Antimicrobial And Antioxidant Activities of *Adhatoda Vasica* Leaf Extract on Clinical Pathogens

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Abstract:

Leaf extract of Adhatodavasicawas tested on pathogenic bacteria species eg. E-coli and Bacillus subtillis pure culture were obtained from the Institute of microbial technology, Chandigarh by using disc diffusin method. The extract prepared from the leaves of Adhatodavasica in different solvents such as ethanolic, methanolic and water have been found to inihibit the growth of bacterial strains. Ethanolic extract was showed strong inhibition compare to other solvents on both clinical pathogens. Both are pathogenic bacteria that cause diarrhea and many more uncontrolled diseases due to resistance. Ethanolic extract showed significant inhibition effect against gram nagetive bacteria E-coli and hydro extract showed significant inhibation effect against Bacillus subtillis.

Keyword:*-Pharmacauticals, Adhatodavasica, antibacterial, E-Coli,Bacillussubtillis.*

Introduction:

AdhatodaVasicanees(Acanthaceae) commonly known as vasaka,its also called Malabar nut tree and well known throughout India. It is tall, with several branches, is dense and is an evergreen shrub. The plant is used in the indigenous system of medicine in India and is a well-known expectorant in both Ayurvedic and Unani Systems of Medicine. (Chopra R.N. et al., 1992, Kapoor L.D. et al., 2001) India, a country of immense biotic wealth, has more than 7000 plant species reportedly used for medicinal purposes most of which are being exploited recklessly for the extraction of drugs. In Ayurvedic medicine, Adhatodavasica has been used for a multitude of disorders including bronchitis, leprosy, blood disorders, heart troubles, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumors, mouth troubles, fever and gonorrhea. It is useful in treating bronchitis, tuberculosis and other lung and bronchiole disorders. A decoction of Vasaka leaves may be used to help with cough and other symptoms of colds. The juice of Vasaka leaves softens the bronchial tube. It is also useful in reducing aggravation of pitta and discomfort due to jaundice. The root and bark, well-known for their expectorant properties (Sampath et al. 2010). It has also been used to speed delivery during childbirth. Antioxidant and anti-inflammatory activity was attributed to an isolated compound, vasicine (Srinivasarao et al. 2006). Many higher plants accumulate extractable organic substances in sufficient quantities to economically manage diseases. The plant compounds to treat infections are an age old practice in developing countries where there is dependence on traditional medicine for a variety of diseases (Shibata et al. 2005; Pieboji et al. 2006).

Presently, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases, but are also often with adulterations and side effects (Babayil et al. 2004).In recent times, the rapid development of multi-resistant bacterial and fungal strains of clinically important pathogens has stimulated scientists to develop newer broad spectrum antimicrobial agents (Rojas et al. 2003).Plants are rich in a wide variety of secondary metabolites, such as tannins, ter-penoids, alkaloids and flavonoids, which have been found in vitroto have antimicrobial properties (Lewis and Ausu-bel 2006). Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Abu-Sha-nab et al. 2004;

Shiotaet al.2004). Throughout the history of mankind, many infectious diseases have been treated with herbal remedies and researchers are increasinglyturning their attention to folk medicine. Continuous search leads to the development of better drugs against microbial infections (Benkeblia 2004).The current investigation aimed to explore the antibacterial potential of different extracts of A. vasica.

Material and Method:

Sample Collectionof plant material and Preparation:

The leaves of the plant Adhatodavasica 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, Seth G.L.BihaniP.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. 250 ml of organic solvents ethanol, methanol and water is taken and 25 gm of leaf powder is soaked in it. Extracts obtained are made solvent free and concentrated by rotary evaporatorat 40°C and 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:

25 gram of dried leaf powder was taken in a separate container. To this 250 ml of ethanol was added and kept for 24 h with periodic shaking, then filtered and the filtrate was collected. The procedure was repeated three times with fresh volume of ethanol. The filtrates were pooled. After 4 hour of incubation, filter paper discs of 6 mm diameter, impregnated with different concentration (2000, 1000, 500, 250 and 125 mg/ml) were placed by using forceps aseptically. One disc of known concentration was loaded in a Petri plate. Discs are arranged in approximately centre of microbe-seeded agar plates. Positive control disc were prepared by using standard antibiotic,Streptomycin (10µg/ml). Solvent as negative control and antibiotic streptomycin (10µg/ml) as positive controls were used. The extract loaded and control loaded discs were incubated at 37°C for 24 h in an incubator. After incubation period the plates were observed and the diameters of the zone of inhibition were measured and recorded. The inhibition zones produced due to the inhibitory activity of the extract is the total inhibition zone excluding the 6 mm diameter of filter paper disc.

Preparation of Tested Organisms:

Four microorganisms representing Gram-positive and Gram-negative bacteria were used. The two gram-positive bacteria were- Staphylococcus aureusand Bacillus subtilisand the two gram-negative bacteria were-Escherichia coli andPseudomonas 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 AdhatodaVasica Sterile filter paper disk of 6mm diameter were impregnated with the different concentration of solvent extracts of AdhatodaVasica 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 Vasicaethanol, 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 AdhatodaVasica.

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 thepetriplates 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.

Results And Discussion:

The plant extract of Adathodavasica was found to be active against all bacterial organisms tested. All the concentrations (2000µg to 125µg) of leaf extract inhibited the bacterial growth. In vitro screening showed a strong antibacterial activity of Adhatoda Vasica against the bacteria Staphylococcus aureus(28mm) followed by Pseudomonas aeruginosa (18mm) Bacillus subtilis (24mm,) E. coli (22mm) table 1 to 4. Pseudomonas aeruginosa andE. coli(gram negative strain) exhibited slightly lesser activity compaired to other organisms i.e (18mm) and (22mm) respectively. Similarly leaf extract of AdhatodaVasica showed 100% inhibition against all tested bacteria. In the present study, 2000µg/ml concentration of ethanol extract of Adathodavasica showed the maximum inhibition showed against all tested Bacteria. Suggesting the leaf extract of Adthodavasica possessed stronger disease control, its showed highly inhibiting activity.

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 Zone of inhibition Streptomycin against four types microbes

Pathogens	Concentration of Streptomycin (µg/ml)	Zone of Inhibition in (mm)				
		2000	1000	500	250	125
Escherichia coli		51	55	47	45	44
Bacillus subtilis		52	57	47	45	44
Pseudomonas aeruginosa		54	52	47	45	44
Staphylococcus aureus		52	55	47	45	44

Table 2 Zone of inhibition Methanolic extract against four types microbes

Pathogens	Concentration of Streptomycin (µg/ml)	Zone of Inhibition in (mm)				
		2000	1000	500	250	125
Escherichia coli		22	19	16	11.5	10
Bacillus subtilis		20	19	17	12	11
Pseudomonas aeruginosa		14	11.5	10	9	8
Staphylococcus aureus		24	19	17	14	10

Table 3 Zone of inhibition Ethanolic extract against four types microbes

Pathogens	Concentration of Streptomycin (µg/ml)	Zone of Inhibition in (mm)				
		2000	1000	500	250	125
Escherichia coli		18	14	11	10	8
Bacillus subtilis		24	21	18	14	8
Pseudomonas aeruginosa		18	14	12	8	6
Staphylococcus aureus		28	27	25.5	20	19

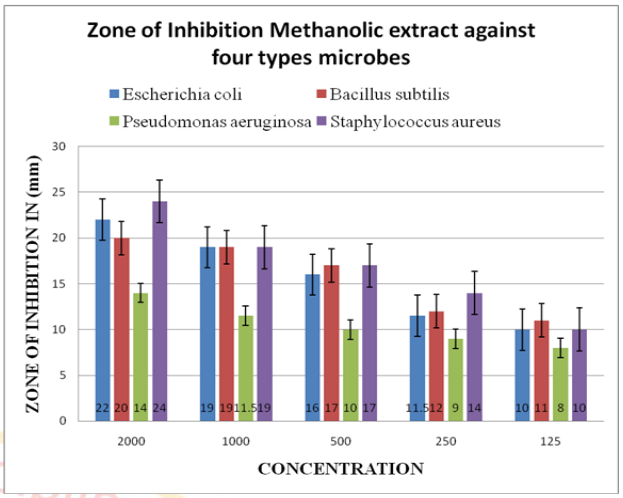
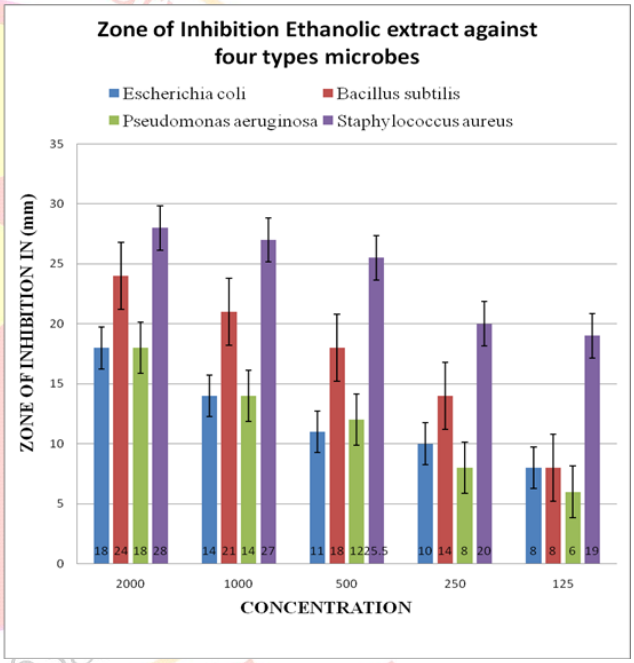
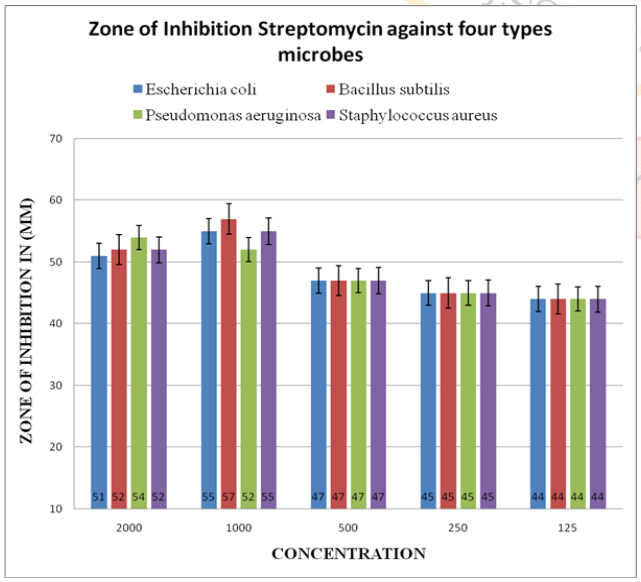
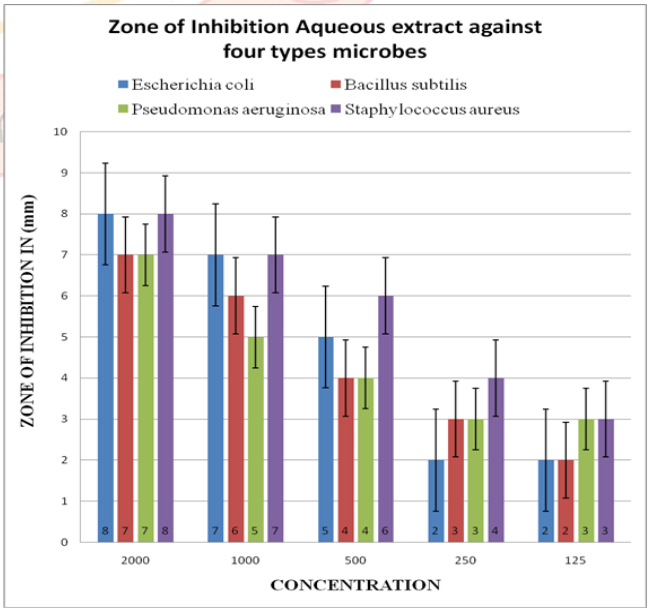


Table 4 Zone of inhibition Water against four types microbes

Pathogens	Concentration of Streptomycin (µg/ml)	Zone of Inhibition in (mm)				
		2000	1000	500	250	125
Escherichia coli		8	7	5	2	2
Bacillus subtilis		7	6	4	3	2
Pseudomonas aeruginosa		7	5	4	3	3
Staphylococcus aureus		8	7	6	4	3







Conclusion:

Plant leaf extracts of Adathodavasica 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 *Adhatodavasica* 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 Adhatodavasica has excellent antibacterial activity against the all tested bacteria. (Sagar et al., 2013),(Josephin et al., 2012). Thus, there is a possibility of developing Adathodavasica 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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