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Antibacterial activity of *Adhatoda vasica* leaves extract against multidrug resistant Gram-negative bacteria

Dr. Amar Singh Mounpria
Associate Professor & Head,
Department of Zoology,
S.N.D.B. Govt. P.G. College, Nohar (Raj.)

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 antibacterial 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 extract 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-negative bacteria i.e. E-coli and Pseudomonas aeruginosa.

Keywords: *Pharmacauticals, Adhatoda vasica, antibacterial, E-Coli,Pseudomonas aeruginosa.*

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 Collection of plant material and Preparation

The leaves of the plant Adhatoda vasica were collected from the open fields of Jhunjhunu and Hanumangarh 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 Ganganagar, 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 powdered sample 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 rotator 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 *Pseudomonas aeroginosa* and *E-coli*, were obtained from the Microbial Type Culture Collection (MTCC)/Institute of microbial technology (IMTECH), Chandigarh. The Gram-negative bacterial strains such as E. coli (MTCC 443) and Pseudomonas aeruginosa (MTCC 4673) were used for antimicrobial assay. All the strains were grown in NB medium and incubated at 37ºC for overnight. The entire microorganisms were subculture 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 *Pseudomonas aeruginosa* and *E-coli* 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 negative 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 22mm).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 *E. coli*, and *Pseudomonas aeruginosa* is 8mm, 7mm and 18mm, 18mm, 22mm, 14mm (table 1B, and 2B) respectively. Thus our studied plant is more effective and can be used as drug formulation with other medicinal plant or individually in the treatment of asthma.

Table 1(B) Average Zone of inhibition (ZOI) Ethanolic extract, Methanolic extract, Aqueous extract and control streptomycin against *E-coli* in vitro

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	51	22	18	8
2	1000	1000	55	19	14	7
3	500	500	47	16	11	5
4	250	250	45	11.5	10	2
5	125	125	44	10	8	2

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

S. NO.	CON. OF EXTRACT µg/ml	CON. OF STREPTOMYCIN µg/ml	AVERAGE ZONE OF INHIBITION(mm)			
			CONTROL	METHANOLIC EXTRACT	ETHANOILC EXTRACT	AQUEOUS EXTRACT
1	2000	2000	54	14	18	7
2	1000	1000	52	11.5	14	5
3	500	500	47	10	12	4
4	250	250	45	9	8	3
5	125	125	44	8	6	3

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).

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