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# Study of Antibacterial Activity of β-Sitosterol Isolated From The Leaves of Nyctanthes Arbor Tristis

KEYWORDS: Antibacterial, sitosterol,Nyctanthes arbor tristis,leaves.

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**ABSTRACT** *Nyctanthes arbortristis Linn. belonging to family Oleaceae is a well known medicinal plant.The stem bark extracts of the plant were tested for their in vitro antimicrobial activity by cup plate method. The test organisms were Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger. The zone of inhibition and Minimum Inhibitory Concentration (MIC) of the extracts were determined and compared with the standard drugs ciprofloxacin and fluconazole. The chloroform extract was found to have both antibacterial and antifungal activity whereas the petroleum ether and ethanol extracts possess only antibacterial activity.*

*The antibacterial potentialof a sitosterol isolated from the ethanolic extract of leaves of Nyctanthes arbortristis was evaluated on gram-positive (Staphylococcus aureus) and gram-negative (Escherichia Coli,Pseudomonas aeruginosa) bacteria.The compound was analysed for its antibacterial potential in terms of zone of inhibition of bacterial growth and showed the significant antibacterial activity against gram-negative bacteria.*

**Introduction:**

Nyctanthes arbor tristis Linin is a small scared ornamental tree known across the country for its fragnant white.It is a large shrub growing to 10 m tall, with flaky grey bark,stiff whitish hair , young branches and rough leaves, commonly known as Night Jaismine or Parijata [1,2].Herbal medicine has become an integral part of standard healthcare based on a combination of time honored traditional usage and ongoing scientific research. Literature survey shows that the extract of different parts of the plant have been reported to possess antibacterial activity. The ethanolic and hydro-alcoholic extracts of the leaves were investigated for its antibacterial performance against both antibiotic resistant and nonresistant strains of Staphylococcus aureus[3]. A benzofuranone derivative,3,3a,7,7a-tetrahydro-3a-hydroxy-6(2H) benzo-furanone, isolated from the flowers showed significant antibacterial activity against both gram positive and gram negative bacteria.[4].The antibacterial potential of different parts of the plant was evaluated gram-positive (Staphylococcus aureus) and gram-negative (Escherichia Coli,Pseudomonas aeruginosa) bacteria[5].The zone of inhibition and minimum inhibitory concentration (MIC) of the extracts of stem bark of the plant were determined and compared with the standard drugs ciprofloxacin and fluconazole. the chloroform extract was found to have both antibacterial and antifungal activity whereas the petroleum ether and ethanol extracts possess only anti bacterial activity[6].Various extracts of bark showed significant positive control in vitro antifunal and antibacterial activity when compared withKetoconazole and ciprofloxacin as standard antifungal and antibacterial drug,respectively[7].Experimental results showed that the chloroform and ethyl acetate extracts of fresh leaves, seeds and fruits have significant bacterial activity against gram negative bacteria (Escherichia Coli,Pseudomonas aeruginosa) and gram positive bacteria (Staphylococcus aureus), where as dried extracts of chloroform and ethyl acetate shown significant antibacterial activity against Pseudomonas aeruginosa [8]. Antimicrobial efficacy of ethanol, methanol, petroleum ether and aqueous extracts of the leaves exhibited varying degree of inhibitory effect against different pathogenic strains. The most susceptible bacterium and fungi were Pseudomonas aeruginosa and Rhizopus stolonifer, respectively. Petroleum ether and aqueous extracts of the leaves exhibited varying degree of inhibitory effect against different pathogenic strains. The most susceptible bacterium and Fungi were Pseudomonas aeruginosa and Rhizopus stolonifer, respectively. Petroleum ether extract of leaves responded more effectively to antimicrobial activity as compared to other extracts [9].The present study was undertaken to investigate the antibacterial activity of a sitosterol isolated from the leaves of Nyctanthes arbor tristis in view of its diverse pharmacological applications in ancient and modern system of medicine.

**2. Materials And Methods:**

**Collection and authentication of plant material:**

The plant Nyctanthes arbortristis was collected from the local areas of Muzaffarnagar (UP) and identified by the courtesy of Dr. Veena Chandra Scientist . The leaves were washed thoroughly with distilled water and dried in shade at room temperature and mechanically crushed. The stem bark of *N. arbortristis* Linn. was collected from Sonipat, India in June, 2008. The bark was shade dried at room temperature (30-40 °C). The plant was authenticated at the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, under voucher specimen number NISCAIR/RHMD/Consult/-2008-09/1058/89.

**Extraction and isolation of β-sitosterol:**

**Microorganisms :**

The test organisms were *Staphylococcus aureus* (MTCC 737), *Micrococcus luteus* (MTCC *106), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Candida albicans* (MTCC 3017) and *Aspergillus niger* (MTCC 1344).The microorganisms were availed from M.T.C.C. Institute of Microbial Technology, Sector 39-A, Chandigarh-160036, India. ST No: 02/ ST/Sci & Tech/STC/Chd/2002. Invoice No.

6kg of dried leaves were exracted with ethanol for 24 hours. The extract was distilled under vaccum to give a dark green brown mass (68gm) and designated as NLE (Nyctanthes arbortristis leaves ethanol extract) . Ethanolic

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extract, NLE was partitioned successfully with petroleum ether,Chloform and methanol.The percentage yield of the fractions were 15%,80% and 5% respectively. Chloroform extract (NLEC) was obtained in good yield and it gave positive test for the presence of steroids, terpenoids, alcohol, iridiods, flavanoids and glycosides. This fraction was chromatographed over acolumn of silica gel (60-120mesh,1.5kg).The column was eluted with solvent system of increasing polarities. Fractions eluted with petroleum ether (100%),petroleum ether :chloroform (75:25 & 50-50 respectively),showed a single spot on TLC plate with same Rf value.These fractions were added together to give one fraction.This fractions gave pink colour , changing to blue and the green in the Liebermann-Burchard test indicating its steroidal nature[10].On TLC plate this fraction appeared as brown colour spot on spray with 50% H2SO4 with developing phase ethyiacetate : toluene (1:9) Rf 0.53 (Lit-137-138UC)[12].Rf value and melting point corresponded to the literature value of asitosterol. Structure of the isolated compound was elucidated on the basis of melting point corresponded to the literature value of a sitosterol. Structure of the isolated compound was elucidated on the basis of melting point determination and using various spectroscopic techniques (IR,NMR & Mass) and confirmed with available data [13,14].

**Microorganisms**
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**Composition of culture media**
The media used for growth of bacteria were,1.Nutrient broth medium 2.Nutrient agar medium.The media were sterilized by autoclaving at15 Ib/sqinch pressure at 1210C for 15 minutes.

**Nutrient broth medium**
Beef extract-10.0g

Peptone- 10.0gm

Sodium Chloride – 5.0g

Distilled Water – 1000ml

The pH was adjusted to 7.2-7.4

All the weighed ingredients were mixed in water and dissolved with the aid of heat with stirring.

**Nutrient agar medium**
Beef extract-10.0g

Peptone- 10.0gm

Sodium Chloride – 5.0g

Agar –agar – 1-2%(w/v)

Distilled Water – 1000ml

The pH was adjusted to 7.2-7.4

All the above weighed ingredients were mixed in water and heated on water bath stirring till agar completely dissolved.Most of the agar dissolved to give a clear liquid.

**Preparation of test solution**
The test solution of the compound was prepared in choloroform and filter paper disc soaked in choloroform was used as control one. Streptomycin was used as standard.

**Antibacterial activity**
The antibacterial activity of β –sitosterol isolated from the ethanolic extract of leaves of Nyctanthes arbor tristis was evaluated by agar well diffusion method[15].Twenty Four hours broth cultures of the bacteria used for the assay. A sterile cotton swab was dipped in to the bacterial suspension and evenly streaked over the entire surface of sterile nutrient agar plate to obtain uniform inoculums. Wells were punched on the seeded plates using sterile borer (8mm). The plates were allowed to dry for 5 min.The compound (200 and 300µl)was dispensed in to each well using sterile micropipette.The plates were incubated overnight at 370C. Antibacterial activity was determined by measuring the diameter of zone of inhibition(mm).

**3.Result**
Atibacterial potential of the compound was assessed in terms of zone of inhibition of bacterial growth.The results of the antibacterial activity was presented in table 1.The antibacterial activity of the compound increased linearly with increase in volume of extract 200il and 300 il volume of the compound showed significant antibacterial activity against the microorganism tested. The results revealed that the gram negative (Escherichia Coli, Pseudomonas aeruginosa) bacteria were more sensitive as compared to those of gram positive bacteria. The growth inhibition zone measured ranged from 15 to 25mm for all the sensitive bactertia.

**Table .1: Antimicrobial activity (zone of inhibition) of stem bark extracts of N. arbortristis Linn.**

| Extracts/ Standards | PE | | | | CH | | ETH | | | Cipro | | | Fluco | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. (mg/ml) | 10 | 20 | 40 | 50 | 10 | 20 | 40 | 50 | 10 | 20 | 40 | 50 | 5 | 5 |
| Microorganisms | | | | | | | Zone of inhibition (mm) | | | | | | | |
| S. aureus | 4 | 5 | 8 | 9 | 7 | 9 | 13 | 15 | 1 | 2 | 4 | 5 | 26 | NA |
| B. subtilis | 4 | 6 | 7 | 8 | 5 | 6 | 8 | 10 | - | 2 | 3 | 5 | 32 | NA |
| M. luteus | 3 | 5 | 5 | 6 | 6 | 8 | 11 | 16 | 2 | 3 | 6 | 7 | 14 | NA |
| P. aeruginosa | 4 | 5 | 8 | 7 | 9 | 11 | 14 | 19 | 6 | 8 | 11 | 12 | 25 | NA |
| E. coli | 3 | 4 | 6 | 6 | 8 | 9 | 11 | 13 | 4 | 5 | 6 | 7 | 22 | NA |
| C. albicans | - | - | - | - | 3 | 6 | 8 | 11 | - | - | - | - | NA | 13 |
| A. niger | - | - | - | - | 4 | 6 | 8 | 10 | - | - | - | - | NA | 14 |

**PE = Petroreum ether extract, CH = Chloroform extract, ETH = Ethanol extract, Cipro = ciprofloxacin, Fluco = fluconazole, *S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis, M. luteus = Micrococcus luteus, P. aeruginosa= Pseudomonas aeruginosa, E. coli = Escherichia coli, C. albicans = Candida albicans, A.niger = Aspergillus niger,* NA = Not Applicable**

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**4. DISCUSSION**

The interest in medicinal and aromatic plants has been shown all over the world because of safe and effective constituents of plant products and in particularly the presence of active principles of medicinal plants. Table 1 shows the result of antimicrobial activity against the tested microorganisms. Isolated compound , a sitosterol showed higher zone of inhibition against Pseudomonas aeruginosa as compared with the other tested bacteria . In the present study observed antibacterial activity of the isolated compound justifies the traditional use of the plant for the treatment of different bacterial infections. Thus further test is recommended on large number of bacterial strains to decide their potential as candidates in development of antibacterial drugs.

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