Assesment of Antibacterial Properties of *Adhatoda vasica* Various Extracts against Gram Positive and Gram Negative bacteria

Amar Singh Mounpria
Associate Professor
Department of zoology
S.N.D.B. Govt. College, Nohar
e-mail: asmunparia@gmail.com

Abstract:

The leaves of Justicia Adhatoda Vasica have been in use in Indian systems of medicine for the last more than 2000 years. J. adhatoda is well known in the indigenous systems of medicine for its beneficial effects, particularly in bronchitis (Claeson and Malmfors, 2000). The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping-cough (Dhuley, 1999) and chronic bronchitis and asthma as sedative-expectorant, anti-spasmodic and as anthelmintic. The bronchodilatory and expectorant properties of the leaves are attributed to vasicine (Chatterjee, 1999). Warm Decoction of the leaves was expectorant and cured asthma patients.

During the present investigation, the antimicrobial activity of methanol, ethanol and water leaf extracts of Adhatoda Vasica was tested on Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and E-coli by the disc diffusion method (Atlas et al., 1995). Ethanolic extract showed maximum activity against Staphylococcus aureus followed by Bacillus subtilis, Pseudomonas aeruginosa and E-coli

Keyword: *disc diffusion, Adhatoda vasica, antibacterial, Decoction,expectorant, spasmodic, Staphylococcus aureus, E-Coli.*

Introduction:

Adhatoda Vasica belongs to the family of Acanthaceae commonly known Malabar nut or adulsa, native to Asia.. It is an erect, terrestrial, perennial shrub. The leaves are dark green above and pale yellow below. *Adhatoda Vasica nees* commonly known as vasaka distributed throughout India. the leaves, flowers, fruit, and roots are extensively used for treating cold cough, whooping cough, chronic bronchitis and asthma as sedative, expectorant and antispasmodic. It is a primary herb of the ayurvedic system.

The important active components include alkaloids vasicine and vasicinone. The whole plant is useful in the removal of intestinal parasites. *Adhatoda Vasica* according Ayurveda, flowers are used for treating tuberculosis. *Adhatoda Vasica* poultice from the leaves is applied for healing wounds, rheumatic pains and edema, whereas a warm decoction of the leaves is useful in treating scabies and other skin diseases. World Health Organization (WHO) has defined medicinal plants as that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs. Medicinal plants constitute an important component of flora and are widely distributed in India.

In the present study, we have chosen leaves of *Adhatoda Vasica* as herbal medicine to determine their antibacterial property. Evidently, there are not sufficient scientific studies that confirm the antimicrobial activity of this plant. This study looks into the in vitro antimicrobial activity of this plant for different solvent extracts against some Gram-positive and Gram-negative pathogenic microorganisms that causes the most common cases of infectious disease.

Materials and Methods:

Collection of 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(chopra *et.al*.,1956). The plants were identified at the Department of Botany. 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. Soxhlet extractor method is used to preparation of extracts. Extracts obtained are made solvent free and concentrated by rotary evaporator at 40°C, and resultant kept at 4°C in refrigerator in airtight bottle until further use (akhter et al, 2014 And charan et al.,2018)

Preparation of Ethanol Extraction:

100gs of coarse powder of leaves was packed in a muslin cloth bag and placed in the body of Soxhlet extractor. Then, 500 ml of ethanol (solvent) was poured in the round-bottom flask. The apparatus was then fitted with the help of clamps and stand to support the Soxhlet extractor, round-bottom flask, and condenser. The rubber tube connected to the tap water was attached to the condenser for continuous flow of water. The solvent was heated using the isomantle, which began to evaporate, moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the plant extract. Once the level of solvent reached the siphon, it poured back into the flask and the cycle began again. The process was made to run for a total of 6 h. Finally, the extract was collected in the round-bottom flask. Once the process was finished, the ethanol was evaporated using rotary evaporator at 40°C. and extract was kept in the refrigerator till further use.

Preparation of Tested Organisms:

Four microorganisms representing Gram-positive and Gram-negative bacteria were used. The two gram-positive bacteria were- Staphylococcus aureus and Bacillus subtilis and the two gram-negative bacteria were-Escherichia coli and Pseudomonas aeruginosa.

Collection of Microorganisms:

Pure bacterial cultures of Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeroginosa and E-coli, (all clinical isolates) were obtained from the Microbial Type Culture Collection (MTCC)/Institute of microbial technology (IMTECH),Chandigarh. The bacterial strains such as E. coli (MTCC 443), Bacillus subtilis (MTCC 441), Pseudomonas aeruginosa (MTCC 4673), 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 by Streak plate method or 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

Screening for Antibacterial Analysis:

The antibacterial activity of Adhatoda Vasica ethanol, methanol and water leaf extract against Staphylococcus aureus, Bacillus subtilis, 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 solvent 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 Discucussion

The antibacterial activity of ethanolic extract of *Adhatoda Vasica* against all Gram-positive and Gram-negative tested microorganisms was greater than the antibacterial activity of methanol, 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.

The present study was conducted to investigate the in vitro antibacterial activity of *Adhatoda Vasica*. But over all ethanol extract of *Adhatoda Vasica* showed maximum photochemical, antioxidant enzymes and antimicrobial assay as compared to methanol and aqueous extract of *Adhatoda Vasica*. In-vitro screening showed a strong activity of *Adhatoda's* alkaloids against the bacteria *Staphylococcus aureus*. Significant antibacterial activity against the Gram-positive and other Gram-negative bacterial strains *Streptococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus* and *E. coli* were also noted by Patel VK and Venkatakrishna Bhatt H, 1984. On the other hand work done by Aibinu *et al.,* 2007, aqueous and Methanol extract of Bryophyllum pinnatum leaves showed antibacterial activity against E. coli and Klebsiella pneumoniae was 0mm,0mm and 16mm,12mm respectively.

While our observed aqueous, ethanolic and methanolic leaf extract of *Adhatoda Vasica* showed antibacterial activity against *E. coli(*8mm, 18mm and 22mm*), Staphylococcus aureus(*8mm, 28mm, 24mm*), Bacillus subtilis(*7mm, 24mm, 20mm*)* and *Pseudomonas aeruginosa* is(7mm, 18mm and 14mm) respectively(table 1, 2 and 3). Thus our studied plant is more effective and can be used as drug formulation with other medicinal plant.

Adhatoda Vasica exhibited the antimicrobial activity against various organisms by means of zone inhibition method. Then findings from the current study *Adhatoda Vasica* plant extract exhibited the antimicrobial activity against four pathogens like *B. subtilis*. *S. aureus, E. coli and P. aeruginosa* (table 1, 2 and 3) and (Fig.1 to 5) All extract showed antibacterial activity but not same level. The ethanol extract of leaves of A.Vasica showed significant antibacterial activity ,comparable to other extract solvent of A. Vasica.

In this thesis disc methed was used to evaluate the Zone of inhibition against the test organisms. These assays are based on the use of discs as reservoir containing solution of the substances to be examined.

Table 1 Average Zone of inhibition (ZOI) Aqueous extract against Gram positive and Gram Negative Bacteria in vitro condition

NEGATIVE BACTERIA	ZONE OF INHIBITION IN (mm)				
	2000	1000	500	250	125
Escherichia coli	8	7	5	2	2
Pseudomonas aeruginosa	7	5	4	3	3
POSITIVE BACTRIA					
Bacillus subtilis	7	6	4	3	2
Staphylococcus aureus	8	7	6	4	3

Table 2 Average Zone of inhibition (ZOI) Ethanol extract against Gram positive and Gram Negative Bacteria in vitro condition

NEGATIVE BACTERIA	ZONE OF INHIBITION IN (mm)				
	2000	1000	500	250	125
Escherichia coli	18	14	11	10	8
Pseudomonas aeruginosa	18	14	12	8	6
POSITIVE BACTRIA					
Bacillus subtilis	24	21	18	14	8
Staphylococcus aureus	28	27	25.5	20	19

Table 3 Average Zone of inhibition (ZOI) Methanol extract against Gram positive and Gram Negative Bacteria in vitro condition

NEGATIVE BACTERIA	ZONE OF INHIBITION IN (mm)				
	2000	1000	500	250	125
Escherichia coli	22	19	16	11.5	10
Pseudomonas aeruginosa	14	11.5	10	9	8
POSITIVE BACTRIA					
Bacillus subtilis	20	19	17	12	11
Staphylococcus aureus	24	19	17	14	10

Fig.1 Zone of maximum inhibition (57mm) of streptomycin against *Bacillus subtilis(Positive Control)*

Fig.2 Zone of inhibition (ZOI) of different concentration methanolic extract on *Bacillus subtilils*

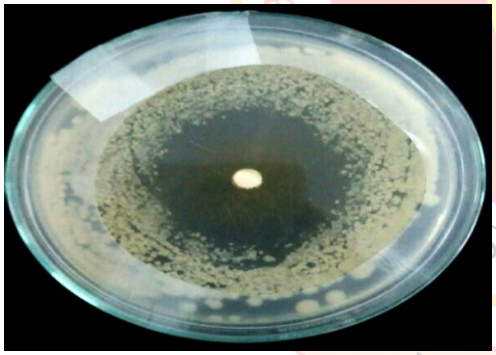

Fig.3 Zone of maximum inhinbition (28mm) of ethanolic Extract against *staphylococcus aureus*

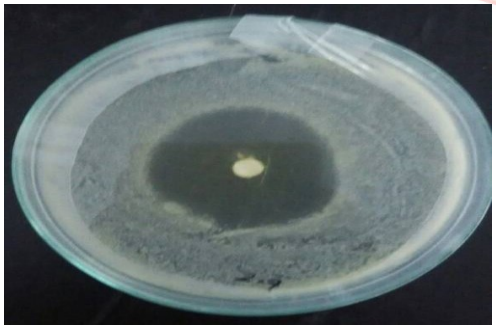

Fig.4 Zone of maximum inhinbition ethanolic extract (18mm) against *Psuedomonas aerugino*

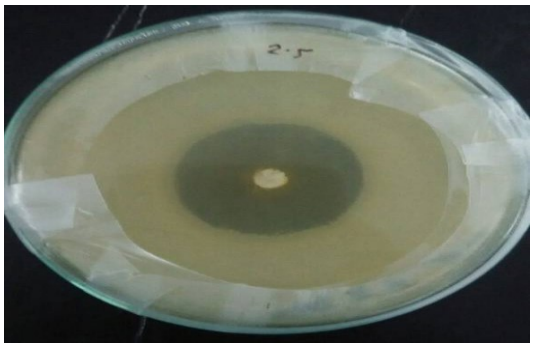

Fig.5 Zone of maximum inhinbition ethanolic extract (20mm) and methanolic extract (22mm) against *E-coli*

Conclusion

The present study we can conclude that the leaf extract of *Adhatoda Vasica* has excellent antibacterial activity against the tested bacteria. (Sagarvijayrao Kathale, 2013, Josephin Sheeba *et al.,* 2012) Plant leaf extracts of Adathoda vasica was found to have significant antibacterial activity. From the results we can conclude that *Adathoda vasica* has potent antimicrobial activity. 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 Bacillus subtilis, S. aureus, E. coli and Pseudomonas aeruginosa was effective against all of these micro-organisms. 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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