Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 1 (4): 149-153, 2007
ISSN 1819-3455

Preliminary Phytochemistry and Antimicrobial Properties of *Stachytarpheta jamaicensis* (Linn.) Vahl. Stem

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Abstract: The phytochemical analysis on stems of *Stachytarpheta jamaicensis* proved the presence of secondary metabolites, including; tannins, saponins and flavonoids Crude concentrations of aqueous extract of stem showed antimicrobial activity on *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aureginosa* and slight activity on *Proteus vulgaris* while the alcoholic extract had almost similar activity, but lesser activity was observed on *Escherichia coli*.

Key words: Phytochemical analysis, antimicrobial, *Stachytarpheta jamaicensis* (Linn.) Vahl. stem

INTRODUCTION

Man since ancient time has been dependent on plants for food, drinks, shelter, clothing, equipment, dental care and medicine (Sofowora, 1986). It has often been said that all plants are potential medicines for one disease or the other. As a result, traditional healers put forward many claims about the healing power of the plant world, some of which have been investigated and substantiated scientifically (Idu *et al*., 2006).

Stachytarpheta jamaicensis (Bastard vervain or Brazillian tea) belongs to the family verbanaceae. 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 and rounded to broadly acute at the apex, widely at the margins, smooth and both surface with short petioles. The inflorescence is made up of slender spikes and a long and swollen rhachis about 30-40 cm long. The flowers are reddish purple to deep blue in color. It has a tubular corolla about 100 mm long and lobes about 3 mm long. They are more or less sparsely grouped and immersed in the axis of the inflorescence (Akobundun and Agyakwa, 1998).

Ethnobotanically, *S. jamaicensis* is an antacid, analgesic, antihelmithic, 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 colds, flu asthma, bronchitis and others, it is also 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 both hypotensive and abortive (Taylor, 2005). In some communities in Nigeria it is known for treatment of diabetes, hypertension and bacterial infection (Ataman *et al*., 2006). Some plants have been discovered in many researches to be rich in secondary metabolites including; tannins, alkaloids, flavonoids, phenols, steroids and volatile oils which are responsible for therapeutic activities (Cowan, 1999; Rabe and Vanstoden, 2000). Also,

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the use of different plant parts, mostly their decoctions, infusions, oral administration and others have been used as popular medicine for various diseases. Some of these plant parts have also been used as antimicrobial agent since time past (Ikenebomeh and Metitir, 1998; Okemo *et al*., 2001).

This study is aimed at determining the phytochemistry and antimicrobial activities of the stems of *S. jamaicensis* against the following organisms; *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aureginosa*, *Proteus vulgaris*, *Klebsiella arogenes* and *Proteus mirabilis*.

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 off the stems and the latter dried in an oven at 40°C before pulverizing.

Extraction of Plant Material for Chemical Analysis

Text for alkaloids, saponins, tannins, flavonoids and anthraquinones, was according to procedure outlined by Trease and Evans (1996). 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 screening. One hundred gram of stems was boiled by decoction in 1000 mL of water for 30 min and 150 g of stems was soaked in 1000 mL of ethanol for 48 h and then filtered. The filtrates were placed in evaporator to gradually drive off the solvents. The pastes formed were kept in two containers and labeled as follows; WS-water extract of stem or aqueous extract and AS-Alcoholic extract of stem.

Determination of Antimicrobial Activity

The organisms used for study were *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aureginosa*, *Proteus vulgaris*, *Klebsiella arogenes*, *Proteus mirabilis* and high resistance strain of standard *Eschericia coli* (J62K12) served as control.

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}.

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 saponin which was confirmed present after it was mixed with sulphuric acid and 90% ethanol, initial frothing disappeared. The test for tannins gave a bluish precipitate which confirmed it's 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 showed that WS was active on all the organisms except *K. arogenes*. There was slight activity on *P. vulgaris*. It showed no activity on J62k12.

From Table 3, the inhibition zone measured showed that WS had highest measurement at 14.0 mm and lowest 11.5 mm on *E. coli* and *P. aureginosa*, respectively. The inhibition zone measured for AS had highest measurement at 13.5 mm and lowest of 11 mm on *P. aureginosa* and *S. aureus*, respectively.

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

Secondary metabolites tested	Stem
Alkaloid	-
Saponins	+
Tannins	+
Anthraquinones	-
Flavaoinoids	+

+: Indicate presence, -: Indicate absence

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

	Extract activity	
Organisms	WS	AS
Bacillus subtilis	++	++
Escherichia coli	++	++
Candida albcans	++	++
Staphylococcus aureus	++	++
Pseudomonas aureginosa	++	++
Proteus vulgaris	+	++
Kebsiella arogenos	-	-
Proteus mirabilis	++	++
J62 K_{12}	-	-

++: Indicates presence of inhibition, +: Indicates minute presence of inhibition, -: Indicates absence of inhibition, WS = Water extract of stem, AS = Alcoholic extract of stem

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

Organisms	WS	AS	Gentamycin	Water
Bacillus subtilis	-	-	5	-
Escherichia coli	14.0	-	5	-
Candida albicans	-	12.0	5	-
Staphylococcus aureus	12.5	11.0	5	-
Pseudomonas augreginosa	11.5	13.5	5	-
Proteus vulgans vulgaris	-	12.0	5	-
Kebsiella arogenes	-	-	5	-
Proteus mirabilis	-	12.0	5	-

-: Implies no zone of inhibition, WS = Water extract of stem, AS = Alcoholic extract of stem

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

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

-: Implies no zone of inhibition, WS = Water extract of stem, AS = Alcoholic extract of stem

All extracts were active on the organisms at high concentrations (Table 4). WS had its MIC activity on *S. aureus* at 500 mg mL^{-1}. AS showed activity on *P. aureginosa*, *P. vulgaris*, *C. albicans* and *P. mirabilis* at 500, 125, 500 and 500 mg mL^{-1}, respectively.

DISCUSSION

The preliminary phytochemical investigation carried out on the stems of *S. jamaicensis* showed it consists of secondary metabolites such as saponins, tannin and flavonoids. These metabolites have been shown to be responsible for therapeutic activity of plants (Trease and Evans, 1996). Also plants containing these metabolites usually demonstrate stronger antimicrobial properties than others (Geyid *et al*., 2000). Tannins have been reported to inhibit growth of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Ogunleye and Ibitoye, 2003). Saponins are special class of glycosides that have been shown to be an antifungal agent (Sodipo *et al*., 1991). Plant phenolic compounds especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (Rauha *et al*., 2000).

The results obtained from the gutter method using aqueous stem extract (WS) showed that at very high concentration, the extract was active on all test organisms except *K. arogenes*. But at lower concentrations the effectiveness became more selective. For instance, only *S. aureus* was inhibited at extract concentration of 500 mg mL^{-1} (Table 4). The results were similar for the alcoholic stem extract, however, the MIC was lower for *P. vulgaris* (120 mg mL^{-1}) and at 500 mg mL^{-1} there were activities recorded against *C. albicans*, *P. aureginosa* and *P. mirabilis*. Although unlike the aqueous extract, it showed no activity against *S. aureus*.

CONCLUSION

The results obtained from phytochemical analysis has shown the presence of some secondary metabolites which proves that the stem could be of great medicinal value. Also the plant being resistant to some strains of clinical microorganisms especially fungi and bacteria at high concentration ascertain that it is a potential antibiotic which is dose dependent.

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