

Full Length Research Paper

Evaluation of antibacterial activity and acute toxicity of the hydroethanolic extract of *Stachytarpheta angustifolia* (Mill) Vahl.

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Accepted 13 May, 2008

The aim of this study was to evaluate antibacterial activity, acute toxicity in mice and phytochemical profiles of hydroethanolic extract of *Stachytarpheta angustifolia* plant. The plant *S. angustifolia* has attracted the attention of the researchers because of its use as an anti-infection agent. The aqueous ethanol (80%) extract of the powdered dried whole plant was obtained by maceration. The bacteria organisms tested include *Shigella dysenteriae* (ATCC 32412), *Salmonella typhi* (ATCC 213415), and the following clinical isolates: coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella* species and *Escherichia coli*. Susceptibility test, acute toxicity test and phytochemical screening of the plant extract were performed using standard procedures. The results showed that the extract had a good antibacterial activity against *S. aureus*, *S. dysenteriae*, coagulase-negative *Staphylococcus* and *Proteus mirabilis*. The minimum inhibitory concentration (MIC) was found to be between 11.6 and 14.0 mg/ml for the susceptible organisms. The extract exhibited minimum bactericidal concentration (MBC) of 150 mg/ml against *S. dysenteriae* only while other susceptible tested bacteria strains required higher concentrations. The median acute toxicity value (LD₅₀) of the extract was determined to be 8.721 g/kg body weight indicating the extract as being slightly toxic. The extract contained triterpenoid saponins as the major bioactive constituent.

Key words: *Stachytarpheta angustifolia*, acute, toxicity, antibacterial and phytochemical.

INTRODUCTION

Plant-based products had primarily served from the outset as the most important and indispensable sources of food. The knowledge that plant derived compounds could also serve as therapeutic weapons available to man to fight various human and animal diseases has made plants a *sine qua non* to human and animal lives. Plant drugs popularly known as herbal remedies are relied upon for the treatment of all sorts of diseases. Their use to treat and manage ailments continues unabatedly to date in most Nigerian communities as well as in the other developing countries. Also increasing reliance on the use of medicinal plants in the industria-

lized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (Hoareau and DaSilva, 1999). Plants, therefore, remain the main source of the active drugs from a natural source and are still indispensable in the traditional medicine for treating a number of diseases.

Herbal remedies have many traditional claims and are employed in the treatment of diseases of diverse origins. They contain active constituents with useful physiological and pharmacological activities. Consequently, attention is now focused in the exploration of herbal remedies as alternatives in the treatment of infectious diseases since pathogens of have been found to develop multiple resistances to most of the currently used synthetic anti-

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biotics (Olukoya et al., 1993).

Stachytarpheta angustifolia is a valuable medicinal plant and remedies prepared with it are used locally as anti-infection agent. The leaves on squeezing produce a foamy juice and the local herbalists probably use it in the treatment of various diseases including sexually transmitted diseases (STDs). *S. angustifolia* is a seasonal weed growing along the banks of rivers and streams, constituting menace in the farmlands during the rainy seasons, in the southern parts of Nigeria. It is also popularly used by children and local farmers as a soap for bathing in the locality where it is available. The preliminary phytochemical analysis of the extract indicated positive results for polyphenols and triterpenoid saponins. For a plant or herbal preparation containing active organic principles to be identified for use in the traditional medicine, a systemic approach is required for the evaluation of efficacy and safety through experiment and clinical findings (Mythilypriya et al., 2007).

The aim of this study was to evaluate the antibacterial activity, phytochemical profile and safety of *S. angustifolia* extract by carrying out acute toxicity study in the animals. Acute toxicity evaluation is required to establish potential adverse effects of this valuable medicinal plant.

MATERIALS AND METHODS

Plant material

S. angustifolia (Mill) Vahl (Fam. Verbanaceae) is a seasonal plant growing mostly in the farmlands at the bank of river Niger and streams, in southern Nigeria, during the rainy seasons. The plants were collected from Atani, a town located at the bank of river Niger in Anambra State of Nigeria, and was authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan and voucher specimen deposited at the Institute's herbarium. The plants were washed, cut into small pieces and dried at an ambient temperature between 35 – 45°C in an oven for 5 days, and powdered into coarse particles. 500 g of the powder was macerated with ethanol (80%) at room temperature for 7 days with constant stirring. After filtration, the solvent was removed under reduced pressure in a rotary evaporator at a temperature below 50°C and dried to a constant weight of 29.45 g (5.89% yield)

Acute toxicity study

The toxicity study was carried out using thirty five (35) male and female Swiss albino mice weighing 20 – 25 g, obtained from Laboratory Animals Center, College of Medicine, University of Lagos. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. They were maintained on animal cubes (Feeds Nigeria Ltd), provided with water *ad libitum* and were allowed to acclimatize for 7 days to the laboratory conditions before the experiment. After starving the animals overnight, the control group received 0.3 ml of normal saline orally. Each treated group received orally the hydroalcoholic extract suspension-prepared by dispersing 8 g of the extract with 5 ml normal saline, thoroughly mixed and the volume made up to 10 ml with normal saline, in different doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg. The animals were observed continuously for the first 4 h and then for each hour for the next 24 h and at 6 hourly interval for the next 48 h after administering of the extract to

observe any death or changes in general behavior and other physiological activities (Shah et al., 1997; Bnrger et al., 2005)

Antibacterial screening

The human pathogenic test organisms used were *Shigella dysenteria* (ATCC 32412), *Salmonella typhi* (ATCC213415), Coagulase-negative *Staphylococcus* (clinical isolate), *Staphylococcus aureus* (clinical isolate), *Proteus mirabilis* (clinical isolate), *Klebsiella* spp. (clinical isolate) and *Escherichia coli* (clinical isolate). The strains were re-identified and characterized, maintained on Mueller-Hinton agar plates and suspended in Mueller-Hinton broth prior to use for antimicrobial susceptibility testing.

Determination of antimicrobial activity

The agar well diffusion method according to Malcolin and Sofowora (1969), Perez et al. (1990) and Olukoya et al. (1993) was modified and used. The dried extract was reconstituted with sterile distilled water to obtain a stock solution of 250 mg ml⁻¹ from which various concentrations; 250, 125, 62.5, 31.25 and 15.625 mg ml⁻¹ were prepared. Overnight broth cultures of the respective bacteria strains were adjusted to turbidity equivalent to 0.5 McFarland standard, to yield approximately 1.0 x 10⁸ colony forming unit (cfu)/ml. Mueller-Hinton agar plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacteria strains. Six wells (6 mm in diameter) were made equidistance in each of the plates using a sterile cork borer. Up to 100 µl (0.1 ml) of each concentration of the extract were respectively introduced into the wells using sterile automatic pipettes, with the stock solution in the center well. It was allowed to diffuse at room temperature for 2 h. The plates were incubated at 37°C for 24 h. The solvent control and the control antibiotic discs, ciprofloxacin disc (5 µg/ml) were used. Diameters of the inhibition zones were measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract.

Determination of MIC and MBC

The aqueous ethanol extract of *S. angustifolia* showed good activity in four bacteria strains (*S. dysenteria*, coagulase-negative *Staphylococci*, *S. aureus* and *P. mirabilis*) in the solid agar well diffusion test. Those concentrations giving an inhibitory zone of 11.00 mm diameter were chosen to assay for the minimum inhibitory concentration (MIC) with the agar dilution method according to NCCLS (1997) guideline. The inoculums were prepared from an overnight broth cultures and adjusted to turbidity equivalent to 0.5 McFarland standards. A stock solution 350 mg ml⁻¹ was made and the serial dilutions of 300, 200, 150, 100 and 50 mg/ml were prepared from it. A 4 ml of each dilution was incorporated in 16 ml of the appropriate melted agar medium and poured into each of the seven petri dishes. Each petri dish was divided into four sections. A loopful of the diluted culture of each test organisms was inoculated by streaking on the surface of each sectioned of the petri dish. The petri dishes were incubated at 37°C for 24 h. A control was also set which contained only nutrient agar and the test organism. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each organism.

The minimum bactericidal concentration (MBC) was determined by sub-culturing the plates that does not show bacterial growth from the results obtained in MIC determination. The organisms were sub-cultured on Muller-Hinton agar and incubated at 37°C for 24 h, after which the viable cells were counted. The MBC was determined as the lowest concentration of the antimicrobial agent giving 99.9%

Table 1. Acute toxicity of the aqueous ethanol extract of *H. glabra* in mice.

Number of mice (n)	Doses of extract (g/kg)	Number of mice dead	Percentage of mice dead
5	0.60	0	0
5	1.5	0	0
5	3.0	0	0
5	6.0	0	0
5	9.0	1	12.5
5	12.0	2	37.5
5	20.0	5	100.0

Control group received orally 0.3 ml normal saline.

reduction in the number of colony forming unit (cfu) from that of the original inoculum.

Phytochemical evaluation of the crude extracts

Phytochemical screening of the extract for the presence of secondary metabolites was performed using the following reagents and chemicals: alkaloids with Mayer's and Dragendorffs reagents (Farnsworth, 1966; Harborne, 1998), flavonoids with the use of Mg and HCl (Silva et al., 1993; Houghton and Raman, 1998), tannins with 1% gelatin and 5% ferric chloride solution, and saponins with ability to produce suds (Houghton and Raman, 1998). Liebermann-Buchard test consisting of a mixture of glacial acetic acid and sulphuric acid (19:1) was used to differentiate the types of triterpenoids and steroidal nuclei present (Farnsworth, 1966).

Statistical analysis

All data collected were summarized as mean \pm SEM. Significant differences were determined using a Student's t-test and the differences were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

In recent time there is increasing awareness and interest in herbal medicines. Consequently, herbal medicines have received greater attention as an alternative to clinical therapy leading to increasing demand (Mythilypriya et al., 2007). The exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is still very common in rural Nigerian communities. Experimental screening method is, therefore, important in order to ascertain the safety and efficacy of herbal products as well as to establish the active component of these herbal remedies.

In the acute toxicity study of the extract, no changes in the behavior and the sensory nervous system responses were observed in the animals (Table 1). Also no adverse gastrointestinal effects were observed in male and female mice used in the experiment. All the mice that received 20.0 g/Kg dose of the extract died within 4 h while the animal that received 6.0 g/kg dose survived beyond the 24 h of observation. The median acute toxicity value (LD_{50}) of the extract was determined to be 8.721 g/Kg

body weight. According to Ghosh (1984) and Klassen et al. (1995), the extract can be classified as being slightly toxic, since the LD_{50} was found to be between 5 -15.0 g/Kg. The gram equivalent of the LD_{50} in an adult man would amount to 523.25 g, making *S. angustifolia* relatively safe. The viscera of the animals did not show any macroscopic changes compared with the control that could point to the cause of the death. However, since the animals did not convulse before dying, it could be postulated that the extract did not kill the mice by some action on the nervous system (Ogwal-Okeng et al., 2003).

The result of the susceptibility profile of the various test organisms is presented in Table 2. The extract was found to inhibit four bacteria strains tested; *S. dysentria* (ATCC32412), and three clinical isolates: *P. mirabilis*, *S. aureus* and coagulase-negative *Staphylococci*, with a measurable zone of inhibition. The standard positive control (ciprofloxacin 5 μ g/ml), showed inhibition diameter ranging between 19.0 and 40.0 mm against gram-negative bacteria and 33.0 mm against gram-positive bacteria. The extract also proved to have higher inhibitory activity against the gram-negative bacteria than the gram-positive bacteria with *S. dysentria* (ATCC32412) and *P. mirabilis* being the most sensitive strains. *S. dysentria* (ATCC32412) and *P. mirabilis* exhibited the maximum zone of inhibition diameter of 14.02 ± 0.33 mm and 14.05 ± 0.26 mm with the extract at concentration of 300 mg/ml, while ciprofloxacin at concentration of 5 μ g/ml exhibited inhibition diameter of 37.01 ± 1.30 mm and 19.20 ± 0.5 mm against *S. dysentria* (ATCC32412) and *P. mirabilis*, respectively. The coagulase-negative *Staphylococci* was the least sensitive strain to the extract, exhibiting zone diameter of 11.6 ± 0.88 mm at concentration of 300 mg/ml and insensitive to the standard positive control drug showing no zone of inhibition. Other bacteria strains tested (*Salmonella typhi*, *Klebsiella* spp. and *E. coli*) were insensitive to the extract and did not exhibit any zone of inhibition while only *S. typhi* was sensitive to ciprofloxacin at concentration of 5 μ g/ml with a diameter of 40.20 ± 0.62 mm zone of inhibition.

The MIC and MBC results of *S. angustifolia* extract are presented in Table 3. The MIC of the extract ranged bet-

Table 2. The antibacterial susceptible pattern of the hydroethanolic extract of *S. angustifolia* plant.

Microorganism	Mean diameter of zone of inhibition in mm (mean± sem)	
	Aqueous extract (300 mg ml ⁻¹)	Standard drug control Ciprofloxacin (5 g ml ⁻¹)
Gram positive bacteria		
<i>S. aureus</i>	13.6 ± 0.12	33.01 ± 0.03
Coagulase-negative <i>Staphylococcus</i>	11.60 ± 0.88	NZ
Gram negative bacteria		
<i>S. dysentriae</i>	14.02 ± 0.33	37.01 ± 1.30
<i>P. mirabilis</i>	14.05 ± 0.26	19.20 ± 0.50
<i>Klebsiella</i> spp	NZ	NZ
<i>E. coli</i>	NZ	NZ
<i>S. typhi</i>	40.20 ± 0.62	NZ

Values of inhibitory zone diameter (n = 5) were expressed as mean ± sem for *S. angustifolia* at 250 g/ml. NZ = no zone of inhibition.

Table 3. Minimum Inhibitory concentration (MIC) and minimum bactericidal count (MBC) of *S. angustifolia* extract against bacterial organisms.

Microorganism	Mean(± sem) of MIC and MBC in mm	
	MIC (mg ml ⁻¹)	MBC
Gram positive bacteria		
<i>S. aureus</i>	250 mg/ml	300 mg/ml
Coagulase-negative <i>Staphylococcus</i>	250 mg/ml	300 mg/ml
Gram negative bacteria		
<i>S. dysentriae</i>	150 mg/ml	250 mg/ml
<i>P. mirabilis</i>	200 mg/ml	300 mg/ml

GPB = Gram positive bacteria, GNB = Gram negative bacteria.

ween 150 and 350 mg/ml. The MBC of the extract was 250 mg/ml for *Shig. dysentriae* (ATCC32412), while other sensitive bacteria strains, *P. mirabilis*, *S. aureus* and coagulase-negative *Staphylococci* required higher concentrations of the extract.

The extract of was found to have high activity against two gram-negative bacteria strains, *S. dysentriae* (ATCC 32412) and *P. mirabilis*, and moderate activity against gram-positive bacteria strains, *S. aureus* and coagulase-negative *Staphylococci*. *Klebsiella* spp and *E. coli* were not susceptible to both the extract and the standard control drug while *S. typhi*, was sensitive only to the control drug. *S. dysentriae* and *P. mirabilis* exhibited the highest zone of inhibition at the concentration used followed by *S. aureus* and coagulase-negative *Staphylococci*. The reason for the differential sensitivity pattern between gram-positive and gram-negative bacterial strains could not be ascribed to their morphological differences nor be attributed to their chemical compositions. Gram-negative bacteria have an outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impenetrable to antimicrobial agents (Nikaido and Vaara, 1985), while the

gram-positive bacteria should be more susceptible having only an outer peptidoglycan, which is not an effective permeability barrier (Scherer and Gerhardt, 1971). In spite of the permeability differences, the aqueous ethanol extract of *S. angustifolia* exerted a broader spectrum of inhibitory activity on gram-negative bacteria than on gram-positive bacteria strains. The higher activity of the extract against *P. mirabilis* and *S. dysentriae* which are at times responsible for the pathogenesis of wound, urinary and enteric infections provides a scientific evidence for the efficacy of *S. angustifolia* in treating such infections (Ramesh et al., 2001). The study showed that *S. aureus* and coagulase-negative *Staphylococci* had a higher MIC value which indicates that a higher concentration of the extract is required to inhibit the organisms' growth, while *S. dysentriae* and *P. mirabilis*, with a lower MIC values would require a low extract concentration of the extract to inhibit their growth. The minimum bactericidal concentration (MBC) value of the extract was low for *S. dysentriae*. The other tested bacteria organisms had a viable colony count after they have been re-cultured in nutrient agar. Thus, it could be explained that higher concentrations of the extract above those used in the experiment would be

Table 4. Phytochemical screening of the hydroethanol extracts of *S.angustifolia*.

Alkaloids	Presence
Cardiac glycosides	-
Anthraquinone glycoside	++
Free anthraquinone	+
Saponin glycosides	+++
Steriodal nucleus	-
Triterpenoid nucleus	+++
Cyanoglycoside	-
Polyphenols	++
Flavonoids	-
Red cell Haemolysis	+++

- = Absent, + = Slightly present, ++ = fairly present, +++ = abundantly present.

required to inhibit the growth of the organisms. Hence, the extract at low concentration was bacteriostatic to *Proteus mirabilis*, *S. aureus* and coagulase-negative *Staphylococci* but was bactericidal against *S. dysentria*. The variation in the quantity of the active ingredient required to effect inhibition may not matter much since medicinal plant have been reported to have little or no side effects (Hussain and Eshrat, 2002).

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one that predominates over the others. It may also be used to search for bioactive lead agents that could be used in the partial synthesis of some useful drugs (Yakubu et al., 2005). Phytochemical screening of the plant (Table 4) revealed the presence of saponins as the major active secondary metabolite, while free anthraquinones and polyphenols other than flavonoids as minor constituents. Alkaloids and organic bases were conspicuously absent. Saponins are characterized by their surface-active properties and they dissolve in water to form foamy solutions and because of surface activity some drugs containing saponins have a very long history of usage (Jean, 1999). Saponins have been implicated as a bioactive antibacterial agent of plants containing them (Mandal et al., 2005; Manjunatha, 2006). The exhibited antibacterial properties of *S. angustifolia* can be attributed to the presence of pentacyclic saponins and polyphenols in the plant. Polyphenols have been associated with antioxidant activities and were earlier reported to have some antibacterial activities (Tomas-Barberan et al., 1990), and might have complimented or potentiated the saponins in the antibacterial activities exhibited by *S. angustifolia* extract.

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