Trends in Medical Research

ISSN 1819-3587

Academic Journals Inc.

www.academicjournals.com

Trends in Medical Research 2 (4): 193-198, 2007
ISSN 1819-3587

Preliminary Phytochemistry, Antimicrobial Properties and Acute Toxicity of *Stachytarpheta jamaicensis* (L.) Vahl. Leaves

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Abstract: The phytochemical analysis carried out on the leaves of *Stachytarpheta jamaicensis* showed the presence of secondary metabolites including tanins, saponins and flavonoids which have great medicinal property. Crude concentrations of aqueous extract of leaves showed varying activities on *Bacillus subtillis*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aureginosa*, *Proteus vulgaris*, *Klebsiella arogene*, *Proteus mirabilis* and J62K$_1$ serving as control. Similar trend applied to the alcoholic extract of leaf but slight inhibition was observed on *Staphyllococcus aureus* and *Proteus vulgaris* thus indicating it's antimicrobial effect but at high concentration. The acute toxicity of the aqueous extract on Winstar rats revealed no mortality even up to dose of 4 g kg^{-1} body weight and no significant changes in body weight $p>0.05$. Also, eye color was normal and loss of hair was absence. Thus indicating that the plant is therapeutically safe for use even at high concentration, though the chronic effect was not investigated.

Key words: Phytochemical analysis, antimicrobial, acute toxicity, *Stachytarpheta jamaicensis* (Linn.) Vahl. leaves

INTRODUCTION

Benefits derived from using medicine obtained from plants are that they are relatively safer than synthetic alternative by offering profound therapeutic benefits and more affordable treatment (Iwu *et al*., 1999). Furthermore, it has been found that some drugs are synthesized from plants. Infact it is estimated that plant materials are present in, or provide the models for more than 50% of western drugs (Robbers *et al*., 1996).

Stachytarpheta jamaicensis (Bastard vervain or Brazillian tea) belongs to the family Verbanaceae which consists of 2600 species and 100 genera. It is an annual weedy herbaceous plant, sometimes perennial, that grows 60-120 cm tall and is reproduced from seeds. The stem is smooth and somewhat woody especially at the base. It is dark green, often covered with powder which gives it a bluish shine. The leaves are opposite, rounded to broadly acute at the apex, smooth on both surfaces and with short petioles. The inflorescence is made up of flowers in slender spikes on a long and swollen rhachis about 30-40 cm long. The flowers are bluish with a white throat or could be seen as reddish purple to deep blue in colour. It has a tubular corolla about 10 mm long and lobes about 3 mm long. They are more or less sparsely grouped along and immersed in the axis of the inflorescence (Akobundun and Agyakwa, 1998).

Ethnobotanically, *S. jamaicensis* is an antacid, analgesic, anti-helmithic, anti-inflammatory, diuretic, hypotensive, laxative, lactogogue, purgative, sedative, stomachictonic, spasmogenic, vasilator, vulnerary and vermifuge (Schapoval, 1998). It is used for allergies and respiratory conditions such as

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colds, flu, asthma, bronchitis and others. It is used for digestive problems such as indigestion, acid reflux, ulcers, constipation, dyspepsia and slow digestion. Pregnant patients and patients with low blood pressure are advised not to use this plant because it is abortive and hypotensive (Taylor, 2005).

Some plants have been discovered to be rich in secondary metabolite, such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids and volatile oil. These compounds are responsible for their therapeutic activities (Cowan, 1999; Rabe and Vanstoden, 2000). Also, since times past, some plant parts have been used as antimicrobial agents, especially their extracts either as decoctions, infusions, or oral administration (Okemo *et al*., 2001). Importantly, plants have been known to exhibit medicinal properties on internal organs in animals. If the toxic effect after administration is low, there is a possible chance of introduction of such drugs for therapeutic purpose (Ibeh, 1998).

This study is aimed at testing the phytochemistry, antimicrobial activities and acute toxicity levels of the aqueous and alcoholic extracts of the leaves of *S. jamaicensis*.

MATERIALS AND METHODS

Sample Collection

S. jamaicensis was collected in the month of March around Ugbowo area in Benin-City, Edo State, Nigeria from a growing site. It was identified properly by comparing with the leaves, inflorescence and stems in standard text (Akobundun and Agyakwa, 1998) and further substantiated by Prof. M. Idu of Botany Department, University of Benin. The leaves were plucked from the stem using knife and shears, they were cleaned of debris and dried in an oven at 40°C for 48 h then grinded.

Extraction of Plant Material for Chemical Analysis:

Twenty gram of grinded plant sample was dissolved in 200 mL of water and allowed to boil for 30 min. The sample was left to cool and filtered with Whatman No. 1 filter paper.

Water and ethanol were used for extraction to obtain samples for the antimicrobial and acute toxicity tests. One hundred gram of leaves was boiled by decoction in 1000 mL of water for 30 min and 150 g of leaves was soaked in 1000 mL of ethanol for 48 h, then filtered. The filtrates were placed into evaporators to drive off the solvents. The pastes formed were kept in two containers and labelled thus: WL-Water extract from leaves and AL-Alcoholic extract from leaves. Tests for alkaloids, saponins, tannins, flavonoids and anthraquinones, were according to procedures outlined by Trease and Evans (1996).

Determination of Antimicrobial Activity

The organisms used for study were *B. subtillis*, *E. coli*, *C. albicans*, *S. aureus*, *P. aureginosa*, *P. vulgaris*, *K. arogene*, *P. mirabilis* and high resistance standard strain of *Escherichia coli* ($J62k_{12}$). The gutter and punch hole methods were applied.

Gutter Method

This was used to determine preliminary activities of the extracts on the organisms. Sterilized nutrient agar was poured into four Petri dishes and allowed to set. The plates were partitioned into four segments using a marker pen. With the aid of a sterilized loop, each organism was streaked across a segment on the agar surface. A sterilized spatula was then used to cut out a gutter across the streaks. The extracts were poured into the gutters and the Petri dishes kept in an incubator for 24 h at 37°C to allow the organisms grow. Clearance of streak growth from the gutter margins indicated inhibitory activity of the extract on the organism.

Punch Hole Method

Punch hole method (Stoke, 1975) was used to measure the zone of inhibition. Eight Petri dishes were poured with already sterilized nutrient agar to the level of obtaining a standard well and allowed

to set. The organisms, dissolved in nutrient broth were poured into set Petri dishes and uniform distribution was ensured. Sterile cork borer of 10 mm in diameter was used to punch holes in the agar. Each of the holes (numbering 2) were filled with extracts 1000 mg mL^{-1} and kept in an incubator for 24 h at 37°C for the organisms to grow.

The active extracts showed zones of inhibition which were measured using meter rule by measuring 2 points across the zone and the average diameter was taken.

Minimum Inhibitory Concentration (MIC)

Agar plates were prepared and two for each of the extracts were flooded the with same organism. Two holes were punched in each plate and filled with 0.2 mL of extracts of different dilutions.

Double dilution of the extract was carried out. Double strength nutrient broth of 5 mL was pipette into universal bottles and each were labeled N, 2, 4, 8. Using a sterile graduated pipette, 5 mL of the extract was measured into the bottle labeled 2 and mixed. Same was done for 4 and 8 using fresh pipettes. In another bottle, broth only was put in without the extract, this served as the control. The plates were incubated at 37°C for 24 h. The order of concentration were N-1000, 2-500, 4-250 and 8-125 mg mL^{-1}.

Acute Toxicity Test

Twenty male wistar rats, five weeks old, were obtained from a single source to reduce the influence of variablity. They and were fed with mouse cubes and had unrestricted access to water.

The animals were divided into groups of five each and the groups labeled as I, II, III and IV respectively. All animals of the respective groups were fasted overnight before proceeding with the experiment.

Doses of 1, 2 and 4 g kg^{-1} of aqueous extract of the leaves were administered intraperitoneally to the animals in group I, II and III respectively and 0.5 mL of Normal saline was administered to the animals in group IV which served as control.

The animals were observed for physical signs of toxicity such as weight change and mortality observed for 24 h and beyond but not more than 48 h. Mortality was calculated as percentage death of the animals used.

RESULTS

Phytochemical Analysis

Results from alkaloidal test proved negative. The presence of frothing when filtrate was shaken was a preliminary evidence of the presence of saponins which was confirmed after it was mixed with sulphuric acid and with 90% ethanol added, frothing disappeared. The test for tannins gave a bluish precipitate which confirmed its presence. Anthraquinones was absent. Flavonoids was confirmed present by the change from colourless to yellow coloration on addition of hydrochloric acid (Table 1).

Antimicrobial Activity

Table 2 shows that WL was active on all organisms while AL was also active on all organisms but slight activity on *S. aureus* and *P. vulgaris*. From Table 3, inhibition zones measurement for WL revealed highest diameter of 18 mm for *P. mirabilis* and lowest diameter of 12 mm for *P. aureginosa* while the AL showed higher inhibition zone for *P. aureginosa than P. mirabilis.* Table 4 shows minimum inhibition concentration of extracts were obtained at high concentrations, while the resistant *E. coli*, $J62k_{12}$, was not inhibited by any of the extracts.

Acute Toxicity

This is presented in three sections.

Table 1: Summary of phytochemical analyses of *S. jamaicensis* leaves

Secondary metabolites	Leaves
Alkaloids	-
Saponins	+
Tannins	+
Anthraquiones	-
Flavonoids	+

+: Indicates present, -: Indicates absent

Table 2: Antimicrobial activity of extracts of *S. jamaicensis* leaves using Gutter method

	Extract activity	
Organisms	WL	AL
Bacillus subtilis	++	++
Escherichia coli	++	++
Candida albicans	++	++
Staphylococcus aureus	++	++
Pseudomonas aureginosa	++	+
Proteus vulgaris	++	+
Klebsiella arogenes	++	++
Proteus mirabilis	++	++
J62 k_{12}	-	-

++: Indicates presence of inhibition, -: Indicates absence of inhibition, +: Indicates minute presence of inhibition, WL = Water extract of leaves, AL = Alcohol extract of leaves

Table 3: Zone of inhibition diameter (mm) produced by extracts of *S. jamaicensis* leaves using Punch hole method

Organisms	WL	AL	Gentamycin	Water
Bacillus subtilis	-	-	5	-
Escherichia coli	13.5	-	5	-
Candida albicans	-	-	5	-
Staphylococus auaus	-	-	5	-
Pseudomonas aureginaosa	12.0	13.0	5	-
Proteus vulgaris	-	-	5	-
Klebseia arogenes	-	-	5	-
Proteus mirabilis	18.0	12.0	5	-

-: Shows absence, WL = Water extract of leaves, AL = Alcohol extract of leaves

Table 4: Minimum Inhibition Concentration (MIC) of extracts of *S. jamaicensis* leaves on the microorganisms

		500	250	125	62.5
Organism	Extract	(mg mL^{-1})			
Bacillus subtilis	WL	-	-	-	-
	AL	-	-	-	-
Escherichia coli	WL	+	+	-	-
	AL	-	-	-	-
Candida albicans	WL	-	-	-	-
	AL	-	-	-	-
Staphylococcus aureus	WL	-	-	-	-
	AL	-	-	-	-
Pseudomonas aureginosa	WL	+	-	-	-
	AL	+	-	-	-
Proteus vulgaris	WL	-	-	-	-
	AL	-	-	-	-
Klebsiella arogenes	WL	-	-	-	-
	AL	-	-	-	-
Proteus mirabilis	WL	-	-	-	-
	AL	+	+	-	-

-: Zone of inhibition absent, +: Zone of inhibition present, WL = Water extract of leaves, AL = Alcohol extract of leaves

Table 5: Weekly weights (g) of wistar rats treated intraperitoneally with aqueous extract of *S. jamaicensis* leaves

Groups	Initial weights	Final weights	Change in weights (%)
1	223.33±39.30^{a}	225.00±25.00^{a}	0.74
2	200.00±28.82^{a}	218.33±18.33^{a}	8.39
3	206.60±23.33^{a}	236.67±7.27^{a}	12.60
Control	210.00±0.00^{a}	240.00±0.00^{a}	12.50

Values with same superscripts mean no significant differences in weights

Table 6: Mortality rates of wistar rats due to intraperitoneal administration of aqueous extract *S. jamaicensis* at different does

Groups of rats	Doses	No. of deaths	Mortality (%)	Total No. of rats
1	1 g kg^{-1}	0 of 5	0	5
2	2 g kg^{-1}	0 of 5	0	5
3	4 g kg^{-1}	0 of 5	0	5
Control (Normal saline)	0.5 mL	0 of 5	0	5

Physical Characteristics/observation of Experimental Rats

There was no noticeable difference in feeding habit throughout the 3 weeks. There was no obvious hair loss in all the rats and eye colour remained normal throughout period.

Body-weight

The average weights in groups I, II and III were taken and no significant weight loss ($p>0.005$) was observed between the initial and final periods (Table 5).

Mortality

None of the rat observed died during the period of this experiment (Table 6).

DISCUSSION

The preliminary phytochemical investigation carried out on *S. jamaicensis* showed that it consists of metabolites such as saponins, tannins and flavonoids. These metabolites have been shown to be responsible for the therapeutic activity of plants (Trease and Evans, 1999; Rabe and Vanstoden, 2000). The tannins have been traditionally used on inflammed surfaces of mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea and as antidote in heavy metal poisoning (Sodipo *et al*., 1991). Tannins have been reported to inhibit growth of micro-organisms by precipitating microbial protein and making nutritional proteins unavailable to them (Ogunleye and Ibitoye, 2003). Saponins are special class of glycosides that have shown to be an antifugal agent (Sodipo *et al*., 1991). The class of alkaloid is among the powerful poisons known (Fluck, 1973). Plant phenolics especially flavonoids have health promoting properties (Rauha *et al*., 2000). The absence of alkaloids in this plant maybe responsible for it's non-toxicity.

The antimicrobial results from the aqueous extract (WL) showed activity on *B. subtillis*, *E. coli*, *C. albicans*, *S. aureus*, *P. aureginosa*, *P. vulgaris*, *K. arogenes* and *P. mirabilis*. Minimum Inhibitory Concentration (MIC) was attained at 0.25 g mL^{-1} for *E. coli* and 0.5 g mL^{-1} for *P. aureginosa*.

The result from the alcoholic extract (AL) showed antimicrobial activity on *B. subtillis*, *E. coli*, *C. albicans*, *P. aureginosa* and *P. mirabilis*, while at very high concentration. The Minimum Inhibitory Concentration (MIC) was obtained at 0.5 g mL^{-1} for *P. aureginosa* and 0.25 g mL^{-1} for *P. mirabilis*.

Ataman *et al*. (2006) reported that rat showed variations in physical signs/body appearance and mild histhopathologic lesions such as congesting fatty tissue changes and necrosis in selective tissues such as liver, blood vessels, kidney, lungs and testis, but the brain, eyes, intestine and heart tissues were essential indicators to the toxicity of drugs due either to dosage or duration of use.

The results of the present research showed that there was no mortality, no changes in eye colour, no loss of hair and no significant weight loss ($p>0.005$) in the wistar rats even at 4 g kg^{-1} dose. Food intake remained normal. All these show that the plant extracts were non-toxic. This research is similar to that reported by Taylor (2006) stating that *S. jamaicensis* had no toxicity even at 2 g kg^{-1} body weight by intraperitoneal administration.

CONCLUSIONS

The results obtained from phytochemical analysis has shown the presence of some secondary metabolites which proves that the plant is of great medicinal values. The plant being resistant to some

strains of clinical microorganisms especially the fungi and bacteria at high concentration ascertain that it is a possible antibiotic which should be administered high doses.

Finally, its having no toxicity at high doses shows that it is relatively safe for therapeutic cure of diseases and could be potentially beneficial to human. Thus more investigation should be carried out scientifically to confirm and prove the efficacy of this plant as well as further studies on its chronic toxicity effects.

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