



EVALUATION OF ANTIBACTERIAL STUDIES IN HARSINGAAR

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ABSTRACT

Plants are rich source of antibacterial agents, which could be control human diseases. Present investigation was an undertaken to evaluate the antibacterial of Harsingar (*Nyctanthes arbortristis* Linn) against five important human pathogenic bacteria i.e. *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* by filter paper disc diffusion method. Dried leaves of *Nyctanthes arbortristis* were collected, washed, shade dried and powdered. Aqueous and methanol extracts were prepared and evaluate their antibacterial activity. The significant result of antibacterial activity was observed in aqueous as well as methanol leaves extract. The strongest antibacterial activity of aqueous leaves extract was observed in *Pseudomonas aeruginosa* with (22.00 mm) zone of inhibition while the methanol leaves extract showed strongest antibacterial activity against *Staphylococcus aureus* with (28.00 mm) maximum zone of inhibition.

Keywords: Human Pathogenic bacteria, *Nyctanthes arbortristis* Linn., antibacterial activity.

INTRODUCTION

Medicinal plants contain some organic compounds which provide definite physiological action on the human body as well as their physiological activities due to the presence of bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids¹. These phytochemical compounds are synthesized by secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas². A large number of phytochemicals belonging to several chemical classes has been shown to have inhibitory effects on all types of microorganisms in vitro³. Plants and their products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds⁴. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances these chemical substance use as an antibacterial agents⁵. Harsingar (*Nyctanthes arbortristis* Linn.) belongs to Family Oleaceae, is a well documented herb as well as tree plant. It is a native of India, distributed wild in sub-Himalayan region and also found in Indian garden as ornamental plant. The indigenous people of India especially Andhra Pradesh widely use the whole plant for treatment of cancer, root for fever, sciatica, anorexia. The bark is use as expectorant; Leaf for control fever, diabetes and as cholagogue, diaphoretic and anthelmintic. Various extracts of the plant is used to treat arthritis⁶, malaria, intestinal worm's tonic and laxative, antitypanosomal, anti-inflammatory and antioxidant activity⁷. In the light of these above mentioned facts, the present investigation was carried out on antibacterial evaluation of *Nyctanthes arbortristis* L. against five human pathogenic bacteria namely, *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh leaves of *Nyctanthes arbortristis* were collected from various places of Agra (U.P.), India. The leaves were washed under running tap water and shade dried for three weeks. The dried leaves were then homogenized by using a grinder to make fine powder and stored in air tight bottles.

Preparation of Aqueous Extract

The plant samples were air dried for 48 hours and ground into uniform powder using a grinder. 15g of dried powder was taken in 250 ml distilled water in separate conical flasks, air tight with cork and then kept on a shaker for 8 hours. After it the extract were filtered by using a vacuum filtration system and stored at 4°C degree in airtight containers.

Preparation of Methanol Extract

The collected leaves were washed twice in running tap water and once with sterile distilled water subsequently. The leaves were shade dried for three weeks and made to coarse powder. The powder of leaves was passed through whatman filter paper No. 40 to achieve uniform particle size and then used for extraction process. A weighed quantity of the powder was subjected to continuous hot extraction in soxhlet apparatus with 85 % methanol solvent. The extract was dried using rotatory vacuum evaporator and they give molten extract and store at 4°C until further use.

Microorganism and Culture Condition

Present investigations were carried out on five human pathogenic bacteria viz. *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *proteus vulgaris* and *Pseudomonas aeruginosa*. Bacteria cultured were maintained on Muller Hinton (MH) medium. The antibacterial activity was observed for aqueous and methanol leaf extract of *Nyctanthes arbortristis*.

Antibacterial Evaluation

Valuation of antibacterial activity was carried out by paper disc method⁸. High media sterile disc were used for activity, saturated disc with the extract (0.04 ml) and known quantity

of standard reference antibiotic separately were air dried at room temperature. The molten Muller Hinton (hi media) was inoculated with the 100 ml of the inoculums and poured into sterile Petri plates (borosil). The disc with test compound placed on the upper surface of sterilized Muller Hinton plate that had been inoculated with the test organism (using a sterile swab) and air dried to remove the surface moisture.

The thickness of MH medium was kept equal in all Petri plates and the standard disc (tetracycline) was used in each plate as control. The plates were inoculated 24 hours at 37°C in incubator. After 24 hours growth of bacteria was measured for its zone of inhibition. The results were obtained by measuring the zone diameter. The experiment was conducted in replicates of three and the mean value is presented.

Table 1: Antibacterial Potential of Aqueous Leaves Extract of *Nyctanthes arbortristis* L against Five Human Pathogenic Bacteria

Bacteria	Zone of inhibition (mm)		
	Aqueous Extract	Antibiotic (Chloramphenicol)	Control (water)
<i>Escherichia coli</i>	20.00	17.00	0
<i>Staphylococcus aureus</i>	19.00	21.00	0
<i>Salmonella typhimurium</i>	16.00	17.00	0
<i>Proteus vulgaris</i>	16.00	13.00	0
<i>Pseudomonas aeruginosa</i>	22.00	18.00	0

Table 2: Antibacterial potential of Methanol Leaves Extract of *Nyctanthes arbortristis* against Five Human Pathogenic Bacteria

Bacteria	Zone of inhibition (mm)		
	Methanolic extract	Antibiotic (Strptomycine)	Control (water)
<i>Escherichia coli</i>	23.00	20.00	0
<i>Staphylococcus aureus</i>	28.00	24.00	0
<i>Salmonella typhimurium</i>	22.00	19.00	0
<i>Proteus vulgaris</i>	22.00	20.00	0
<i>Pseudomonas aeruginosa</i>	19.00	18.48	0

RESULTS AND DISCUSSION

The results obtained from antimicrobial activities in various solvent of leaves extract of *Nyctanthes arbortristis* L. by disc diffusion have been described. The antibacterial activity in aqueous as well as methanol leaf extract of *Nyctanthes arbortristis* L. against five human pathogenic bacteria i.e. *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *proteus vulgaris* and *Pseudomonas aeruginosa* were summarized in Table 1 and 2. The present observations clearly indicate that the tested extracts exhibit potential antibacterial activity against all the tested bacteria. The aqueous leaves extract showed maximum antibacterial activity against *Pseudomonas aueruginosa* with (22.00 mm) maximum degree of zone of inhibition and least antibacterial activity was observed against *Proteus vulgaris* with (16.00 mm) minimum zone of inhibition>however, the normal antibacterial activities were observed in *Salmonella typhimurium*, *Escherichia coli* and *Staphylococcus aureus*. The methanols leaves extract of this plant also have potential to show antibacterial activity against all the test bacteria. The methanol extract exhibits highest antibacterial activity against *Staphylococcus aureus* with (28.00 mm) maximum zone of inhibition. However, the lowest antibacterial activity was recorded against *Proteus vulgaris* with (23.00 mm) minimum zone of inhibitory growth. The all zone of inhibition produced by aqueous as well as methanol leaves extract were higher or similar than corresponding zone of antibiotic. The result of the present investigations on methanol leaves extract of *Nyctanthes arbortristis* L. also revealed the significant antibacterial activity against all test human pathogens. Methanol leaves extract of *Nyctanthes arbortristis* L. exhibited the highest antibacterial activity against *Staphylococcus aureus* with (26.00) maximum inhibitory growth. The various workers have been made intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine.^{9,10} All the leaves extract of *Nyctanthes arbortristis* were able to inhibit the growth of all tested human pathogenic bacteria. This activity may be due to the presence of the active antibacterial principles of the plant and

their parts. Plants are important source of active principle responsible for antibacterial activities and in the development of new drugs for the therapeutic use in human beings. Some of these findings have helped in search of new active principle for infectious diseases causes by tested bacteria. Now a days the discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases in human beings. However, the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency.¹¹ There is a universal problem that the antibiotics are sometimes associated with adverse side effects on the host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reactions.¹² The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection.¹³ The development of new antibiotics should be continued as they are of primary importance to maintain the effectiveness of antimicrobial treatment by plant extracts like The discovery and development of antibiotics are among the most powerful and successful achievements of modern science or the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency.¹¹ The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase uses of has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection.¹³ However, the development of new antibiotics should be continued as they are of primary importance to maintain the effectiveness of antimicrobial treatment by plant extracts like *Nyctanthes arbortristis* L. The present paper concluded that the methanol extracts were

more effective than aqueous extracts of *Nyctanthes arbortristis* L. showed strong antibacterial activity against all the human pathogenic tested bacterial strains. Hence, *Nyctanthes arbortristis* L. can be used to bioactive natural phytochemical compounds that may serve as leads in the development of new antibacterial activities.

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