RESEARCH PAPER

Phytochemical Analysis and Antimicrobial Efficacy of an Aromatic Weed: Stemodia viscosa Roxb. (Scrophulariaceae)

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ABSTRACT

Stemodia viscosa is one of the common aromatic weed presents in the agricultural field. This current study is intended to identify the phytochemicals present in *S. viscosa*; to quantify the phenol, flavonoids and tannins content; and to screen the antibacterial activity. The quantitative analysis of present study exposed that both stem and leaves of *S. viscosa* contain more tannin content than total phenolics and flavonoids. FTIR spectra showed the presence of the functional group in both stem and leaf extracts which have medicinal properties and can be used as antimicrobial, antiinflammatory and antidiabetic agents. The antibacterial activity of *S. viscosa* was tested by disc diffusion method. The dried stem and leaf samples were mixed in different solvents namely acetone, methanol and water. All extracts were showed antibacterial activity against the human pathogenic bacterial strains namely *Bacillus substilis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi and Serratia marcescens.*

Keywords: Aromatic weeds, *Stemodia viscosa*, phytochemical analysis, FTIR analysis, antibacterial activity.

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INTRODUCTION

India is rich in biodiversity and is endowed with many useful plants. Out of 2,50,000 plant species, weeds constitute about 250 species which are prominent in agricultural and non-agricultural system (Cowan, 1999) [1]. These weeds possess non-nutritive plant chemicals that contain disease protective and disease preventing compounds, therefore which acts against various microorganisms. Many of the weeds contain therapeutic property, so that they are used in the field of medicine. *Stemodia viscosa* (Scrophulariaceae) is an aromatic weed of agricultural field, distributed in tropical and subtropical regions of the world. The volatile oil and essential oil were extracted from this plant. It is one of the medicinal plants and used to heal wounds, diabetes, cold, flu, etc. and it possesses antilipidemic activity. The fragrant leaves of this herb are placed in pillows to induce a restful sleep, or crushed and mixed with fat to make a rubbing medicine to treat cold and flu symptoms. It is used as an aboriginal healing rub along with olive oil and beeswax (Mammen and Daniel, 2012)[2]. Infectious diseases and food borne illnesses can cause severe health effects and can even lead to death among the residing population, especially in the developing regions of the world. The continual emergence of antibiotic resistant microorganisms has prompted researchers' world over to search for new antimicrobial agents that are more effective against the resistant microbial pathogens (Gislene *et al.,* 2000; Thaller *et al.,* 2010)[3, 4]. Therefore this study is aimed to qualitatively and quantitatively analyze the phytochemicals and to test their antimicrobial efficacy.

MATERIALS AND METHODS

Collection of plant materials

The field grown *Stemodia viscosa* were collected from agricultural field of Kulayankarisal village in Thoothukudi District. The mature and healthy plants were collected from the field and the leaves and stems were separated before shade drying and powdered separately.

Preparation of extracts for phytochemical screening and antimicrobial activity

The coarse powder (100g) was extracted successively with petroleum ether, acetone, chloroform, methanol and water, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for phytochemical screening and antibacterial activity.

Qualitative phytochemical tests

Phytochemical analyses of the crude extracts (petroleum ether, acetone, chloroform, methanol and water) were conducted in accordance with standard protocols (Harbourne, 1973; Trease and Evans, 1978; Philip *et al*. 2011; Damodaran and Manohar, 2012)[5-8]. All tests were replicated twice. Detailed methodologyare as follows:

Test for alkaloids

Hager's test

One ml of extract was mixed with one ml of Hager's reagent (1 g of picric acid in 100 ml distilled water). The formation of a yellow precipitate indicated the presence of alkaloids.

Test for amino acids

Ninhydrin test

Two drops of Ninhydrin reagent were added to two ml of dilute extract. A deep purple colour change indicated the presence of amino acids.

Test for proteins

Xanthoproteic test

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Test for carbohydrates

Molisch's test

Molisch's reagent (15 g α-naphthol dissolved in 100 ml ethanol) was added to five ml crude plant extract. A brownish red colour reaction indicated the presence of polysaccharides.

Test for glycosides

Modified Borntrager's test

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Test for cardiac glycosides

Keller-Killiani test

5 ml of plant extract was mixed with 2 ml glacial acetic acid. Two drops of ferric chloride were added, followed by the addition of concentrated sulphuric acid such that the acid remains underneath. The presence of glycosides was indicated by the formation of a brown ring at the junction of the two layers and a blue green ring at the upper surface.

Test for coumarins

1 ml of 10% NaOH was added to 1 ml extract. The development of a yellow colour indicates a positive reaction for coumarins.

Test for flavonoids

Shinadow's Test

To a few mg of the powder, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of red colour shows the presence of flavonoids.

Test for tannins

Lead acetate test

To 1 mL of the extract, 2 drops of lead sub acetate solution was added. A coloured precipitate indicates the presence of tannins.

Test for phlobatannins:

Few drops of 2% hydrochloric acid were added to 1ml of the extract. Appearance of red colour precipitate indicates the presence of phlobatannins.

Test for quinones

1 ml concentrated sulphuric acid was added to equal amount of extract. The development of a red precipitate indicated the presence of quinones.

Test for anthraquinones

A few drops of 2% hydrochloric acid were added to one ml of extract. The formation of a red precipitate indicates the presence of anthraquinones.

Test for saponins

Foam test

The extract (5 ml) was diluted with distilled water to 20 ml. The solution was shaken in a graduated cylinder for 15 minutes. The presence of saponins in the extract was identified by the formation of a persistent two cm foam layer.

Test for steroids

Lieberman-Burchard test

The extract was dissolved in water and then treated with chloroform. The liquids were separated using separating funnel. The chloroform portion was collected and then divided into 2 portions and was used for the test.

A few drops of acetic anhydride was added to the filtrate in a test tube, then followed by the addition of conc. sulphuric acid by the wall of the test tube. The formation of brown ring at the junction indicates the presence of phytosterols.

Test for terpenoids

Salkowski's test

5 ml of crude extract was mixed with 2 ml chloroform. Concentrated sulphuric acid (3 ml) was then carefully added to the mixture forming distinct layers. The presence of terpenoids was indicated by

the formation of a reddish brown colour at the interface between the two solutions.

Test for diterpenes

Copper acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Vitamin C

1 ml of sample extract were mixed with few drops of Dinitrophenyl hydrazine following the addition of 1 ml concentrated H_2SO_4. Appearance of yellow precipitate indicates the presence of Vitamin C.

Quantitative analysis

Estimation of flavonoids

The total flavonoid content in the sample was estimated by the method of Chang *et al.* (2002) [9]. A volume of 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 µl of 5% sodium nitrite was added and after six minutes 0.15 ml of aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read at 510 nm along with standard quercetin at 5 - 25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent / gm of dried sample.

Estimation of Total Phenolic Content

Total phenolic content of extract was determined according to the Folin-Ciocalteau method of Slinkard and Singleton (1977) [10] with some modifications. Briefly, 0.1 ml of extract (200, 600 and 1000 µg/ml), 1.9 ml distilled water and 1 ml of Folin-Ciocalteau's reagent were seeded in a tube, and then 1 ml of sodium carbonate was added. The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared with catechol calibration curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

Total Tannins Content

Tannins were determined by the method of Peri and Pompei (1971) [11]. 1 ml of the sample extracts of concentration 1mg/ml was taken in a test tube. The volume was made up to 1ml with distilled water and 1 ml of water serves as the blank. To this 0.5 ml of Folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 min. Blue colour was formed and the colour intensity was read at 640 nm. A standard graph (gallic acid - 1 mg/ml) was plotted, from which the tannin content of the extract was determined. The total tannin content was expressed in mg/g of extract.

FTIR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iDl transmission, between 4000- 400 cm^{-1} (Kareru *et al.* 2008) [12].

Antimicrobial Activity

Antimicrobial study of acetone, methanol and water extracts of Stem and leaves of *Stemodia viscosa* was carried out by disc diffusion method (Barry and Thornsberry, 1985) [13] against the pathogens viz *Bacillus substilis*; *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Serratia marcescens.* A loopful of bacteria was taken from the stock culture and dissolved in 0.1 ml of saline. All the tests were done by placing the disc (^mm diameter) impregnated with (20 mcg) respective different extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram Positive and Gram Negative bacteria. Respective solvents without plant extract served as negative control. Standard antibiotic streptomycin (30 mcg/disc) was used as reference or positive control. Plates were incubated at 37° C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone and antibacterial activity against the pathogenic bacteria was recorded.

RESULT AND DISCUSSION

Qualitative analysis

The preliminary phytochemical screening of petroleum ether, acetone, chloroform, methanol and water extracts of stem and leaf of *Stemodia viscosa* is depicted in Table 1. It revealed the presence of proteins, carbohydrates, cardiac glycosides, coumarins, flavonoids, tannins, quinones, steroids, terpenoids, diterpenes and vitamin C in different extracts of stem and similar solvent extracts of leaves of *S. viscosa* divulged the presence of proteins, carbohydrates, cardiac glycosides, coumarins, flavonoids, tannins, phlobatannins, quinones, steroids,

terpenoids, diterpenes and vitamin C. So, it can be effectively used for the treatment of various diseases.

Table 1: Qualitative phytochemicals analysis of *Stemodia viscosa* Roxb. stem and leaves

Bioactive components	Petroleum ether		Acetone		Chloroform		Methanol		Water	
	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
Alkaloids	-	-	-	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-	-	-	-
Proteins	-	-	-	+	-	-	+	-	+	+
Carbohydrates	+	+	-	-	+	+	-	-	+	+
Glycosides	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	-	-	+	+	-	-	-	-	-
Coumarins	-	-	+	+	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	-	+	+	-	-
Tannins	-	-	-	+	-	-	+	+	+	+
Phlobatannins	-	-	-	+	-	-	-	-	-	-
Quinones	+	-	+	+	+	-	-	-	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	-	+	+	+	-	-	-
Terpenoids	-	+	-	-	-	+	-	+	+	+
Diterpenes	-	-	+	+	-	-	+	+	-	-
Vitamin C	-	+	+	+	+	+	+	+	+	+

+ : Present ; - : Absent

Quantitative analysis

Total phenolics, flavonoid and tannin content of stem and leaves of *Stemodia viscosa* are presented in Figure 1. Aqueous extract of *S. viscosa* leaves possess more amount of total phenolic content (1.674±0.012 g $100g^{-1}$) than stem (1.159±0.011 g $100g^{-1}$). Total flavonoid content of aqueous extract of *S. viscosa* leaves (0.676±0.0009 g $100g^{-1}$) is more than that of stem flavonoid content (0.665±0.002 g $100g^{-1}$). The aqueous extract of leaves contain more tannin content (5.482±0.009 g $100g^{-1}$) than the stem (3.033±0.014 g $100g^{-1}$). On the whole the aqueous extract of *S. viscosa* leaves contained more amounts of total phenolics, flavonoids and tannin content than the stem aqueous extract.

The phenolic compounds are one of the largest and most ubiquitous group of plant metabolites. A number of studies have focused on the biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiantherosclerosis, cardiovascular protection and improvement of the endothelical function as well as inhibition of andiogenesis and cell proliferation activity (Han *et al.*, 2007) [14]. Phenolic compounds have been extensively used in disinfections (Okwu 2001) [15]. Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as antiinflammatory agent (Frankel 1995) [16]. The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al.,* 2003 and Cook and Samman 1996)[17, 18]. Tannins are complex moieties produced by majority of plants as protective substances; they have wide pharmacological activities and have been used since past as tanning agents. Tannins contribute property of astringency i.e. faster the healing of wounds and inflamed mucous membrane and have received considerable attention in the fields of nutrition, health and medicine largely due to their physiological activity such as antioxidant, antimicrobial and antiinflammatory properties [19] (Killedar and More 2010).

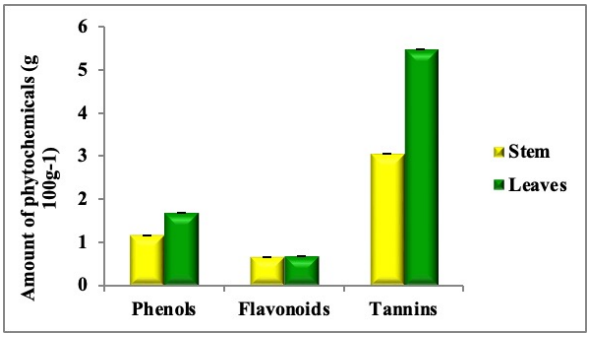

Figure 1: Total phenolics, flavonoid and tannin content of stem and leaves of *Stemodia viscosa* Roxb.

FTIR analysis

The FTIR analysis was carried out to predict the functional groups present in the methanolic extracts of stem and leaves of *Stemodia viscosa*. FTIR spectrum of stem and leaves of *S. viscosa* are shown in Figure 2 and 3 respectively. From the spectral data, presence of C-Br, C-C, =C-H, C-Cl, C-F, C-N, C=C, C=O, C-H, O-H and N-H were identified. These bonding are responsible for the presence of alkyl halide, alkyne, alkane, halo compound, conjugated alkene, amine, cyclic anhydride, ester and carbonyl compound in the stem of *S. viscosa*. The spectral data revealed the presence of C-I, C-Br, C-Cl, =C-H, C-Cl, C-O, C-N, O-H, C=O, N-H and O=C=O in the leaves of *S. viscosa* (Table 2). These bonding are responsible for the presence of alkyl halide, alkene, halo compound, ester, alcohol, amine, phenol, alkane, aromatic compounds, carbonyl compounds and carbon dioxide. Thus, the study exposed that the *S. viscosa* contain a considerable amount of secondary metabolites and it may considered in future to be used in human disease management.

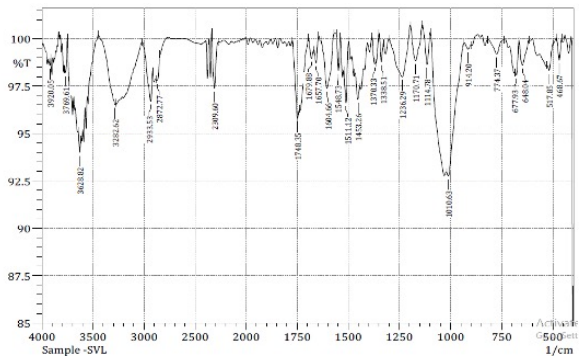

Figure 2: FTIR spectrum of stem of *Stemodia viscosa* Roxb.

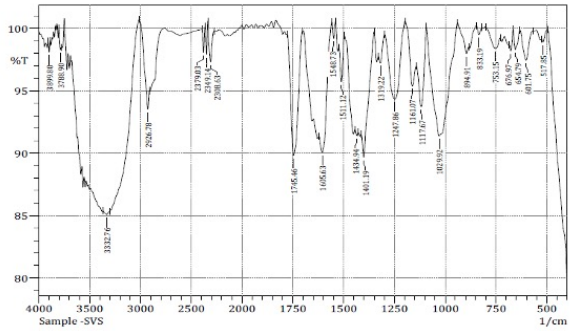

Figure 3: FTIR spectrum of leaves of *Stemodia viscosa* Roxb.

Table 2: FT-IR spectroscopic data of stem and leaf extracts of *Stemodia viscosa* Roxb.

S. No	Stem		Leaf	
	Stretching Frequency (cm^{-1})	Functional Group	Stretching Frequency (cm^{-1})	Functional Group
1.	517.85	Alkyl Halide C-Br	468.67	Alkyl Halide C-I
2.	601.75	Alkyl Halide C-C	517.85	Alkyl Halide C-Br
3.	654.79	Alkyne	648.04	Alkyl Halide C-Cl
4.	676.97	Alkene =C-H	677.93	Alkene =C-H
5.	753.15	Alkyl Halide C-Cl	774.37	Halo compound C-Cl
6.	833.19	Halo compound C-Cl	914.2	C-H out-of-plane bending
7.	894.91	Symmetric CH stretching	1010.63	Ester C-O
8.	1029.92	Alkyl Halide C-F	1114.78	Alcohol C-O/ Ether C-O

9.	1117.67	Carboxylic acids	1170.71	Amine C-N/ Ether C-O
10.	1161.07	Isopropyl	1236.29	Amine C-N/ Ether C-O
11.	1247.86	Alkyl Halide C-F/ Amine C-N/ Ether C-O	1338.51	Phenol O-H
12.	1319.22	Alkyl Halide C-F/ Amine C-N	1370.33	Alkane –C-H/ Phenol O-H
13.	1401.19	Alkane – C-H	1453.26	Aromatic C=O
14.	1434.94	Alkane – C-H	1511.12	Tetrazoles C-H
15.	1511.12	Tetrazoles C-H	1548.73	Cis amides (band absent)
16.	1548.73	Cis amides (band absent)	1604.66	Amine N-H
17.	1605.63	Conjugated alkene C=C	1657.7	Aromatic compound C-H
18.	1745.46	Carbonyl C=O/ Ester C=O/ Cyclic Anhydride	1679.88	Aromatic compound C-H
19.	2308.63	Amino acids (in hydrochlorides)	1748.35	Carbonyl C=O
20.	2349.14	Sulphinic acid (-SO_2H)	2309.6	Carbon dioxide O=C=O
21.	2379.03	Sulphinic acid (-SO_2H)	2872.77	Azoles N-H
22.	2926.78	Alkane C-H/ Carbonyl O-H	2933.53	Azoles N-H
23.	3332.76	Alcohol O-H (H-bonded)/ Amine N-H	3282.62	Alcohol O-H (H-bonded)/ Amine N-H

Antibacterial actvity

The zone of inhibition of various extracts of stem and leaves of *S. viscosa* values were compared to the standard antibiotic values (Figure 4). The acetone extracts of *S. viscosa* stem showed maximum inhibitory activity (12 mm) against *Bacillus substilis* than methanol and aqueous extracts. Methanol extracts of *S. viscosa* stem (13 mm) possessed higher inhibitory activity against *E. coli* which is followed by acetone extracts (12 mm). Acetone and methanolic extracts showed maximum inhibitory activity (2 mm) against *S. typhi* than water extract and streptomycin. Maximum inhibitory activity against *S. marcescens* was observed in methanolic extract (6 mm) of *S. viscosa* stem than other solvents. Methanolic extract of *S. viscosa* leaves inhibited growth of *B. substilis* and *E. coli* to the maximum (2 mm and 10 mm respectively) than other solvents. Aqueous extract of *S. viscosa* leaves showed maximum inhibitory activity against *K. pneumonia* (10 mm) and methanolic extract showed maximum inhibitory zone (2 mm) but these are less than standard zone formation. Methanol extracts of *S. viscosa* leaves (13 mm) possessed

higher inhibitory activity against *S. marcescens* when compares to other solvents and streptomycin.

The medical properties are not essentially limited to a single compound present in plant. But various phytocostituents combinely showed antimicrobial activity (Pasquale, 1984) [20]. The presence of proteins or peptide may act directly on microorganisms on results in growth inhibition by distrupting cell membrane synthesis of essential enzyme. This may lead to inhibit the growth of pathogens taken into an account for the study (Devendra *et al*., 2011) [21]. Flavonoids, another constituent found in plants extracts and various organic fractions showed a broad range of biological properties like antimicrobial activity and antioxidant potential (Hodek *et al.*, 2002) [22]. Anthraquinones possessed antiinflammatory and bactericidal effects (Feroz *et al.*, 1993) [23]. Tannins were toxic to bacteria, fungi and viruses and inhibit their growth (Scalbert 1991) [24]. The earlier reports showed that growth of microorganism are inhibited very effectively by plants extracts due to the presence of phenolics and flavonoids (Mori *et al.*, 1987) [25].

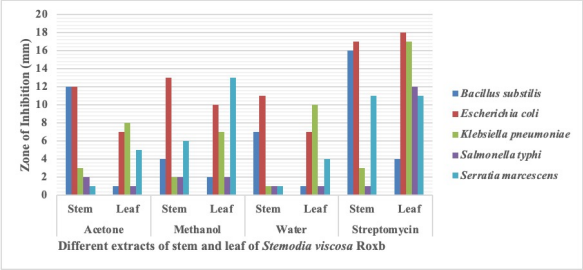

Figure 4: Antibacterial activity of stem and leaf extracts of *Stemodia viscosa* Roxb

CONCLUSION

Phytochemicals are the intermediary components of the plant metabolism, which play a vital role to control many diseases and microorganisms. The qualitative analysis of *S. viscosa* revealed various phytochemicals and these components are useful to treat disease causing pathogens and ailments. The quantitative analysis of present study exposed that both stem and leaves of *S. viscosa* contain more tannin content than total phenolics and flavonoids. The major activity of tannin is to heal wounds and it also acts on the inflammation, microorganisms and so on. Following to the tannin content, phenols placed as a major component. Phenolic components are widely used as disinfectant and it also used as a anticarcinogen, antiapoptosis, antiaging, etc. The FTIR analysis has been used to identify the complicated structures of plant secondary metabolites (Hori and Sugiyama 2003)[26]. FTIR spectra showed the presence of the functional group in both stem and leaf extracts which have medicinal properties and can be used as antimicrobial, antiinflammation and antidiabetic agents.The antibacterial activity of *S. viscosa* was tested by disc diffusion method. The experimental observations proved that the plant *S. visocsa* showed antibacterial activity against different bacterial strains at all concentrations.

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