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Research Paper

Evaluation of Antibacterial Activity of Crude Extracts of Nyctanthes Arbor-*Tristis* Leaves Against Staphylococcus Aureus, Escherichia Coli, Pseudomonazs Aeruginosa and Bccillus Subtilis

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ABSTRACT

The management of various diseases and the age-old subject of treatment along with Ayurveda and home remedies play a crucial role in the Human therapy. Therefore, adopting an idea from home remedies and Ayurveda, these studies was designed and conducted to demonstrate the anti-bacterial application of Nyctanthes arbor-tristis leaves scientifically. Solvents acetone, ethanol, acetic acid, and water were utilized to prepare the extracts.Antibacterial activities of various extracts were performed against four bacteria, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bccillus subtilis, by agar cup diffusion technique. Zone of inhibition caused by various extracts were quantified. Acetic acid extract of Leaves of Nyctanthes arbor tristis exhibited antibacterial activity against all tested bacteria in the sequence of sensitivity as Staphylococcus aureus>Pseudomonas aeruginosa>Escherichia, coli>Bacillus subtilis. Antibacterial effect of Staphylococcus aureus was sensitive having 8, 12, 24 and 6 mm zone of inhibition at 10 mg mL-1 of Acetone, Ethanol, Acetic acid and Water extracts respectively. Zone of inhibition is 10, 12, 20, 8 mm for Acetone, Ethanol, Acetic acid and Water extracts respectively for, Pseudomonas aeruginosa. Escherichia coli demonstrated no zone of inhibition for water extract while it has 2, 10, 18 mm zones of inhibition at 10 mg mL-1 for Acetone, Ethanol, Acetic acid respectively. Bacillus subtilis exhibited 4, 9, 20 and 3 mm zone of inhibition at 10 mg mL-1 for Acetone, Ethanol, Acetic acid and Water extracts. The leaves of the plant are observed to act as the highest green constituent with anti-bacterial activity against various Staphylococcus Aureus, Escherichia Coli, Pseudomonazs Aeruginosa and Bccillus Subtilis pathogens studied.These findings indicated that the Acetic acid extract possesses a broad spectrum of antibacterial activity compared to the Acetone, Ethanol and Water extracts.

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INTRODUCTION

Medicinal plant life possesses varied antimicrobial marketers, which may be effective against a broad range of microbes, that's why maximum medicinal plant life is widely utilized for disease control as a whole and, at times, the unique constituents of the plant [1].At times samples of different solvents are arranged to check the antimicrobial activity (anti-bacterial activity). Such medicinal plant species exhibit their importance because of the reason that due to constant use of selective antimicrobial marketers, the microbes will be likely to develop resistance homes of their own due to the R-gene donation because the greater chromosomal DNA of Bacteria and other microbes, in this regard, maximum of the medical categories are looking for special medicinal plant species to harvest special active compounds and special homes to formulate new composition and target the various, unique constituents of microbes, primarily microorganism to kill and prevent the growth of micro organism to control bacterial disease [2]. Nyctanthes arbor tristis, also referred to as Harsingar and Parijat, is from the own family Oleaceae [3]. Nyctanthes arbor-tristis has been proven via numerous prior researches to possess anti-bacterial, antifungal, antiviral, analgesic, and antipyretic properties [4]. These flowers also possess antimalarial, antihelminthes, and antiallergic properties [5]. Various recent researches exhibit that this flower also possesses anti-oxidant and liver-protecting properties. In this research work, the leaves of those plants which survive from unique geographical locations are accumulated and analyzed for antimicrobial activity, primarily anti-bacterial residences against Staphylococcus aureus, Escherichia coli, Pseudomonczs aeruginosa and Bccillus subtilis [6-10].

Nyctanthes arbor-*tristis*

Fig.1 Leaves of Nyctanthes arbor *tristis*

Nyctanthes arbor tristis is also referred to as night flowering jasmine, parijat, and shiuli. It is a member of its own family of Oleaceae [11]. The plant is found in South Asia and Southeast Asia. It is a small tree 10-30 ft long [12]. Their leaves are simple and opposite 6-12cm long and 2-6. Five cm wide with the entire margin [13]. This tree is at times referred to as the Tree of Sorrow because the plant life of this plant becomes dull in the evening. This plant has further mentioned its role and significance in our Veda [14]. The leaves of this plant are used in ayurvedic medicines and homeopathy and domestic medications to cure pain and fever, and various infections. Nyctanthes arbor tristis, contains mannose and glucose that forms a mucilaginous layer around the urinogenital, gastrointestinal, and respiratory tract upon oral consumption [15-17]. The layers catch the microbial flora and render them incapable of invading the system. Hence, the bacteria are unable to develop in the media with Nyctanthes arbor tristis extract. The leaves of this species contain many active compounds such as D-mannitol, β-Sitosterol, Flavanol, Glycosides, Nicotiflorin, Oleanolic acid, Tannic acid, Ascorbic acid, Methyl salicylate, glycosides, carotene, glucose, and benzoic acid [18-20].

Collection and Authentication of Plant:

Leaves of the plant of Nyctanthes arbor- *tritis* L. were collected from the Company Garden, Prayagraj, U.P., India, during the month of

October. Taxonomically identified and verified by Botanical survey of India, Prayagraj and herbarium specimen was prepared and depicted in the department herbarium.

Preparation of extracts

Washed thoroughly Nycanthes arbor tristis Leaves, dried on paper towel, and then oven-dried at 600 C. The dried powder of the plant (20 g) was taken and dissolved in 100 mL of different solvents Acetone, Ethanol, Acetic acid and Water (from Lower to Higher polarity) and stored for 72 h, the extract was filtered and collected. This process is done three times for effective extraction. The collected extracts were combined, dried to dryness using rotary evaporator. The residues were then resuspended in DMSO stock concentration of 100 mg mL-1.

Qualitative Phytochemical Test:

Various tests
are performed to examine for the presence of anthraquinone, terpenoids, saponin, flavonoid, and glycosides as per try that is standard.

Anthraquinone Test

1 ml of leaves extract is boiled with 2 ml of sulfuric acid (H_2SO_4) and filtered hot. Now, the filtrates are shaken with 2 ml of chloroform. The chloroform layer is removed carefully by Pipette and 2 ml of ammonia, and blue or red color change is observed with the presence of anthraquinone [21].

Test for Terpenoids:

1 ml of leaves extract had been mixed with 4 ml of chloroform. Now carefully concentrate (H_2SO_4) 6 ml to prepare a layer. The development of a reddish-brown color the definite presence of terpenoids.

Saponin test:

0.5ml of leaves extract is mixed with 2 ml of distilled water within a test shake and pipe regularly to observe mixture development. Now the mixture is mixed with 3 drops of essential olive oil and shakes regularly, and watches the synthesis of emulsion that shows the formation of saponins.

Flavonoid test:

2 ml of diluted ammonia is added along with all the extracts of various solvents of leaves, and now 1 ml of concentrated H2SO4 is added. Yellow precipitates that fade with time indicate the presence of flavonoids.

Test for Glycosides:

100µl of extracts taken in test tubes and add 2.0 ml of chloroform then 3.0 ml of concentrated H_2SO_4. Reddish-brown shows the presence of terpenoids and greenish yellowish formation the presence of steroids.

Microorganism Tested

Following microorganisms were utilized: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bccillus subtilis.
They were collected in December 2009 from National Collection of Industrial Microorganisms (NCIM) National Chemical Laboratory, Pune and cultured on nutrient agar. The four bacteria mentioned above cause nosocomial infections.

Determination of Antimicrobial Activity:

Antibacterial susceptibility tests were conducted employing agar diffusion method. The microorganisms were grown overnight at 370 C in nutrient agar. Bacterial inoculants final cell concentrations were 106 -107 CFU mL-1. Petri plates of 60 mL sterile nutrient agar were taken

with the correct bacterial suspension. The extracts were added to wells and the plates were incubated at 370C for 24 h, this occurrence of zone of inhibition was considered as the occurrence of antimicrobial action and antimicrobial activity was quantified in terms of average diameter of the zone of inhibition in millimeter. Each test was performed in triplicate.

RESULTS:

Total plant extracts of Nyctanthes arbor tristis Leaves (Aqueous and Ethanolic) are found to inhibit gram positive bacteria, S. aureus and B. subtilis, but the inhibitory activity was extremely low in aqueous extract (6-3 mm, Table 1) compared to acetic acid extract (24-20 mm), Ethanol extract (12-9 mm) and Acetone extract (8-4 mm). Similar trend was also found with gram negative bacteria, E. coli, P. aeruginosa (Table 1), relatively higher MIC values were found for gram negative bacteria E. coli (Table l) with acetic acid extract. Interestingly, there has been no inhibitory action observed for aqueous extract, this may be due to active component of Nyctanthes arbor tristis Leaves getting extracted in acetic acid instead of water. Finding indicates that the glacial acetic acid extracts have immense inhibitory action on gram positive bacterium, S. aureus followed by B. subtilis (Fig. 1). Among gram negative bacteria maximum inhibitory effect was noted with Nyctanthes arbor- tristis, then E. coli (Fig. l)

In this paper, we have described that acetic acid extract of Nyctanthes arbor tristis Leaves possesses very good antibacterial activity towards gram negative as well as gram positive bacteria with a very low MIC.

Table 1: Antibacterial activity of Nyctanthes arbor tristis Leaves extracts: Acetone, Ethanol, Acetic Acid, and Water. Minimum inhibitory concentration (MIC) of Acetic acid plant extracts against human pathogenic bacteria

Minimum Inhibitory Concentration of Bacteria						
Type	**Bacteria Zone of inhibition (mm)**	**Acetone Extract**	**Ethanol Extract**	**Acetic Acid Extract**	**Water Extract**	**MIC for Acetic Acid Extract (mg mL^{-1})**
Gram Positive	Staphylococcus aureus	8.0	12	24	6.0	0.349
	Bacillus subtilis	4.0	9.0	20	3.0	0.273
Gram Negative	Escherichia coli	2.0	10	18	-	0.78
	Pseudomonas aeruginosa	10	12	20	8.0	0.349

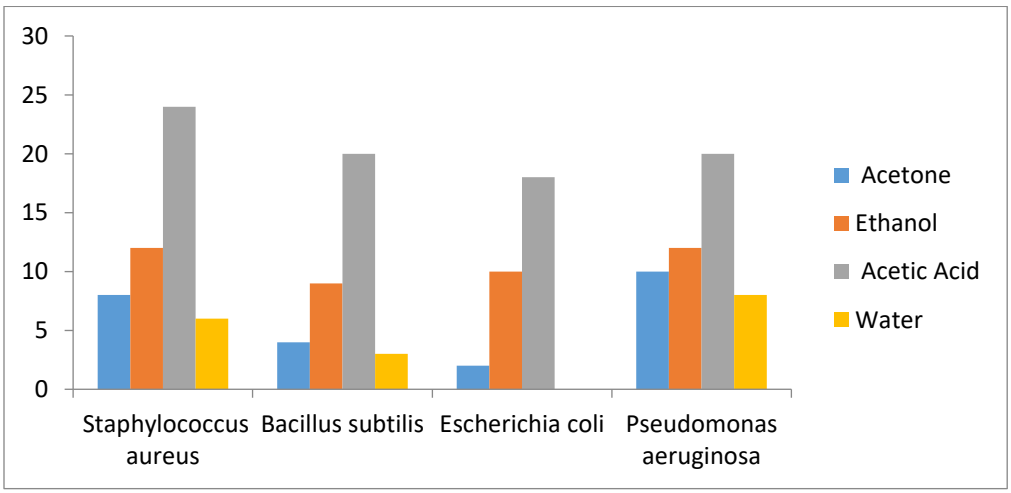

Fig: 2 Inhibitory effect of Acetone, Ethanol, Acetic acid, and Water Extracts of Nyctanthes Arbor tristis Leaves on different bacterial pathogens

DISCUSSION:

The current research strongly proved that Nyctanthes arbor tristis Leaves possesses intense antibacterial activity. The above findings indicate that acetic acid extract possesses maximum antibacterial activity. The result indicates that the phytoconstituents causing antibacterial activity are polar in nature and extracted in the polar solvents only. The chemical constituents, flavonids, terpenoids, saponins, and glycosides, mannans and saponins are the primary and secondary phytochemicals which are accountable for the various medicinal property found in Nyctanthes arbor tristis.

CONCLUSION:

The above findings indicated that Nyctanthes arbor tristis possess very strong antibacterial activity. Therefore, from the above study it can be inferred that the plant Nyctanthes arbor tristis can be utilized as a strong antimicrobial agent in the treatment of diseases. Therefore, further study can be performed on the isolation process for determining the precise active moiety involved in biological activity.

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CONFLICT OF INTEREST

I hereby declare that there is no conflict of interest.

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