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Antibacterial activity of *Adhatoda vasica* leaves extract against multidrug resistant Gram positive bacteria-*Bacillus subtilis and Staphylococcus aureus*

Dr. Amar Singh Mounpria
Head &Associate Professor
Department of Zoology
S.N.D.B. Govt.PG College, Nohar
asmunparia@gmail.com

Abstract

Pathogenic Bacteria have always been considered as a major cause of morbidity and mortality in humans. Even though Pharmaceutical companies have produced a number of new antibacterials in the last years resistance to these drugs has increased and has now became a global concern.

Adhatoda vasica belongs to the family Acanthanceac and is commonly known as malabar nut / Vasaka and it a traditional medicinal plant native to Asia, widely used in Siddha, Ayurvedic and unani systems of medicine. The present study evaluation of antibacterial activity of leaves extr act of Adhatoda vasica on E-coli and Pseudomonas aeruginosa.

The result of the present study support the traditional use of studied plants in the treatment of bacterial infections. It also provide an important basis for the use of leaves extract of Adhatoda vasica used to control infectious deseases caused by Gram-positive bacteria i.e. Bacillus subtilis and Staphylococcus aureus.

Keyword *- Pharmacauticals, Adhatoda vasica, antibacterial, Bacillus subtilis and Staphylococcus aureus.*

Introduction

Adhatoda vasica, an important Indian medicinal plant has long been used in ayurvedic system of medicine. The plant has been found to diverse number of pharmacological activities include Respiratory tract infection, cough formulation, expectorant, anti-spasmodic and bleeding pills. (Ahmad Sayeed et al.,2009) Recently various researchers have found greater interest in anti-microbial activity against several species in different studies.

Due to its medicinal properties *Adhatoda Vasica* (Linn.) Nees has been recommended by Ayurvedic physicians for management of various types of respiratory disorders. Vasaka belongs to the plant family Acanthaceae, its botanical/taxonomic name is Justicia adhatoda(Linn.) Some of its synonyms are *Adhatoda Vasica* Nees and Adhatoda zeylanica Medicus. Vasaka appears to be the most common name for this plant. The crude drug may be derived from powdered dry leaves or from extracts of fresh leaf juice. Other parts of plants that are also used are – roots, bark, flowers. (Ahmad S. *et al.*, 2009) In our study, only fresh wet leaves and their extract were used to detect antimicrobial activity of Vasaka. The nature of constituents is quinazoline alkaloid among which vasicine is the chief principle. Formulation comprises fresh juice, decoction, infusion, powder, alcoholic extract, liquid extract or syrup but are also given along with other expectorants. Drug from Vasaka act as a sedative,expectorant, antispasmodic & antihelmenthic. Expectorant activity is due to the essential oils present in leaves. (Chatterjee S. *et al.*, 1999) Leaf extract has been used for treatment of bronchitis, asthma, fever, jaundice, diarrhoea, dysentery, glandular tumour, cough and breathlessness. Large doses of fresh juices of leaves have been used in tuberculosis. Due to strong coagulation activity it minimizes blood loss. It has uterine stimulatory activity. It acts as a uterotonic & is also useful to control post-partum haemorrhage. (Chatterjee S.*et al.*, 1999) It also acts as antimicrobial & anticancer agent.

Material And Method

Sample Collectionof plant material and Preparation

The leaves of the plant Adhatoda vasica were collected from the open fields of jhunjhunu and Hanuman garh District, Rajasthan, India in the March and April. The plants were identified at the Department of Botany and , Seth G.L.Bihani P.G. College Sri Ganga nagar, Rajasthan, India. The fresh leaves were washed with distilled water and the leaves were separated and kept in a clean shaded place for 9-10 days, grounded to a powder and weight the whole powder .Cold Maceration method is used to prepare extracts. Extracts obtained are made solvent free and concentrated by rotary evaporator at 40°C and kept at 4°C in refrigerator in airtight bottle until further use (akhter et al.,2014)

Preparation of Ethanol extraction

A powedred samle of 25g was weighed and soaked in 250 ml of 95% ethanol in a separating funnel for 24 hours, with intermittent shaking. The plant extract was collected and then filtered using Muslin cloth and 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 methanol and water as solvents, using 25gm of the fresh ground sample, for each extraction. All the extracts were cooled at room temperature.

Collection of Microorganisms

Pure bacterial cultures of *Bacillus subtilis and Staphylococcus aureus*, were obtained from the Microbial Type Culture Collection (MTCC)/Institute of microbial technology (IMTECH),Chandigarh.The Gram-positive bacterial strains such as *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 3160) were used for antimicrobial assay. All the strains were grown in NB medium and incubated at 37ºC for overnight. The entire microorganisms were subcultured in 30 days.

Maintenance of Microbial Culture

Nutrient agar for bacterial strains was prepared by autoclaving them at 121°C at 15 lbs/sq-inch presence for 30 minutes. The medium was poured in Petri plates and allowed to solidify. Microbial culture dilution was prepared by taking a loop full of microbial culture and mixing it with distilled water, for uniform distribution of microorganism in Petri plate.

Agar Disc Diffusion Method

Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37° C for 48 hrs. The agar plates of the above media were prepared and One drop of bacterial strain was spread over the medium by rod. each plate was inoculated with 18h hold cultures and spread evenly on the plate. All the plates were incubated at 37°C for 24 hrs and the diameter of Zone of Inhibition (ZOI) were noted. The inoculation of microbes was prepared from bacterial culture.

The control disc were filled with Streptomycin along with solvent. The anti microbial activity was tested against (methanol ethanol and water), leaves of *Adhatoda Vasica* Sterile filter paper disk of 6mm diameter were impregnated with the different concentration of solvent extracts of *Adhatoda Vasica* like 125µg, 250µg, 500µg, 1000µg and 2000µg. 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

Antibacterial Analysis

The antibacterial activity of *Adhatoda Vasica* ethanol,methanol and water leaf extract against *Bacillus subtilis and Staphylococcus aureus* was determined using the agar disk discussion method. The results of antibacterial screening, the agar plates showing the zone of inhibition (ZOI) of ethanol leaf extract of *Adhatoda Vasica*.

Screening for antimicrobial activity of antibiotics

The antimicrobial activity studies were carried out by disc diffusion method. Streptomycin (10µg/ml) was used. Three disc were placed on the plates of seeded organisms using sterile forcep of 6 mm in diameter and different concentration of antibiotics were placed on the disc in different plates with a control disc with DMSO. All bacterial plates were incubated at 37°C for 24 hrs. The zone of inhibition was measured in mm.

Result And Discussion

The comparison of two positive strain

The plant extract of Adathoda vasica was found to be active against all bacterial organisms tested. various concentration of leaf extract (2000µg to 125µg). All the concentrations of leaf extract inhibited the bacterial growth. Maximum activity was observed at 2000µg/ml concentration of extract. With different concentrations of extract tested, the inhibition zone varied from (2mm to 28mm).The antibacterial activity of methanol extract of *Adhatoda Vasica* against all tested microorganisms was greater than the antibacterial activity of ethanolic, aqueous extract of *Adhatoda Vasica*. *Adhatoda's.* antibacterial properties have been clinically evaluated by Brantner AH and Chakraborty A, 1998. Generally, plants extracts are usually more active against gram positive bacteria than gram negative bacteria.

While our studied aqueous, ethanolic and methanolic leaf extract of *Adhatoda Vasica* showed antibacterial activity against *Bacillus subtilis and Staphylococcus aureus* is 7mm, 24mm and 20mm & 8mm, 28mm, 24mm (table 1B, and 2B) respectively. Thus our studied plant is more effective and can be used as drug formulation with other medicinal plant. The analysis revealed the presence of alkaloids, flavonoids, triterpenoids, sterols, tannins and glycosides. In particular, the ethanolic extract tested positive for most of the secondary metabolites tested. The leaf ethanolic extracts were hence chosen for further evaluation of antibacterial activity. Among all the bacterial strains used, S. aureus was most susceptible to the ethanol extract. The results were more promising against the Gram-positive bacteria S. aureus. This could be attributed to the fact that the cell wall in Gram-positive bacteria has a single layer where-as the Gram-negative cell wall is a multi-layered structure (Yao et al. 1995; Ozcelik 1998),

Table 1(B) Average Zone of inhibition (ZOI) Ethanolic extract, Methanolic extract, Aqueous extract and control streptomycin against *Bacillus subtilis* in vitro condition

S. No.	Con. Of Extract µg/Ml	Con. Of Streptomycin µg/Ml	Average Zone Of Inhibition (Mm)			
			Control	Methanolic Extract	Ethanolic Extract	Aqueous Extract
1	2000	2000	52	20	24	7
2	1000	1000	57	19	21	6
3	500	500	47	17	18	4
4	250	250	45	12	14	3
5	125	125	44	11	8	2

Table 2(B) Average Zone of inhibition (ZOI) Ethanolic extract, Methanolic extract, Aqueous extract and control streptomycin against *Staphylococcus aureus* in vitro condition

S. NO.	CON. OF EXTRACT µg/ml	CON. OF STREPTOMYCIN µg/ml	AVERAGE ZONE OF INHIBITION (mm)			
			CONTROL	METHANOLIC EXTRACT	ETHANOLIC EXTRACT	AQUEOUS EXTRACT
1	2000	2000	52	24	28	8
2	1000	1000	55	19	27	7
3	500	500	47	17	25.5	6
4	250	250	45	14	20	4
5	125	125	44	10	19	3

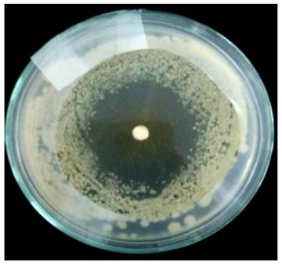

Fig. 1(A) Zone of maximum inhibition (55mm) of streptomycin against Bacillus subtilis(Positive Control)

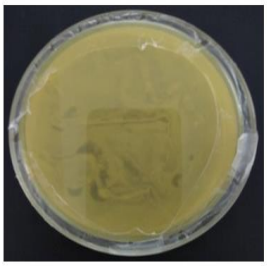

Fig. 1(B) Initial growth of Bacillus subtilis (stage - I)

Fig 1(C) Zone of inhibition (ZOI) of different concentration methanolic extract on *Bacillus subtilis*

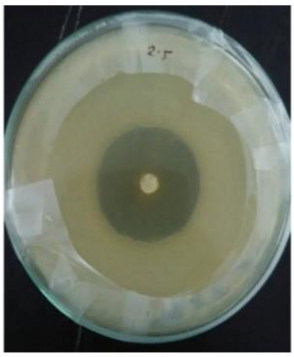

Fig. 2(A) Zone of maximum inhinbition (28mm) of ethanolic Extract against *staphylococcus aureus (Conc.)*

Fig. 2(B) Zone of minimum inhinbition ethanolic extract (19mm) and methanolic extract (10mm) against *Staphylococcus aureus(Conc. 125)*

Conclusion:

Plant leaf extracts of Adathoda vasica was found to have significant antibacterial activity.The obtained results support the use of these plants in traditional medicine. The potential for developing antimicrobials from higher plants appears rewarding as it leads to the development of new drugs which is needed today. Further research is necessary to find the active compounds within these plants with their full spectrum of efficacy. However, the present study of antibacterial activity of *Adhatoda vasica* form primary platform for further phytochemical and pharmacological studies. From the results obtained during the present study, we can conclude that the leaf extract of Adhatoda vasica has excellent antibacterial activity against the all tested bacteria. (Sagar et al., 2013),(Josephin et al., 2012). Thus, there is a possibility of developing Adathoda vasica as an important source of biopesticide and that could be useful for an important and antibacterial agent.(K. Ilango et al., 2009).

In the present investigation, aqueous, ethanolic and methanolic leaf extract of *Adhatoda Vasica,* was evaluated for anti-microbial activity through zone inhibition method against Gram Positive bacteria i.e. Bacillus subtili & S. aureus. These results indicate that the anti-bacterial activity of these extracts might be due to the presence of phytochemicals i.e. alkaloids, saponins, flavonoids, tannins, terpenoids, amino acids etc.

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