaf decoction is taken to cure haemorrhoids.

The phytochemical analysis of the roots and stem bark extracts by qualitative study showed the presence of steroids, triterpenoids, saponins, polyphenols, flavonoids, tannins, alkaloids and quinine (Kone *et al*., 2011). Quantitative estimation proved that both extracts of roots and stem bark have considerably high constitutions of phenolic compounds. Similar total phenolic contents were obtained for the stem bark and roots with respectively 254.46 and 254.96 µg/g GAE (Kone *et al*., 2011).

Materials and Methodology

Sample collection

Sample of stem bark of the plant (*Lannea barteri*) was collected by making longitudinal and transverse incisions through the outer layers of the stem of the plant followed by feeling; it was collected in the month of August 2014 from Bomo Villlage of Sabon Gari Local Government, Kaduna

state. The plant material was identified as *Lannea barteri* at herbarium unit of the department of Biological sciences, Ahmadu Bello University, Zaria and was assigned Voucher specimen number of 06872.

The stem bark collected was further dried at room temperature for about 3 weeks and then pounded with the aid of mortar and pestle to obtain the semi powdered form of the plant material which was stored in air – tight container prior to use.

Extraction and partitioning

Two (2) kg of powdered stem bark of *Lannea barteri* was subjected to maceration with 5 liters of methanol for 5 days at room temperature. The mixture was filtered using Whatman filter paper and the filtrate was dried on water bath at 40ºC to obtain a dark brown gummy residue.

Solvent – solvent fractionation was carried out using protocol design by Kupchan and Tsou (1973) and modified version of Wagenen *et al*. (1993).

50 g of the crude extract was suspended in hot water. The prepared solution was then partitioned successively using the following solvents of increasing polarity; N-hexane (500 ml X 2), ethyl acetate (500 ml X 2) and n-butanol (500 ml X 2) (Fig. 1). All the fractions were evaporated to dryness at low temperature of 39ºC and kept in air tight container for further analysis.

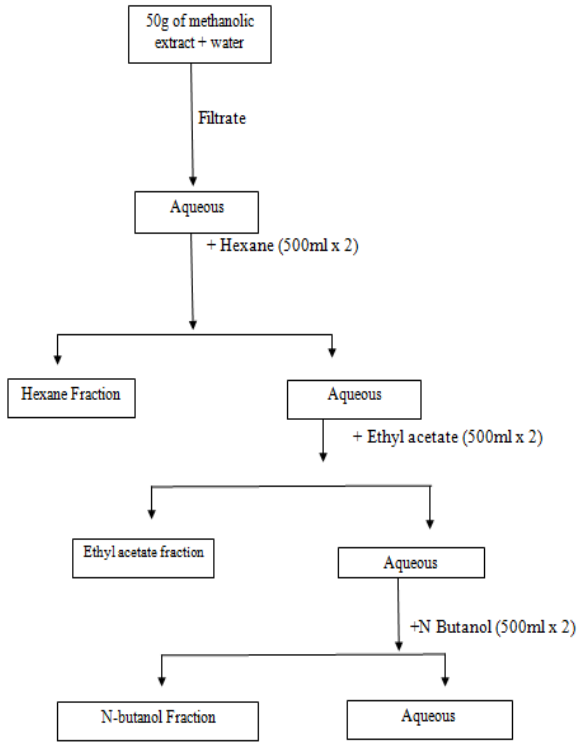

Fig. 1: Fractionating chart for crude extract of *L. barteri* stem bark

Sensitivity test

Test organisms and culture media

The test organisms used for this analysis were clinical isolates of bacteria obtain from department of microbiology, Ahmadu Bello University, Zaria. The isolates were; *Staphylococcus aureus, Bascillus subtilis, Escherichia coli* and *Salmonella typh*i.

The culture media used for the analysis include Mueller Hinton agar (MHA), Mueller Hinton broth (MHB) and nutrients agar. The mentioned media were used for sensitivity test, determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC). All media were prepared according to manufacturer's construction and sterilized by auto cleaving at 121ºC for 15 min.

Determination of inhibitory activity (Sensitivity test) of the fractions using agar well diffusion method

The standard inucula of the bacterial isolates were streaked on sterilized Mueller Hinton agar using sterile swab stick. Four well were punched on each inoculated agar mate with a sterile cock borer and were properly labelled according to different concentration of the extract prepared which was 100, 50, 25 and 12.5 mg/ml, respectively. Each well was filled up with approximately 0.2ml of the extract. The inoculated plates with the extract were allowed to stay for one hour and then incubated at 37ºC for 24 h. At the end of the incubation period, the plates were observed for any evidence of inhibition which appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of the zones was measured using a transparent ruler calibrated in millimetre and the result was recorded.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the fractions was determined using the dilution method with the mueller hinton broth as a diluent. The lowest concentration of the extract and tested fractions showing inhibition for each organism tested were serially diluted in the test tubes containing mueller hinton broth. The organisms were inoculated into each tube containing the broth and the extract. The inoculated tubes were then incubated at 37ºC for 24 h. At the end of the incubation period, the tubes were examined/observed for the presence or absence of growth using turbidity as a criterion, the lowest concentration on the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration (MIC). The result was also recorded.

Minimum bactericidal concentration (MBC)

The result from the minimum inhibitory concentration (MIC) was used to determine the minimum bactericidal concentration (MBC) of the extract. A sterilized wire loop was dipped into the test tubes that did not show turbidity (clear) on the MIC test and aloop was taken and streaked on a sterile nutrient agar plates. The plates were incubated at 37ºC for 18-24 h. At the end of incubation period, the plates were examined / observed for the presence or absence of growth. This is to determine whether the antibacterial effect of the extracts is bactericide or bactericidal.

Results and Discussion

The results on Table 1 indicates that all the tested fraction were active on gram positive strains with the exception of hexane which was active on *E coli* with diameter zone of inhibition of 19 mm at 100 mg/ml and 14 mm at 50 mg/ml and this may be attributed to the differences in the composition of the secondary metabolites as the antibacterial activity of plant extracts is believed to be due to the presence of secondary metabolites such as flavonoids, tannins and alkaloids (Draughon, 2004).

Highest activity was recorded for Ethyl acetate fraction (22 mm) and this may be attributed to the fact that the partitioning of the crude extract enhanced the antibacterial activity of the ethyl acetate fraction and reduced those of other fractions. This indicates that the active principles might be more concentrated in ethyl acetate fraction and more diluted in other fractions.

Good antibacterial activity should be above 10 mm zone of inhibition (Abdallah, 2014). The standard drug (Ciprofloxacin) which was used as control at the concentration of 40 µg/ml was sensitive against all the test organisms with diameter zone of inhibition ranging from 38 to 45 mm (Table 1). Thus, this showed that the fractions and the crude extract possess a modest activity against the tested bacterial strains when compared with standard drug used.

Table 1: Zone of inhibition (mm) at varying concentration (mg/ml) of the extract and fractions

Organism	Conc. In Mg/ml	Crude	Hexane extract	Ethyl acetate extract	N-Butanol extract	Ciprofloxacin (Conc. 40 µg/ml)
S. aureus	**100**	20±0.81	14.70±1.24	20±0.81	18.0±0.81	
	50	16.50±6.12	11.70±1.24	19.0±0.81	15.1±5.52	
	25	15.33±5.53	-	14.7±6.43	14.0±5.05	39.66±1.24
	12.5	14.40±5.25	-	14.0±5.32	12.74±4.90	
B. subtilis	**100**	15.33±1.24	12.0±0.81	20±0.81	15.0±0.81	
	50	12.70±4.50	12.33±0.50	17.0±6.17	12.85±4.62	
	25	11.70±4.20	-	15.70±5.70	12.02±4.02	43.66±1.24
	12.5	-	-	14.44±5.41	-	
E. coli	**100**	-	20.0±0.81	-	-	
	50	-	14.22±6.60	-	-	
	25	-	-	-	-	35.66±1.69
	12.5		-	--	-	
S. typii	**100**		-	-	-	
	50	-	-	-	-	38.0±1.63
	25	-	-	-	-	
	12.5	-	-	-	-	

Butanol, Ethylacetate fractions and crude extract of *L. barteri* had an MIC of 12.5 mg/ml against gram negative bacterial isolates (Fig. 2). N-Hexane fraction showed activity against both gram negative (*S. aureus*) and gram positive (*E. coli*) with MIC of 12.5 mg/ml (Fig. 2). The MIC values obtained (Fig. 2) on the corresponding (sensitive) bacterial strains confirm the bactericidal effects of the concerned samples (Tamokou *et al*., 2009). This is interesting in view of the perspective of developing new antibacterial drugs from natural products.

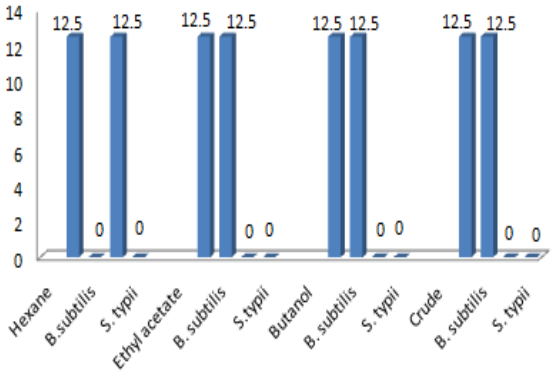

Fig. 2: Minimum inhibitory concentration mg/ml (MIC)

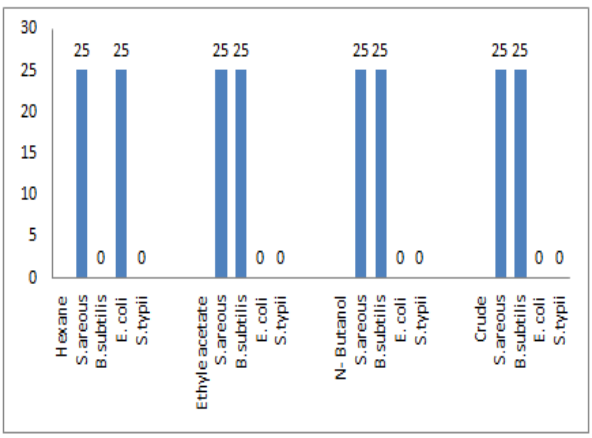

Fig. 3: Minimum bactericidal concentrations mg/ml (MBC)

Butanol, Ethyl acetate fractions and the crude extract of *L. barteri* had MBC of 25 mg/ml against gram negative bacterial isolates tested (Fig. 3). No activity was recorded against gram positive test organisms. N-Hexane fraction showed activity against both gram negative (*S. aureus*) and gram positive (*E. coli*) with MIC of 25 mg/ml (Fig. 2).

Our findings are in agreement with Manjunatha (2006) who studied the bactericidal potential of bark of many taxon. Stem bark extract of *Pterocarpus santalinus* that showed maximum activity against *Bacillus subtilis*, 17.0 mm. The methanolic extract of stem bark of *Tetracarpidium conophorum* inhibited the growth of *B. subtilis*, 12.3 mm (Kilani, 2006). According to Manjunatha (2006) the stem bark extract of *Pterocarpus santalinus* inhibits the growth of *S. aureus* (16.05 mm). Methanolic extracts of stem bark of *Vitex doniana* also noticed to possess bactericidal potential against *S. aureus* (Kilani, 2006). Stem bark extract of *Holarrhena antidysenterica* possess antibacterial potential against enteric pathogen *E. coli* (Ballal *et al.*, 2001). Doughari *et al*. (2008) noticed that stem bark of *Cochlospermum planchoni* inhibited the growth of *P. aeruginosa* (26 mm). Sangetha *et al*. (2008) noticed that methanolic extracts from the stem of *Cassia fistula* and *Cassia surattensis* arrested the growth of *S. typhi* (19 mm).

Currently, the antibiotics are losing their effectiveness worldwide and necessitating surveillance program and research interest (Abdallah, 2014); thus, the overall results of this study can be considered as very promising in the perspective of new antibiotic drugs discovery from plant sources, especially when the medical importance of tested microorganisms is considered. *S. aureus* and *B. Subtilis* are major cause of community and hospital-associated infection with an estimated prevalence of (27.5%) in Nigerian teaching hospitals (Lateef *et al.*, 2003).

Conclusion

The anti-bacterial activity carried out in this research shows that the stem bark extracts of *L. barteri* posses activity on *S. areous, B. subtilis, E. coli and S. typhi*. This further justifies the traditional claim on the wound healing activity of the stem-bark of *L. barteri*. Pharmacological and toxicological studies, currently going on in our laboratory, will be necessary to confirm this hypothesis.

Conflict of Interest

Authors declare that there are no conflicts of interest

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