

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: -www.journalijar.com</p> <h2>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</h2> <p>Article DOI: 10.21474/IJAR01/7090 DOI URL: http://dx.doi.org/10.21474/IJAR01/7090</p>	
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RESEARCH ARTICLE

EXTRACTION AND ANTIMICROBIAL ACTIVITY OF CESTRUM NOCTURNUM.

Pragati Rokade¹, Dipali Rokade², Pallavi Dhekale¹.

Department of Pharmaceutical Chemistry, Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara, 415015, Maharashtra, India.

Manuscript Info

Manuscript History

Received: 13 March 2018
Final Accepted: 15 April 2018
Published: May 2018

Keywords:-

Cestrum nocturnum, antimicrobial activity, agar plate diffusion method.

Abstract

Night blooming jasmine, botanically known as "Cestrum nocturnum" is an evergreen shrub that grows in tropical and sub-tropical regions throughout the world. Cestrum nocturnum is a popular ornamental plant due to its showy and fragrant white flowers. It is also used as a hedge plant and cultivated as a medicinal plant. The medicinal properties of night blooming jasmine include antioxidant, anti-hyperlipidemic, hepatoprotective, analgesic, antifungal, anti-convulsant, anti-HIV and larvicidal activities.^[1]

The crude methanol extract of plant of Cestrum nocturnum L. (Solanaceae) and its subsequent fractions were tested against various bacterial and antifungal strains with the exception of staphylococcus aureus, Escherichia coli. The zone of inhibition ranged from 19 to 280 µg/ml. The crude extract and fractions were also susceptible to Candida species and Asper species. The zone of inhibition for various fungi ranged from 170 to 290 µg/ml.

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Introduction:-

The genus Cestrum nocturnum contains more than 300 species, and most of them are native to warm subtropical and tropical areas of America. Cestrum nocturnum L. (Solanaceae), commonly known as night blooming jasmine is an evergreen shrub with glossy, smooth, simple leaves, vine like stems and greenish, creamy white tubular flowers. The flowers volatile compound were identified as phenylacetylaldehyde and linalol(Li ET AL.) The leaves of C. nocturnum have pharmacological significance in Chinese folks medicine and have been used for the treatment of burns and swelling. The leaves of plant have shown significant analgesic and bactericidal activity. Local anaesthetic effect, inhibitory effect on central nerve system and cardiac arrhythmic effect of plant is also documented.

Mature leaf holds a calcinogenic glycoside that escort to vitamin D toxicity and is accountable for elevated serum calcium level .some of glycosides such as (25R) -spirot -5-ene-2R3-diol. Pentaglycosides (nocturnoside A), and (25R)-spirot-5-en-3-ol tetraglycosides (nocturnoside B) and phenolic glucosides (cesternoside A and B) two new flavonol glycosides and seven steroidal saponins including four new ones and eight new steroidal glycoside have been isolated from the leaves of C. nocturnum. In the present study the antibacterial and antifungal activities of C. nocturnum.

Corresponding Author:-Pragati Rokade.

Address:-Department of Pharmaceutical Chemistry, Gourishankar Institute of Pharmaceutical and Research, Limb, Satara, 415015, Maharashtra, India.

Material and Methods:-

Plant material:-

The fresh leaves of *Cestrum nocturnum* free from disease was collected in the month of August. The leaves were washed with tap water and then deionised water and shade dried. The plant material was regularly checked for fungal growth or rotting. After the plant material was dried it was powdered to obtain a uniform fine particle size. The plant material was stored in airtight container.

Extraction:-

Methanol extract:-^[2, 4, 6, 8]

After drying the plant material was powdered in fine particle. Exposure to sunlight was avoided to prevent loss of active constituents. Methanol extraction fluid 200ml was mixed with 20gm of each of powdered plant material. The mixture were then kept for 7days in tightly sealed vessel at room temperature, protected from sunlight, and stirred thoroughly several time a day with sterile glass rod. The mixture thus obtained were filtered through Whatman No 1 filter paper and residue adjusted to the required concentration (50ml of methanol for residue of 20g powdered material) with extraction fluid for further extraction) This was repeated three times, and colourless supernatant extraction liquid was finally obtained. The extracted liquid was subjected to rotary evaporation in order to remove methanol. The semisolid extract produced were kept at 80° C in freezer overnight and then subjected to freeze drying for 24 h at 60°C at 200ml vacuum. Then the extract was stored in an airtight container at 4°C in the refrigerator until further use.

Determination of antimicrobial activity:-

Antibacterial assay^[2, 4, 6, 8]:-

The crude extract and its various fractions in the concentration of 3mg/ml were screened against various human pathogens by agar well diffusion method. In this method 10ml aliquots of nutrients broth was inoculated with test organism and incubated at 37°C for 24h. Using a sterile pipette, 0.6ml of the broth culture of the organism was added to 60ml of molten agar, mixed well the and poured into a sterile petri dish (for a 9ml petri dish, 0.2ml of the culture was added to 20ml of agar). Duplicate plates of each organism were prepared. The agar was allowed to set and harden and the required number of wells was dug in the medium with the help of a sterile metallic cork borer. Agar plugs were removed. Stock solution of the test samples in the concentration of 1 mg/ml was prepared in the sterile dimethyl sulfoxide (DMSO) and 100 and 200µg/ml of each dilution was added in their respective wells. Control well received only 100 and 200µg/ml of DMSO. The plates were left at room temperature 2h to allow diffusion of the sample and then incubated in an upward direction at 37°C for 24 h. The diameter of the zone of inhibition was measured to nearest mm (the well size also noted).

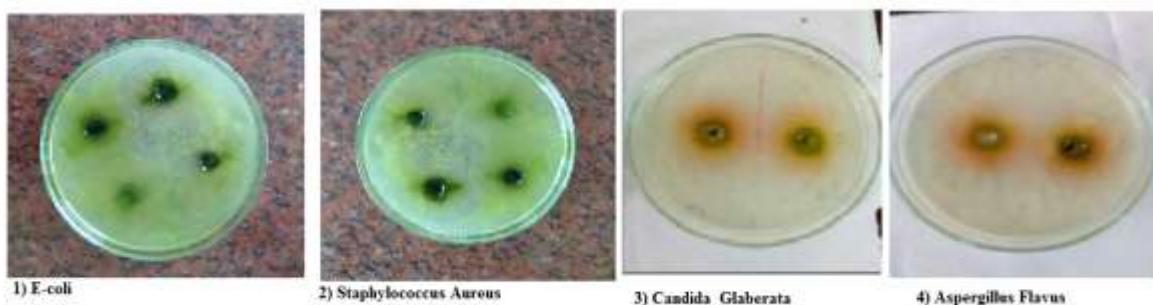
Antifungal assay^[2, 4, 6, 8]:-

Antifungal activity of the crude extract and various fractions were evaluated by agar disc diffusion method. The sample in the concentration of 10mg/ml was dissolved in the sterile dimethyl sulfoxide (DMSO) which served as stock solution. Nutrients agar was prepared by mixing 0.4gm beef extract, 0.4gm peptone, 1.6gm of agar-agar, 0.4 gm. sodium chloride in 100 ml distilled water. It was then stirred to dissolve it. Media were autoclaved at 120°C for 15min and then cooled to 15°C. Plates were then allowed to solidify at room temperature. Each plate was inoculated with a piece of inoculum removed from a seven days old culture of fungi for non-mycelial growth an agar surface streak was employed. Other media supplements with dimethyl sulfoxide and reference antifungal drugs served as negative and positive control respectively. Inhibition of fungal growth was observed after 24 hrs of incubation at 28 °C. Humidity was controlled by placing an open pan of water in the incubator. After incubating for 24 hrs the plates were analysed for the visible growth of the microorganisms.

Result and Discussion:-

Determination of zone of inhibition^[2, 4, 6, 8]:-

To determine the zone of inhibition extract (10 mg/ml) were dissolved in DMSO and serially diluted with sterile water in micro plates in an aseptic condition. The same volume of an actively growing culture of the tested pathogen was added to the different wells and culture were grown overnight in 100% relative humidity at 37°C. The following morning, Growth was indicated by a violet colour of the culture. Zone of inhibition was rated by the lowest concentration of the test solution that inhibited growth. The negative control acetone had no influence on the growth at the highest concentration used; Imipenem, Amphotericin-B and Miconazole were used as control for comparison.

Fig 1:-Zone of inhibition of *Cestrum nocturnum***Antibacterial activity:-****Table 1:-**Zone of inhibition of *Cestrum nocturnum* leaf extract in mm

Sr.no	Temperature	PH	E -coli	S. Aureus
1	37°C	7.3 - 7.5	16 mm	12mm
2	37°C	7.3 - 7.5	14 mm	15 mm
3	37°C	7.3 - 7.5	15 mm	16 mm

Antifungal activity:-**Table 2:-**Zone of inhibition of *cestrum nocturnum* leaf extract in mm

Sr. No	Temperature	PH	Aspergillus flavus	Candida glaberata
1	120°C	7.3 - 7.5	24 mm	25 mm
2	120°C	7.3 - 7.5	25 mm	30 mm
3	120°C	7.3 - 7.5	22 mm	21mm

Conclusion:-

The leaf extract of *cestrum nocturnum* shows considerable antibacterial and antifungal activity. The highest zone of inhibition shown on antifungal strain is *Aspergillus flavus*, *candida glaberata*.

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