

***Adhatoda vasika* N. leaves extract: Phytochemical analysis and antibacterial activity**

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-----ABSTRACT-----

Adhatoda vasica N. belongs to the family Acanthaceae and is commonly known as Malabar nut/vasaka, and is a traditional medicinal plant native to Asia, widely used in Siddha, Ayurvedic and Unani systems of medicine. The present study deals with the phytochemical analysis and evaluation of antibacterial activity of ethanol, acetone, ethyl acetate and petroleum ether extracts of the leaves of *A. vasica*. The extracts were tested against Gram negative (*Klebsiella pneumoniae* and *Escherichia coli*) and Gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) bacterial strains. The phytochemical investigation showed the presence of alkaloids, flavonoids, terpenoids, tannins and saponins. Although, all extracts exhibited dose dependent antibacterial activity, however, highest antibacterial activity was observed in case of Gram positive bacterial isolates. As a result, *A. vasica* leaves extract could be a green alternative to hazardous artificial/synthetic antibacterial agents through more research and systematic clinical trials.

Keywords - *Adhatoda vasica*; plant extract; phytochemical analysis; antibacterial activity.

I. INTRODUCTION

In recent years, the infectious diseases remain the leading cause of death worldwide and infections due to antibiotic resistant ability of some microorganisms. However, synthetic antimicrobial agents provide broad spectrum characteristics, but often associated with the adverse effects on the host, including immune suppression, hypersensitivity and several allergic responses [1-3]. Generally, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality [4]. This situation reinforced the scientist communities looking for eco-friendly alternatives so that novel bioactive therapeutic agents can be made. Medicinal plants bears potent antimicrobial potential, many of them used in traditional system of medicine, which are readily available in rural areas at relatively cheaper than modern medicine [5].

Adhatoda vasica, native to Asia is a well known plant in Siddha, Ayurveda and Unani systems of medicine. Various parts of this plant have been used to treat of several ailments as herbal remedy such as, cold, cough, whooping cough, chronic bronchitis, fever, jaundice asthma as sedative expectorant, diarrhoea and dysentery and rheumatic painful inflammatory swellings [6-8]. Literature survey shows that the leaves of *A. vasica* contains many secondary metabolites and phytochemicals

(Fig. 1) such as, vasicine, vasicinone, vasicine acetate, 2-acetyl benzyl amine, vasicinolone, vasicol, vasicoline, vasicolinone and adhatodine [8,9-11] responsible for its biological properties [12-14].

Nevertheless, some reports [15-18] are available towards the antibacterial potentiality focused on *A. vasica* extracts, but more research is required to assess its antibacterial efficacy. The present paper discusses the comparative suitability of antibacterial potential of *A. vasica* leaves extract as a cleaner substitute to the eco-unsafe antibacterial agents that is a need for the society of eco-preservation and environmental safety.

II. MATERIALS AND METHODS

Materials

Strains and media

Four bacterial strains were selected due to their popularity and suitability. The pure cultures of organisms, Gram negative (*Klebsiella pneumoniae* and *Escherichia coli*) and Gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) were sub-cultured in McConky agar (HiMedia, India) nutrient broth based on previously used method [19]. They were inoculated, separately, into nutrient broth and kept at 37°C for 24 hours. Then, they were kept at 4°C until further use.

Chemicals and reagents

All the chemicals and reagents in this study were of Laboratory grade and used without further purification.

Methods

Plant sample collection and preparation of extracts

Fresh and healthy leaves of *A. vasica* were collected from college garden, Nuh, Haryana. Thereafter the leaves were air-dried in shade at room temperature for 4 weeks and ground with an electric grinder to obtain fine powder. The powdered leaves were stored in a sealed bottle at room temperature.

100 g of powdered sample was transferred into round bottom flask (capacity 1000 mL) fixed with a magnetic stirrer and 750 mL solvents were (Ethanol, Acetone, Ethyl acetate and Petroleum ether) added for 72 h. The plant extract was then collected and filtered through Whatman No.1 filter paper. The filtrates were concentrated *in vacuo* using a rotary evaporator at 45 °C followed by dried in a desiccator and crude extracts stored in refrigerator for use.

Phytochemical analysis

The stock solution was prepared from each of the crude extracts. The obtained stock solutions were subjected to phytochemical analysis based on standard methods described [20-22].

Test for alkaloids

15 mg of each extract was separately stirred with 6 mL 1% dil. HCl on a water bath for 5 min and filtered. These filtrates were divided into three equal parts.

(a) *Dragendorff's test*: To one portion of the filtrate, Dragendorff's reagent (Potassium bismuth iodide solution) (1 mL) was added; an orange red precipitate shows the presence of alkaloids.

(b) *Mayer's test*: To one portion of filtrate, Mayer's reagent (Potassium mercuric iodide solution) (1 mL) was added. Formation of cream coloured precipitate gives an indication of the presence of alkaloids.

(c) *Wagner's test*: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water and the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to the filtrate; a brown coloured precipitate indicates the presence of alkaloids.

Test for steroids

The crude extracts (0.1 gm) were dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall side. The colour of the upper layer turned red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for flavonoids

A few drops of dil. sodium hydroxide solution were added to the stock solution of crude extracts (0.5 ml). An intense yellow colour appeared in the plant crude extract, which became colourless upon the addition of a few drops of dil. H₂SO₄ acid. This shows the presence of flavonoids.

Test for terpenoids

(a) *Salkowski test*: The crude extract (about 100 mg) was separately shaken with chloroform (2 mL) followed by the addition of concentrated H₂SO₄ (2 mL) along the side of the test tube, a reddish brown colouration of the interface indicates the presence of terpenoids.

(b) *Liebermann-Burchard test*: Each extract (100 mg) was shaken with chloroform in a test tube; few drops of acetic anhydride was added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated H₂SO₄ (2 mL) was added along the side of the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids while formation of deep red colour indicates the presence of triterpenoids.

Test for tannins

Extract (100 mg each) was separately stirred with distilled water (5 mL) and then filtered. A few drops of 5% ferric chloride solution then added. Black or blue-green colouration or precipitate was taken as positive result for the presence of tannins.

Test for Saponins

The stock solution from each crude extract (50 mg) was dissolved in distilled water (10 ml) and then the test tube was shaken by hand for 15 min. The formation of a foam layer on the top of the test tube showed the presence of saponins.

Tests for glycosides

(a) *Anthraquinone glycoside (Borntrager's test)*: To the extract solution (1 mL), 5% H₂SO₄ (1 mL) was added. The mixture was boiled in a water bath and then filtered. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red colour of the ammonia layer gives indication of anthraquinone glycosides.

(b) *Cardiac glycoside (Keller-Killiani test)*: Extract (1 mL) was shaken with distilled water (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed by

H₂SO₄ (1 mL) along the side of the test tube. The formation of brown ring at the interface gives positive result for cardiac glycoside.

Antibacterial activity assay

Antibacterial assessment was carried out by means of disc diffusion assay method [3, 23]. Negative controls were prepared by using the same solvents employed to dissolve the samples. Inhibition zones were measured and compared with Ceftriaxone as standard reference antibacterial agent. Whatman No. 1 sterile filter paper discs (4 mm in diameter) were impregnated with extracts and placed on the inoculated agar. All the plates were incubated at 37 °C for 24 h.

The diameter of zone of inhibition was recorded in mm after 48 h, clear zone of inhibition was measured and compared with that of control. The experiment was performed for both the Gram (–ve) & Gram (+ve) bacterial strains. Index of sensitivity is defined as:

$$\text{Zone diameter (mm)/concentration } (\mu\text{g/mL}) = \text{clearing (mm}/\mu\text{g)}$$

Evaluation of antibacterial activity was measured as the diameter of the zones of inhibition against the tested microbes. Each method in this experiment was replicated three times. Values were shown in terms of Mean \pm SD error of all three respective categories.

III. RESULTS AND DISCUSSION

Phytochemical analysis

The leaves extracts (ethanol, acetone, ethyl acetate and petroleum ether) of *A. vasica* have been analyzed for the presence of phytochemicals. From the Table 1 it can be observed that the extracts of *A. vasica* leaves, contain phytochemicals (Fig. 1) such as, alkaloids, flavonoids, terpenoids, tannins and saponins. Therefore, tannins and saponins were observed in ethanol and petroleum ether extracts only.

Table 1: Phytochemical analysis of *A. vasica* leaves

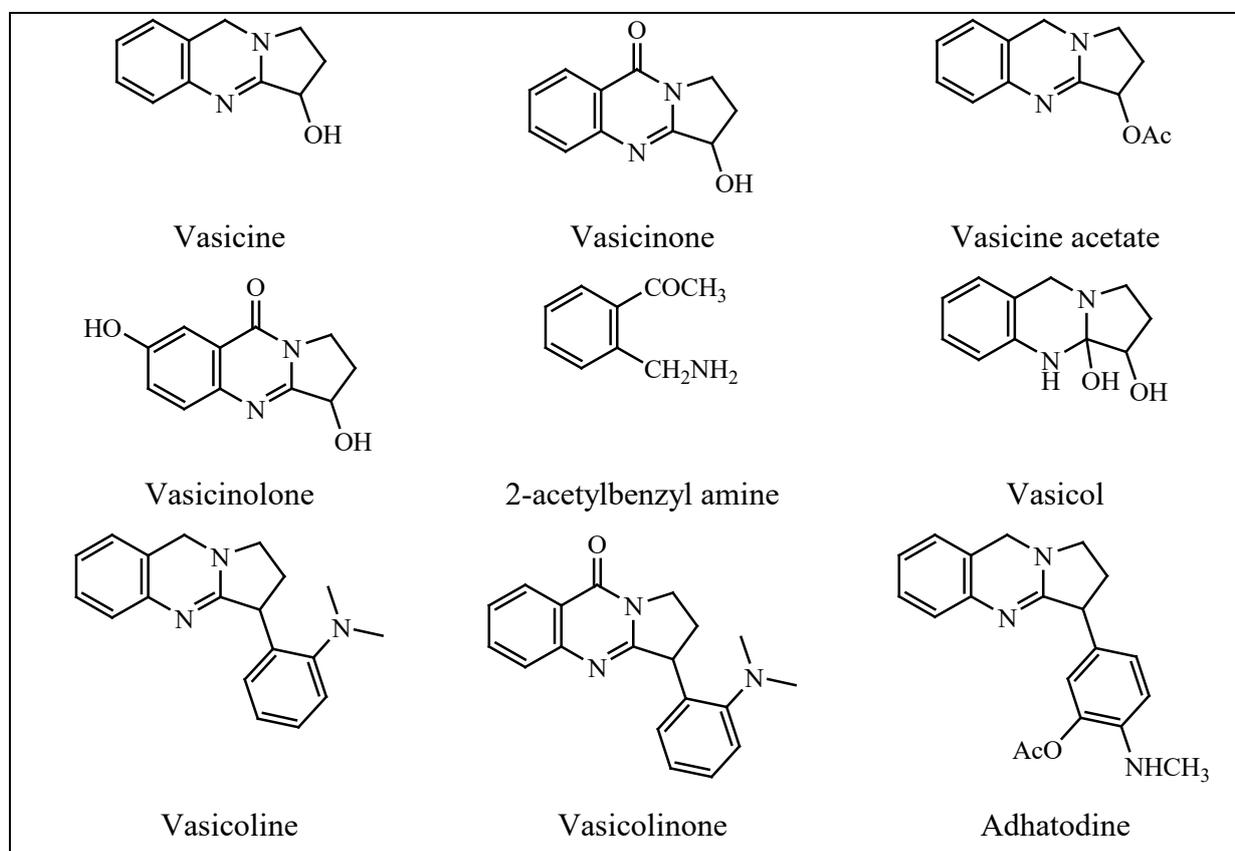
Phytochemicals	EE	AE	EAE	PEE
Alkaloids				
<i>Dragendorff's test</i>	+	+	+	+
<i>Mayer's test</i>	+	+	+	+
<i>Wagner's test</i>	+			
Steroids	-	-	-	-
Flavonoids	+	+	+	+
Terpenoids				
<i>Salkowski test</i>	+	+	+	+
<i>Liebermann-Burchard test</i>	+			
Tannins	+	-	-	+
Saponins	+	-	-	+
Glycosides	-	-	-	-
<i>Borntrager's test</i>	-	-	-	-
<i>Keller-Killiani test</i>	-			

EE = Ethanol extract, AE = Acetone extract, EAE = Ethyl acetate extract, PEE = Petroleum ether extract, + = presence and - = absence

Table 2: Antibacterial activity of various extracts of *A. vasica* leaves

Micro-organisms tested	Zone of inhibition (1000 µg/mL)				Ceftriaxone (1000 µg/mL)
	EE	AE	EAE	PEE	
<i>Kiebsiella pneumoniae</i>	12.5	9.5	9.7	9.8	17.5
<i>Escherichia coli</i>	10.5	7.6	8.2	8.8	16.9
<i>Staphylococcus aureus</i>	13.9	10.2	10.1	10.5	19.1
<i>Enterococcus faecalis</i>	15.5	11.1	12.5	11.6	28.5

EE = Ethanol extract, AE = Acetone extract, EAE = Ethyl acetate extract, PEE = Petroleum ether extract
Zone of inhibitions were expressed as mean±SD error (0.5) of three replicates.

Figure 1. Chemical structure of phytochemicals found in *A. vasica* leaves

Antibacterial activity

In the present study different extracts of *A. vasica* leaves were assessed for their antibacterial potential against Gram negative and Gram positive bacterial strains using different solvent systems viz. ethanol, acetone, ethyl acetate and petroleum ether. Table 2 represents the zone of inhibition of various extracts with ceftriaxone as a standard reference. From Table 2 it can be observed that ethanol extract of *A. vasica* leaves is found to be most effective, and least effectiveness was observed

for acetone extract. The order of considerable antibacterial efficacy is ethanol > petroleum ether > ethyl acetate > acetone.

The infections caused by Gram negative bacteria were found to be multi-drug resistant, difficult to treat with conventional antibiotics [24, 25]. However, Gram negative bacteria have showed resistance effect in all cases (extract as well as ceftriaxone). All extracts have remarked antibacterial activity against *Enterococcus faecalis* possessing maximum zone of inhibition. The variation of the susceptibility of microorganisms towards the *A. vasica* leaves extract attributed to the presence of several bio-active phytochemicals and their intrinsic properties that are related to the permeability to the cell surface of micro-organisms [3, 17]. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [1-3]. Due to the emergence of the antibiotic resistant pathogens, medicinal plants have found a better platform and could be an excellent alternates to combat the spread of multi drug resistant micro-organisms [1, 2, 19]. Thus, the result of the present study provides an account towards medicinal plants that possess synergized potentiality for biological characteristics.

IV. CONCLUSION

From the present study it was noticed that *A. vasica* leaves have significant antibacterial activity against for both Gram negative as well as Gram positive bacterial strains. The antibacterial activity of leaves extract of *A. vasica* was found to be quite satisfactory comparable with the standard antibiotics screened under related conditions. As the leaves extracts exhibited pronounced activity comparable with standard antibacterial agent (ceftriaxone) towards tested microbial isolates, it can be used as an eco-safe, biodegradable alternative in prevention and treatment of bacterial infections.

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