ORIGINAL ARTICLE

Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica*. L

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Preliminary phytochemical and antibacterial investigations were carried out of the crude extracts obtained from the leaf of *Adhatoda vasica,* using solvents of varied polarity. The presence of phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars was indicated by the tests conducted. The effect of ethanol, petroleum ether and water extracts were tested on *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Pseudomonas aeroginosa, Proteus vulgaris, Klesiella pneumoniae* and *Candida albicans*. The minimum inhibitory concentration of the crude extracts was determined for various organisms.

Key words: *Adhatoda vasica*, antibacterial activity, minimum inhibitory concentration

INTRODUCTION

Adhatoda vasica belongs to the family of 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. It is a primary herb of the ayurvedic system used in the treatment of coughs, bronchitis, asthma and symptoms of common cold. A yogic practice is to chew the leaf buds alone, or with a little ginger root, to clear the respiratory passages in preparation for the vigorous breathing exercises. It is used as an ingredient in numerous popular formulations, including cough syrups, in which it is frequently combined with tulsi (holy basil) and ginger. Its main action is as an expectorant and antispasmodic (bronchodialator). The important active components include alkaloids vasicine (aka peganine) and vasicinone. The former is under development as a herbal drug in India, as are the semi-synthetic derivatives of alkaloids, bromhexine and ambroxol. A secondary property of the herb is that it helps to stop bleeding. The roots, leaves and flowers of the plant are used for the extraction of volatile oils (heptanone) and alkaloids, which have great medicinal importance.[1]

MATERIALS AND METHODS

Sample Collection and Preparation

The leaves of the plant *Adhatoda vasica* were collected from the open fields of Narthamalai, Pudukkottai District, Tamil Nadu, India. The plants were identified at the Department of Botany, JJ College of Arts and Science, Pudukkottai District, Tamil Nadu, India. The fresh leaves were dried for 6 hours at 50-60°C. The dried samples were then crushed into powder using an electronic blender. The powdered sample was stored in a bottle at room temperature, prior to analysis.

Preparation of Extracts

Ethanol extraction

A powdered sample of 100 gm was weighed and soaked in 250 ml of 95% ethanol in a separating funnel for 24 hours, with intermittent shaking. The plant extract was then collected and filtered through Whatman No.1 filter paper. The extract was concentrated at 50°C using a rotatory evaporator and then air-dried. The dried powder was stored at 40°C in an airtight bottle. Similarly, the procedure was repeated with petroleum ether and water as solvents, using 100 gm of the fresh ground sample, for each extraction. All the extracts were cooled at room temperature.[2]

Phytochemical Analysis

The extracts were analyzed for the presence of phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars, using the standard procedure.[3]

Agar-well Diffusion Method

The antimicrobial activity was carried out by the agar-well diffusion assay using Muller Hinton agar plates.[4] The plates were swabbed with *S. aureaus,*

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Table 1: Phytochemical analysis of *Adhatoda vasica* leaf extract

Type of extract	Phenols	Tannins	Alkaloids	Saponins	Flavanoids	Reducing sugar	Amino acid
Ethanol	+	+	+	+	+	+	+
Pet. ether	+	+	–	–	–	+	–
Water	–	–	–	–	–	+	+

(+) - Presence of phytochemical compounds; (−) - Absence of phytochemical compounds

S. epidermidis, B. subtilis, E. faecalis, E. coli, P. aeroginosa, P. vulgaris, K. pneumoniae and *C. albicans*. Wells were punched out from a swabbed solid agar using a sterile cork borer. A crude extract of 100 µl from the stock solution was added into the wells. The plates were then incubated at 37°C for 24 hours. The clear zone of inhibition was measured.

Minimum Inhibitory Concentration (NCCLS, 1990)

In determining the minimum inhibitory concentration (MIC), solutions of varying concentrations (25, 50, 75 and 100 mg/ml) were prepared. These dilutions were taken in separate test tubes and labelled respectively. To each of the test tubes, 3 ml of Muller Hinton broth, 0.5 ml of bacterial suspension and 0.1 ml of plant extract were added. A positive control tube containing the growth medium and the bacterial suspension, without the extract, was also prepared. The tubes were incubated at 37°C for 24 hours. After 24 hours the turbidity was measured spectrophotometrically at 520 nm. The turbidity measurement was taken as an indicator of bacterial density. The rate of inhibition was found to be directly related to the turbidity of the medium. The lowest concentration, which did not permit any visible microbial growth when compared with that of the control, was recorded as the MIC value.

RESULTS AND DISCUSSION

The results of the phytochemical analysis [Table 1] show that phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars are present in the leaves of *Adhatoda vasica*. It has also been shown that tannins are biologically active, against *E. coli, S. aureus, S. paratyphi* and *C. albicans*.[5] These classes of compounds in the bark extracts are known to show curative effects against several pathogens.[6,7] The results of the agar-well diffusion method show that the crude ethanolic extract of the leaf exhibits an antimicrobial activity against the test organisms; *S. aureus*, *S. epidermidis*, *B. subtilis*, *P. vulgaris* and *C. albicans*, with a maximum diameter of the zone of inhibition ranging from 19 mm, 18 mm, 14 mm, 15 mm and 12 mm. Similarly the antimicrobial activity of petroleum ether extract against *S. aureus* is observed with a 16 mm zone of inhibition. Other test organisms are highly resistant to petroleum ether and aqueous extracts [Table 2]. This justifies the traditional use of ethanol in extracting the leaf components, to control the pathogenic organisms.[8] The MIC of the ethanolic extract of *Adhatoda vasica* against bacterial pathogens, such as, *S. aureus*, *S. epidermidis*, *B. subtilis* and *P. vulgaris* is observed at 75 mg/ml. For *C. albicans* it is observed to be 100 mg/ml. For petroleum ether it is found at 25 mg/ml [Table 3]. Similar reports have been well documented earlier, which state that a great number of medicinal plants are less active against gram negative than gram positive organisms.[2,9] The inhibitory activities of the extracts live up to their potential in the treatment of microbially induced ailments or diseased conditions, in line with the traditional use of plant extracts.

Table 2: Antibacterial activities of *Adhatoda vasica* leaves by Agar well diffusion assay

Test organisms	Agar-well diffusion (Zone of inhibitions in mm)			
	Ethanol mg/ml	Pet. Ether mg/ml	Water mg/ml	Positive control mg/ml
S. aureus	19	16	-	20
S. epidermidis	18	-	-	19
B. subtilis	14	-	-	16
E. faecalis	-	-	-	16
E. coli	-	-	-	13
P. aeroginosa	15	-	-	10
P. vulgaris	15	-	-	16
K. pneumoniae	-	-	-	10
C. albicans	12	-	-	14

(-) - No activity

Table 3: Minimum inhibitory concentration of ethanol and petroleum ether extracts of *Adhatoda vasica* by agar-well diffusion method

Test organism	Concentrations (mg /ml) (Zone of inhibition in mm)			
	100	75	50	25
S. aureus	12	9	-	-
S. epidermidis	12.5	10	-	-
B. subtilis	13	10	-	-
P. vulgaris	12	7	-	-
C. albicans	12	8	-	-
S. aureus *	13	10	10	10

(-) - No activity; (*) - For petroleum ether extract of leaf

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