RESEARCH ARTICLE

Invitro studies on the primitive pharmacological activities of *Adhatoda vasica*

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ABSTRACT

Adhatoda vasica is an indigenous herb belonging to family Acanthaceae has been used in the Indian system of medicine. It was spread all over the India and in some other Asian countries. It contains some phytochemicals such as alkaloids, tannins, flavonoids, terpenes, and glycosides. Alkaloids like vasicine, vasicinone, and vasicinol from leaves and roots, flavonoids like apigenin, kaempferol, astragalin, etc, and triterpenes like daucosterol from flowers have been isolated for the study of pharmacological activities such as Antioxidant, antibacterial, antifungal and anti-inflammatory activities. In the present study we clearly investigate the in vitro activity of *Adhatoda vasica* against bacteria, fungi and oxidative stress. Our research reveals that the aqueous, ethanol and chloroform extracts shows maximum inhibitory effect against various bacteria and fungi. In addition to that we proved that these extracts also have antioxidant activity.

Key words: Antioxidant, Antibacterial, Antifungal, DPPH.

INTRODUCTION

Adhatoda vasica belonging to the family Acanthaceae as a herb and is spread all over the world especially in Asian countries such as India, Bangladesh and Indonesia (Stepanovic, 2003). Its cultivated more over all parts of India. From ancient times it was used for a variety of ailments such as bronchitis, fever, asthma, jaundice etc. and its leaves & roots are effectual for coughs, arthritis, dysentery and diarrhea (Kumar *et al*., 2013). It contains the phytochemicals such as alkaloids, tannins, flavonoids, and terpenes because of that it exhibits pharmacological activities such as antioxidant, anti-inflammatory, and anti-microbial and in ancient traditional medicine it was used to treating rheumatic painful inflammatory swellings, anthelmintic, cold, cough, sedative expectorant, whooping cough, chronic bronchitis, rheumatism asthma, and antispasmodic.in addition to that the alkaloids like vasicine, lvasicinone,

maiontone, and vasicinol from leaves and roots, and triterpenes like daucosterol from flowers. And finally flavonoids like apigenin, kaempferol, astragalin were isolated to investigate the pharmacological activities such as antioxidant, antimicrobial (Rashmi and Linu Mathew, 2012) and anti-inflammatory activities. In the pharmaceutical industry drug development is depend on the natural products especially the plants even though the origin of all modern drugs were natural product but mechanistic study of them were very low (Palombo and Semple, 2001).

So our research mainly focused on to find the mechanism of the action of the drug compounds present in the *Adhatoda vasica* in order to find that we plan to first examined the antioxidant, anti-inflammatory, and anti-microbial in vitro.

MATERIAL AND METHODS

Plant collection:

We have selected the healthy, disease free and mature plants of *Adhatoda vasica* from Thiruvarur and Thanjavur districts of Tamilnadu, India. All the chemicals were analytical grade from hi-media and all the glass wares were completely sterilized before and after every process.

Extraction:

The leaves of *Adhatoda vasica* were washed with distilled water and 100 gm of fresh leaves were crushed using mortor and pestal and the extract was filtered through Whatman No. 1 filter paper and centrifuged up to the complete removal of debris. The whole process was repeated three times and finally, the concentrated extract was collected in closed container and kept in a refrigerator at 4 °C.

Bacterial strains:

Bacillus subtilis, Bactere maserins, Candida albicans, Escherichia faecalis, Pseudomonas aeroginosa, Proteus vulgaris, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus heamophytenus, Serratries masciens, and Vibrio cholorae were the bacterial strains used for the in vitro activity of *Adhatoda vasica*.

Antibacterial test:

Test samples were prepared by dissolving 80 mg of each of the aqueous, ethanol, and chloroform in 2 ml of respective solvents. The resulting solution contains 0.8 mg/50 µl. For the preparation of sample disc, paper discs of 5 mm diameter were made from Whattman filter paper by punch machine and then autoclaved at 121°C for 15 min. 50 µl of extract was applied to the paper discs under aseptic conditions. Blank discs were also prepared using solvents only. Chloramphenicol discs (30 µg/disc) were used as standards.

For each of the test organisms, the pre-culture was taken from stock cultures and was grown in nutrient broth at 37°C for 24 h. using sterile forceps, both the sample and blanks discs were placed on the marked positions on the seeded petri dishes maintaining an aseptic condition. The standard discs were placed separately onto another set of seeded Petri dishes. The plates were kept at 4°C for 24 h to allow sufficient time for the test material to diffuse to a considerable area of the medium. Afterwards, they were incubated at 37°C for 24 h. The resulting clear zones were measured by a transparent scale (Meignanalakshmi *et al*., 2013; Samy and Ignacimuthu (2000).

Fungal strains:

Aspergillus fumigatus, Botryodipodia theobromae, Colletotrichum corchori, Curvularia lunata, and Fusarium equiseti were the fungal strains used for the in vitro activity of *Adhatoda vasica*.

Antifungal test:

The anti-mould screening was done by poisoned food technique while disc diffusion method was followed for anti-yeast screening. 80 mg of each extract was mixed uniformly with 100 ml of sterile PDA to get a concentration of 0.8 mg/ml and immediately poured into the Petri dishes aseptically. After solidification, 10 mm agar blocks of the test mould were placed in the center of the treated plate. For each of test moulds, one such plate was prepared. Similarly, another set of plates were prepared using a standard antibiotic clotrimazole at a concentration of 80 µg/ml. A set of control plates were also prepared using PDA plates alone. All of the plates were incubated at 24°C for 4 days after which the radial growth of fungal colony was measured with a transparent scale in mm and the percentage of inhibition of mycelial growth was calculated. For anti-yeast activity, the same procedure as of antibacterial screening was followed with few exceptions: such as PDA was used as a medium, and plates were incubated at 25°C, finally the antibiotic clotrimazole was employed as a standard for comparison (Rashmi and Mathew, 2012).

Antioxidant Test:

The antioxidant activity of *Adhatoda vasica* extract was assessed in comparison to standard antioxidant ascorbic acid depending on the scavenging effect of 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical. Ascorbic acid solution (5 ml) and different concentrations of extract (100, 200, 400 and 800 μg/ml in methanol) solutions (5 ml) were mixed with 3 ml of 0.4 mM (0.004 %) DPPH solution. The mixtures were kept in dark for 30 min to measure the absorbance at 517 nm using a UV-Visible spectrophotometer and ascorbic acid was used as a positive control. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The degree of de-colorization of DPPH from purple to yellow indicates the scavenging efficiency of the extract. The scavenging activity against DPPH was calculated (Kumar *et al*., 2013).

RESULTS AND DISCUSSION

Antibacterial activity:

The aqueous, ethanol, and chloroform extracts of *Adhatoda vasica* were tested for antibacterial activities against nine pathogenic bacteria including gram-positive and gram-negative using disc diffusion method and the results were discussed in Table 1. The zone less than 7mm was considered as resistant. All the studied pathogens were found to be moderately susceptible to aquous extract with a zone of inhibition ranging from 8 to 17 mm. The highest activity was found against *Bacillus subtilis* (with a zone of inhibition of 17 mm). The Ethanol extract also found to have potential antibacterial activity against some of the bacteria were studied. The highest activity was found against *Staphylococcus aureus* (with a zone of inhibition of 15 mm). The chloroform extract also found to have potential antibacterial activity against some of the bacteria were studied. The highest activity was found against *Serratries masciens* (with a zone of inhibition of 15 mm). From above result proved that aqueous extract had a stronger antibacterial activity than Ethanol and chloroform extracts. However, the standard antibiotic chloramphenicol showed very strong inhibition against almost all of the test bacteria. Figure 1 shows the graphical representation of the antibacterial activity of aqueous, ethanol, and chloroform extract of *Adhatoda vasica* compared with the stranded drug chloramphenicol.

Table -1: Antibacterial activity of *Adhatoda vasica*

Name of the bacteria	Aqueous Extract	Ethanol Extract	Chloroform Extract	chloramphenicol
Bacillus subtilis	**17**	**12**	**-**	54
Bactere maserins	**9**	**-**	**9**	45
Candida albicans	**8**	**9**	**-**	42
Escherichia faecalis	**12**	**-**	**11**	24
Pseudomonas aeroginosa	**10**	**13**	**-**	35
Proteus vulgaris	**8**	**12**	**-**	26
Staphylococcus aureus	**13**	**15**	**-**	45
Staphylococcus epidermidis	**9**	**13**	**12**	22
Staphylococcus heamophytenus	**8**	**-**	**11**	25
Serratries masciens	**9**	**-**	**15**	**30**
Vibrio cholorae	**11**		**10**	**42**

Table - 2: Antifungal activity of *Adhatoda vasica*.

Zone of inhibition				
Name of fungus	**Aqueous extract**	**Ethanol extract**	**Chloroform extract**	**Clotrimazole**
Aspergillus fumigatus	10	6	10	85
Botryodipodia theobromae	9	10	3	80
Colletotrichum corchori	9	13	6	70
Curvularia lunata	12	3	7	60
Fusarium equiseti	10	12	9	95

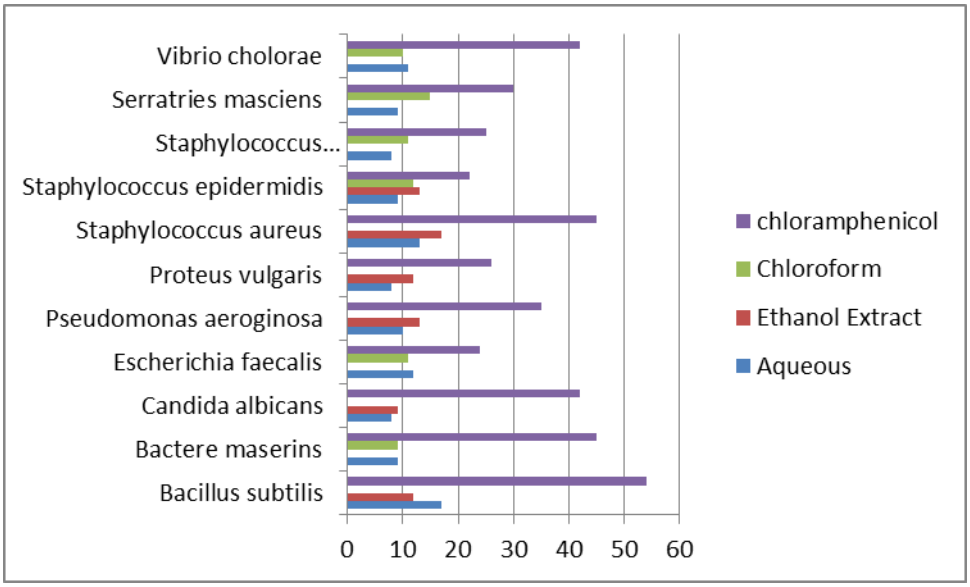

Fig-1: Antibacterial activity of *Adhatoda vasica*.

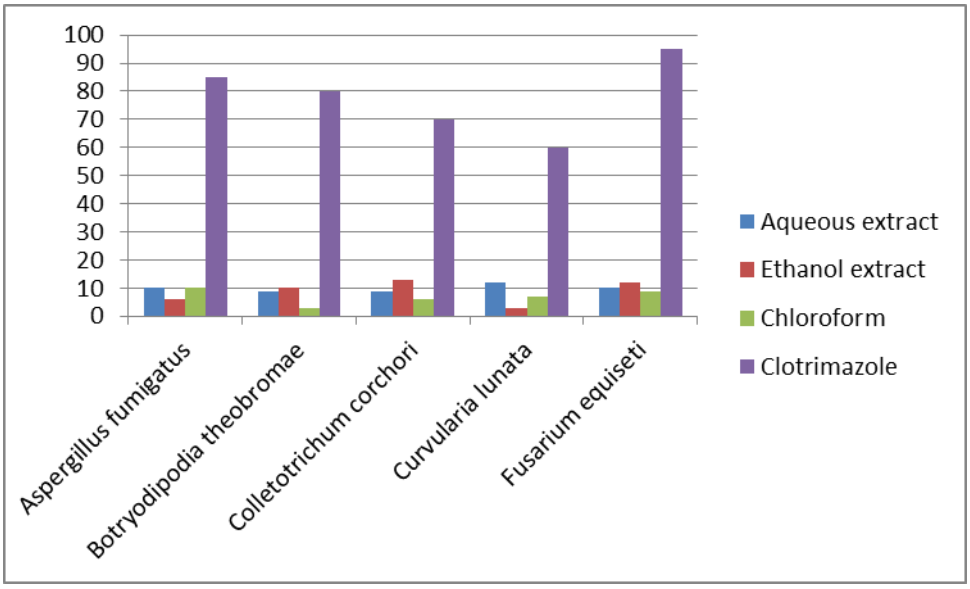

Fig-2: Antifungal activity of *Adhatoda vasica*.

Antifungal activity:

All the three extracts were tested for antifungal activities against five moulds and two yeasts. However, these extract showed activity against the yeasts and moulds where studied. As well as the standard clotrimazole also showed very strong activity against both yeasts and moulds (Table 2). Figure 2 shows the graphical representation of the antifungal activity of aqueous, ethanol, and chloroform extract of *Adhatoda vasica* compared with the stranded drug Clotrimazole.

Anti-oxidant activity:

The DPPH free radical scavenging activity of the *Adhatoda vasica* ethanolic extract and ascorbic acid is tabulated in Table 3. Both *Adhatoda vasica* ethanolic extract and ascorbic acid showed a dose-dependent activity. Among the four different concentrations (100, 200, 400 and 800 µg/ml) *Adhatoda vasica* ethanolic extract showed scavenging activity 55.20, 75.80, 80.60 and 82.40 percentages respectively. Among the above mentioned four different concentrations the highest scavenging activity of *Adhatoda vasica* ethanolic

extract was 82.40% at concentration 800 µg/ml. While ascorbic acid showed 80.70, 85.40, 90.30 and 93.10 percentage scavenging activity at 100, 200, 400 and 800 µg/ml respectively. 93.10% at concentration 800 µg/ml was the highest one. Figure 3 and 4 shows the graphical representation of *Adhatoda vasica* ethanolic extract and Ascorbic acid antioxidant activity respectively.

Table -3: Antioxidant activity of *Adhatoda vasica* ethanolic extract *and* Ascorbic acid

Test material	Concentration	Scavenging activity
Adhatoda vasica ethanolic extract	100	55.20
	200	75.80
	400	80.60
	800	82.40
Ascorbic acid	100	80.70
	200	85.40
	400	90.30
	800	93.10

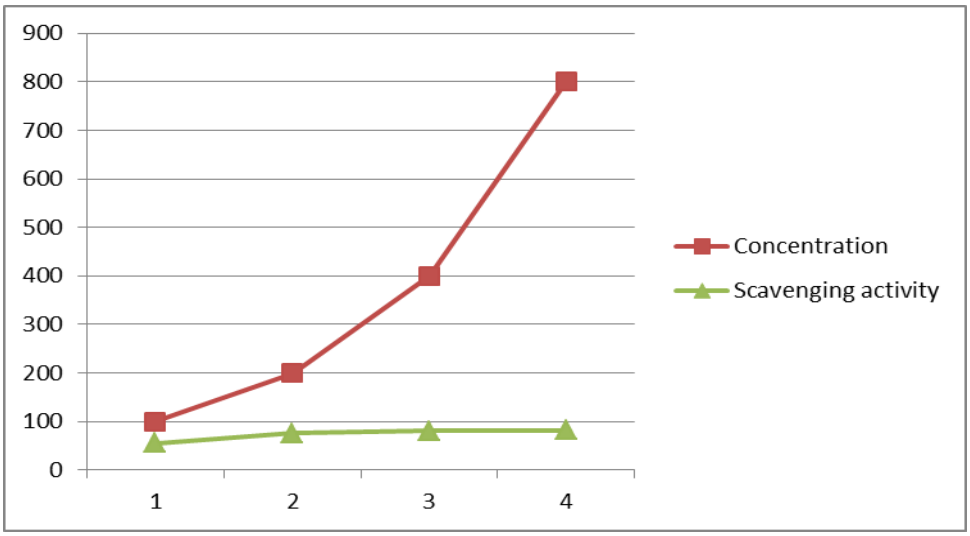

Fig-3: Antioxidant activity of *Adhatoda vasica*.

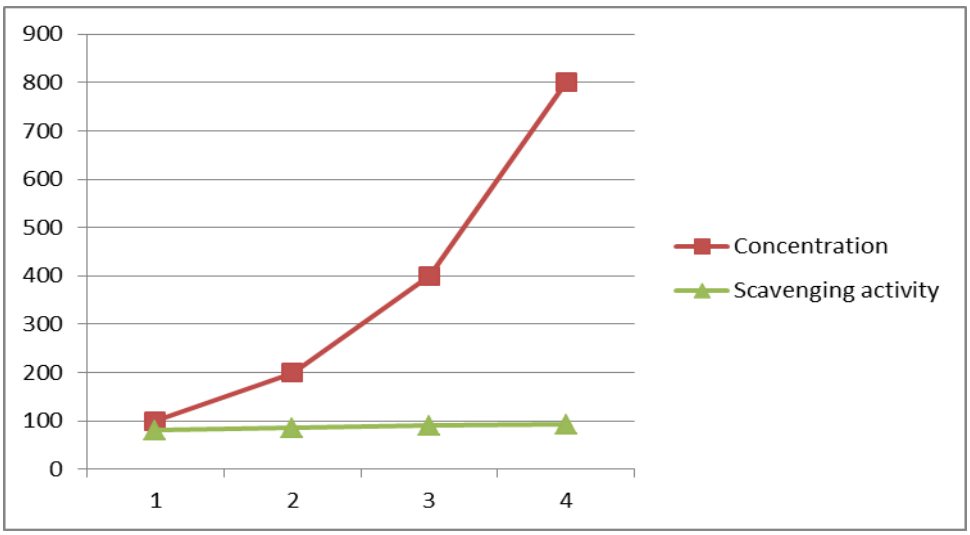

Fig-4: Antioxidant activity of ascorbic acid.

CONCLUSION AND FUTURE PERSPECTIVES

Finally we concluded that the results of the study reveles that the ethanol extract of *Adhatoda vasica* exhibits a very potential antioxidant effect. In addition to that aqueous extract, ethanol extract, and chloroform extract of the *Adhatoda vasica* shows antibacterial activity. These results can be the strong scientific evidence for the use of this plant as a useful source of antioxidant, antibacterial and antifungal references. Our team regularly studied the different pharmacological activities of various medicinal plants such as *Allivum sativum*, (Rathnasamy. S *et al*., 2014) *Solanum trilobactum* (Balakrishnan P *et al*., 2015) and proteins from animals Sundaramoorthy M *et al*., 2014) and *L.aspera* (Suganthi.N and Boominathan. M 2016) Furthermore, all our studies were in-vitro for the better observation of its activities we need to study the in vivo activities of *Adhatoda vasica*.so we continue this work on in-vivo studies.

Conflicts of interest: The authors stated that no conflicts of interest.

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