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SCREENING OF ANTIBACTERIAL AND PHYTOCHEMICAL ACTIVITY OF *ADATHODA VASICA* L AGAINST CLINICALLY ISOLATED RESPIRATORY PATHOGENS

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Abstract

Antibacterial activity and phytochemical analysis were carried out in *Adathoda vasica* L against isolated respiratory pathogens such as Escherichia coli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogens*. Soxhlet and crude method were carried out with the help of solvents like Ethyl acetate, Ethanol, Methanol and Aqueous. Crude Aqueous extract shows highest zone of inhibition in *Streptococcus pyogens* (20mm).Phytochemicals such as flavonoids, terpenoids, lignin, inulin and saponin were found to be present in the plant extracts.

INTRODUCTION

The Respiratory tract is the most common site for infection by pathogens. This site becomes infected frequently because it comes into direct contact with physical environment and exposed to microorganism in the air. It has been calculated that the average individual ingests about 8 microorganisms per minute or 10,000 per day (Chamber Lain, 2009). Respiratory diseases range from mild and self limiting such as the common cold to life threatening such as bacterial pneumonia or pulmonary embolism Upper respiratory infections are usually benign, transitory self limited illness (Toshiharu Matsushima, 2002).

Some respiratory diseases are caused by bacteria. For instance, *Staphylococcus aureus* causes pneumonia (Rubinstein et al., 2008), Escherichia coli causes community-acquired pneumonia (Tillotson and Lerner, 1967), and *Pseudomonas aeruginosa* typically infects the pulmonary tract and cause pneumonia (Grant et al., 2000).

In recent years, because of the costs as well as serious side-effects of a number of modern drugs, attention has turned back to medicinal plants as a source for discovery of newer drugs with low cost and side effects. It has been reported that about 64% of the total world population is using traditional medicine to satisfy their health-care needs (Cotton, 1996).

The medicinal plants are rich source of secondary metabolites like alkaloids, glycosides, steroids, and flavonoids, which are potential source of drugs. In India, around 20,000 medicinal plants have been recorded however traditional communities are using only 7,000 - 7,500 plants for curing different diseases. Plant derived medicines have been the first line of defense in maintaining health and combating diseases. In the last century, roughly 121 pharmaceutical products have been discovered based on the information obtained from the traditional healers (Anesini and Perez, 1993).The history of use of medicinal plants by human beings to

treat diverse ailments goes back to thousands of years ago (Sofowara, 1982).

Use of plant extracts and phytochemical compound establish the antimicrobial properties lead to great significance in preventive and therapeutic measures (Thenmozhi et al., 2011).The plant chosen for our study was *Adatoda vasica* (Leaves) belongs to the family of Acanthaceae. It is a small evergreen, subherbacious bush. The Leaves are 10 to 16 cms in length, minutely pubescent and broadly lanceolate. The inflorescence is dense, short pedunculate, bractate and spike terminal. The corolla is large and white with lower lip streaked purple. The fruit is a four-seeded small capsule. It is a primary herb of the ayurvedic system used in the treatment of coughs, bronchitis, asthma and symptoms of common cold.

MATERIALS & METHODS

Plant Materials

The plant material was collected from Trichy Districts of Tamilnadu in India. The plant was authenticated by Mr. John Britto, and it has been deposited in the Herbarium, Department of Rapinant herbarium and center for molecular systematic, st. Joseph's college, Tiruchirapalli, Tamil Nadu, India, for reference.

Preparation of Extracts:

Fresh *Adatoda vasica* leaves were washed thoroughly in tap water and with distilled water and air dried in the shade at room temperature for five days. Dried leaves were taken separately and powdered for extraction. The Plant Powders (100 g) were successively extracted by Soxhlet and Cold extraction methods with Ethyl acetate, Ethanol, Methanol and Aqueous. The extracts were dried in vaccum desicator and were stored in a sterile container for further use (Kelmanson et al., 2000).

Collection of Samples

Hundred sputum samples were collected from clinically diagnosed patients from Government Hospital, Srirangam. Samples were collected in the sterile containers and transported to laboratory for further analysis.

Isolation and identification of pathogens from sputum samples

For the isolation of causative agents the sputum samples were inoculated in Blood agar and MacConkey agar. Plates were incubated at 37ºC for 24-48 hrs. Colonies

were analyzed by physiological and biochemical test for conformation. (Koneman et al., 1998).

Agar Well Diffusion Assay

Agar well diffusion method was followed Muller-Hinton Agar (MHA) plates were seeded with 18 hours old culture of the isolates. The organic fractions were dissolved in Dimethyl sulfoxide (DMSO) and sterilized by using sortorious syringe filter of pore size 0.22µm. various concentrations of the extracts (250µl, 500µl, 750µl and 1000µl) were added into the sterile 8mm diameter well. Incubation was made at 37^0C for 24hrs. Antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well using standard (Hi-Media) scale. The experiment was repeated thrice and the average values were calculated for antibacterial activity (Perez et al., 1990).

Phytochemical screening

The herbal extracts were subjected to various phytochemical tests separately. Different qualitative chemical tests were performed for determining the phytoconstituents present in the plant extracts. Phytochemical analyses were done according to the procedure of phytochemical methods by Harborne, 1984 and Sofowara, 1993.

Thin layer chromatography

Preparation of TLC Plates

25x10 cm glass plates were washed with distilled water followed by smearing with acetone. After drying the plates were placed on the template in row. The slurry of silica gel G prepared with glass distilled water in the ratio 1:2 (w/v) was poured in the applicator. The glass plates were immediately coated with a layer of silica gel in 500µm thickness. The coated plates were activated at 80°c for 3 hours. Then the plates were stored in a plate chamber for further study. In that study chloroform and methanol (solvent) was used in 96:4 ratios.

Loading of substances

The concentrated plant extract of 2.5 mg was loaded on the TLC plates just above 2 cm from the bottom using a capillary tube. The plates were reserved in a developing jar containing the solvent mixture. After, the solvent front reached approximately 18cm height. The plates were removed and allowed at room temperature for 30 min. Then the plates were also observed under

UV light (240 and 300 nm) and recorded the Rf value of fluorescence substances (Anushia et al., 2009).

RESULTS AND DISCUSSION

Antibacterial activity

The antibacterial activity against Escherichia coli, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Streptococcus pyogens* was carried out. Under crude method aqueous extracts shows best activity than methanol, ethanol and ethyl acetate. *Streptococcus pyogens* shows 20mm as highest zone under aqueous extract. In ethyl acetate *Pseudomonas aeruginosa* was not sensitive at low concentrations but it shows zone at 1000µl concentration. Rest of the organisms shows sensitivity in all the concentrations. *S. pyogens* shows highest zone(14mm) followed by *S. aureus* (13mm), *E. coli* (12mm), *K. pneumonia* (11mm) and *P. aeruginosa* (7mm). Ethanol extract shows 14mm zone of inhibition in *S. pyogens* whereas 12mm in both *S. aureus* and *K. pneumonieae*. 10mm in E. coli and 6mm in *P. aeruginosa*. 13mm of zone were recorded in *E. coli*, *P. aeruginosa, S. aureus* 10mm in *S. pyogens* and 15mm in *K. pneumoniae* under methanolic extract. Aqueous extract shows higher rate of inhibition in *S. pyogens* followed by E. coli, *P. aeruginosa*, *K. pneumoniea*, and *S. aureus* as 20mm, 16mm, 15mm, 15mm and 13mm respectively (Table 1). In soxhlet method ethanolic extract shows high inhibition than ethyl acetate, methanol and aqueous extractions. Under ethyl acetate 12mm of zone was recorded as highest in *Streptococcus pyogens*. *Staphylococcus aureus* shows 12mm as highest zone in both methanol and aqueous extractions. *Staphylococcus aureus* (14mm) shows overall highest concentration in Aqueous extract under soxhlet method (Table 2).

5.2 Phytochemical Analysis:

Carbohydrate, lignin and saponin were present in all the extracts. Under soxhlet extract flavonoids, flavones and inulin present in ethyl acetate extract, flavonoids and tannins present in ethanolic extract whereas flavones and inulin found to be present in Aqueous extract (Table 3). Table 4 shows the presence of phytochemicals in crude extractions. Carbohydrate, Flavones, Lignin, inulin and saponin were found to be

present in all the four extracts. Flavonoids and tannin present in Aqueous extract, whereas terpenoids present in all the extract except aqueous extract.

Thin layer Chromatography

Table 5 shows the TLC result of crude extractions under various solvents .Green spots were identified in both ethyl acetate and ethanolic extractions under various Rf values like 0.63 and 0.55 respectively. In methanolic extract brown spot shows 0.81 as Rf values whereas in aqueous extraction brown spot (Rf 0.94) were observed.

Under soxhlet extraction ethyl acetate shows green spot (Rf 0.76), Ethanolic extracts shows olive green spot (Rf 0.86), methanolic extract shows pale yellow spot(Rf 0.68) and in aqueous extract 0.55 Rf value were recorded under brown spot observation(Table 6).

CONCLUSION

Adathoda vasica L is an excellent herbal medicine for respiratory diseases. Crude and soxhlet methods were carried out in the dried leaves power of *Adathoda vasica* L. Extracts were prepared by using various solvents like Ethyl acetate, Ethanol, Methanol and Aqueous. Aqueous extracts of *Adathoda vasica* L shows highest activity when compared with all the extracts and crude method is best when compare with soxhlet method. Phytochemicals such as flavonoids, terpenoids, lignin, inulin and saponin were found to be present in the plant extracts. Many tribal peoples used this plant for respiratory tract problems. Aim of our study is to create awareness about herbal plants to cure diseases. We suggest that *Adathoda vasica* L leaves is excellent remedy for respiratory problems.

Table 1

Antibacterial Activity of *Adathoda Vasica* L against Respiratory Pathogens by Crude Method

Sr. No	Organisms	Concentration of Extracts(µl) /Zone of inhibition in mm/Crude method															
		Ethyl acetate				Ethanol				Methanol				Aqueous			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
1.	*Escherichia coli*	5	8	11	12	4	6	8	10	5	7	10	13	9	11	14	16
2.	*Pseudomonas aeruginosa*	-	4	6	8	-	-	-	6	5	10	11	13	4	8	10	15
3.	*Staphylococcus aureus*	6	8	10	13	4	6	8	12	8	10	11	13	8	10	11	13
4.	*Streptococcus pyogens*	6	10	12	14	6	9	12	14	4	5	7	10	4	10	16	20
5.	*Klebsiella pneumoniae*	4	6	9	11	4	7	9	12	6	8	11	15	4	7	10	15

Table 2

Antibacterial Activity of *Adathoda Vasica* L against Respiratory Pathogens by Soxhlet Method

Sr. No	Organisms	Concentration of Extracts(µl) /Zone of inhibition in mm/Soxhlet method															
		Ethyl acetate				Ethanol				Methanol				Aqueous			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
1.	*Escherichia coli*	-	-	6	10	-	4	7	9	-	-	9	11	4	6	9	11
2.	*Pseudomonas aeruginosa*	-	-	4	11	-	-	5	10	-	4	6	8	-	-	5	10
3.	*Staphylococcus aureus*	-	-	5	8	-	4	6	14	5	9	10	12	4	6	9	12
4.	*Streptococcus pyogens*	7	10	11	12	-	4	7	11	-	5	8	11	-	-	5	10
5.	*Klebsiella pneumoniae*	-	-	5	8	-	-	4	9	-	4	6	9	-	-	4	7

Table 3

PHYTOCHEMICAL ANALYSIS OF *Adathoda vasica L* LEAF IN CRUDE EXTRACTS.

Sr. No	Tests	Ethylacetate extract	Ethanolic extract	Methanolic extract	Aqueous extract
1	Alkaloids	-	-	-	-
2	Flavonoids	-	-	-	-
3	Steroids	-	-	-	-
4	Carbohydrates	+	+	+	+
5	Tannins	-	-	-	-
6	Flavones	+	+	+	+
7	Terpenoids	+	+	+	-
8	Amino Acid	-	-	-	-
9	Protein	-	-	-	-
10	Lignin	+	+	+	+
11	Inulin	+	+	+	+
12	Saponin	+	+	+	+

(+) Positive ; (-) Negative.

Table 4

PHYTOCHEMICAL ANALYSIS OF *Adathoda vasica L* LEAF IN SOXHLET EXTRACTS.

Sr. No	Tests	Ethyl Acetate extract	Ethanolic extract	Methanolic extract	Aqueous extract
1	Alkaloids	-	-	-	-
2	Flavonoids	+	-	-	-
3	Steroids	-	-	-	-
4	Carbohydrates	+	+	+	+
5	Tannins	-	+	-	-
6	Flavones	+	-	-	+
7	Terpenoids	-	-	-	-
8	Amino Acid	-	-	-	-
9	Protein	-	-	-	-
10	Lignin	+	+	+	+
11	Inulin	+	-	-	+
12	Saponin	+	+	+	+

(+) Positive ; (-) Negative.

Table 5

THIN LAYER CHROMATOGRAPHY OF *Adathoda vasica L* UNDER CRUDE METHOD

Sr. No	Extracts	Observation	Rf values
1	Ethyl acetate	Green	0.63
2	Ethanol	Green	0.55
3	Methanol	Pale yellow	0.81
4	Aqueous	Brown	0.94

Table 6

THIN LAYER CHROMATOGRAPHY OF Adathoda vasica L UNDER SOXHLET METHOD

Sr. No	Extracts	Observation	Rf values
1	Ethyl acetate	Green	0.76
2	Ethanol	Olive green	0.86
3	Methanol	Pale yellow	0.68
4	Aqueous	Brown	0.55

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