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ANTIMICROBIAL ACTIVITY OF ALKALOIDS EXTRACTED FROM *ADHATODA VASICA*

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ABSTRACT

Adhatoda vasica, a popular Indian medicinal plant known locally as Adulsa in Maharashatra has been explored for various biological activities. The present paper gives an account of the antimicrobial activity of alcoholic extracts and alkaloids extracted from this plant which have not been studied extensively. The hot and cold Methanolic extracts of *Adhatoda vasica* and alkaloids isolated from the hot methanolic extract, were evaluated for antimicrobial activity against clinically important bacteria such as *Staphylococcus aureus ATCC 25923, Staphylococcus aureus NTCC 3750, Escherechia coli ATCC 25922, Proteus mirabilis, a Clinical isolate, Salmonella typhi NTCC 786, Pseudomonas aeruginosa ATCC 27853, Candida albicans MTCC 183 and Cryptococcus neoformans NCIM 3542. In vitro* antimicrobial activity was performed using agar cup diffusion method. Both the (hot and cold) methanolic extracts of *Adhatoda vasica* were found to be active only against *S. aureus* and *P. aeruginosa*, but alkaloids isolated from these extracts` exhibited excellent antimicrobial activity against organisms investigated.

KEYWORDS: ***Adhatoda vasica,*** **Alkaloids, Antimicrobial activity, Clinical strains**

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INTRODUCTION

The indiscriminate use of antibiotics has led to the development of multidrug resistant pathogens causing infections worldwide. In spite of the progress made in the understanding of microbiology and control of infectious diseases, incidences of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease causing microbes, pose enormous public health concern[1]. Plant derived medicines have made large contributions to human health. Plants are rich in a wide variety of secondary metabolites. They are important source of potentially useful structures for the development of new chemotherapeutic agents such as tannins, alkaloids, terpenoids and flavonoids[2]. The first step towards this goal is the *in vitro* antibacterial activity assay. Alkaloids, as active principles of many plants, exhibit extraordinary spectrum of pharmacological activities such as antibacterial, antiprotozoal, astringent, tonic, vasomotor and circulatory stimulant[3]. *Adhatoda vasica* Nees., (Adulsa, Malabar Nut) (Family—Acanthaceæ) is known in India and other countries for thousands of years for its medical properties, mainly as a bronchodilator and for antihistaminic effects attributed to its active alkaloid content vasicine[4-5]. Uterine stimulant activity and moderate hypotensive activity of the alkaloids have also been reported[6]. However, literature survey carried out during the present study did not reveal systematic studies on antimicrobial activity of alkaloids extracted from *Adhatoda vasica.* Hence, an investigation was under taken to screen hot methanolic and cold methanolic extracts of leaves of *Adhatoda vasica* and alkaloids isolated from these extracts, for their antimicrobial activity.

MATERIALS AND METHODS

Plant material

The air-dried coarsely ground leaves of *Adhatoda vasica* were procured from local shop, Mumbai. A botanist authenticated the raw material. Voucher specimens were deposited at Department of Botany, Shri C. B. Patel Research Centre, Vile Parle (W), Mumbai.

Preparation of extract: Hot methanolic extraction (HME)

Hot Methanolic Extracts were prepared using soxhlet apparatus. Cold methanolic extraction (CME): Cold methanolic extracts were prepared on a mechanical shaker, set at 200 RPM, at 30^{o}C ± 2. These extracts were dried and residues were used to extract alkaloids and also to determine its antimicrobial activity

Extraction of Alkaloids from Adhatoda vasica

The alkaloids were extracted from the dry residue of the HME, according to the method described by Kokate[7]. with modification. It was validated at Shri C. B. Patel Research centre. Briefly the procedure used was as follows. To 1 gram of dried residue from hot methanolic extract, 4 ml of diluted HCl (not more than 1.0 M and not less than 0.1 M. pH 0-1), was added. Activated charcoal was added to this mixture (0.1 gram of activated charcoal per 10 grams of dried residue or 10 ml of extract). The mixture was boiled for 10 minutes and filtered. Residue obtained was washed with fresh diluted HCl before discarding. The HCl extract was basified with 5 M NaOH to adjust pH well above 7. The basified fraction was then extracted three times with chloroform, using a separating funnel. From the two separated layers, lower chloroform layer was collected in a clean, pre-weighed beaker. Chloroform was allowed to evaporate to obtain alkaloids. The extracted phytochemical was confirmed as alkaloids, using standard chemical analysis as described by Kokate, *et al.,* (2000) [8] and also using Thin Layer Chromatography[9]. The dried plant extracts and isolated phytochemicals were reconstituted in methanol up to a concentration of 50 mg/ml.

Organisms used

In order to screen and determine antimicrobial effect of Hot and cold methanolic extracts of *Adhatoda vasica* and alkaloids isolated from hot

methanolic extract of *Adhatoda vasica*, eight different organisms representing different groups were used. Two strains of *Staphylococcus aureus, Staphylococcus aureus ATCC 25923* and *Staphylococcus aureus NTCC 3750,* representing group of gram positive pathogenic organisms were tested. Three other organisms selected were the members of Enterobactericeae family, *Escherechia coli ATCC 25922, Proteus mirabilis,* a Clinical isolate obtained from Department of microbiology, Lilawati hospital, and *Salmonella typhi NTCC 786.* Another gram negative organism selected was *Pseudomonas aeruginosa ATCC 27853,* a most common cause of hospital infections. In addition to above bacterial species, two fungal representatives *Candida albicans MTCC 183* and *Cryptococcus neoformans NCIM 3542* have also been used in the present study.

Antimicrobial assay

The agar cup diffusion method was employed to study the antimicrobial activity against the selected group of pathogenic bacteria. The experiment was performed essentially according to the method described in European Pharmacopeia with slight modification in inoculums size[10]; where instead of 0.5 ml of 10^4 – 10^5 cells/ml, 0.1 ml of testing innoculum with 10^7 –10^8cells/ml was swabbed on solidified Muller Hinton agar for bacteria and Sabouraud's dextrose agar for yeasts. Since methanol was used as a solvent for reconstituting the plant extracts, control wells were maintained, to test the antimicrobial activity in each plate. Results were noted after the appropriate incubation period by measuring the diameter of zone of Inhibition in millimeter (mm) scale.

RESULTS AND DISCUSSION

Extractive Value

Percentage yield of alkaloids from *Adhatoda vasica* is reported to be between 0.25 – 1.0 % by various researchers[5, 11-12]. During the present study, the observed value of alkaloid yield from the HME of *Adhatoda vasica* was 0.768 % which is in comparison with the reported alkaloid content. Table I shows antibacterial activities of crude extracts and alkaloids extracted from *Adhatoda vasica* analyzed during the present study. It was clearly observed that, all crude extracts of *Adhatoda vasica*, and alkaloids obtained during the present study, were having antibacterial property against the test organisms.

Table I

Screening of CME and HME of Adhatoda vasica and alkaloids isolated from Adhatoda vasica, to detect antimicrobial activity.

Name of the organisms	Zone of inhibition (mm) Mean ± S.D.*			
	Control	CME	HME	Alkaloids
S. aureus ATCC 25923	NI	14.5±0.5	17.67±0.29	27.16±0.76
S. aureus NTCC 3750	NI	11.83±0.76	13.83±0.29	20.83±0.58
E. coli ATCC 25922	NI	11.67±0.29	13.66±0.29	20.50±1.32
P. mirabilis Clinical isolate	NI	NI	NI	17.17±0.76
P. aeruginosa ATCC 27853	NI	11.83±0.76	13.17±1.26	15.83±0.58
S. typhi NTCC 786	NI	NI	NI	18.16±0.29
C. albicans MTCC 183	11.33±1.04	11.33±0.29	12.5±0.5	15.33±0.58
C .neoformans NCIM 3542	12.17±1.04	12.17±1.04	11.00±0.87	16.5±0.5

All the values are mean ± standard deviation of three determinations
NI – No inhibition
mm – Millimeters

Antimicrobial activity of HME and CME of Adhatoda vasica

The HME of dry leaves of *Adhatoda vasica*, was found to be effective against *S. aureus*, *E. coli* and *P. aeruginosa*. The same extract did not have any activity against *S. typhi*, *P. mirabilis*, *C. albicans* and *C. neoformans*. In case of CME of dry leaves of *Adhatoda vasica*, the activity pattern was similar to HME but was less effective as compared to HME. These results are in agreement with the studies reported in wealth of India[12]. Recent report on antimicrobial effect of Ehanolic extracts of leaves of *Adhatoda vasica* by Kathale revealed that it is effective against Gram positive as well Gram negative bacteria such as *Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*[13].

Antimicrobial activity of Alkaloids obtained from Adhatoda vasica

As mentioned earlier, alkaloids are known to be broad-spectrum antimicrobials. Hahn, *et al.,* (1976) and Kaneda, *et al.,* (1991) have reported antimicrobial activity of berberine, which is the isoquinoline alkaloid[14-15]. Berberine exhibits a broad spectrum antibiotic activity. Morphine, isolated in 1805 from the opium poppy *Papaver somniferous*, is the first medically useful example of an alkaloid. Codein and heroin are both derivatives of morphine[16]. Diterpenoid alkaloids, commonly isolated from the plants of Ranunculaceae or buttercup family are commonly found to have antimicrobial properties[17-19]. Also a glycol alkaloid from the berries of *Solamum khasiamum* and other alkaloids are reported to have activity against HIV infection[20-21]. Mc Devitt *et al.,* 1996 has reported activity of alkaloids against intestinal infections associated with AIDS[22.] Alkaloids have also been found to have antimicrobial effect against *Giardia* and *Entamoeba species*[23]. There are mixed reports regarding antimicrobial activity of *Adhatoda vasica*. Thus while alkaloids of *Adhatoda vasica* were ineffective against *Streptococci* and *Staphylococci,* its essential oils were shown to be effective against *M. tuberculosis*[11, 24]. A phyto-pharmacological overview on *Adhatoda zeylanica. syn. A. vasica* (Linn.) Nees. by Sayeed Ahemad does not reveal detailed study of antimicrobial activity of this plant[25].

However, alkaloids isolated from *Adhatoda vasica* during the present study, exhibited good antibacterial activity against all the organisms under investigation. The alkaloids were effective with a maximum activity against *S. aureus*. They revealed good activity against *S. typhi, P. aeruginosa* and *C. neoformans*. It was also observed that the alkaloids inhibited pigment production and formation of metallic sheen up to a zone with diameter 35 mm in case of *P. aeruginosa*. The extract was less effective against *E. coli* and *C. albicans*.

CONCLUSION

From the results obtained during the present study, we can conclude that the alkaloids from *Adhatoda vasica* have excellent antibacterial activity against the most resistant bacteria such as *S. aureus*, *P. aeruginosa* and the highly pathogenic bacteria like *S. typhi*. As mentioned earlier, there is a need for special attention in research strategies to look into phytochemicals which are active against such resistant and pathogenic bacteria. These substances further can be subjected to carry out pharmacological evaluation.

ABREVIATIONS

MTCC- Microbial Type Culture Collection (Chandigarh, India), ATCC – American Type Culture Collection, HME - Hot methanolic extraction, CME - Cold methanolic extraction, NI – No Inhibition, mm – Millimeters

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