



Assessing the curative properties of *Adathoda vasica* against Respiratory infections

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ABSTRACT

Adathoda vasica is a well-known plant drug in Ayurvedic and Unani medicine. It has been used for the treatment of various diseases and disorders, particularly for the respiratory tract ailments. In the present study, the antibacterial and phytochemical analysis of *Adathoda vasica* was thoroughly studied for fourteen different solvents against drug resistant human pathogens. The different extract was tested for antibacterial activity by disc diffusion method. The different dosage was determined by broth dilution method. Based on the zones of inhibition and MIC values, it can be concluded that *Adathoda vasica* can be used as a therapeutic drug. Antibacterial activity of ethanol extract of *Adathoda vasica* leaves were tested against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *E.coli* through disc diffusion assay. Qualitative analysis reveals positive results for pharmacological constituents. These reports are very encouraging and indicate that the herb should be studied more extensively for its therapeutic benefits. To determine the growth inhibition against target respiratory isolates, minimal inhibitory concentration has been carried out by broth dilution method. The ethanol extract was found to be more effective in inhibiting the bacteria at a concentration of 3.125mg. As a result the study provides scientific evidence on traditional use of *Adathoda vasica* leaf extract in treating bacterial infections.

Key word: *Adathoda vasica*, MIC, respiratory isolates, sputum

INTRODUCTION

Infections often treated with antibiotics are the second most common cause of adverse drug events in the elderly, with a risk comparable to insulin, warfarin, and digoxin. Moreover, concurrent use of warfarin and any antibiotic is associated with an increased risk of bleeding. The herbal healing for various infections and diseases was passed from generation to generation by word of mouth. This study was undertaken to determine if there is any evidence for the clinical efficacy of the chosen herbal preparations for the treatment of both upper and lower respiratory infections. Hence, alternate and safe therapy is needed. With this concept, we have decided to explore an Indian traditional plant which has been used as a folklore medicine against respiratory infections. *Adathoda vasica* belongs to the family Acanthaceae. It is an erect, terrestrial, perennial shrub. The leaves are dark green above and pale yellow below. The flowers are typical, white, arranged in a pedunculated spike. *Adathoda vasica* (Acanthaceae) commonly known as vasaka distributed throughout India up to an altitude of 1300m. The leaves, flowers, fruit, and roots are extensively used for treating cold cough, whooping cough, chronic bronchitis and asthma as sedative, expectorant and antispasmodic[1]. The plant is recommended for a variety of ailments such as bronchitis, asthma, fever, jaundice etc. The leaves & roots are efficacious in coughs, arthritis, diarrhoea and dysentery have the best chemostatic quality. Leaves are anti-inflammatory, and effective in skin disorders and cardiotoxic[2]. This is one of

the most potent anti tuberculosis drug[3]. Vasicine is also reported for its anthelmintic and hypertensive activity[4]. It is a small evergreen bush, which grows all over the plains of India and is extensively used as a remedy for colds, bronchitis and asthma. In acute stages of bronchitis, the juice of the plant gives unfailing relief, especially where the sputum is thick and sticky. In the present study, the traditional claims of *Adathoda vasica* leaves in treating respiratory infections was evaluated by determining its antibacterial property. Evidently, there are not sufficient scientific studies that confirm the antimicrobial activity of this plant. This study looks into the *in vitro* antimicrobial activity of this plant for different extracts against a few Gram-positive and Gram-negative pathogenic bacteria that cause the most common cases of infectious diseases. Given the alarming incidence of antibiotic resistance in bacteria, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop safer and newer alternative antimicrobial drugs for the treatment of infections. Several screening studies have been carried out in different parts of the world. It has been suggested that aqueous and ethanol extracts from plants used in allopathic medicine are potential sources of antiviral, antitumor, and antimicrobial agents[5]. In less developed states of India, low-income people such as farmers, people of small isolate villages and native communities use herbal medicines for the treatment of common infections. It is necessary to evaluate, in a scientific base, the potential use of herbal medicine for the treatment of infectious disease produced by common pathogens.

EXPERIMENTAL SECTION

Preparation of plant extracts

Leaves of *Adathoda vasica* were collected from Vayallur road sides at Tiruchirappalli district, Tamilnadu, India and the plant was authenticated by the Botany department of St. Joseph's College and the voucher specimen was deposited (001). The plant chosen for the study had been washed, macerated and lyophilized. About 500g of all the three plant leaves yielded 33g powder respectively. The procedure was repeated to collect the needed quantity. 100g powdered plant was extracted with 1L of 12 different solvents in Soxhlet apparatus for 18hrs the filtrate was stored in an air tight container for future use.

Culture

Several respiratory isolates such as *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris* (Gram negative) *Streptococcus pneumonia*, *Staphylococcus aureus* (Gram positive) were obtained from the microbiology department of a local hospital, Tiruchirappalli, India. These clinical isolates were collected and stored in semi-solid agar and were plated out on nutrient agar and Macconkey agar plates to check the viability of the bacteria.

Screening the best solvent

Several solvents were chosen for the study based on solubility. Based on the results of preliminary antibacterial screening, the best solvent was chosen based on the result of antimicrobial zone. The filtrate was evaporated to dryness under reduced pressure and at a lower temperature in a rotary evaporator. The dried residues were stored in airtight containers for further use.

Antimicrobial activity testing

Disc diffusion Test

The antibacterial activity of the extracts was determined by the disc diffusion method. The zones of inhibition were calculated by measuring the diameters of the zone around the disc.

Determination of Minimal Inhibitory Concentration (MIC)

The chosen ethanol extract was subjected for determining the minimal inhibitory concentration (MIC) by broth dilution method. The minimum inhibitory concentration of the extract was estimated for each of the test organisms in triplicates. To 100 μ l - 200 μ l of varying concentrations of the extracts, 100 μ l of nutrient broth was added and then a loopful of the test organism previously diluted, the microwell plate was incubated for 24hrs and each dilution was plated and observed for the bacterial growth inhibition. The MIC values were interpreted as the highest dilution of the sample, which showed no growth. Results of minimum inhibitory concentration (MIC)

Phytochemical Analysis

The chosen extract was screened to determine the presence of bioactive chemicals constituents using phytochemical tests to detect alkaloids, flavonoids, carbohydrate, cardiac glycosides, phenols, phlobatannis, saponins, sterols, tennis, quinines, oxalate, reducing sugar, amino acids, anthraquinones, triterpenoids, leucoantholyanin, coumurins,

fatty acids, Diterpenes, physterols, protein, lactones, anthrocyanins, xanthoprotein, carboxylic acid, vitamin-c, starch, and catechin compounds by the following procedure.

RESULTS AND DISCUSSION

Among the 12 solvents tested, ethanol was found to be the most effective solvent against targeted respiratory isolates on agar by the standard disc diffusion method. The diameter of the zones of inhibition of growth was compared with standard antibiotics and results were recorded. The result from the current study ethanol extract of *Adathoda vasica* showed better activity against various Gram-positive and Gram-negative organisms by means of agar disc diffusion method. So from the above study reveals that ethanol extract of *Adathoda vasica* has effective capability to fight against respiratory isolates (Table 2). The ethanol extract was evaluated for presence of various phytochemical constituents, qualitative chemical tests shown the presence of secondary metabolites anthraquinones, glycosides, saponins, glycosides, saponins, tannins, flavonoids, Terpenoids, coumurins as seen in (Table 1). Prevalence of antibiotic resistance in chosen bacterial isolates has been increasing since last few decades and this has raised the demand for the scientific community to search for new anti-bacterial components. Treatment of infections is compromised worldwide by the emergence of bacteria that are resistant to multiple antibiotics. There are several reasons for development of multidrug resistance. *Adathoda vasica* has demonstrated severe antibacterial activity against Gram positive and Gram-negative bacteria.

Table 1 Phytochemical analysis of *Adathoda vasica*

S.No	Phytochemicals	Ethanol extract
1	Steroids	+
2	Tannin	+
3	Saponin	+
4	Anthocyanin	+
5	Coumarins	+
6	Emodins	-
7	Alkaloids	+
8	Proteins	-
9	Aminoacids	-
10	Diterpenes	+
11	Phytosterol test	+
12	Phenols	+
13	Phlobatannin	-
14	Leucoanthocyanin	-
15	Cardial glycosides	+
16	Flavonoids	+
17	Terepenoids	+
18	Carbohydrate	+

Table 2 Antibacterial activity of *Adathoda vasica* leaves by Disc diffusion method

Bacterial sps	Acetone	DMSO	Ethylacetate	Chloroform	Propanol	Hexane
<i>P.aeruginosa</i>	0.00	0.00	20±0.19	17±0.28	10±0.18	0.00
<i>S.aureaus</i>	0.00	0.00	10±0.23	0.00	10±0.23	0.00
<i>K.pneumoniae</i>	17±0.23	0.00	12±0.23	0.00	14±0.20	0.00
<i>P.vulgaris</i>	10±0.23	0.00	0.00	0.00	6±0.18	0.00
<i>S.pneumoniae</i>	10±0.18	10±0.18	0.00	0.00	6±0.18	0.00
<i>E.coli</i>	0.00	0.00	0.00	17±0.28	0.00	0.00

Table 2 Antibacterial activity of *Adathoda vasica* leaves by Disc diffusion method

Bacterial sps	Butanol	Methanol	Ethanol	Isoamylalcohol	Pet.ether	Aqueous
<i>P.aeruginosa</i>	0.00	20±0.20	21±0.19	14±0.20	0.00	9±0.23
<i>S.aureaus</i>	0.00	14±0.20	20±0.20	0.00	0.00	5±0.18
<i>K.pneumoniae</i>	12±0.23	0.00	26±0.17	0.00	0.00	10±0.18
<i>P.vulgaris</i>	0.00	0.00	0.00	0.00	0.00	15±0.29
<i>S.pneumoniae</i>	0.00	0.00	15±0.29	0.00	8±0.15	13±0.19
<i>E.coli</i>	0.00	0.00	20±0.22	12±0.23	0.00	0.00

Table 3 Minimal inhibitory concentration of *Adathoda Vasica* (in mg)

Bacterial sps	MIC (mg)
<i>E.coli</i>	3.125
<i>P.aeruginosa</i>	100
<i>S.aureus</i>	100
<i>K.pneumoniae</i>	100
<i>P.vulgaris</i>	100
<i>S.pneumoniae</i>	100

The ethanol extract of *Adathoda vasica* exhibited effective activity against the tested organisms among the 11 tested extracts of *Adathoda vasica*. The plant showed the antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae*. Ethanol extract of *Adathoda vasica* demonstrated activity against *S.aureus*, *P.vulgaris*, *E.coli*[6]. Medicinal plants are of prime importance to the health of individuals and communities and the medicinal value of these economically important plant species is due to presence of some chemical substances which produce a definite physiological action on human body like alkaloids, tannins, flavonoids and saponin etc[7]. Medicinal properties of plants are due to the secondary metabolites present in different plant parts[8]. The ethanol extract of *Adathoda* revealed the presence of 5 phytochemicals and 8 constituents in *Adathoda zeylanica* ethanol extract[9]. In the present investigation, the ethanol extract of *Adathoda vasica* indicate that the anti-bacterial activity might be due to the presence of phytochemicals like alkaloids, saponins, flavonoids, tannins, terpenoids, amino acids etc. In contrast in our study *E.coli* was inhibited at MIC value 3.125mg/ml.

CONCLUSION

Among the 12 solvents tested, ethanol was found to be the most effective solvent against targeted respiratory isolates on agar (Table 1) by the standard disc diffusion method. The diameter of the zones of inhibition of growth was compared with standard antibiotics and results were recorded. The result from the current study ethanol extract of *Adathoda vasica* showed better activity against various Gram-positive and Gram-negative organisms by means of agar disc diffusion method. So from the above study reveals that ethanol extract of *Adathoda vasica* has effective capability to fight against respiratory isolates. The ethanol extracts revealed the presence of secondary metabolites anthraquinones, glycosides, saponins, glycosides, saponins, tannins, flavonoids, Terpenoids, coumursins. The above study reveals that the ethanol extract of *Adathoda vasica* is effective to fight against respiratory isolates. The ethanol extract was found to be more effective in inhibiting the bacteria at a concentration of 3.125mg. As a result the study provides scientific evidence on traditional use of *Adathoda vasica* leaf extract in treating bacterial infections.

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