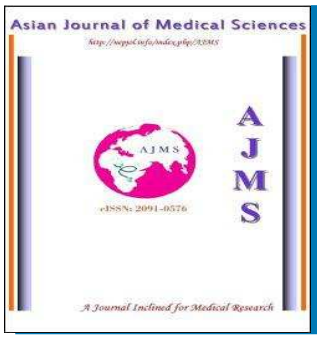

ANTIBACTERIAL POTENTIAL OF THE METHANOL STEM BARK EXTRACT OF *STACHYTARPHETA INDICA*

ORIGINAL ARTICLE,Vol-4 No.4

Asian Journal of Medical Science, Volume-4(2013) http://nepjol.info/index.php/AJMS

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"The search for new drugs to counter the challenges posed by resistant strains of bacteria might have started yielding results as the investigation of this plant has demonstrated enormous therapeutic potential."

ABSTRACT

Objective: There is increasing need for potent antimicrobial agents to tackle the problem of diseases in man. In view of this, the activities of methanol stem bark extract of *Stachytarpheta indica* was evaluated against some disease causing microorganisms.

Method: The activity of the extract against *S. aureus, P.aeruginosa, E. coli, S. typhi* and *Shigella spp* was determined using agar diffusion technique.

Results: The methanol stem bark extract demonstrated significant activity against the test organisms. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the stem bark extract ranged from 12-60 µg/ml.

Conclusion: Further isolation of active compound responsible for the antibacterial activity could be the potential sources of new antibacterial agents.

Key words: Antibacterial agent; *Stachytarpheta indica*; Medicina plant; Stem bark; Methanol; *in vitro*

INTRODUCTION

Antibacterial resistance has become a global problem. Strategies to improve the current situation include research in finding new antibacterial agent of plant origin. Plants have great potential for producing new drugs of great benefit to mankind. Natural plants have been seen as a valuable source of medicinal agents with proven potential for treating infectious diseases with lesser side effects compared to synthetic agents[1,2]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as animals, microorganisms, soil and plants. One of such sources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds[3, 4].Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing; imposing the need for a permanent search and development of new antimicrobial agent of natural origin [5, 6]. With increase in antibiotic resistance, cost and inaccessibility (especially in rural areas) to some orthodox modern antibiotics, medicinal plants are fast gaining popularity even to urban and civilized dwellers. There is an urgent need to evaluate the plants used in traditional medicine, with the aim of establishing their potential antibacterial effects and identifying the compounds responsible for their antimicrobial properties [7, 8]. *Stachytarpheta indica* Vahl (Verbenaceae) is commonly known as snake weed. A well branched herb, 2-3 ft high with very long narrow spikes; flowers deep blue with white centre. The plant is known by various names in different parts of Nigeria, such as 'Tsarkiyar kuse' (Hausa), 'Iru amure' (Yoruba) [9]. It has been used locally as an abortifacient, and in the management of asthma, headache, alopecia, bronchitis, bruises, constipation, diarrhoea, skin sore, dysentery, dysmenorrheal, fever, inflammation, liver disease, poisoning ,tumor, venereal diseases, cataract, sedative, anti-fertility and rheumatism [10, 11]. In Northern Nigeria, a decoction of the leaves with natron is given for dysentery in human and for similar condition in horses[9].The study was designed to investigate the antibacterial activity of the methanol stem bark extract of *Stachytarpheta indica*. The result is hoped to substantiate or otherwise, the ethnomedicinal use of the stem bark extract for the treatment of bacteria and possibly pave the way for its wider acceptability as antibacterial agent.

MATERIALS AND METHODS

Plant collection

The stem bark of *Stachytarpheta indica* was collected from plants growing within the campus of University of Calabar, Nigeria. The plant material was authenticated by Mr. Frank I. Apejoye of the Department of Botany, University of Calabar, where a voucher spacemen (No.133) is maintained. The international plant name index is Verbenaceae *Stachytarpheta indica* Vahl. Enum.pl.1:206.1804. The stem bark was cut into smaller pieces, dried at room temperature for 7 days and pulverized to dry powder using a mortar and pestle.

Extraction of plant material

Five hundred grams (500 g) of the dry stem bark powder was extracted with methanol by cold maceration for 48h with constant shaking. The methanol extract was concentrated to dryness in vacuum at 40^{o}C. The yield was (12% w/w). The dry extract was stored in a refrigerator at 4^{o}C until use for the experiment.

Phytochemical screening

The methanol stem bark extract of *Stachytarpheta indica* was subjected to qualitative phytochemical screening according to standard methods [12, 13]

Test Organisms

Clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Shigella spp.* obtained from

Department of Microbiology, University of Calabar Teaching Hospital, Calabar, Nigeria, were used for the experiment. Purity plates of each bacterial isolates were obtained by culturing on their respective selective media. Biochemical tests were performed to re-identify and confirm the identity of the isolates. Fresh plates of the test organisms were made from the isolate cultures obtained on agar slants. Discrete colonies of fresh cultures of different bacterial isolates were then picked and suspended in 5 ml nutrient broth in Bijou bottles and incubated for 24 h at 37^{o}C prior to antimicrobial susceptibility testing.

Determination of antibacterial activity

The antibacterial activities of the methanol stem bark extract of *Stachytarpheta indica* was determined by agar well diffusion method of [14, 15, 16].Broth cultures of the test isolate (0.5 ml) containing 1x10^{5} cfu/ml of organism was introduced into sterile petri-dish and 15 ml of Muller Hinton agar was added. The content was properly mixed and allowed to solidify. Holes were bored on the plates, using a standard sterile cork borer of 6mm in diameter and the stem bark extract reconstituted in distilled water at varying concentrations of 25, 50, 75,100 and 125 µg/ml were transferred into the wells with the aid of micropipette. The experiments were carried out in duplicate. The plates were allowed to stand for 1 h for prediffusion of the extract to occur and incubated at 37^{o} C for 24 h. At the end of the incubation, the diameter of the zones of inhibition were measured and recorded in mm.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by the method as described by Andrews [17]. Six sterile tubes were arranged in a test tube rack and 0.5 ml of sterile nutrient broth was transferred into each test tube. Thereafter, there was a serial dilution of the extract to obtain concentrations of 6.25, 12.5, 25, 50, 75 and 100 µg/ml respectively. The test tube organism (0.5 ml) was taken and transferred into each of the test tubes containing the mixture of the broth and the extract and then incubated at 37^{o}C for 24 h. The MIC was recorded as the least concentration of plant extract that completely inhibited the growth of the organism.

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration of the plant extract on the clinical bacterial isolates was carried out according to National Committee for Clinical Laboratory Standard [18]. A loopful of broth was collected from the determination of MIC tubes which did not show any growth and streaked on a sterile nutrient agar. All the plates were then incubated at 37^{o}C for 24 h. The least concentration of the stem bark extract with no visible growth after incubation was taken as the minimum bactericidal concentration.

Statistical analysis

The mean of the reading measured for each zone in the sensitivity test was taken to be the zone of inhibition of the bacterial isolates. Two ways ANOVA was used to compare means and differences were considered significant at $p<0.05$.

RESULTS

Phytochemical studies

Phytochemical screening of the stem bark extract revealed the presence of saponins, flavonoids, steroids, terpenoids, alkaloids, tannins and reducing sugars, while Phlobatannins and phenols were not detected. These classes of compounds are reported to show important biological activities[19,20,21] (table 1).

Antibacterial activity of the stem bark extract

The results of antibacterial activity of the methanol

stem bark extract of *Stachytarpheta indica* against the test organisms are shown in table 2. The zones of inhibition of the isolates are a function of the antibacterial activity of the extract. The activity of the stem bark extract was shown to be concentration dependent. The extract showed significant inhibition against all the test organisms. Distilled water used as respective controls were inactive against the bacteria.

Minimum inhibitory concentration (MIC) and Minimum bactericidal Concentration

Table 3 shows the results of MIC and MBC determination on the test organisms. The lowest MIC and MBC of 12 and 25 µg/ml was demonstrated against *S. aureus*, while the MIC and MBC values ranging between 15-60 µg/ml were demonstrated against the rest of the test organisms.

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [22]. Many reports are available on the antibacterial, antiviral, antifungal, anthelmintics and anti-inflammatory properties of plants [23, 24, 25, 26,27.] Some of these observations have helped in identifying the active principle responsible for such activities and in developing drugs for the therapeutic use in human. The phytochemical investigation revealed the presence of saponins, flavonoids, steroids, terpenoids, alkaloids, tannins and reducing sugars (Table 1). These bioactive components which are naturally occurring in most plant materials, are known to be bactericidal, pesticidal or fungicidal in nature[28,29]. Thus, the presence of the above phytochemical components may account for the high antimicrobial activity of the stem bark extract against some selected microorganisms. The methanol stem bark extract showed good antimicrobial activity against pathogenic bacterial strains like *Shigella spp, Salmonella typhi, Pseudomonas aeruginosa Staphylococcus aureus* and *Escherichia coli*. Plant based products have been effectively proven for their utilization as source for antimicrobial components. The fact that the methanol stem bark extract of *Stachytarpheta indica* showed activity against both gram-negative and gram-positive bacteria tested may indicate a broad spectrum of activity [30]. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. However, the very high significant antibacterial shown by the stem bark extract of *Stachytarpheta indica* is beneficial as it indicates probably the emergence of a new antibiotic with such a wide spectrum of activity.

The fact that treatment of infections caused by organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Shigella spp* are increasingly becoming difficult further strengthens the importance of these present findings and the need for a continuous search for chemotherapeutic agents. Apart from antimicrobial activities, the stem bark extract of *Stachytarpheta indica* was found to possess analgesic, anti-inflammatory and antipyretic activities [31].

In conclusion, the present study has shown that the stem bark extract of *Stachytarpheta indica* possesses antimicrobial properties; hence could be of considerable interest to the development of new drugs. The bioassay-guided fractionation and further characterization of the active principles responsible for the antimicrobial potential of the plant is underway in our laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

Table 1. Phytochemical constituents of the methanol stem bark extract of *Stachytarpheta indica*

Test	Methanol extract
Saponins	++
Flavonoids	++
Steroids	++
Tannins	+++
Terpenoids	++
Alkaloids	+
Reducing sugars	+++
Phlobatannins	-
Phenols	-

+ = Slight presence; ++ = Medium presence; +++ = Heavy presence; - = Absence

Table2. Antibacterial potency of the methanol stem bark extract of *Stachytarpheta indica* (mm)

Dose (µg/ml)	*Shigella spp.*	*S. typhi*	*S. aerus*	*P. aeruginosa*	*E. coli*
12.5	9.53±3.0	7.53±3.2	13.0±4.7	9.78±6.0	9.0±3.0
25	11.56±3.3	9.81±5.0	17.8±5.9	16.63±4.6	11.9±5.0
50	15.63±6.4	13.63±4.0	22.13±5.8	17.0±0.0	13.0±5.0
75	18.50±2.8	17.50±2.5	25.0±0.0	17.50±4.9	16.63±5.0
100	22.75±3.9	22.0±4.4	27.74±2.0	20.50±0.0	19.25±1.8
125	33.50±5.3	32.50±4.0	47.0±3.0	29.50±0.0	29.50±0.0

Values are shown in mean ± SEM

Table 3. Antibacterial effect (MIC and MBC in µg/ml) of methanol stem bark extract of *Stachytarpheta indica*

Test organisms	MIC	MBC
Staphylococcus aureus	20	40
Salmonella typhi	12	25
Shigella spp.	15	30
Pseudomonas aeruginosa	20	40
Escherichia coli	30	60

ACKNOWLEDGEMENT

The authors are grateful to Mr Frank I. Apejoye of the Department of Botany, University of Calabar, Nigeria for his botanical assistance.

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