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Antimicrobial activity of *Adhatoda vasica* against clinical pathogens

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ABSTRACT

The antimicrobial activity (MIC) of Adhatoda vasica was assessed against clinical pathogen solvents like methanol, ethanol, acetone, chloroform, diethyl ether and water were used for the preparation of plant extracts in various concentrations by disc diffusion method the antimicrobial activity (MIC) was measured. From this, solvents showed higher activity in the order of diethyl ether > methanol > ethanol > acetone > Chloroform> water. The plant extract of Adhatoda vasica showed higher activity for different clinical pathogens in the order of Klebsiella pneumoniae>Staphylococcus aureus > Proteus valgaris > Pseudomonas aeroginosa > Streptococcus Pyogens.

Key words: *Adhatoda Vasica*, Antibacterial activity.

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have made large contributions to human health and well-being. In our country, we are using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicines obtained from plant resources [26].

Nowadays, multiple drug resistance has developed due to the indiscriminate use of the commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression, and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medicinal importance there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious from medicinal plants. Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world [9].

World wide, infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United states [21].

Herbal have become increasingly popular and their use is wide spread. Clear-cut proof of their efficacy in microorganisms inducing pathogens is yet to be explored. Various medicinal plants have been used for years in daily life to treat disease all over he world. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of

natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral, and antimicrobial agents. 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 treatement 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[11].

Microorganism and medicinal plants are rich sources of secondary metabolites, which are potential sources of useful drugs and other useful bioreactive product. Medicinal aid for various diseases. Scientific experiments on the antimicrobial properties of plant components were first documented in the 19th centaury. In India from ancient times, different parts of medicinal plants have been used to cure specific diseases. Today there is wide spread interest in drugs. Microorganisms are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. Plants are used as medicines since time immemorial. Reported for its anthelmintic and weak hypotensive activity. India has rich heritage of using medicinal plants in traditional medicines such as siddha, ayurvedha, and unnani.. Antibacterial properties of various plant parts like leaves, seeds, and fruits have been well documented for some of the medicinal plants for the past two decades. Antibiotic principles are the distributed widely among angiosperm plants. A variety of compounds is accumulated in plant parts accounting for their constitutive antimicrobial activities[29].

Adhatoda vasica nees (Acanthaceae) commonly known as vasaka distributed throughout India up to an attitude 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. The study aims at making a qualitative and quantitative analysis of certain chemicals in *Adhatoda vasica(20)*.

Antifedent and toxic activity damaging potential, photosynthetic activities of *A. vasica* is available. The study aims at making qualitative and quantitative analysis of certain chemicals in *Adhatoda vasica* to quantify the phytochemical variation in different season of the year, to establish the fact that there is annual post rotation in *adhatoda* altering the quality of the active ingredients and to study the damaging potential of the various insect, pests in different seasons[16].

The plant is recommended for a variety of ailements such as bronchitis, asthma, fever, jaundice etc. The leaves &roots are efficacious in coughs, arthritis, diarrhoea and dysentery and have the best chemostatic quality. Leaves are anti-inflammatory, analgesic effective in skin dis orders, cardiotonic. This is one of the most potent anti tuberculosis drug. Vasicine is also reported for its anthelmintic and weak hypertensive activity[14].

In the present study, we have chosen *Adhatoda vasica* as herbal medicine to determine their antibacterial property. Evidently, there are not sufficient scientific studies that confirm the antimicrobial activity of this plant. This study looks into the invitro antimicrobial activity of this plant for different solvent extracts against some Gram-positive and Gram-negative pathogenic microorganisms that causes the most common cases of infectious disease [1].

MATERIALS AND METHODS

Antimicrobial activity determination

Selection of medicinal plant:

In the present work, an ***Adhatoda vasica*** plant was screened for potential antibacterial activity.

Adhatoda vasica

English name : Malabar nut
Sanskrit name : vasaka
Family : acantheceae
Parts used : leaf

Identification and preservation of plant material:

Fresh plants were collected from the different areas around Monday market on 12/01/11. The taxonomic identities of this plant were determined. Plant parts were washed with 70% alcohol and then rinsed with sterilized distilled water, air-dried and stored in airtight bottles at 4^{o}c for further use.

Preparation of extracts:
Clean dry plant samples collected in a cotton bags. The materials were grinded to fine powder with the help of the mixer grinder. Then these powdered materials used for the preparation of aqueous methanol, ethanol, acetone, chloroform and diethyl ether extracts.

a)Preparation of aqueous extract:
2 gm of powdered materials mixed with 20 ml of sterile distilled water and kept on rotary shaker for 12 hours at 30ºc. There after, it was filtered with the help of Whatman no: 1 filter paper. The filtrate then centrifuged at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4ºc for future use.

b)Preparation of methanol extract:
10gm of powdered materials were soaked in 30 ml of 70% methanol kept at 30ºc for 12 hours on a rotary shaker. After 12 hours, the previous portion of added methanol were evaporated so to make the same volume methanol was added and then it was placed on a rotary shaker for another 12 hours, at 30ºc. After that, it was filtered through Whatman no: 1 filter paper. The filtrate is centrifuged at 2000 rpm for 10 min. the supernatant was collected and the supernatant was allowed to evaporate until completely dry. The extracts kept sterile bottles under refrigerated condition until use. Then 100µg, 200µg, 300µg, 400µg, 500µg/ml.

Like methanol extract, the other solvents like ethanol, acetone, chloroform, diethyl ether and water was used for extraction of *Adhatoda vasica.*

Bacterial strain used for assay:
Staphylococcus aureus, Streptococcus pyogens, Proteus vulgaris, Escherichia coli, pseudomonas aeruginosa, and Klebsiella pneumonia.

Antimicrobial activity:
The media and the test bacterial cultures were poured in to Petri dishes (Muller Hinton agar media). The test strain (0.2ml) was inoculums size (108 cells/ml) care was taken to ensure proper homogenization. The plant extracts were tested for antimicrobial activity in the agar well diffusion assay against *Staphylococcus aureus, Streptococcus pyogens, Proteus vulgaris, Escherichia coli, and Pseudomonas aeruginosa and klebsiella pneumonia.*

Agar disk diffusion method:
The anti microbial activity was tested against (methanol ethanol, acetone, chloroform, diethyl either and water) , leaves and stem of *Adhatoda vasica*. The inoculation of microbes was prepared from bacterial culture. About 15-20ml of Muller- Hinton agar medium was poured in the sterilized Petri dish and allows solidifying. One drop of bacterial strain was spread over the medium by rod.. Sterile filter paper disk of 6mm diameter were impregnated with the different concentration of solvent extracts of *Adhatoda vasica* like 100µg, 200µg, 300µg, 400µg and 500µg. The paper discs were allowed to evaporate and after that placed on the surface of the inoculated agar plates. Then the plates were incubated over night at 37ºc for 24 hrs. At the end of the incubation period, the antibacterial activities were evaluated by measuring inhibition zone diameters.

RESULTS

The isolated organisms like*E.coli,Staphylococcus aureus,Klbsiella pnumoniae,Proteus vulgaris,Streptococcus pyogens,Pseudomonas aeruginosa* exhibited the antimicrobial activity for different concentrations like 100µg, 200µg, 300µg, 400µg and 500µg of different polar, unipolar& bipolar solvents extracts of *A.vasica.*

Methanol extract:
The methanol extract of *A.vasica* showed highest activity against *E.coli, Staphylococcus aureus, Klebsiella pneumoniae and Proteus vulgaris than Streptococcus pyogenes and Pseudomonas aeruginosa.* (Table1).

Ethanol extract:
The ethanol extract of *A.vasica* showed maximum activity against *Staphylococcus aureus, Streptococcus pyogenes and Klebsiella pneumoniae* than *Proteus vulgaris and Pseudomonas aeruginosa*. The least activity against *E.coli*. (Table 2).

Acetone extract:
Acetone extract of *A.vasica* showed highest activity against *Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa than Streptococcus pyogens, Escherichia coli, and Proteus vulgaris,* (Table 3)

Chloroform extract:
Chloroform extract of *A.vasica* showed maximum activity *Staphylococcus aureus, Klebsiella pneumoniae, E.coli than Pseudomonas aeruginosa*. Least activity was showed against *Streptococcus pyogens* and *Proteus vulgaris*. (Table 4).

Diethyl ether extracts:
Diethyl ether extract of *A.vasica* showed maximum activity against *Staphylococcus aureus, Streptococcus pyogens, E.coli and Klebsiella pneumoniae than Proteus vulgaris and pseudomonas aeruginosa*. (Table5).

Aqueous extract:
Aqueous extract of *A.vasica* showed no activity in 100µg. Then the maximum activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* than *Pseudomonas aeruginosa, Streptococcus pyogens, E.coli* and *Proteus vulgaris.* (Table6) .

Table -1 methanolic extract of *adhatoda vasica* against clinical pathogens (µg/ml)

Pathogens	Zone of inhibition in (mm)				
	100µg	200µg	300µg	400µg	500µg
Staphylococcus aureus	1	2	3	3.5	4
Streptococcus pyogens	NA	1	1	2	3
Escherichia coli	1	1	1.5	2	3
Klebsiella pneumoniae	1	2	2	4	6
Proteus vulgaris	1	1.5	2.5	2.5	3
Pseudomonas aeruginosa	NA	1	1	2	2

Table -2 ethanolic extract of *Adhatoda vasica* against clinical pathogens (µg/ml)

Pathogens	Zone of inhibition in (mm)				
	100µg	200µg	300µg	400µg	500µg
Staphylococcus aureus	1	1	2	2	3
Streptococcus pyogens	1	2	2	3	3
Escherichia coli	NA	NA	0.5	1	2
Klebsiella pneumoniae	1	2	3	6	7
Proteus vulgaris	0.5	0.5	1	1	2
Pseudomonas aeruginosa	1	1	1	1	1

Table -3 Acetone extract of *Adhatoda vasica* against clinical pathogens (µg/ml)

Pathogens	Zone of inhibition in (mm)				
	100µg	200µg	300µg	400µg	500µg
Staphylococcus aureus	1	1	2	2	4
Streptococcus pyogens	1	1.5	2	2	3
Escherichia coli	0.5	1	1	2	4
Klebsiella pneumoniae	1	2	2	4	6
Proteus vulgaris	NA	NA	1	1	3
Pseudomonas aeruginosa	1	1	2	3	4

Table -4 Chloroform extract of *Adhatoda vasica* against clinical pathogens (µg/ml)

Pathogens	Zone of inhibition in (mm)				
	100µg	200µg	300µg	400µg	500µg
Staphylococcus aureus	0.5	0.5	1	2	4
Streptococcus pyogens	NA	NA	0.5	1	1
Escherichia coli	NA	0.5	1	1	1.5
Klebsiella pneumoniae	1	2	2	4	5
Proteus vulgaris	NA	NA	NA	1	1
Pseudomonas aeruginosa	NA	1	1	2	2

Table -5 Diethyl ether extract of *Adhatoda vasica* against clinical pathogens (µg/ml)

Pathogens	Zone of inhibition in (mm)				
	100µg	200µg	300µg	400µg	500µg
Staphylococcus aureus	2	4	5	8	10
Streptococcus pyogens	1	3	3	4.5	5
Escherichia coli	2	4	4.5	6	6
Klebsiella pneumoniae	1	2	2.5	4	7
Proteus vulgaris	NA	1	1.5	2	3
Pseudomonas aeruginosa	NA	1	2	3	4

Table -6 Aqueous extract of *Adhatoda vasica* against clinical pathogens (µg/ml)

Pathogens	Zone of inhibition in (mm)				
	100µg	200µg	300µg	400µg	500µg
Staphylococcus aureus	NA	0.5mm	1	2	2.5
Streptococcus pyogens	NA	NA	1.5	2	3
Escherichia coli	NA	NA	1	1.5	2
Klebsiella pneumoniae	NA	1	2	2	3.5
Proteus vulgaris	NA	NA	1.5	2	2.5
Pseudomonas aeruginosa	NA	0.5	2	2.5	3

DISCUSSION

Different solvent extract of Adhatoda vasica exhibited the antimicrobial activity against isolated pathogens (Table 1,2,3,4,5&6)

Adhatoda vasica has the antibacterial activity against Gram positive and Gram-negative bacteria. The methanol extract of Adhatoda vasica exhibited high activity against the tested organisms rather than aqueous extract of Adhatoda vasica. This is an agreement with the current study (i.e.) Adhatoda vasica showed the antimicrobial activity *against Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumoniae* which also exhibited the highest activity in methanol extract than the aqueous extract. (Table 2,6)[24]. the crude extracts obtained from the leaf of Adhatoda vasica using solvents of varies polarity such as ethanol, petroleum ether and water extracts exhibited the activity against *Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumoniae*. The result from the current study Adhatoda vasica showed the better activity in ethanol, diethyl ether and water against Gram-positive and Gram-negative organisms, (Table 3&6) [16].

Adhatoda zeylanica posses' important activities like antitisssue, antibacterial, antiulcer, abortifacient, and antioxidant. This is an agreement with findings of this study *Adhatoda vasica* showed the antibacterial activity. (Table 2,3,4,5&6) [2].

Adhatoda zeylanica exhibited the antimicrobial activity against various organisms by means of agar diffusion method. Then findings from the current study Adhatoda vasica plant extract exhibited the antimicrobial activity against six pathogens like *Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, and Klebsiella pneumoniae.* (Table2,3,4,5&6) [14].

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