ANTIBACTERIAL POTENTIALS OF STEM BARK EXTRACTS OF *LEPTADENIA LANCIFOLIA* AGAINST SOME PATHOGENIC BACTERIA

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Summary

Antibacterial activity the phytoconstituents of stem bark extracts of *Leptadenia lancifolia* extracted with various solvents (water, acetone and methanol) were investigated using the filter paper disc diffusion method. The phytocostituents detected (alkaloids, tannins, saponins, phenols and glycosides) demonstrated activity against *Streptococcus pyogenes, Staphhylococcus aureus, Salmonella typhi, Escherichia coli, Proteus vulgaris, Pseudomonas aerugenosa* and *Shigella flexnerri* with the ethanol extracts demonstrating the highest activity against *S. aureus* (22 mm zone diameter of inhibition, MIC 0.08 mg/ml, MBC 0.1 mg/ml), while the least activity was demonstrated by the aqueous extracts against *Pseudomonas aeruginosa* and *Shigella flexnerri* (6 mm zone diameter of inhibition in each case, and MIC 0.5 and 1.0 mg/ml, and MBC 1.0 and 2.0 mg/ml respectively). The antibacterial activity of the plant was concentration dependent slightly higher than cotrimoxazole and increased with increased temperature and reduced (acidic) pH. The MIC and MBC values of the extracts against the test bacteria ranged between 0.06-8.0 mg/ml while those of cotrimoxazole ranged between 0.06-10.0 mg/ml. *Leptadenia lancifolia* can be used to source antibacterial substances for development of novel drugs for the treatment of urinary, respiratory and gastrointestinal tract infections.

Key words: Antibacterial, *Leptadenia lancifolia,* plant extracts, phytochemical, cotrimoxazole.

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Introduction

Leptadenia lancifolia (Per) Decne, (Asclepiadaceae R.Br. Ceropegieae) is a common herb with several creeping stems, succulent leaves, with unbranching and inflorescence flowers and a dry one-celled follicle fruit commonly found in tropical dry land of Africa. Commonly called yadiya (Hausa, Nigeria), sabato kusubi (Fulani, Nigeria), nawonu (Chamba) and Ding (Vere) (1), the plant has various food and medicinal uses. The leaves are often prepared with groundnut mash as local salad, and also as a local soup called "demiska" (Nigeria) or as "hunger food" (Niger). Its medicinal uses include relief for labour pains (boiled leaves), cure for stomach ache and treatment of fungal infections (ring worm) (1-3). Plant product drugs and herbal remedies have been employed since prehistoric times to treat human and animal diseases, and several countries still rely on plants and herbs as the main sources of drugs. This is because of the presence of various inherent phytoconstituents such as protoberberines, alkaloids, picralima-type indole alkaloids, phenols and anthraquinones in different parts of the plant. These phytoconstituents confer antimicrobial properties to plant and thus are used as natural protective agents against fungal bacterial and pesticidal attacks on the plants. Consequently various parts of different plants have been used traditionally in form of decoctions, concoctions, diffusions or infusions for the treatment of various diseases. However due to global rise in reported cases of multidrug antimicrobial resistance against various existing drugs, scientific investigations into medicinal plants is on the rise in order to find more effective remedies that will be readily available cheap and affordable with possibly novel mechanisms of action against the recalcitrant microbes. In the present study, we report the antibacterial potentials of stem bark extracts of *Leptadenia lancifolia* and the phytochemical profile of the extracts screened using percolation with various organic solvents which, to the best of our knowledge, have not been studied previously.

Materials and Methods

Collection of Plant Samples

The plant was collected from the wild in Bole Ward of Yola South Local Government Area of Adamawa State, Nigeria, and authenticated by Mr. Joseph Adamu, of the Department of Forestry Ministry of Environment Yola, Adamawa State, Nigeria where a voucher specimen was deposited.

Test Organisms

Clinical isolates of *Staphyloccocus aureus, Salmonella typhi,* and *Streptococcus pyogenes* were obtained from the Microbiology Laboratory of the Specialist Hospital Yola, Adamawa State. While *Escherichia coli, Proteus vulgaris* and *Pseudomonas aerugenosa* was obtained from the Microbiology Laboratory of the Department of Microbiology, Federal University of Technology Yola, Nigeria.

Preparation of the Plant and Plant Extracts

The plant was collected, washed with clean tap water to remove dirt and stem barks removed and dried under shade to constant weight for 5 days. The dried stem barks was then reduced to pieces using mortar pestle and later grinded to powder using an electric mill. The powdered material was then kept in air tight containers until use. For extraction of bioactive components 20 g each of the powdered samples were soaked into 100 ml of water, acetone and methanol for 72 h with stirring at 24 h interval. The mixtures were then filtered using Whatman no. 1 filter paper and the filtrates then concentrated under vacuum at 40^{o}C (4,5).

Preliminary Phytochemical Analysis

The freshly prepared stem bark extracts were subjected to standard analyses for the presence of phytoconstituents as described by (6).

Evaluation of antibacterial activity

This was carried out using the filter paper disc diffusion method as described elsewhere (7). Filter papers (2 mm in diameter) were cut using a paper punch and then sterilized by autoclaving. The sterilized filter papers were then soaked in different concentrations of extracts (10-40 mg/disc) and then allowed to dry. To test for susceptibility, 0.5 ml McFarland turbidity

standard of test bacteria were seeded on to sterile Mueller Hinton agar plates and spread out using sterile glass rod in order to achieve confluent growth. The plates were left on the table for 5 min to dry. Sterile filter paper discs soaked in the extract solution at different concentration (10, 20, 30, 40 mg/disc) were placed on the different Mueller Hinton agar plates earlier seeded with different test organisms. The plates were then incubated at 37ºC for 24 h.

Effect of temperature and pH on the activity of extracts

This was determined as earlier described (7). 50 mg/ml concentration of extracts was reconstituted and 5 ml of the suspension was transferred into four different test tubes and treated at 4°C in the refrigerator and 30 (ambient), 50 and 100°C in a water bath respectively for 1 h and tested for antifungal activity. To determine the effect of pH, the extract was treated at pH ranges of 2.0 to 10 using 1N HCl and 1N NaOH solutions respectively in a series of test tubes for 1 h. After 1 h of treatment, each of the extracts was neutralized (pH 7) once again using 1 N HCl and 1 N NaOH as the case may be and then tested for antimicrobial activity.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of extracts

Plant extracts were reconstituted to varying concentrations of 0.02, 0.04, 0.06, 0.08, 0.1, 0.5, 1.0, 1.5, 2.0, 3.0, 6.0, 12.0, 24.0 and 48 mg/l in series of test tubes and 1 ml of Nutrient Broth (NB, Oxoid) was dispensed to each extract suspension and a loop-full of test bacterial isolates (0.5 McFarland turbidity standard) was added. A set of test tubes containing NB only were seeded with the test fungi and used as control. The tubes were incubated at 37ºC for 24 h. The MIC was defined as the lowest drug concentration allowing no visible fungal growth. The concentration at which no visible growth (in terms of turbidity) was observed after incubation period was noted as the MIC. For MBC determination, a loopful of culture from each set of tubes that did not show any visible growth in the MIC determination were subcultured on to fresh plates of MHA and incubated at 37ºC for 24 h. The concentration at which no visible growth was observed after incubation period was noted as the MBC (8).

Evaluation of the combined effect of cotrimoxazole and plant extracts or phytochemicals on the test organisms

This evaluation was carried out as described elsewhere (9). 0.5 ml of resistant bacterial cultures (0.5 McFarland turbidity standard) grown in 10 ml of NB for 6 h were inoculated in MHA supplemented with 0.50 mg/ml cotrimoxazole with varying concentrations (5 to 50 mg/ml) of plant extracts, based on MIC values that had previously been evaluated. The cultures were then incubated at 37ºC for 24 h. After 24 h, the optical density of each sample was determined and compared to those of MIC to verify any synergistic effect among the tested compounds.

Results

Results of phytochemical analysis of the stem bark extracts of *Leptadenia lancifolia* showed the presence of alkaloids, tannins, saponins, phenols and glycosides (Table 1). Results also show that the plant has antibacterial activity against the test bacteria and the activities were concentration dependent. The highest activity (22 mm zone diameter of inhibition, MIC 0.08, MBC 0.1 mg/ml) was demonstrated by the methanol extracts followed by the acetone extracts (20 mm zone diameter of inhibition, MIC 0.08, MBC 0.1 mg/ml).

Table 1: Phytochemical components of stem bark extracts of *Leptadenia lancifolia*

Phytochemical components	Stem bark extracts
Alkaloids	+
Tannins	+
Saponins	+
Cardiac glycosides	-
Phenols	+
Volatile oils	-
Glycosides	+

Key: + = Present; - = Absent

The water extracts demonstrated the least activity (6 mm zone diameter of inhibition, MIC 1.0, MBC 2.0 mg/ml) against *S. aureus* (Table 2). Cotrimoxazole demonstrated a slightly lower activity (16 mm zone diameter zone of inhibition, MIC 0.8, MBC 1.2 mg/ml) than the plant extracts against *S. aureus.* Results of effect of temperature and pH showed that, activity of the extracts against the test bacteria increased significantly when the temperature was increased

and as the pH was adjusted towards acidity (Table 3 and 4). Results of effect of combination of aqueous extracts (5-50mg/ml) with cotrimoxazole (0.5 mg/ml) is shown in Table 5. When combined with cotrimoxazole, activity of the extracts against the test bacteria decreased significantly at all the tested concentrations. For instance at 50 mg/ml, the activity of the extracts alone against *S. aureus* was 22 mm (zone of inhibition), but this decreased to 10 mm when combined with cotrimoxazole. A similar trend was observed with the other test bacteria.

Table 2: Antimicrobial activity of stem bark extracts (50 mg/ml) of *Leptadenia lancifolia*

Organisms	Zone of inhibition (mm)					MIC	MBC	*MIC	*MBC
	AQ	AE	EE	ME	Cot				
Streptococcus pyogenes (SP 003 MBFTY)	4	8	12	-	8	4.0	8.0	8.0	10.0
Staphhylococcus aureus (SA012 MBFTY)	-	20	22	-	16	0.08	0.1	0.8	1.2
Salmonella typhi (ST 008 MBFTY)	-	19	20	-	22	0.06	0.08	0.06	0.08
Escherichia coli (EC 002 MBFTY)	-	10	24	8	20	0.06	0.06	0.6	1.0
Proteus vulgaris (PV 001 MBFTY)	-	8	18	8	16	2.5	3.0	3.0	4.0
Pseudomonas aerugenosa (PA 005 MBFTY	6	12	18	10	14	0.5	1.0	1.5	3.0
Shigella flexnerri (SF 002 MBFTY)	6	8	16	6	18	1.0	2.0	1.0	2.0

AQ = aqueous extracts; Cot = cotrimoxazole; AE = acetone extracts; EE = Ethanol extracts; ME= methanol extracts; - = no measurable zone of inhibition; MIC = minimum inhibitory concentration for ethanol extracts; MBC = minimum bactericidal concentration for ethanol extracts; *MIC = minimum inhibitory concentration for cotrimoxazole; MBC = minimum bactericidal concentration for cotrimoxazole

Table 3: Effect of temperature on the activity of stem bark extracts (50 mg/ml) of *Leptadenia lancifolia*

Organisms	Diameter of zone of inhibition (mm)				
	* 28^{o}C	4^{o}C	50^{o}C	70^{o}C	100^{o}C
Streptococcus pyogenes (SP 003 MBFTY)	12	12	13	13	16
Staphhylococcus aureus (SA012 MBFTY)	22	22	22	14	16
Salmonella typhi (ST 008 MBFTY)	20	20	24	24	26
Escherichia coli (EC 002 MBFTY)	24	22	24	24	30
Proteus vulgaris (PV 001 MBFTY)	18	19	20	20	22
Pseudomonas aerugenosa (PA 005 MBFTY	18	20	23	25	25
Shigella flexnerri (SF 002 MBFTY)	16	16	18	18	20

*Untreated sample; no measurable zone of inhibition

Table 4: Effect of pH on the activity of extracts (50 mg/ml) of *Leptadenia lancifolia*.

Organisms	Diameter of zone of inhibition (mm)				
	pH 5.0*	pH 2.0	pH 6.0	pH 8.0	pH 10.0
Streptococcus pyogenes (SP 003 MBFTY)	12	12	14	10	6
Staphhylococcus aureus (SA012 MBFTY)	22	22	24	22	18
Salmonella typhi (ST 008 MBFTY)	20	20	22	20	16
Escherichia coli (EC 002 MBFTY)	24	26	26	24	18
Proteus vulgaris (PV 001 MBFTY)	18	20	20	20	18
Pseudomonas aerugenosa (PA 005 MBFTY	18	18	20	20	16
Shigella flexnerri (SF 002 MBFTY)	16	18	18	16	8

* Untreated; - = No measurable zone of inhibition

Table 5. Combined effect of extracts on test bacteria

Test Bacteria	Extract**	**Cot	*zone diameter of inhibition (mm) of Combined extract-cotrimoxazole concentration (mg/ml)				
			5	10	15	25	50
Streptococcus pyogenes (SP 003 MBFTY)	12	8	-	-	6	6	8
Staphhylococcus aureus (SA012 MBFTY)	22	16	6	6	8	8	10
Salmonella typhi (ST 008 MBFTY)	20	22	8	8	10	10	12
Escherichia coli (EC 002 MBFTY)	24	20	8	10	10	12	12
Proteus vulgaris (PV 001 MBFTY)	18	16	5	6	8	8	10
Pseudomonas aerugenosa (PA 005 MBFTY)	18	14	5	5	5	6	8
Shigella flexnerri (SF 002 MBFTY)	16	18	4	4	6	6	8

*0.5 mg/ml Cotrimoxazole + various concentrations of methanol extracts; **uncombined activity; *ZI = zone of inhibition diameter (mm) of combined extract (5-50 mg/ml) and cotrimoxazole (0.5 mg/ml); - = no measurable zone; cot = Cortimoxazole

Discussion

Preliminary Phytochemical analysis revealed the presence of alkaloids, tannins, saponins, phenols and glycosides. These bioactive substances are reported to confer resistance to plants against bacteria, fungi and pests (10, 11). Polyphenols were earlier reported to have some antimicrobial activities (12). The presence of these compounds in the extracts of *Leptadenia lancifolia* were responsible for the exhibition of antibacterial properties. Among all the solvents used, methanol extracts showed the highest activity against *S. aureus*. Different solvents have been demonstrated to have the ability to extract different phytoconstituents which depends on their polarity and solubility in the solvents (13). Except for the aqueous extracts, all organisms were generally susceptible to the extracts. This could be an indication of the potential of this plant as an effective source of antibacterial substances. The ethanol extracts also generally demonstrated slightly higher activity compared to cotrimoxazole (Table 2). The efficacy of the extracts as antibacterial agent was further demonstrated by the lower MIC (0.06-2.5 mg/ml) and MBC values (0.06-3.0 mg/ml) compared to the values of cotrimoxazole (0.06-10.0 mg/ml). This also justifies the ethnobotanical usage of the plant as health remedy. MIC is the lowest concentration that completely inhibits visible growth of the organism as detected by the unaided eye, usually after 16 to 24 h for antibiotics and also an *in vitro* reference value that describes the activity of antimicrobial agents against microorganisms (14 -16). The MIC corresponds to the net result of microorganism growth and kill over the

selected period of time (16). Optimum activity of the plant extracts were achieved with increase in temperature and acidic pH. This is an indication that the plant constituents are heat stable and acid stable. The decrease in activity with alkaline pH is an indication that the constituents are labile to alkaline conditions. In a traditional setting, the highly alkaline potash may have deleterious effect on the bioactive constituents (17, 18). The pH stability is an indication that the constituents in this plant are acid stable and may possibly resist the acidic pH of the stomach, hence permitting the development of oral antibiotics from them. An increase in activity of phytoconsituents at acidic pH has earlier been reported (11).

Conclusion

Leptadenia lancefolia has demonstrated antifungal activity against some pathogenic fungi in this study, justifying its traditional application as health remedy against various fungal infections. The study has paved way for the possibility of the use of *Leptadenia lancifolia* in drug development for human consumption and to source novel classes of antifungal substances that could serve as selective agents for chemotherapy and control of mycotic diseases including dermatophytosis, crytococcusis and systemic mycoses. More research should however be carried out to identify the various phytoconstituents and determine their chemical structure and their respective roles in antimicrobial activity with the view to purifying them for drug development. Toxicological studies to determine their safety and effect of the plant against a wider range of fungi and bacteria should also be carried.

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