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# *In-Vitro* Phytochemical and Antimicrobial activity of *Nyctanthes arbortristis Linn* against human pathogens

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## ABSTRACT

*Nyctanthes arbortristis Linn. (Fam. Oleaceae), commonly known as Harsingar or Night Jasmine, is a common wild hardy large shrub or small tree. The decoction of Nyctanthes arbortristis Linn. (Harsingar) leaves are widely used in Ayurvedic system of medicine for the treatment of sciatica, arthritis, fevers, various painful conditions and as laxative. It has been reported that the seeds, flowers and leaves of Nyctanthes arbortristis possesses immunostimulant, hepatoprotective, antileishmanial, antiviral and antifungal activities. The aim of the present investigation is to characterize the antimicrobial property of the leaves of Nyctanthes, and find out its effect at on the microbes on their different concentration. In the present study, Phyto chemical analysis revealed the present of various compounds like phenols, flavanoids, proteins, carbohydrates. The antimicrobial test conducted for the leaves of Nyctanthes arbortristis showed interesting antibacterial activity against some gram-positive and gram-negative microorganisms (chloroform and ethyl acetate extracts). Thus anti-microbial activity in leaves of Harsingar supports its use in various conditions by the followers of the Ayurvedic system of medicine. The immuno stimulant substances found in N. arbortristis are believed to play a role in its antiamoebic, antileishmanial, antiviral and certain other activities.*

***Keywords*** *- Nyctanthes arbortristis Linn, Antimicrobial, Secondary metabolites, Solvent extracts.*

## INTRODUCTION

Plants readily synthesize substances for defence against attack by insects, herbivores and microorganisms. Many plants extracts owe their potency to the presence of substances such as tannins, phenolic compounds, alkaloids & so on. These substances are found in various parts of the plants like roots, leaves, shoots and bark. Many of the plants have therefore become a source of important drugs and the pharmaceutical industries have to come to consider traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicine.

Tranquilizing, antihistaminic and purgative activities were also exhibited by the leaves extract[1]. Roots of it are used for emaciation. Stem bark of this plant is taken to cure dysentery, ulcer of palate and internal injuries[2]. It has been reported that *Nyctanthes* possesses analgesic, antipyretic, ulcerogenic, anti-inflammatory[3] antileishmanial, antiviral, antifungal[4] antibacterial properties[5] Antispermatogenic[6] Antiplasmodial[7]. Stem bark extract of NAT contain compounds like b-amyrin, Arbotristoside –A1, oleonic acid, nyctoside-A2, and Nyctanthic acid[8]. In recent years, secondary plant metabolites (phytochemicals), previously with known pharmaceutical activities, have been extensively investigated as a source of medicinal agents[9]. Thus it is anticipated that phytochemicals with adequate antibacterial and antifungal efficacy will be used for the treatment of bacterial and fungal infections[10]. The aim of the present study was to evaluate the susceptibility of various disease causing bacterial and fungal strains to different alcoholic extracts of leaves and bark of *Nyctanthes* for antibacterial and phyto-chemicals or secondary metabolites present in it. The extracts were also run on TLC and column

chromatography to separate the pigments or compounds present in the extracts. Initial purification of the secondary metabolites was done by column chromatographic techniques using silica gel as packaging material and various combinations of solvents as eluents in the column.

## MATERIAL AND METHODS

### Preparation of plant material

The leaves and seed samples were collected in the month of January. The time around which the samples were collected was selected to be before 8 AM in the morning. Only the fresh leaves and seeds were collected and screened. The samples were then washed thoroughly under running tap water several times. After which the leaves and seeds were placed under shade conditions for drying. Drying process for leaves took around 5 days while for seeds it extended up to 15 days. After the samples were completely dried, they were then crushed to powdery form using a pre sterilized mixer grinder. The samples were then sealed in air tight bottles and were further analysed.

### Extraction

In this case the samples were put through the second process. In this process, 20 grams of leaves and seed samples were weighed separately. They were then mixed with different solvents in a dark coloured tight sealed glass bottles. These bottles were then placed in the shaker incubator overnight and the temperature was maintained at 25 degree C.

### Filtration

After overnight incubation, the bottles were shaken properly to mix the contents. They were then filtered using Whatmann filter paper. The filtrate were then concentrated on boiling water bath till the solvents evaporated. Finally the decoction was stored at 4 degree C for further analysis.

### Antibacterial assay (primary screening)

Culture suspension was made by inoculating loop full of the strain in 5ml sterile distil water. 100 micro litre of culture suspension was spread onto MH agar plates. Wells were made with the help of sterile metal- borer. (1 for sample /extract and 1 for respectively extract solvent as control). Then required amount of the extracts diluted and were taken in vials Using micropipette 70 microliter from the vials were introduces into the wells.

Plates were incubated at 37C for 24 hrs. Microbial growth was determined by measuring the diameter of zone of inhibition.

### Identification of secondary metabolites

The methods described by Odebiyi and Sofowara [11] were used to test for the presence of saponins, tannins, alkaloids, flavonoids and glycosides in the test samples.

**Table 1: Qualitative analysis**

| | Test | Observation | Inference |
|---|---|---|---|
| **1**. | 0.5gm of extract + 20ml boiled water in test tube + filtered + few drops of 0.1 ml Ferric chloride added. | Brownish green or blue black Colouration. | Tannin present. |
| **2**. | 2gms of extract + 20ml d/w boiled in water bath & filter + 10ml filterate + 5ml d/w & shaken vigourously for stable persistant froth. The froth is mixed with 3 drops olive oil & shaken vigourously. | Formation of emultion | Presence of saponin. |
| **3**. | 5ml of each extract + 2ml of chloroform + conc. Sulphuric acid carefully added to form a layer along sides. | Reddish brown colouration of the interface. | Presence of terpenoids. |
| **4**. | 5ml of extracts + 2ml of glacial acetic acid containing 1 drop of ferric chloride solution + 1ml of conc. Sulphuric acid. | a. A brown ring at the interface indicates a deoxysugar characteristic of carbenoids<br>b. Development of violet indicates the presence of glycosides. | Presence of glycosides. |
| **5**. | Extract + 10ml ethyl acetate over a steam bath for 3 mins and filtered. 4ml of filterate shaken with 1ml of dil. ammonia solution. | Yellow colouration. | Flavonoids present. |
| **6**. | Mayer's test 500mg potassium iodide + 136mg mercuric chloride mixed in 10ml d/w. + extract. | Turbid pink precipitate. | Alkaloids present. |

## RESULT AND DISCUSSION

Qualitative phytochemical analysis yielded positive results for most of the solvents in both the leaf and seed extracts. The phytochemical analysis of the leaf and seed extracts of various solvents showed the presence of Tannin, Saponin Terpenoids, Glycosides, Flavonoids and alkaloids. The presence of these bioactive compounds also account for the antimicrobial activity of the plant species (Table II and III).

**Table II: Phytochemical components of the plant extracts. (LEAF)**

| Chemical constituents | Acetone | Methanol | Ethanol | Hexane | Diethyl ether | Ethyl acetate | 2-Butoxy ethanol |
|---|---|---|---|---|---|---|---|
| ***Tannin*** | -- | + | -- | -- | -- | + | -- |
| ***Saponin*** | + | + | + | -- | = | -- | + |
| ***Terpenoids*** | + | + | + | + | -- | + | + |
| ***Glycosides*** | -- | + | + | + | + | + | -- |
| ***Flavonoids*** | + | -- | + | ++ | ++ | -- | -- |
| ***Alkaloids*** | + | -- | + | + | + | + | + |

+ = positive , -- not detected , = trace elements

**Table III. Phytochemical components of the plant extracts. ( SEED )**

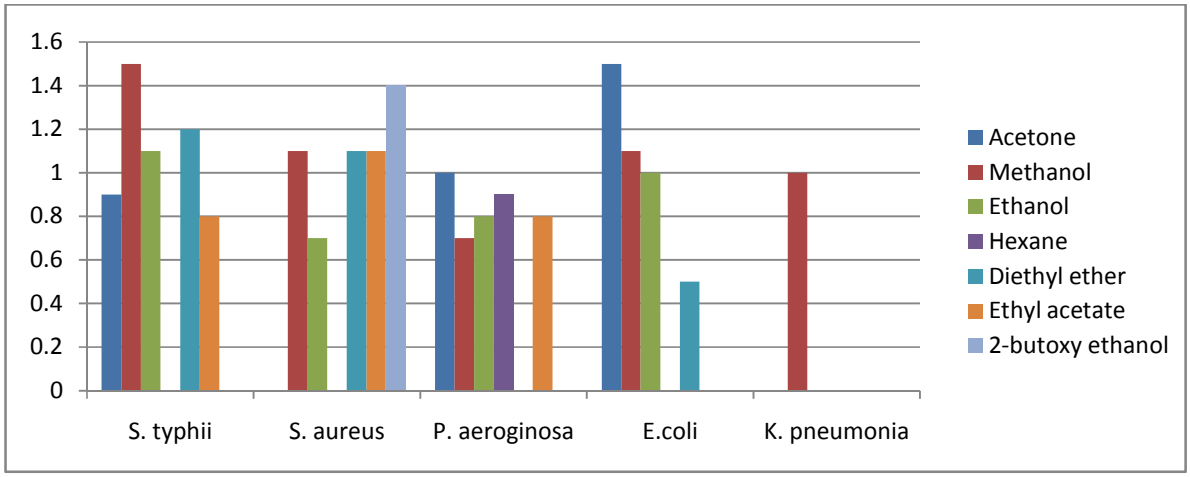
| Chemical constituents | Acetone | Methanol | Ethanol | Hexane | Diethyl ether | Ethyl acetate | 2-Butoxy ethanol |
|---|---|---|---|---|---|---|---|
| ***Tannin*** | + | + | -- | -- | -- | + | -- |
| ***Saponin*** | + | + | + | -- | = | -- | + |
| ***Terpenoids*** | + | + | + | + | = | -- | + |
| ***Glycosides*** | ++ | ++ | ++ | -- | = | ++ | ++ |
| ***Flavonoids*** | + | + | + | -- | = | + | + |
| ***Alkaloids*** | + | + | -- | + | + | ++ | -- |

+ = positive , -- not detected , = trace elements, ++ strongly positive

### Antimicrobial assay

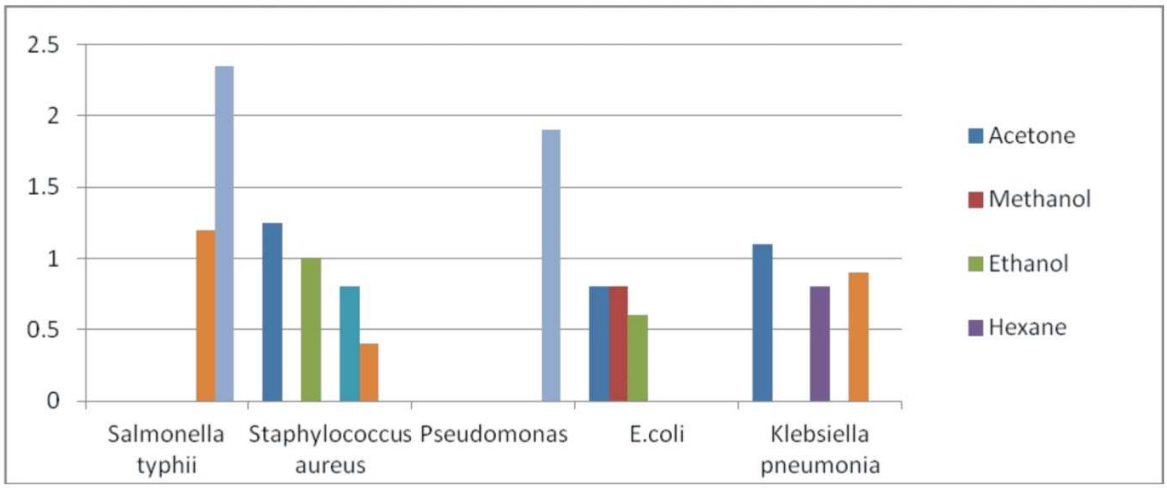
Antimicrobial activity of the extracts against human pathogens were conducted using Muller Hinton agar. The results of which are represented. The zone of inhibition was calculated by subtracting the zone obtained in the control. Acetone and Methanol extract showed maximum zone of inhibition of 1.5 cm was in *E. coli* and S. *typhii*. 2-Butoxy ethanol showed zone of inhibition only against *S. aureus*. Diethyl ether extract showed minimum zone of inhibition against *E. coli.* Results from Figure-1 shows that seed extract prepared in Ethyl acetate as solvent had shown positive result on Salmonella typhii and Klebsiella pneumoniae and was less effective on Streptococcus aureus. E.coli and Streptococcus showed similar positive result in case of Ethanol seed extract. Hexane seed extract was found to give positive results with only Klebsiella pneumoniae. Similarly Methanol seed extract was effective against E.coli. While Acetone seed extract was effective against Klebsiella, Streptococcus and E.coli. Diethyl ether seed extract showed positive result only with Streptococcus. 2- Butoxy ethanol seed extract showed results with Salmonella and Pseudomonas.

**Figure 1: Graph for Antibacterial activity of leaf extract**



*P. Aeroginosa* showed activity only against 2-Butoxy ethanol extract and maximum zone of inhibition of 1.86 cm was also recorded in the same. *S. typhii* showed activity against Ethyl acetate extract. S. aureus recorded minimum zone of inhibition of 0.4 cm against Ethyl acetate extract. From Figure-2, it can be illustrated that Methanol leaf extract has positive result on all the microorganisms. Salmonella typhii and Pseudomonas were affected equally by acetone leaf extract. Ethanol leaf extract affected all the microorganisms except Klebsiella pneumoniae. Hexane leaf extract showed effect only on Pseudomonas. Diethyl ether extract showed almost equal effect on Salmonella typhii and Streptococcus aureus and had less effect on E.coli. Ethyl acetate leaf extract showed positive result with Salmonella, Streptococcus and Pseudomonas but had no effect on Klebsiella and E. coli.

**Figure 2: Graph for antibacterial activity of seed extract**




## DISCUSSION

From the results obtained, it is noted that Leaf extracts showed better zone of inhibition when compared to seed extracts. Also Methanol, Ethanol, Ethyl acetate and Acetone extract showed good results with leaf as well as seed sample. Hexane leaf and seed extract were found to be least effective. One observation that was found to be noticeable was that 2- Butoxy ethanol showed positive with all the microorganisms in its pure form without the extract. It is used as a solvent in spray lacquers, enamels, varnishes, and latex paints and as an ingredient in paint thinners and strippers, varnish removers, and herbicides .

From the following results, it can be ascribed that the antibiotic property of leaf and seed extract of Nyctanthes arbor-tristis is very potent when compared to standard antibiotics present and thus it is highly effective against preventing the growth of microorganisms. The concentration of compounds present in the extract is almost 10- 15 % of the total sample taken. From this observation, the concentration of extracts is nearly 20 microgram. While the concentration of standard antibiotics taken for the comparative study was 400 microgram. Thus, it can be ascribed that at equal concentration, pure extract from Nyctanthes arbor-tristis will have much better activity when compared to the standard antibiotics. This property of the extracts relies on the compounds present in the extract. Further study on this aspect has the potential to lead towards a few more astonishing facts towards the antibiotic property of its extracts.

Further qualitative assessment of the separated compounds in column chromatography can be done by HPLC-MS, NMR etc.

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