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REMARKABLE POTENTIAL OF DIFFERENT EXTRACTS OF *NYCTANTHES ARBOR-TRISTIS* LINN BARK ON VARIOUS HUMAN PATHOGENS

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ABSTRACT

Exact goal of this study is to evaluate the tremendous potential of different extracts of *Nyctanthes Arbor-tristis* Linn bark on against gram positive and gram negative human bacterial pathogens and fungal strains. Disc diffusion test was used to evaluate the anti microbial activity of the chloroform, methanol and aqueous extracts against five Gram positive and five Gram negative bacterial strains and seven fungal strains. All different extracts were subjected to phytochemical analysis. The pattern of inhibition varied with the solvent used for extraction and the microorganism tested. Among these extracts, ethanol and aqueous extracts showed significant antibacterial activity against most of the tested microbes. The most susceptible microorganism was *P. aeruginosa* (31 mm zone of inhibition in Ethanol extract). Pet. ether and ethanol extracts showed encouraging anti fungal activity against the tested fungal pathogens; the most susceptible microorganism was *Candida albicans* (26 mm zone of inhibition in pet. ether extract). Preliminary phytochemical analysis of different extracts revealed the presence of alkaloids, Steroids, glycosides, saponins, phenolic compounds and tannins. The different crude bark extracts of *Nyctanthes Arbor-tristis* Linn were inhibited the growth of bacteria and fungi, it is an indication of its broad spectrum antimicrobial potential, which are comparable with those of standard drugs, which may be employed in the management of microbial infections.

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INTRODUCTION

Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health^[1]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have lead to the screening of several medicinal plants for their potential antimicrobial activity^[2,3]. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being^[4]. Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses^[5].

Nyctanthes arbor-tristis Linn commonly known as Harsinghar or Night Jasmine is one of the well known medicinal plants. Different parts of *Nyctanthes arbor-tristis* are known to possess various ailments by rural mainly tribal people of India (Orissa and Bihar) along with its use in Ayurveda, Sidha and Unani systems of medicines. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic^[6-8]. Leaves are also used in the enlargement of spleen. Traditionally the powdered stem bark is given in rheumatic joint pain, in treatment of malaria and also used as an expectorant^[7]. Leaves extracts was found to have antimicrobial activity^[9] but no report is available on the antimicrobial activity on the stem bark part, so the present study is aimed at the screening of the antimicrobial activity in the stem bark extracts of the plant *Nyctanthes arbor-tristis* Linn.

OBJECTIVES:

To evaluate the tremendous potential of different extracts of *Nyctanthes Arbor-tristis* Linn bark on against gram positive and gram negative human bacterial pathogens and fungal strains.

MATERIAL AND METHODS

Collection of plant

The fresh barks of *Nyctanthes arbor-tristis* Linn were collected in the month of Feb from sangameswarar temple near Bhavani (Erode District) Tamil Nadu. It was then authenticated by Prof. P

Jayaraman, Ph.D., Plant Anatomy Research Centre, Chennai, Tamil Nadu. The voucher specimen was deposited at the department for future reference.()

Extraction of plant material

The bark was collected by felling method. The bark cuttings were collected and washed with water and dried in shade. About 400g of air dried powdered bark was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days. At the end of second day the powder was taken out and it was dried. It was further extracted with chloroform, ethanol and aqueous solvents at ambient temperature. The extracts was filtered and concentrated to a syrupy mass (Yield 1.2, 2.5, 10, 6.3 % w/w respectively) under reduced pressure at 50-55°C.

Preliminary phytochemical analysis of leaves extracts:

Phytochemical examinations were carried out for all the extracts as per the standard methods^[10, 11].

Test microorganisms:

The test microorganisms used in this study was:

Bacterial strains - Gram positive: *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus pyogenes*. Gram negative strains: *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*.

Fungi: *Trichophyton longifusus*, *Candida glaberata*, *Candida albicans*, *Penicillium notatum*, *Aspergillus niger*, *Aspergillus flavus*, *Microsporum canis* were obtained from NCIM (national collection of industrial microorganisms, National chemical laboratory, pune). The bacterial isolates were first subcultured in a nutrient broth (Oxoid) and incubated at 37°C for 18 h while the fungal isolates were subcultured on a Sabouraud dextrose agar (SDA) (Oxoid) for 72 h at 25°C.

Screening for anti bacterial activity:

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method^[12]. The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (106 cfu/ml-1). Two hundred micro liter of the standardized cell suspensions were spread on a Mueller-Hinton agar (Oxoid). Wells were then bored into the agar using a sterile 6 mm diameter

cork borer. Approximately 100 µl of the crude extract at 10 mg/ml were introduced into the wells, allowed to stand at room temperature for about 2h and then incubated at 37°C. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The plates were observed for zones of inhibition after 24 h. The effects were compared with those of ciprofloxacin a concentration of 1 mg/ml

Screening for anti fungal activity:

The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) (Oxoid) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. The harvested fungal spores and bacterial isolates were standardized to an OD_{600nm} of 0.1 before use. One hundred microliter of the standardized fungal spore suspension was evenly spread on the SDA (Oxoid) using a glass spreader. Wells were then bored into the agar media using a sterile 6 mm cork borer and the wells filled with the solution of the extract taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Plates were incubated at 25°C for 96 h and later observed for zones of inhibition.

Table – 1 Anti bacterial activity of different extracts of *Nyctanthes arbor tristis* Linn bark.

Tested bacterial strains	Diameter of zone of inhibition (mm)				
	Standard drug	Pet. Ether extract	Chloroform extract	Ethanol extract	Aqueous extract
<i>Bacillus subtilis,</i>	22	4	9	20	-
<i>Bacillus megaterium</i>	17	-	-	-	14
<i>Staphylococcus epidermidis</i>	29	7	-	25	-
<i>Staphylococcus aureus</i>	28	5	12	24	23
<i>Staphylococcus pyogenes</i>	-	-	-	22	-
<i>Escherichia coli</i>	31	10	7	30	-
<i>Shigella dysenteriae</i>	25	9	0	0	19
<i>Pseudomonas aeruginosa</i>	32	6	9	31	-
<i>Salmonella typhi</i>	-	-	-	-	16
<i>Klebsiella pneumoniae</i>	21	3	8	19	18

Screening for anti fungal activity:

All the four different extracts of *Nyctanthes arbor-tristis* Linn showed encouraging antifungal activities against

The effect of the extract on fungal isolates was compared with miconazole at a concentration of 1 mg/ml.

RESULTS:

Preliminary phytochemical analysis of the extract:

Preliminary phytochemical analysis of the plant extracts (Petroleum ether, chloroform, ethanol and aqueous) showed the presence of alkaloids, Steroids, glycosides, flavanoids, saponins, phenolic compounds and tannins.

Screening for anti bacterial activity:

All the four different extract of *Nyctanthes arbor-tristis* Linn showed varying degree of antibacterial activities against the test bacterial species (Table 1). The antibacterial activities of the ethanol and aqueous extracts compared favorably with that of standard antibiotic. Ethanol extract was found to be active against seven out of ten tested bacterial strains viz *P. aeruginosa*, *E. coli*, *S. epidermidis*, *S. aureus*, *S. pyogenes*, *B. subtilis*, and *K. pneumoniae* with the zone of inhibition of 31mm, 30mm, 25mm, 24mm, 22mm, 20mm and 19mm respectively. On the other hand, the aqueous extract was effective against five out of ten tested bacterial strains viz *S. aureus*, *S. dysenteriae*, *K. pneumoniae*, *B. megaterium* and *S. typhi* with the zone of inhibition of 23mm, 19mm, 18mm, 16mm and 14mm respectively.

the tested fungal species (Table 2). The antifungal activities of the pet.ether and ethanol extracts compared favorably with that of standard antibiotic.

Pet.ether extract was found to be active against *Candida albicans*, *Aspergillus niger*, *Penicillium notatum*, *Candida glaberata* with 26mm, 25mm, 24mm, and 19mm zone of inhibition respectively. Where in turn ethanol extract

was found to be active against *Candida albicans*, *Penicillium notatum*, *Aspergillus niger* with 23mm, 23mm and 21mm zone of inhibition respectively.

Table – 2 Anti fungal activity of different extracts of *Nyctanthes arbor tristis* Linn bark

Tested fungal strains	Diameter of zone of inhibition (mm)				
	Standard drug	Pet.Ether extract	Chloroform extract	Ethanol extract	Aqueous extract
<i>Trichophyton longifusus</i>	24	4	-	4	-
<i>Candida glaberata</i>	21	19	7	3	-
<i>Candida albicans</i>	28	26	8	23	6
<i>Penicillium notatum</i>	25	24	10	23	5
<i>Aspergillus niger</i>	26	25	9	21	8
<i>Aspergillus flavus</i>	23	5	-	-	-
<i>Microsporum canis</i>	19	3	-	2	-

DISCUSSION:

Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects [13, 14]. The present study was to evaluate the different pet.ether, chloroform, ethanol and aqueous extracts of *Nyctanthes arbor tristis* Linn

were evaluated for its antibacterial and antifungal activity against the different pathogens.

In screening for anti bacterial activity, among all tested Gram positive organisms *S. epidermidis*, *S. aureus*, in Gram negative organisms' *P. aeruginosa*, *E. coli* was more susceptible to the ethanol extract. Aqueous extract was active against *S. aureus* in Gram positive organisms and *S. dysenteriae*, *K. pneumoniae* in Gram negative organisms. (Fig -1, Fig-2)

Figure – 1 Anti bacterial activity of different extracts of *Nyctanthes arbor tristis* Linn bark on Gram positive bacterial strains

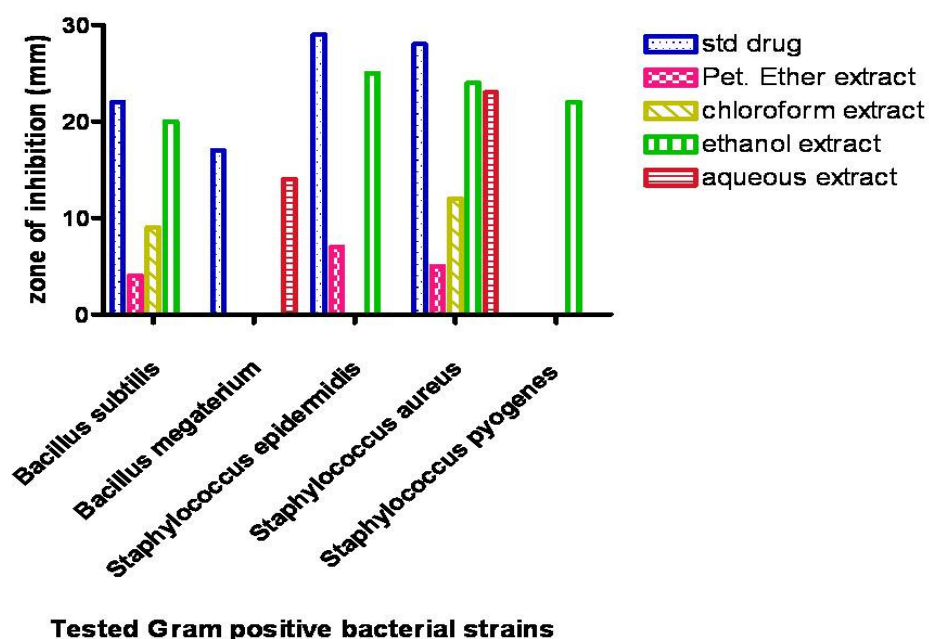
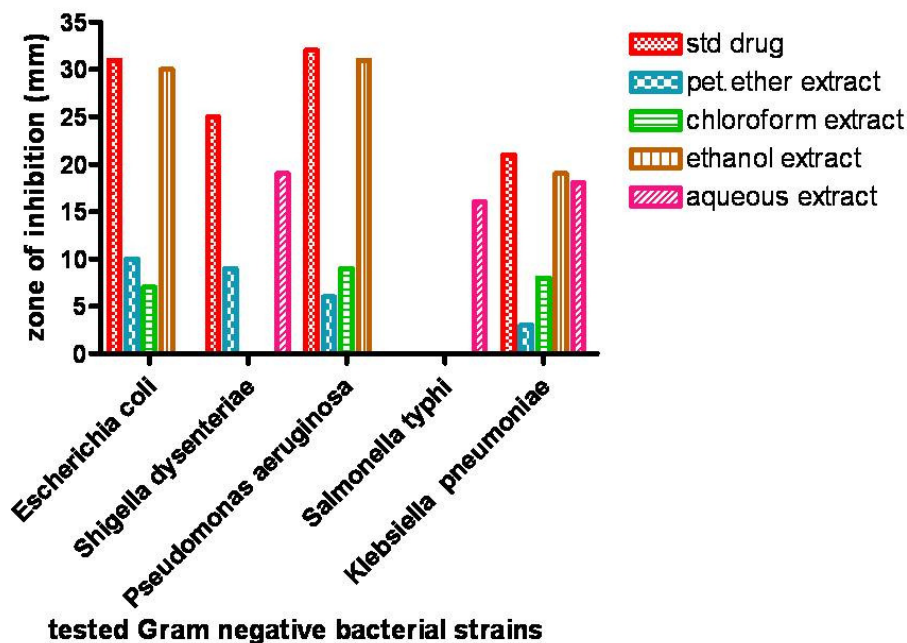


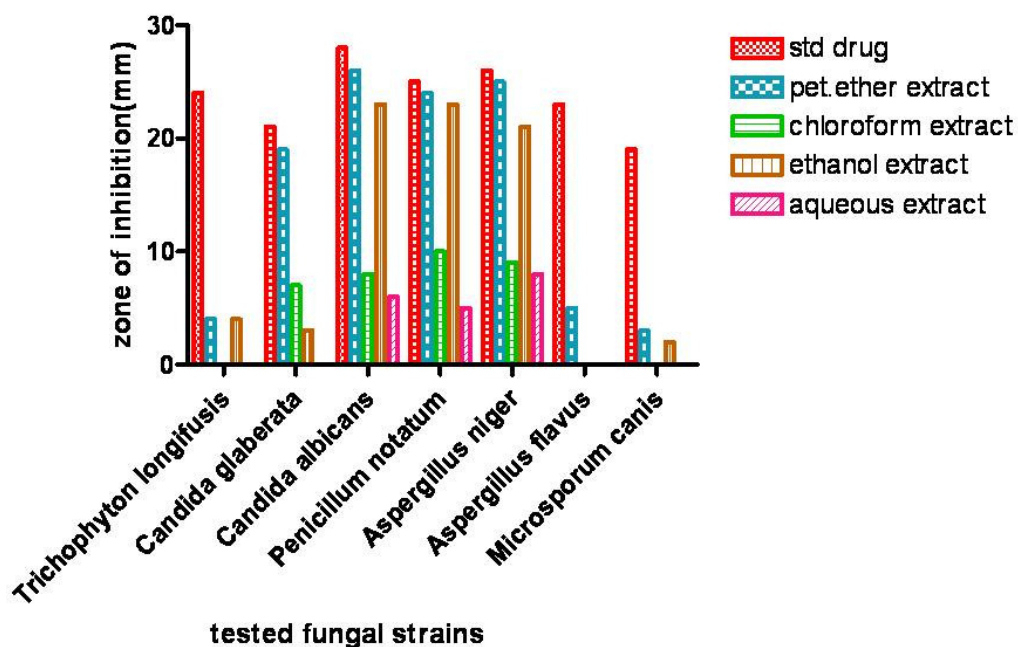
Figure – 2 Anti bacterial activity of different extracts of *Nyctanthes arbor tristis* Linn bark on Gram negative bacterial strains



In Screening for anti fungal activity, pet.ether extract was more susceptible to *Candida albicans*, *Aspergillus niger*, *Penicillium notatum*, *Candida glaberata* among the all tested fungal pathogens, ethanolic extract

was effective against *Candida albicans*, *Penicillium notatum*, *Aspergillus niger* among the all tested fungal pathogens. (Fig-3)

Fig – 3 Anti fungal activity of different extracts of *Nyctanthes arbor tristis* Linn bark



Investigations on the phytochemical screening of *Nyctanthes arbor-tristis* Linn bark extracts revealed the presence of alkaloids, Steroids, glycosides, flavanoids, saponins, phenolic compounds and tannins. These compounds are known to be biologically active and therefore aid the antimicrobial activities of the plant.

Tannins are known for their astringent property and anti microbial activity^[15]. One of the most common biological properties of alkaloids is its toxicity against cells of foreign organisms^[16]. Steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates^[17]. Further study is under process to find out the exact phytoconstituents responsible for the anti microbial activity, to determine the mechanism of action of action.

As an antimicrobial agent, If other related further studies and clinical trials are carried out, it will definitely open up a new vistas in modern medicine.

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