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Research Article

IDENTIFICATION OF THE COMPOUNDS OF *ADHATODA VASICA* BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS AND PROBING: THE MODE OF ACTION OF THE COMPOUNDS BY *IN SILICO* STUDY

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ABSTRACT

Objective: The present study was aimed to investigate the promising compound of ethanol extracts of *Adhatoda vasica* against respiratory isolates by gas chromatography–mass spectrometry (GC-MS) analysis and *in silico* studies.

Methods: The leaves of the chosen plant were extracted with ethanol, evaporated in vacuum, and stored for further study. The ethanol extract was analyzed for antibacterial activity against respiratory pathogens. To determine the dosage of the extracts against target respiratory isolates, minimal inhibitory concentration (MIC) has been determined by broth dilution method. The extract was screened to identify the promising compounds by GC-MS, and docking was performed using Schrodinger software to determine the effective of these compounds with target proteins against respiratory infections.

Results: The ethanol extract of *A. vasica* showed promising antibacterial activity by agar diffusion method. MIC has also been determined by broth dilution method. The ethanol extract was subjected to GC-MS analysis based on the percentage of peak area, compounds were chosen for docking analysis. *In silico* studies had shown effective binding with target protein 2WYP (sialic acid binding protein) and 1X7Y (α-ketoacid dehydrogenase).

Conclusion: The above study concludes that plant *A. vasica* can be used in Ayurvedic treatment and has no side effect to cure both lower and respiratory infections. Thus, we can also extend our study to drug formation.

Keywords: *Adhatoda vasica,* Gas chromatography–mass spectrometry, 2-(4-(but-2-yl)phenyl)propanoic acid, N,N-dimethylglycine n-hexadecanoic acid.

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INTRODUCTION

Infections frequently treated with antibiotics express the threat of materialization of antibiotic resistance. Specified the upsetting occurrence of antibiotic resistance in bacteria, there is a stable necessitate for novel and efficient remedial agents. Hence, there is a necessity to expand safer and novel substitute antimicrobial drugs for the healing of infections. Numerous test studies have been approved out in diverse parts of the world. It has been recommended that aqueous and ethanol extracts from plants worn in allopathic medicine are prospective sources of antiviral, antitumor, and antimicrobial agents [1]. In with a reduction of 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 regular infections. Ethanol may concentrate better antimicrobial molecules contained in the leaves of *Cyanea acuminata*. Indeed Sonibare *et al*. [2], demonstrated that the ethanol extract of leaves inhibits the growth of microorganisms such as *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans*. Crude extract of *Avicennia marina* was subjected to the antimicrobial activity; the results display *Proteus vulgaris* is resistant, and the inhibitory effect of extract is high on Gram-negative culture than Gram-positive culture [3]. Mode of the reported compounds have not been investigated or reported earlier, and hence this study is unique in determining the mechanism by *in silico* analysis.

The herbal curative for different infections and diseases was conceded from generation to generation by word of mouth. This study was undertaken to verify if there is any facts for the clinical worth of the preferred plant for the treatment of respiratory infections. *Adhatoda vasica* is an Ayurvedic medicinal plant which is a habitat therapy for numerous diseases and human rations. *A. 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. *A. vasica* (*Acanthaceae*) universally known as vasaka are scattered all over India up to an altitude of 1300 m. The leaves, flowers, fruit, and roots are widely used for treating cold cough, whooping cough, chronic bronchitis, and asthma as a sedative, expectorant, and antispasmodic [4]. The plant is suggested for a diversity of ailments such as bronchitis, asthma, fever, and jaundice. The leaves and roots are efficacious in coughs, arthritis, diarrhea, and dysentery have the greatest chemostatic excellence. Leaves are anti-inflammatory and effective in skin disorders and cardiotonic [5]. This is one of the mainly powerful antituberculosis drugs [6]. Vasicine is also reported for its anthelmintic and hypertensive activity [7]. Evidently, there are not adequate precise studies that authenticate the compounds dependable for antimicrobial activity of this plant [8]. The current study was undertaken to assess the antibacterial activity of the plant against respiratory infections and to recognize the compounds present and examine its mechanism is warfare respiratory infections.

METHODS

Chosen plant and extract preparation

Leaves of *A. vasica* were collected from local market at Tiruchirappalli District, Tamil Nadu, India, and the plant was authenticated by the Botany Department of St. Joseph College and the voucher specimen (S001) was obtained. The leaves of chosen plant had been washed, macerated, and lyophilized. About 500 g of *A. vasica* was extracted with ethanol, and it yielded 33 g powder, respectively. The procedure was repeated to collect the needed quantity.

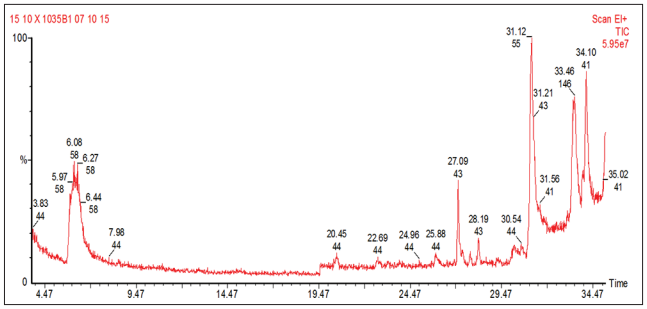

Fig. 1: Chromatogram pattern of ethanol extracts of *Adhatoda vasica* leaves

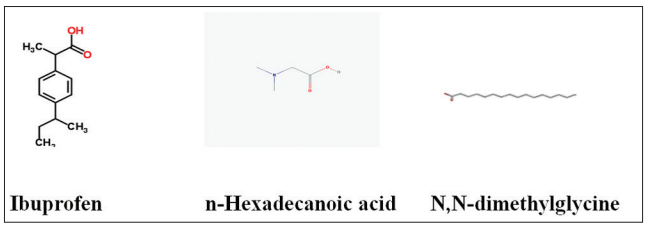

Fig. 2: Structure of the three compounds

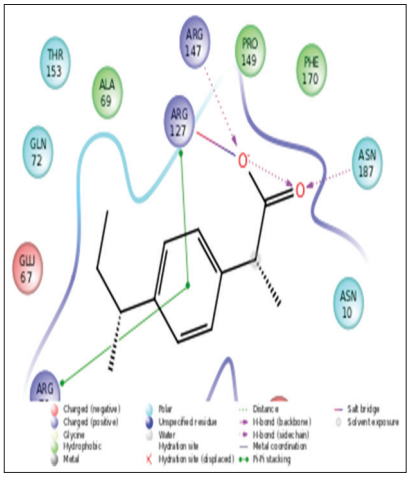

Fig. 3: *In silico* studies of 2-(4-(but-2-yl)phenyl)propanoic acid

Antimicrobial activity testing - disc diffusion assay (1966)

All isolates were tested for susceptibility to the ethanol extract and antimicrobial agent on Mueller-Hinton agar (Hi-Media, India) by the standard disc diffusion method recommended by the National Committee for Clinical Laboratory Standards. The diameter of the zones of inhibition of growth was recorded and interpreted.

Determination of minimal inhibitory concentration (MIC)

The effective dosage of the ethanol extract in controlling the bacterial isolates was obtained by determining the MIC by microbroth dilution assay.

Gas chromatography–mass spectrometry (GC-MS) analysis

GC-MS analysis of the ethanol extract of *A. vasica* was performed using a PerkinElmer GC Clarus 500 system comprising an AOC-20i autosampler and a GC-MS equipped with a capillary column Elite-5 (5% phenyl/95% dimethyl polysiloxane) fused a capillary column (30×0.25 µm ID×0.25 µm). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/minutes, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was

Table 1: Zones of inhibition by ethanol extract of *A. vasica*

S.No.	Bacterial species	Ethanol extract zone in mm	Standard antibiotic streptomycin
1	*E. coli*	24±0.20	20±0.18
2	*S. aureus*	33±0.23	22±0.23
3	*K. pneumoniae*	20±0.19	18±0.15
4	*P. vulgaris*	23±0.24	20±0.18
5	*S. pneumoniae*	20±0.19	19±0.23
6	*P. aeruginosa*	23±0.25	22±0.23

E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, P. vulgaris: Proteus vulgaris, S. pneumoniae: Streptococcus pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, A. vasica: Adhatoda vasica

Table 2: MIC of *A. vasica* (in mg/ml)

S.No.	Bacterial species	MIC (mg)
1	*E. coli*	3.125
2	*S. aureus*	12.5
3	*K. pneumoniae*	3.125
4	*P. vulgaris*	100
5	*S. pneumoniae*	100
6	*P. aeruginosa*	100

E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, P. vulgaris: Proteus vulgaris, S. pneumoniae: Streptococcus pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, A. vasica: Adhatoda vasica, MIC: Minimal inhibitory concentration

maintained at 280°C, the ion source temperature was 200°C, the oven temperature was programed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minutes to 200°C, then 5°C/minutes to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. The solvent delay was 0-2 minutes, and the total GC-MS running time was 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was TurboMass Gold-PerkinElmer, and the software adopted to handle mass spectra and chromatograms was a TurboMass version 5.2.

***In silico* docking**

Docking was performed using Schrodinger software. The compound 2-(4-(but-2-yl)phenyl)propanoic acid has shown effective binding energy −5.301 and −6.99 along with interactive residues ASN147, ARG147, ARG 127, ARG 70, and ARG 394 with a target protein 2WYP (Sialic acid binding protein) and 1X7Y (α-ketoacid dehydrogenase).

RESULTS AND DISCUSSION

Antibacterial activity

The ethanol extract of *A. vasica* was tested against targeted respiratory isolates on agar (Hi-Media India) by the standard disc diffusion method and results were recorded. The ethanol extract of *A. vasica* exhibited effective activity against the tested organisms as *S. aureus, Streptococcus pneumoniae*, as depicted in Table 1. *Escherichia coli, P. aeruginosa, P. vulgaris,* and *Klebsiella pneumoniae*. The results from the current study showed promising activity against various Gram-positive and Gram-negative organisms by means of agar disc diffusion method.

MIC

Among the several isolates chosen for the study, *E. coli* and *S. aureus* were highly susceptible to the extract (MIC=3.125 mg), followed by *P. aeruginosa* (MIC=12.5). The minimal concentration required to inhibit the other isolates was 100 mg/ml (Table 2).

GC-MS analysis

The ethanol extract subjected to the GC-MS analysis revealed the presence of 12 different compounds as seen in Fig 1. Based on the

percentage of peak area, three compounds were chosen for docking analysis (Fig. 2). The compounds included for the study are ibuprofen n-hexadecanoic acid and N,N-dimethylglycine (DMG).

***In silico* docking**

Docking program identified active compounds from a pharmaceutically relevant pool of decoy compounds. The compound 2-(4-(but-2-yl) phenyl)propanoic acid has shown effective binding energy −5.301 and −6.99 along with interactive residues ASN147, ARG147, ARG 127, ARG 70, and ARG 394 with a target protein 2WYP (Sialic acid binding protein) and 1X7Y (α-ketoacid dehydrogenase) (Fig. 3).

Prevalence of antibiotic resistance in chosen bacterial isolates has been increasing for the past few decades, and this has raised the demand for the scientific community to search for new antibacterial components. *A. vasica* has demonstrated severe antibacterial activity against Gram-positive and Gram-negative bacteria. The ethanol extract of *A. vasica* exhibited effective activity against the tested organisms. The plant exhibited remarkable activity against *S. aureus, S. pneumoniae*, *E. coli, P. aeruginosa, P. vulgaris,* and *K. pneumoniae* and is in accordance with earlier report [9]. Ethanol extract of *A. vasica* exhibited better activity against various Gram-positive and Gram-negative organisms by agar disc diffusion method and hence can be effective to fight against respiratory isolates. Results reported that ethanol flower extract (EF) showed a high zone inhibition against *S. aureus*. For *K. pneumoniae*, results showed that EF showed a high zone inhibition. Ethanol extract had a maximum inhibition activity as compared to chloroform, acetone, petroleum ether, and aqueous extract.

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 the presence of some chemical substances which produce a definite physiological action on human body such as alkaloids, tannins, flavonoids, and saponins [10]. Medicinal properties of plants are due to the secondary metabolites present in different plant parts. In the present investigation, the ethanol extract of *A. vasica* indicates that the antibacterial activity might be due to the presence of phytochemicals such as alkaloids, saponins, flavonoids, tannins, terpenoids, and amino acids. Few earlier reports indicate that *A. vasica* extract inhibited bacteria from 4 to 128 mg/ml, but our study showed that *E. coli* was inhibited at MIC value of 3.125 mg/ml.

Among the several compounds screened by GC-MS, the predominant compound was found to be 2-(4-(but-2-yl)phenyl)propanoic acid (ibrufen). It has been reported to possess several medicinal properties. High doses of ibuprofen significantly slowed the progression of lung disease during a period of 4 years in patients. Ibuprofen also prevented increased airspace bronchoalveolar protein and extravascular lung water accumulation, suggesting a protective effect on the alveolar-capillary membrane [10]. Ibuprofen has also been shown to have a direct effect on cystic fibrosis transmembrane conductance regulator (CFTR) function by inhibiting chloride secretion. Whether ibuprofen, by some mechanism, could have had a suppressive effect on the CFTR function making an individual more susceptible to mycobacterial invasion is discussed [10]. Few of the research papers reveals the patients with sepsis, the production of arachidonic acid metabolites by cyclooxygenase increases, but the pathophysiologic role of these prostaglandins is unclear. In animal models, inhibition of cyclooxygenase by treatment with ibuprofen before the onset of sepsis reduces physiologic abnormalities and improves survival. In pilot studies of patients with sepsis, treatment with ibuprofen led to improvements in gas exchange and airway mechanics. Our goal was to achieve the inhibitory effect of ibuprofen on neutrophil activation and migration, which generally occurs at concentrations over 50 μg/mL. *Pseudomonas* infected rats treated with ibuprofen twice daily, a mean peak plasma concentration of 55 μg/mL significantly reduced lung inflammation, as compared with that in controls and resulted in better weight gain without worse infection. High doses of ibuprofen significantly slowed the progression of lung disease during a period of 4 years in patients. *S. pneumoniae* ability to produce biofilms may induce persistent infections and difficulties for eradication *in vivo* ibuprofen at 128 μg/mL significantly reduced biofilm formation [5]. The other major compounds identified were E-14-hexadecenal; undecane; 3, 7, 11, 15 tetramethyl-2-hexadecen-1-ol; heptadecane; and 2, 6, 10, 14-tetramethyl.

DMG has also been shown to be useful in the treatment of diseases of veterinary animals, including horses, having inflammatory conditions. For example, chronic obstructive pulmonary disease (heaves or chronic alveolar emphysema), which is a chronic noninfectious respiratory disease of horses, characterized by labored respirations, chronic cough, unthriftiness, and lack of stamina, has been successfully treated with DMG. This respiratory condition is thought to be allergy related and is accompanied by inflammation and narrowing of the airway passages in the lungs. DMG was demonstrated to reduce the inflammatory condition. Three standard red horses, ages 3-7 years, were diagnosed as having chronic obstructive pulmonary disease, also known as equine asthma or the heaves. Clinical signs included frequent coughing, nasal discharge, increased respiratory rate, and wheezing in the chest area. The horses were treated orally with 1.5 g of DMG hydrochloride twice daily for 10 days. All three horses showed symptomatic relief after 5 days, with improved breathing and less nasal discharge, and by the end of 10 days, the air passages of all three horses had cleared.

The surfaces of all vertebrate cells are decorated with a dense and complex array of sugar chains, which are mostly attached to proteins and lipids. Most soluble secreted proteins are also similarly decorated with such glycans. Sialic acids are a diverse family of sugar units with a nine-carbon backbone that are typically found attached to the outermost ends of these chains [5]. Given their location and ubiquitous distribution, sialic acids can mediate or modulate a wide variety of physiological and pathological processes [5]. Many respiratory pathogens, including *Haemophilus influenzae, S. pneumoniae,* and *P. aeruginosa*, express neuraminidases that can cleave α2,3-linked sialic acids from glycoconjugates. As mucosal surfaces are heavily sialylated, neuraminidases have been thought to modify epithelial cells by exposing potential bacterial receptors. The *P. aeruginosa* neuraminidase has a key role in the initial stages of pulmonary infection by targeting bacterial glycoconjugates and contributing to the formation of biofilm. Inhibiting bacterial neuraminidases could provide a novel mechanism to prevent bacterial pneumonia [5].

However, scoring functions were found to be highly successful at distinguishing the crystallographic conformation from the set of docked poses. Docking programs identified active compounds from a pharmaceutically relevant pool of decoy compounds. In silco study revealed the effective docking score as −5.301 along with interactive residues ASN147, ARG147, ARG 127, ARG 70, and ARG 394 with a target protein 2WYP (Sialic acid binding protein) and 1X7Y (α-ketoacid dehydrogenase) against respiratory infections. Thus, the chosen plant could be more effective and can be used as a drug in the treatment of both lower and upper respiratory infections.

CONCLUSION

The present study demonstrated that the leaf of *A. vasica* promotes curing of both upper and lower respiratory infections. The ethanol extract of the leaf showed the remarkable activity against both Gram-positive and Gram-negative respiratory pathogens. *A. vasica* had shown almost effective antimicrobial activity against all species of bacteria which was chosen in the study as compared with standard antibiotic streptomycin. These results indicate that the antibacterial activity of ethanol extract might be due to the presence of important phytochemicals such as alkaloids, flavonoids, saponins, tannins, terpenoids, and amino acids. The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug. Based on the minimal growth inhibitory effect, 3.25-100 mg/ml ethanol extract of *A. vasica* was found to be effective

against respiratory ailments in infants and adults. GC-MS analysis of ethanol leaf extract revealed the presence of around 12 compounds, and among them, 2-(4-(but-2-yl)phenyl)propanoic acid (ibrufen) found to be shown a highest peak value 61.3117. To determine the binding energy of the selected compound was subjected to *in silico* docking. The study revealed that the target compound is effectively docked with the binding energy score (−5.301) against target protein. This is concluded from our study that plant *A. vasica* can be used in Ayurvedic treatment and has no side effect to cure both lower and respiratory infections. Thus, we can also extend our study to drug formation.

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