

PRELIMINARY PHYTOCHEMICAL SCREENING AND BIOEVALUATION STUDIES OF STEM BARK OF *VENTILAGO MADERSAPATANA* GARTEN

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ABSTRACT

The aim of the present work was to investigate the *in vitro* antimicrobial and antioxidant activity of aqueous and methanolic extracts of stem bark of *Ventilago maderaspatana* Garten. In preliminary phytochemical analysis we observed carbohydrate, alkaloids, saponins, phenolic compounds, proteins, and thin layer chromatography was also performed. Antimicrobial activity was evaluated for eight bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris*, *Bacillus subtilis*, *Bacillus megatherium*, and *staphylococcus aureus* and one fungus *Candida albicans* by using stokes disc diffusion and well diffusion methods. Methanolic plant extract showed a maximum zone of inhibition in *Proteus vulgaris* by disc method but in well diffusion method *E.coli* and *S.aureus* showed maximum inhibitory activity. The antioxidant activity of the plant extract was also determined by DPPH method using ascorbic acid as standard. IC₅₀ values were also calculated.

Keywords: *Ventilago maderaspatana*, Phytochemical, Antimicrobial activity, Antioxidant.

INTRODUCTION

Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern¹. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens². Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional

remedies such as herbs for their basic health care needs. India is rich in flora and fauna. Its civilization is very ancient and the country as a whole has long been known for its rich resources of medicinal plants. Today, Ayurvedic, Homoeo and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Material Media³. There is ample literature on preliminary phytochemical surveys. Plants consist of a number of biologically active compounds ingredients therefore they are used for the treatment of a large number of infectious

diseases⁴⁻⁹. These biologically active ingredients are alkaloids, flavonoids, steroids, glycosides, terpenes, tannins and phenolic compounds¹⁰⁻¹⁴. The evaluation of the antioxidant activities of polyphenols from ethnomedicinal plants may also be necessary because they are among desired medicinal properties of plants due to their nutraceutical effects¹⁵. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. In addition to their individual effects, antioxidants interact in synergistic ways and have sparing effect in which one may protect another against oxidative destruction. These justify the overwhelming interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants¹⁶.

Ventilago maderaspatana is a medicinal herb belonging to family Rhamnaceae. It is distributed in Forests of low elevations-South Greece, India, Indonesia, Myanmar, Sri-lanka. *Ventilago maderaspatana* possess the effects of Kapha, Dyspepsia, Colic Disorder, Leprosy, Scabies, Prurities and other skin disorders, fever and general disability¹⁷.

The present study was carried out to evaluate the antimicrobial efficacy and antioxidant activity of methanol and aqueous extract of *Ventilago maderaspatana* which helps in the development of new, novel drugs.

MATERIALS AND METHODS

Collection of Plant Materials

The study period was from January 2011 to March 2011. The stem bark of *Ventilago maderaspatana* was collected from forest of Chichpalli village near Chandrapur, Maharashtra, India. The identification was carried out by Dr.Arvind Mungole of PGTD, Dept of Botany, RTMNU, Nagpur. The stem bark was washed thoroughly with tap water to remove dust particles then washed with sterile distilled water. The stem bark was again washed with mild detergent tween 20. Again it was washed with Calcium hypochlorite. Then the stem bark was dried, powdered, stored in an air tight containers and was used for the study.

Extraction Procedure

Distilled water and methanol were used as solvents for extraction of the plant materials. The air dried and fine powdered stem bark was extracted with water and methanol using Soxhlet extraction apparatus according to

Soxhlet method where materials are extracted by repeated percolation which lasts about 6-8 hours under reflux in a specialized glassware. The extracts preparations were done as previously described by Alade and Irobi. The aqueous and methanolic extracts obtained were used for study.

Chemicals

All chemicals and reagents used were of analytical grade and obtained from Sigma chemical company and used without further purification.

Test Microorganism used

The various micro organisms such Gram negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris* and Gram positive bacteria like *Staphylococcus aureus*, *Bacillus megatherium* and *Bacillus subtilis* and one fungus *Candida albicans* are procured from department of Microbiology, Guru Nanak College Of Science, Ballarpur, Maharashtra India.

Antimicrobial Agent

The reference standard Chloramphenicol was procured from Chandak Chemist and Drugist, Chandrapur, Maharashtra, India.

Antimicrobial Assay

The antimicrobial activity of the aqueous and methanolic extracts was tested individually by using stokes disc diffusion sensitivity technique and well diffusion methods.

Disc diffusion method

In stokes disc diffusion method, a loop of bacteria from the agar slant stock was cultured in nutrient broth over night and spread with a sterile cotton swap into Petri plates containing 10 ml of Muller Hinton agar No.2 medium. Sterile Whatmann filter paper no.1 discs (6 mm in diameter) impregnated with the plant extract (100mg/ml) were placed on the cultured plates and incubated at 37°C for 24 hrs. The solvent without extracts served as negative control. Standard antibiotic Chloramphenicol (10µg) was employed as positive control. After 24 hrs of incubation an antibacterial activity was assessed by measuring the inhibition zone. For antifungal activity Potato Dextrose agar was used. The diameters of the zones of inhibition by the samples were then compared with the diameters of the zones of inhibition produced by the

standard antibiotic discs. Each experiment was carried out in triplicate and the mean diameter of the inhibition ones was recorded.

Screening of anti bacterial activity was performed by well diffusion technique. The nutrient agar plates were seeded with 0.1 ml of standardized inoculums of each of the eight test micro organisms. The inoculum was spread evenly over plate with loop or sterile glass spreader. The inoculated plates were incubated at 37°C for 20 minutes. After incubation a standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of nutrient agar medium and 100µl of the extracts was introduced in the well and incubated at 37°C for 24 hrs and the zone of inhibition was measured in millimeter (mm). Mean zone of inhibition and standard deviations were calculated

Phytochemical screening

Small quantity of aqueous and methanolic extracts of stem bark of *Ventilago maderaspatana* was dissolved in distilled water and used for detection of phytochemicals such as glycosides, phytosterols, proteins, alkaloids, flavonoids, tannins, saponins, fats & fixed oils, gums and mucilages.

Test for glycosides

The extract was hydrolyzed with HCl for few hours on hot water bath and the hydrolysate was subjected to Fehling's, Benedict's, Barfoed's tests and the results were recorded.

Test for alkaloids

Presence of alkaloids was tested with four reagents:

Mayer's reagent (potassium mercuric iodide solution)

Dragendorff's reagent (potassium bismuth iodide solution)

Hager's reagent (saturated solution of picric acid)

Wagner's reagent (iodine and potassium iodide solution).

Test for phytosterols

Lieberman-Burchard test and Salkowski test was performed to identify the presence of phytosterols. The residue was dissolved in few drops of acetic acid and three drops of acetic anhydride was added followed by few drops of concentrated sulfuric acid. Bluish green colour was formed shows the presence of phytosterols.

Test for fixed oils and fats

A few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats. Spot test was also performed to identify the presence of oil.

Test for gums and mucilages

Test for gums and mucilages was carried on extract by using 90% alcohol and the precipitate was dried in air.

Test for proteins

Small quantity of extract was dissolved in few ml of water and treated with Biuret, Ninhydrin, Xanthoprotein and Million's reagents,

Test of saponins

Foam test was conducted by diluting the extract with 20 ml of distilled water and agitated in graduated cylinder 0.1cm layer of foam was formed and the result was recorded

Test for phenolic compounds

A small quantity of extract was taken in water and FeCl₃ test was performed to identify the presence of phenolic compound.

Thin Layer Chromatography (TLC)

Silica gel coated TLC plates were purchased and was used for the study. A line was drawn on the TLC plate at a distance 2 cm from the base, marks were made on the line for sample application. The sample was spotted on the line with the help of capillary tube and it was allowed to dry. The plate was placed the developing jar with mobile phase. After the solvent reaches $\frac{3}{4}$ th of the TLC plate it is taken out of the jar, the solvent front was drawn. The plates were then kept in iodine jar for few seconds, shaken and taken out. They were examined under the UV/Vis lamp and the spots were circled with pencil. The spots were labeled and the distances from the base lines were measured. The R_f values were calculated by the formula Retention factor (R_f) = Distance travelled by solute from origin / Distance travelled by solvent from origin

Assessment of In Vitro Antioxidant Activity Free radical scavenging activity

Methanolic extract of stem bark of *Ventilago maderaspatana* was used to assess the in vitro

antioxidant activity. Antioxidant scavenging activity was studied using 1, 1-diphenyl, 2-picrylhydrazyl free radical (DPPH) (18). Various concentration of test solution in 0.1ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Methanol only (0.1ml) was used as experimental control. After 30 minute of

incubation at room temperature, the reduction in the number of free radical was measured, reading the absorbance at 517nm. Ascorbic acid was used as reference standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH.

Table 1: Preliminary phytochemical screening of Ventilago maderaspatana

Sr.No.	Phytochemicals	Test	Aqueous	Methanol
1	Carbohydrate	a)Molisch's test	++	+++
		b)Fehling's test	++	+++
		c)Benedict's test	++	+++
		d)Barfoed's test	++	+++
2	Phytosterols	a)Libermann burchard test	+++	+++
		b)Salkowski test	+++	+++
3	Proteins	a)Biuret test	++	++
		b)Ninhydrin test	++	++
		c)Xanthoprotein test	++	++
		d)Millon's test	++	++
4	Alkaloids	a) Mayer's Test	++	++
		b)Drangandroff's test	++	++
		c)Hager's test	++	++
		d)Wagner's test	++	++
5	Flavonoids	a)With aqueous NaOH solution	++	++
6	Phenolic compounds	a)FeCl ₃ test	++	++
7	Tannins	a) FeCl ₃ test	-	+
8	Saponins	a)Foam test	+	+
9	Fats & Fixed Oils	a)spot test	+	++
		b)Saponification test	+	++
10	Gums & Mucilages	a)With 90% alcohol	-	-

Key: +++High, ++=Moderate, += Normal concentration, - = absent



Fig. 1: Zone of inhibition of methanol extract of Ventilago maderaspatana against Gram positive and gram negative micro organisms by Agar well method and Disc diffusion method

The percent inhibition was calculated from the following equation:

$$\% \text{ inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test sample}) / \text{Absorbance control}] \times 100}{1}$$

A dose response curve was plotted to determine the IC₅₀ values. IC₅₀ is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

RESULTS

The results of the phytochemical study are given in table1. TLC fingerprint profiles of both aqueous and methanol extract showed 2 spots. The Rf values are 0.60, 0.34 and 0.56, 0.34 respectively (table2). The extracts showed a broad spectrum of antimicrobial activity by inhibiting the growth of test microorganisms. Methanolic extract of *Ventilago maderaspatana* showed strong antimicrobial activity against Gram positive and Gram negative bacteria. The concentration of 100 mg/ml methanolic extract showed significant rate of inhibition in *P.vulgaris* showing 13.98 mm inhibition zone by disc diffusion method. But in well diffusion method same concentration showed maximum zone of inhibition (20 mm) against *E.coli* and *S.aureus* (table3). Other tested bacteria viz. *Pseudomonas aeruginosa*; *Bacillus subtilis*; *Bacillus megatherium*; *Klebsiella pneumonia*, *Salmonella typhi* showed significant susceptibility to methanolic extract of stem bark of *Ventilago maderaspatana* by disc diffusion method and agar well diffusion method. In vitro antioxidant results showed that although the methanolic extract of stem bark of *Ventilago maderaspatana* showed 100% anti scavenging activity by DPPH method, its IC₅₀ value does not compare well with vitamin C. (table 4).

DISCUSSION

The stem bark of *Ventilago maderaspatana* has been used for thousands of years for its medicinal properties. It is rich in a wide variety of secondary metabolites such as glycosides,

alkaloids, phytosterols, proteins, saponins and phytosterols which has been found invitro to have antimicrobial properties. In this connection the present study on the methanolic and aqueous extracts was conducted to evaluate the antimicrobial activity of stem bark. Aqueous extract of stem bark showed milder antimicrobial activity compared to methanolic extract, which certainly indicates that methanolic extract contain higher concentration of active antimicrobial agents such as alkaloids, glycosides, volatile oils, which are all found in more abundant amount in methanolic extract of *Ventilago maderaspatana*. Preliminary results of the activity of antimicrobial agents such as plant active components, antibiotics are usually expressed invitro as zones of inhibition around the chemical this is in comparable to the work of Gislene et.al.,(2000) on the antibacterial activity of the plant extract and phytochemicals on antibiotic resistance bacteria in rail according to them any chemical that demonstrates activity with zones of inhibition of 7 mm and above is acceptable as being active, the stem bark extract of *Ventilago maderaspatana* showed 8 mm inhibition zone, therefore it contains effective antimicrobial compounds. Stem bark extracts of *Ventilago maderaspatana* showed broad spectrum antimicrobial activity since water, and methanol extracts of stem bark have exhibited antibacterial activities against *E.coli*, *Pseudomonas*. Besides, extract of the entire plant has shown antifungal activity against *Candida albicans*. In addition to these properties, it has also been used as appetite stimulant, treatment for gastrointestinal infection and to lower blood glucose sugar in diabetes. Its use for treatment of certain types of cancer and viral infections has also been reported. It is unclear which active ingredients are having clinical usefulness.

Table 2: TLC profile of *Ventilago maderaspatana*

Extract	No. of spots visible by UV	Rf values
Aqueous extract	2	0.65
		0.33
Methanol extract	2	0.6
		0.33

Table 3: Antimicrobial activity of Ventilago maderaspatana by disc diffusion and well diffusion method

Methods	Extracts (100 mg/ml)	Microorganism/ zone of inhibition in mm*								
		Gram negative bacteria					Gram positive bacteria			Fungus
		EC	PA	PV	KP	ST	BS	BM	SA	CA
Disc Diffusion	Aqueous	8.1±0.5	7.2±0.3	7.2±0.3	7.1±0.2	7.0±0.3	7.1±0.3	9.87±0.3	8.1±0.5	-
	Methanol	11.0 ±0.3	13.08±0.3	13.98±0.2	12.92±0.2	12.98±0.2	12.98±0.2	13.0±0.2	12.03±0.3	11.0 ±0.3
Well Diffusion	Aqueous	9.2±0.5	9.3±0.3	9.2±0.4	9.4±0.3	9.4±0.4	9.3±0.5	10.0±0.3	11.0±0.5	-
	Methanol	20.0±0.3	18.0±0.3	18.0±0.4	19.1±0.3	18.0±0.4	18.0±0.3	18.0±0.4	20.0±0.3	14±0.3
Chloramphenicol (10µg/ml)		24	20	24	20	19	24	24	26	-

Key: EC= *Escherichia coli*; PA= *Pseudomonas aeruginosa*; BS= *Bacillus subtilis*; KP= *Klebsiella pneumoniae*; ST= *Salmonella typhi*; PV = *Proteus vulgaris*; BM= *Bacillus megatherium*; SA= *Staphylococcus aureus*; CA= *Candida albicans*; - = Not tested
*Each value was expressed as the mean±SD. (N=3)

Table 4: Antioxidant activity and IC₅₀ values of methanolic extract of stem bark of Ventilago maderaspatana by DPPH method

Sample Name	% Antioxidant Activity	IC ₅₀ (µg/ml)
V. maderaspatna	100	60.15
Vitamin C	81.78	20.9

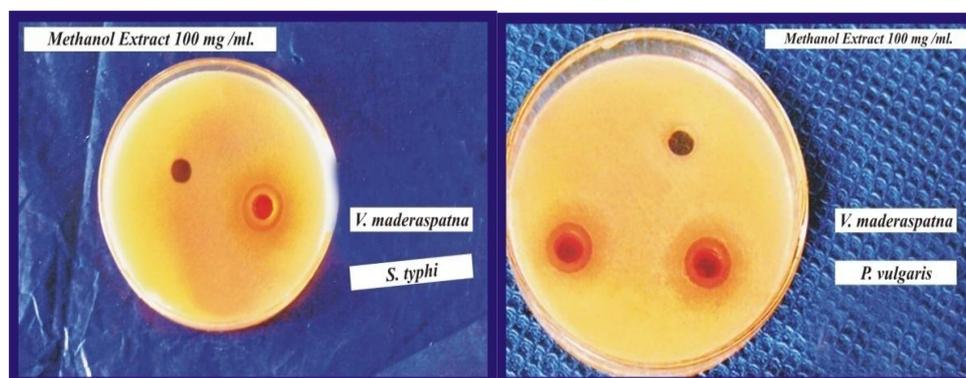
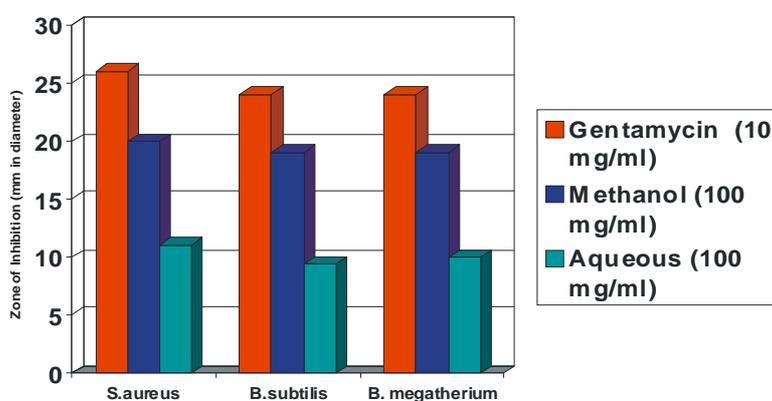


Fig. 2: Zone of inhibition of methanol extract of Ventilago maderaspatana against Gram positive microorganisms by Agar well method

Gram Positive Organism



Gram Negative Organism & Fungus

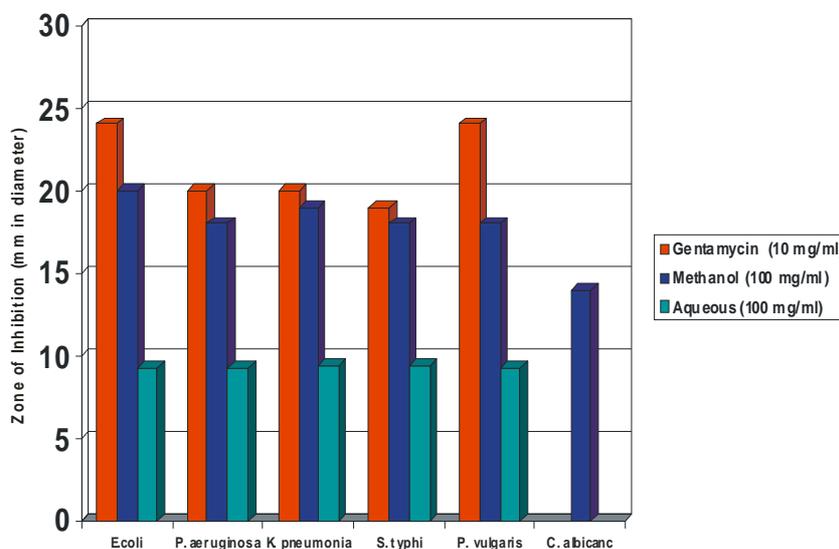


Fig. 3: Zone of inhibition of methanol extract of *Ventilago maderaspatana* against Gram negative micro organisms by Agar well method

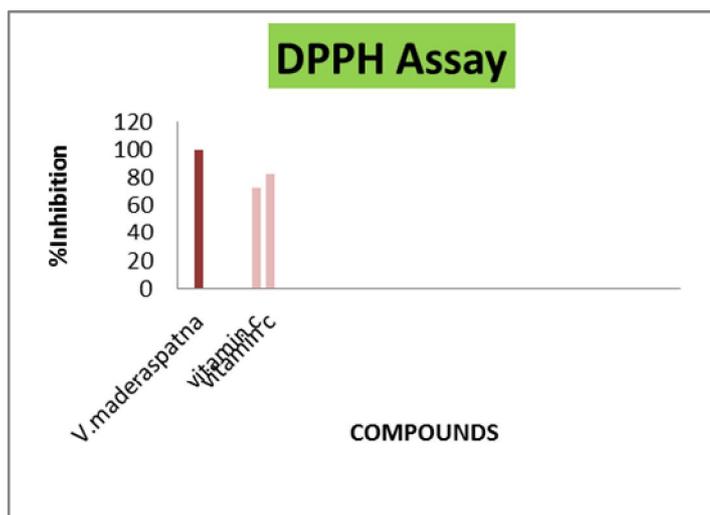


Fig. 4: Antioxidant activity of *Ventilago maderaspatana* by DPPH method

CONCLUSION

From our study and with previous literature survey we can come to conclusion that the stem bark of *Ventilago maderaspatana* is rich in phytochemicals which has free radicals scavenging activity and strong antimicrobial activity against various microorganisms. Further studies can be made to isolate and identify the chemical nature of the antioxidant as well as antimicrobial agent present in the plant.

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