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DETERMINATION OF *IN-VITRO* ANTIBACTERIAL ACTIVITY OF NEEM AND NYCTANTHES FLOWERS

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ABSTRACT

Background: The term "antibacterial" refers to a substance, drug or chemical that can inhibit the growth of bacteria or destroy them completely. Antibacterial agents are most commonly used to treat bacterial infections in the human body, and to disinfect or sterilize surfaces. These agents work against both Gram positive and Gram negative bacteria. The crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs also exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria. In the current study we have selected Neem and Nyctanthes flowers. **Objectives:** To Extract active constituents from plant materials (flowers) using suitable solvents by maceration process and comparing its Antibacterial activity shown in combinational plant extracts with that of single plant extracts. **Methods:** The flowers of *Nyctanthes arbor-tristis* and *Azadirachta indica* were collected, cleaned with water and dried and powdered. The dried plant material was subjected for extraction using ethanol for 7days, Dried and concentrated. The individual plant extracts and combined plant extracts were subjected for evaluation of Antibacterial activity using Agar cup plate method. **Results and Discussion:** The present study shows the ethanolic extracts of both Neem and Nycthanthes flowers containing phytochemicals showed synergistic anti bacterial activity when tested using *in vitro* method using Cefotaxime which is an Antibiotic used to treat a number of bacterial infections such as Pneumonia, lung infections, STD's (like Gonorrhea) etc.

KEYWORDS: Cefotaxime, Pneumonia, Gonorrhea, Anti bacterial activity.

INTRODUCTION

Bacteria are microscopic, single-celled organisms that exist by the millions, in every environment, both inside and outside other organisms. Bacteria are found almost everywhere on Earth and are vital to the planet's ecosystems.[1]

In the human body, some bacteria play a major role in maintaining the health and function of the gastrointestinal tract. These are also known as gut flora or micro flora of the human body. It is estimated that the human body contains more bacterial cells than human cells.[2]

Bacteria come under "Prokaryota" kingdom of the "Two Kingdom Classification". Prokaryotes are organisms that do not possess a defined nucleus or any other membrane bound organelles. They are considered to be the earliest forms of cells to exist on the Earth. There are various types of bacteria such as Sperical shaped (Eg: *Streptococcus group*), Rod shaped (Eg: *Bacillus anthracis or anthrax*), Spiral shape (Eg: *Treponema pallidum sps*).[1]

The term "antibacterial" refers to a substance, drug or chemical that can inhibit the growth of bacteria or destroy them completely.[3] The "activity" of antibacterial drugs refers to the ability of the drug to show effect against bacterial growth/reproduction.[4] These agents are most commonly used to treat bacterial infections in the human body, and to disinfect or sterilize surfaces. They work against both Gram positive and Gram negative bacteria.[5]

The antibacterial agents act by inhibition of cell wall synthesis, inhibition of protein synthesis, Inhibition of Nucleic acid synthesis etc., and can be standardized by microbial assays such as Agar cup plate method, agar disk-diffusion method, agar well diffusion method etc.[6]

The crude extracts like cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs also exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria.[7]

Neem is an omnipotent tree and a sacred gift of nature mainly cultivated in the Indian subcontinent known by

the Botanical name *Azadirachtaindica* (A. indica) A. Juss. belonging to family, Meliaceae. Neem has been used extensively by humankind to treat various ailments from the beginning of history.[8]

It has been used in Ayurvedic medicines for more than 4000years. Nimbin, nimbinene, acetylnimbinase, nimbandial quercetin are important phytoconstituents. Neem phytoconstituents exhibit antihemorroidal, antihelminthic, antileprotic activities. Also used in treatment of seborrheic dermatitis, snakebite, ulcers, ulcers, inflammation etc.[9]

Nyctanthesarbortristis Linn. (Oleaceae) is popularly known as 'Harsinghar' (Hindi), Parijata in Sanskrit and 'Night Jasmine' (English) due to the fact that its flowers emit a very strong and pleasant fragrance during the whole night. The flowers start falling after midnight and by the day break, the plant appears dull.[10]

The generic name 'Nyctanthes' has been coined from two Greek words 'Nykhta' (Night) and 'Anthos' (flower). The specific name 'arbortristis' meaning 'the sad tree' is supposedly derived from dull looks of the tree during daytime.[11,12]

Various chemical constituents such as flavanoids, Glycosides, tannic acid, nyctanthin, nyctanthesideetc exhibit various pharmacological activities such as amoebicidal, anti-ulcer, Hepatoprotective, anti-viral, anti-fungal etc. it is also used in treatment of snakebite, sciatica, bronchitis, alopecia, etc.[13]

In the current study we have studied whether the combined flower extracts of Neem and Nyctanthes possess antibacterial activity or not when compared to the standard drug Cefotaxime which is a Cephalosporin antibiotic which is used to treat a number of bacterial infections such as Pneumonia, lung infections, STD's (Gonorrhea), Meningitis, spinal cord infections etc.

MATERIAL AND METHODS

The flowers of *Nyctanthes arbor-tristis* were collected from Dhulapally area near kompally, Hyderabad in the month of November. The flowers of *Azadirachta indica* were collected from Maisammaguda, Hyderabad. The flowers of both plants were cleaned with water and dried in shade for one week, then powdered and stored in air tight container.

❖ PREPARATION OF EXTRACT

1. Flowers of both plants were powdered and the extracts were prepared by maceration process[14] using ethanol as solvent.
2. The coarsely powdered crude drug is soaked in conical flask with the solvent (Ethanol) and is allowed to stand at room temperature for a period of 7 days with frequent agitation until the soluble matter has dissolved. The mixture after 7 days was strained and filtered using funnel.
3. Individual powdered drug was subjected for Maceration process were the crude drug is dissolved in solvent (Ethanol) for 7 days, filtered and concentrated.
4. And mixture of two plants were subjected for maceration for 7 days, filtered and concentrated.
5. All the three extracts are subjected for Screening of Anti-bacterial activity using Agar Cup Plate method

❖ PRELIMINARY PHYTOCHEMICAL SCREENING[15,16,17]

Table 1

Test	Neem	Nyctanthes	Mixture
Carbohydrates: **Molisch's test** - Extract was shaken with 2 ml Molisch solution. To this 2 ml of concentrated H2SO4 was added from the sides of the test tube.	Positive	Positive	Positive
Proteins: **Biuret test** - To 3 ml extract 4% NaOH solution was added along with a few drops of 1% CuSO4 solution.	Positive	Positive	Positive
Alkaloids: **Dragendorff's test** - To 2 - 3 ml extract a few drops of Dragendorff's reagent are added.	Positive	Positive	Positive
Phenols and Tannins: To 2-3ml extract add few drops of 5% $FeCl_3$.	Positive	Positive	Positive
Flavonoids: Shinoda test - To dry powder or extract, 5 ml of 95% ethanol was added along with few drops of concentrated HCl and 0.5g of magnesium turnings	Positive	Positive	Positive
Glycosides: **Legal's test:** To extract add 1 ml pyridine and sodium nitroprusside.	Positive	Negative	Positive
Steroids: Salkowski test: To the extract, add 2ml chloroform and 2ml conc. Sulphuric acid and shake well.	Positive	Positive	Positive

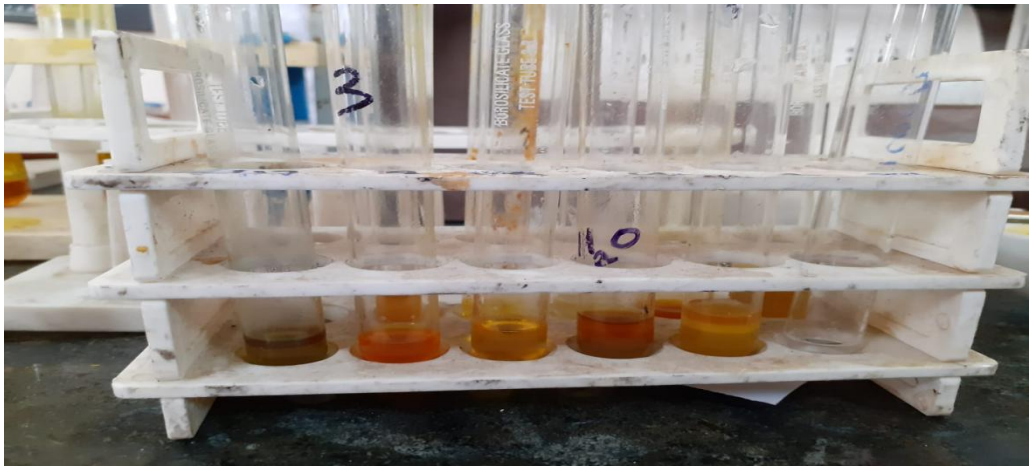

Fig. 1

ANTI BACTERIAL ACTIVITY BY AGAR CUP PLATE METHOD[18]

Principle - This method depends on the diffusion of an antibiotic from a vertical cavity or cylinder, through the solidified agar layer in a petri plate. The growth of test microorganisms is inhibited entirely in a circular area or zone around the cavity or cylinder containing antibiotic solution.

Procedure - Prepare nutrient agar plate inoculated with test organism, with a depth of 4-5mm and then allow it to solidify. Divide the NA plate into four equal portions. And with the help of a sterile borer make four cavities one in each portion. Then fill three cavities with antibiotic solution and in one fill the standard solution. Slowly incubate the plates at 37°C for 24 hours. After incubation measure the zone of inhibition.

- The nutrient agar is melted, cooled and poured into petri dish.
- 0.2ml of known concentration of inoculum was spread on the surface of solidified agar.
- 4 cups or cavities were made by using a sterile borer.
- Three different concentrations of test extracts were poured into cups of agar plates and to the remaining bore standard solution was added.
- The plates were incubated at 37^{o} C.

Fig.2

Fig.3 Neem

Fig. 4 Nyctanthes

Fig.5 Neem + Nyctanthes

RESULTS AND DISCUSSIONS

❖ **Percentage yield of Ethanolic extracts of Individual Plants**

Table 2

S.No	Ethanolic extract	Method of extraction	Colour	Wt of plant material	Wt of the extract	% Yield	Images
1	*Azadirachta indica*	Maceration	Green color liquid	50g	2.38g	4.76%	
2	*Nyctanthes arbortristis*	Maceration	Orange color liquid	50g	3.03g	6.06%	

❖ **Percentage yield of Ethanolic extracts of Two Plants.**

Table 3

S.No	Ethanolic extract	Method of extraction	Colour	Weight of plant material	Weight of the extract	% Yield	Image
1	*Azadirachta indica + Nyctanthes arbor tristis*	Maceration	Brown color liquid	100g	3.97g	3.97%	

❖ **Table showing Anti bacterial activity**

Table 4

Plant extract	1µg/ml	10µg/ml	100µg/ml
Neem	**0.59**	**0.61**	**0.63**
Nyctanthes	**0.64**	**0.66**	**0.71**
Mixture	**0.68**	**0.72**	**0.81***
Standard	**0.66**	**0.70**	**0.78**

CONCLUSION

The available pre-clinical data in the literature indicates the remarkable Pharmacological activities of *Azadirachta indica* and *Nyctanthes arbortristis* which makes suitable to be utilized to treat various diseases. As per our study the selected plants and their extracts show many secondary metabolites such as Phenolic compounds, tannins, flavonoids, glycosides, terpenoids, saponins, resins, carbohydrates etc. And as per our research study we conclude that the Ethanolic extracts of individual plants (flowers) such as *Azadirachta indica* and *Nyctanthes arbortristis* exhibit Antibacterial activity. But the combination of two plants showed significant antibacterial activity when compared to the Standard Cefotaxime by Agar Cup Plate Method when tested against *E.coli* bacteria. And it can be used as easily accessible source of natural antibacterial and as can be used in pharmaceutical industry.

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