Evaluation of Antibacterial activity of *Adhatoda vasica Leaves Extract on* Pseudomonas aeruginpsa

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Abstract-

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 *Pseudomonas aeruginosa*. 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.

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 *Pseudomonas aeruginosa* bacteria.

Keyword - Pharmacauticals, *Adhatoda vasica*, antibacterial, *Pseudomonas aeruginosa.*

Introduction:

Antimicrobial screening is the first stage of antimicrobial drug research to ascertain the susceptibility of pathogenic microorganisms to any plant agent. Herbal industries always use the crude extracts of medicinal plants for their medicine and beautifying constituents. In most cases, they use medicinal plant crude extract without confirming or validating their level of toxicity and active ingredient. However, it is necessary to know which compound is specifically responsible for showing specific activity. Evergreen harbaceous basok (Adhatoda vasica Nees., Acanthaceae) is a medicinal plant native to Asia, found abundantly in wild in all over Nepal, India, Pakistan and Bangladesh. From prehistoric time, plants are used for their medicinal property. The active ingredients present in plant are many but mainly used and important are Alkaloids – bitter tasting chemicals. Glycosides – drug containing these are used to support the beating of the heart and act as diuretics; Polyphenols – drugs used to treat gynaecological problems; Terpenes – strongly aromatic and used by plant to repel het bivores. Leaves, bark, roots, flower of Adhatoda are full of medicinal property. Various extract of plants are used to care pulmonary, bronchial and asthmatic disorders. It is also used to speed childbirth (kanthale et al., 2014). The adhatodha vasica leaves extract were screened for their Phytochemical content. Quantitative test were used to detect the presence of alkaloids, tannins, flavonoids, saponins, phenolic acid. Presence of these phytochemicals in the medicinal plants indicates the presence of antibacterial, antispasmodic and various chest ailments properties against *Pseudomonas aeruginosa and other* micros . Various parts of the plant are used for the preparation of medicines for various ailments. Plants make many chemical compounds to protect themselves against fungi and bacteria. Plants use as drugs has undergone tremendous increase as pathogens are developing resistance against the frequent use of drugs. New drugs with better effect are needed to provide good health facility. Another benefit of these drugs are that they are safe and costeffective, a gift for the non-industrialized and underdeveloped countries. India is a large repository for herbal plants in world. Major diseases are also treated by the herbal plants. AYURVEDA and UNANI works on these medicinal plants help to provide their benefit to people.

Aim of this study is to identify various medicinal properties of Adhatodha vasica plant. Leaf

was the main part of the plant used for the observation of medicinal properties.

Materials And Methods

Plant material and extraction

The dried mature leaves of Adhatodha vasica were collected from local area in Jhunjhunu (Rajasthan), India. 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 evaporator at 40°C ,and kept at 4°C in refrigerator in airtight bottle until further use. (akhter et al, 2014 And charan et al.,2018)

Collection of Microorganisms

Pure bacterial cultures of Pseudomonas aeroginosa were obtained from the Microbial Type Culture Collection (MTCC)/Institute of microbial technology (IMTECH),Chandigarh. The strains were grown in NB medium and incubated at 37ºC for overnight.

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

Antibacterial activities of all the extracts (ethanol, methanol, aqueous) of A. vasica were determined by agar disc diffusion method. In this method DMSO (dimethylsulphoxide) was dissolved in the extract to diferent concentrations. Commercial antibiotic (Straptomycin) and DMSO was taken as positive and negative control respectively. 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. The test was done in triplicates and the final results obtained were presented as the mean zone of inhibition.

Result And Discussion

The inhibitory effects of the extracts from *Adhatoda vasica* on bacterial strains in Mueller Hinton agar (Hi Media) are shown in Table 1. Inhibition zones were measured in mm at 24 hour after incubation. Streptomycin was used as appropriate controls. The results indicated that the maximum inhibition zone is showed by the ethanolic extract against the *Pseudomonas aeruginosa* where as, *In* vivo other extracts showed minimum inhibition zone against tested bacterial strain, while the water showed least activity of tested strain. In *Pseudomonas aeruginosa,* both (Methanol and Ethanol) extract shows similar decreasing pattern but among them ethanol show strong inhibition.

Similar results and method with other plant extract against different bacteria and fungus were reported by others (Saraf *et al.*, 2011). The antimicrobial activity may be due to the presenceof triterpene secondary metabolite in the extract. Similarly, Barre *et al.,* (1997) have reported a bioactive triterpene- 22 beta aceto xylantic acid and other triterpenes which showed antimicrobial activity against Staphylococcus aureus and Salmonella typhii. Antibacterial activity of different plant extract on phytopathogenic Xanthomonas campestris pathovars was studied and reported by other workers (Mazumder *et al.,* 2004). Methanol extract of various parts of Lagerestroemia parviflora Roxb exhibited antifungal activity against Aspergillus and Penicillium species and yielded good zones of inhibition. Thus, the extract was found to be fungistatic in its action (Sharma *et al.,* 1988). Antibacterial activity of Methanolic leaf extract of *P. guajava* was determined against *S. aureus, E. coli, P.aeruginosa*, and the fungus *C. albicans* and *A. niger* by the paper disc diffusion

method supported by the tubidimetric method (Dhiman *et al.,* 2011).

Therefore, present work highlights the use of solvent extracted leaves extracts of *Adhatoda vasica* containing a highly potential phytochemical which could be characterized .

Table 1 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

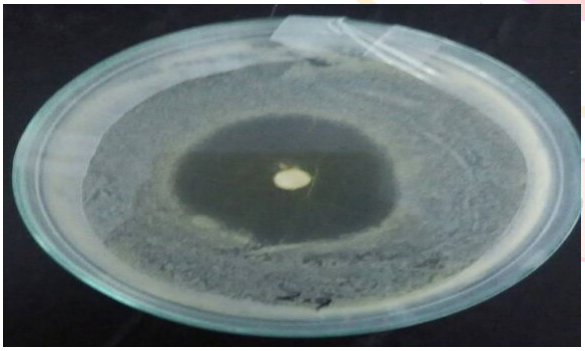

Fig. 5(D)

Zone of maximum inhinbition ethanolic extract (20mm) against *Psuedomonas aeruginosa*

Conclusion

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.

The present study revealed that *Adathoda vasica* has a broad spectrum of antibacterial activity and potential source of antimicrobial agents. From the results obtained during the present study, we can conclude that the leaf extract of Adhatoda vasica has excellent antibacterial activity against the tested bacteria. (Sagar et al., 2013),(Josephin 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).

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