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FORMULATION OF HERBAL SYRUP USING *Nyctanthes arbor-tristis* AND IT'S IN-VITRO EVALUATION AGAINST THROAT INFECTION CAUSING *Streptococcus pyogenes*

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ABSTRACT:

Strep throat is caused by infection with the bacterium known as Streptococcus pyogenes, also Called group A Streptococcus.The Nyctanthes arbor-tristis Works Against Fever, Breathing Problem, Stimulate appetite, digestion problem treats urinary tract disorder.this plant additionally has antioxidant and liver protecting Residences. In this study work, the leaves of those plants, flowers and seeds that Lives from special Geographical Places are accrued and examined for antimicrobial Activity, mainly anti-bacterial Residences towards the Streptococcus. The extract thus obtained may be ready for use as a medicinal agent in The form of fluid extracts.In today's world, the medicine enterprise is among the quickest Developing companies because of outbreaks of diverse sicknesses and antimicrobial Opposition of diverse microbes towards typically utilized antibiotics.The control of Diverse sicknesses and the traditional topic of treatment along with Ayurveda and Home-based treatments also carries out a vital function within the part. realize that is relative been achieved to judge the antibacterial pastime of Nyctanthes arbortristis towards Streptococcus pyogenes from Decided on leaves of Nyctanthes arbor-tristis from unique geographic places.

KEYWORDS: Streptococcus, Nyctanthes, syrup, infection, throat infection and cough

1. INTRODUCTION

Strep throat is caused by infection with the bacterium known as *Streptococcus pyogenes*, also Called group A *Streptococcus*. Streptococcal bacteria are contagious. They can spread through Droplets when someone with the infection coughs or sneezes, or through shared food or drinks. *Nyctanthes arbor-tristis* is a species of *Nyctanthes* native to South Asia and Southeast Asia. It is Commonly known as night-blooming jasmine(parijata).Binomial name: *Nyctanthes arbor tristis* . The Leaves have been used in Ayurvedic medicine and Homoeopathy for arthritis, and fevers. The *Nyctanthes arbor-tristis* Works Against Fever, Breathing Problem, Stimulate appetite, digestion problem treats urinary tract disorder. It has antifungal and antimalarial properties and has Anti allergic properties. *(Anshu Kumar Singh and Shailesh Solanki et al., 2021).*

It was mainly used for the Throat Infection and Cough. Sometimes extracts of various solvents are Organized to test the antimicrobial Activity Several latest researches Display that this plant additionally has antioxidant and liver protecting Residences. In this study work, the leaves of those plants, flowers and seeds that Lives from special Geographical Places are accrued and examined for antimicrobial Activity, mainly anti-bacterial Residences towards the *Streptococcus*. (*Uniyal SK, et al., 2006.) (Agrawal J, Pal A et al., 2016).* Extraction, as the term is used pharmaceutically, involves the separation of Medicinal active Portions of plant or animal tissues from the inactive or inert Components by using the selective Solvents. The products obtained from the plants are impure liquids, semisolids or powders intended Only for oral or external Use. The extract thus obtained may be ready for use as a medicinal agent in The form of fluid extracts, it may be further processed to Be incorporated in Syrup form. Extraction Procedures contribute significantly to The final quality of the herbal syrup. Herbal infused syrups are Concentrated herbal teas, preserved in sugar or honey. *(Kakoti BB.et al.,2013).* Medicinal plant life has Diverse antimicrobial marketers, which can be Powerful towards a wide variety of microbes. Sometimes extracts of various solvents are Organized to test the antimicrobial activity (antibacterial activity). Such medicinal plant life Displays their significance in antimicrobial activity. . These plant life additionally have Antimalarial, antihelminthes, and antiallergic Residences. Several latest researches display that This plant additionally has anti-oxidant and liverprotecting residences. In this study work, the leaves Of those plants that lives from special geographical Places are accrued and examined for antimicrobial Activity, mainly anti-bacterial residences towards *Streptococcus pyogenes.*

In today's world, the medicine enterprise is among the quickest Developing companies because of outbreaks of diverse sicknesses and antimicrobial Opposition of diverse microbes towards typically utilized antibiotics. The control of Diverse sicknesses and the traditional topic of treatment along with Ayurveda and Home-based treatments also carries out a vital function within the part. Consequently, using an idea from home-based treatments and Ayurveda, these Studies was created and done to show the

anti-bacterial usage of Nyctanthes arbortristis leaves scientifically. (*Anshu Kumar Singh and Shailesh Solanki et al., 2021).*

A realize that is relative been achieved to judge the antibacterial pastime of *Nyctanthes arbortristis* towards *Streptococcus pyogenes* from Decided on leaves of *Nyctanthes arbor-tristis* from unique geographic places. *(Jain PK, Pandey A.et al., 2016).* The ethanol extract of leaves has a greater pastime that is antibacterial chosen pathogenic microsystem when compared with the methanol and Chloroform extract. The ethanol extracts of leaves are effective with anti-bacterial Pastime towards all chosen microsystems. The leaves of *Nyctanthes arbor-tristis* have anti-bacterial, antifungal, and anti-inflammatory properties that will be Especially ideal for just about any microbial and condition that is fungal. Consequently, the leaves of *Nyctanthes arbor-tristis* are used to cope with diverse Trivial mycosis and pyogenic transmissions and the enteric temperature. *(Pandeti S,et al.,2015). Nyctanthes arbor-tristis* is a traditional medicinal plant, which belongs to family Oleaceae. The *Nyctanthes arbor-tristis* is a shrub or tree having fragrant flowers. The plant generally grows in the tropical and subtropical regions. The *Nyctanthes arbor-tristis* having various names like Parijat, Night Jasmin, Coral Jasmin, Harsinghar, etc. There are several Hindu religious stories related to the *Nyctanthes arbor-tristis* (Parijat). The holistic connection of the Parijat plant with Bhagwat Purana, The Mahabharata and Vishnu Purana. The Nyctanthes arbor-tristis is called as “Tree of Sorrow” because of the loss of the flowers their brightness during day time. The plant name arbor-tristis means the sad tree. (*Uniyal SK.et al., 2016).*

2. MATERIALS AND METHODS

2.1 Collection of Sample:

The *Nyctanthes arbor-tristis* was collected and authenticated by Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

2.2 Collection Of Bacterial Culture:

Streptococcus pyogenes culture has been purchased from Kovai Medical Centre Hospital, Coimbatore, Tamil Nadu.

2.3 Extraction of plant Sample:

Leaves, flowers and seeds of *Nyctanthes arbor-tristis* was sun dried and powdered. The air- dried leaves of *Nyctanthes arbor-tristis* were extracted with ethanol in Soxhlet apparatus for 15-20 hrs using 200ml of solvent. The extract was stored at 4°C for further use. *(James Redfern., 2014).*

2.4 Concentration of Sample:

The extract was concentrated using Rotary evaporator at 75°c for 30minutes and stored in refrigerator below 4°C for further use. *(Kerry Elgie et al., 2022).*

2.5 Phytochemical Analysis:

To perform phytochemical analysis include tannins ,Saponin ,Terpenoids ,Alkaloid ,Anthraquinone and etc.,

2.6 Antimicrobial Activity

The Antimicrobial activity of *Nyctanthes arbor-tristis* leaves, flowers and seeds are by using the agar diffusion method. A Muller-Hinton agar plate is prepared for Each extract with the different samples for antimicrobial testing. Now, the bacterial sample is Spread over the plate with the help of the sterile cotton swab on the agar plates. Within the agar Plates, the wells are prepared and different concentration of the extracts were poured the plates are now Incubated at 37 °C for 18-24 h. After the incubation Period, the inhibition zone is measured in Micrometres that appear around the disc. The sample having higher antimicrobial activity are selected for further process. (*Anshu Kumar Singh and Shailesh Solanki et al., 2021)*

2.7. Minimal Inhibitory Concentration

After the Dilution preparation and method selection, the extracts and their various concentrations are analysed to check the Minimal inhibitory concentration (MIC) of the lowest amount of extracts that inhibit the growth of selected bacteria. When the extracts are soaked in a plane disc and Placed in a petri plate along with the selected Bacteria, the extracts produce the zone of inhibition around each well of antibiotics.

2.8. Formulation of Herbal Syrup

Herbal syrup was formulated by using the following ingredients: *(Mali et al., 2019)*

Ingredients	**Quantity**
Extract	4 ml
Sugar (sucrose)	6 ml
Preservative (Sodium benzoate)	0.1 µl
Flavouring agent (honey)	4.5 ml

***Table** 1:* Compositions of syrup

2.9. Evaluation parameter

2.9.1. Procedure to determine density

1) Clean thoroughly the specific gravity bottle with chromic acid or nitric acid.

2) Rinse the bottle at least two to three times with distilled water.

3) If required, rinse the bottle with an organic solvent like acetone and dry.

4) Take the weight of empty dry bottle with capillary tube stopper (w1).

5) Fill the bottle with unknown liquid and place the stopper, wipe out excess liquid from outside the tube using tissue paper.

6) Weight bottle with unknown liquid on analytical balance (w2).

7) Calculate weight in grams of unknown liquid (w3).

Formula for density:

Density of liquid under test = weight of liquid under test / volume of liquid under test = w3/v *(Mali et al., 2019).*

2.9.2. Procedure to determine Specific gravity

1) Clean thoroughly the specific gravity bottle with chromic or nitric acid.

2) Rinse the bottle at least two to three times with purified water.

3) If required, rinse the bottle with an organic solvent like acetone and dry.

4) Take weight of empty dry bottle with capillary tube stopper.

5) Fill the bottle with distilled water and place stopper; wipe out excess liquid from side tube using tissue paper (w2).

6) Weight bottle with stopper and water on analytical balance (w2).

7) Repeat the procedure for liquid under test by replacing the water after emptying and drying.

8) Weight bottle with stopper and liquid under test on analytical balance (w3). (*Mali et al., 2019).*

Formula for specific gravity:

Specific gravity of liquid under test (syrup) = weight of liquid under test /weight of water = w5/w4

4.9.3. pH determination:

The pH determination of syrup by using two techniques. *(Mali et al., 2019).*

a) Glass electrode. b) pH paper.

Procedure for glass electrode:

1) Prepare 30ml buffer. The volume of the stock solution to be taken. Prepare the buffer by mixing appropriate volume.

2) Allow the solution for 15minutes to establish equilibrium.

3) Measure the pH of solution using a pH meter

Procedure for pH paper:

pH was dipped in the formulated syrup and the colour was compared with the standard chart.

2.10. Comparative studies:

The formulated syrup was compared with the commercially available syrup by using agar well diffusion method

3 RESULT AND DISCUSSION

3.1 Collection of samples:

Leaves, stem and seeds of *Nyctanthes arbor-tristis* were collected and brought to the laboratory.

***Figure** 2* - Leaves of *Nyctanthes arbor-tristis*

***Figure** 3* - Flowers of *Nyctanthes arbor-tristis*

***Figure** 4* - Seeds of *Nyctanthes arbor-tristis.*

Figure 5 - Streptococcus pyogenes culture

S. pyogenes is the common organism causing Strep throat infection. Obtained culture was the stored in the refrigerator for further analysis.

3.2 Soxhlet extraction process:

***Figure** 6* - Soxhlet apparatus Set-up

Samples were sundried and powdered using mortar and pestle. Powdered samples were extracted using Ethanol as a solvent by Soxhlet extraction method.

***Figure** 7*- Leaf Extract

***Figure** 8*- Flower Extract

Figure 9 - Seed Extract

Extracts of *Nyctanthes arbor-tristis* obtained from Soxhlet extraction process which was then further concentrated using Rotary evaporator

3.3 Rotary Evaporator:

***Figure** 10* - Rotary Evaporator Setup

Samples extracted using Soxhlet apparatus was then concentrated with the help of Rotary evaporator by removing the solvents by evaporation. Extracted samples are then stored at 4°C.

3.4 Phytochemical analysis of leaf, flower and seed extracts of *Nyctanthes arbor-tristis:*

	Leaf extract	**Flower extract**	**Seed extract**
Tannin	+	-	-
Saponin	+	+	+
Terpenoids	+	-	-
Alkaloids	-	-	+
Anthraquinone	+	+	-
Phytosterols	+	+	-
Proteins and amino acids	+	-	+
Carbohydrates	-	-	-
Steroids	+	-	-
Phenol	-	-	+
Glycosides	+	+	-

***Table** 2:* Phytochemical results of leaf, flower and seed extracts

The leaf extract showed positive results for tannin, saponin, terpenoids, anthraquinone, phytosterols, proteins and amino acids, steroids and glycosides. Alkaloids, carbohydrates and phenol were absent. The flower extract results indicate the presence of for saponin, anthraquinone, phytosterols and glycosides. Other phytochemical tests showed negative result. The seed extract showed positive results for saponin, alkaloids, proteins and amino acids and phenols. Tannin, terpenoids, anthraquinone phytosterols, carbohydrates, steroids and glycosides were absent.

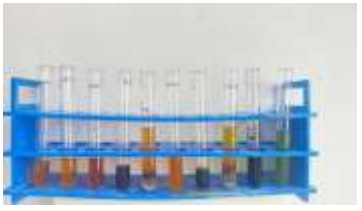

***Figure** 11- Phytochemical results for Leaf Extract*

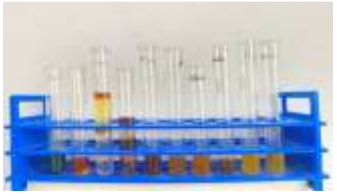

***Figure** 12* - Phytochemical results for Flower Extracts

***Figure** 13 - Phytochemical results for Seed Extracts*

Phytochemical analysis for leaf, flower and seed of *Nyctanthes arbor-tritis* results were shown in the *Figure 12, 13 and 14*

3.5 Antimicrobial activity of leaf, flower and seed extracts against *Streptococcus pyogenes* (performed as triplicates):

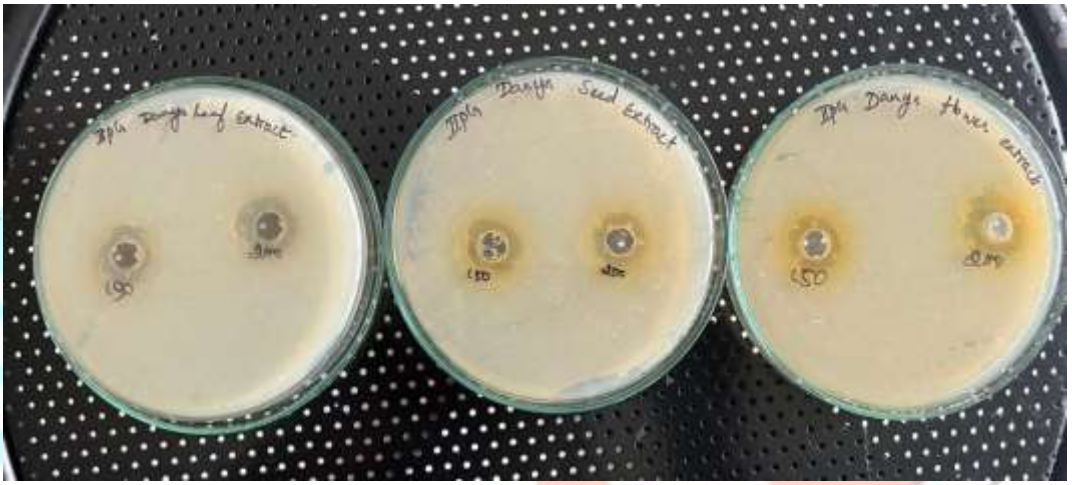

***Figure** 14*- Results for antimicrobial susceptibility test

Extracts	Zone of inhibition (mm)	
	150 µl of extracts	200 µl of extracts
Leaf extract	13	16
Seed extract	7	8
Flower extract	5	7

Table 3: Zone of inhibition against S. pyogenes

The maximum zone of inhibition was observed in the plate with leaf extract of *Nyctanthes arbor-tristis* with the zone measuring about 16mm in 200 µ of the extract

From the results, Leaf extract were found to have more antimicrobial activity against *S. pyogenes* than the seed and flower extracts.

3.6 Minimal Inhibitory Concentration Values:

The lowest concentration of the extract at which the visible growth of the microorganisms was inhibited. Results of Antimicrobial activity against *S. pyogenes* showed that the leaf extract of *Nyctanthes arbor-tristis* maximum zone of inhibition. It is further analyzed for minimal inhibitory concentration

Dilutions	OD Value
20	0.76
40	0.66
60	0.63
80	0.62
100	0.60

***Table** 4*: Results of Minimal Inhibitory Concentration

MIC values of the leaf extract was shown in the *Table 4*. Minimal inhibitory concentration of the leaf extract of *Nyctanthes arbor-tristis* was found to be 0.60

3.7 Formulation of Syrup using leaf extract:

From the results of MIC leaf extracts of *Nyctanthes arbor-tristis* were further selected for the formulation of Herbal syrup

***Figure** 15* - Formulated syrup

3.8 Result for evaluation parameter:

Parameter	Observation
Density	1.05 gm
Specific gravity	0.4685
pH paper	Neutral
pH meter	7.07

Colour	Brownish green
Odor	Aromatic
Taste	Bitter
Appearance	Clear

***Table** 5:* Evaluation of various parameter of Syrup

Formulated syrup using leaf extracts of *Nyctanthes arbor-tristis* were analyzed for the quality parameters which was shown in *Table 5.*

3.9 . Comparative studies:

***Figure** 16* - Antibacterial susceptibility test performed using commercially available syrup

The comparison between the commercially available syrup and the syrup formulated using

Nyctanthes arbor-tristis leaves were done using agar well diffusion method. The commercially available syrup produced the sensitive zone of inhibition whereas the syrup formulated using the leaf extracts of *Nyctanthes arbor-tristis* showed intermediate zone oh inhibition.

4. DISCUSSION:

Nyctanthes arbor-tristis was a traditional medicinal plant which having various medicinal activities but present research was focused on the antimicrobial activity against the *Streptococcus pyogenes* (Strep throat causing microorganisms). The leaves powder was used to formulate syrup. Quality assessment for the formulated syrup shows good results on the several parameters. The compression of formulated syrup, were evaluated and gives satisfactory results. Based on the results it is concluded that the formulation and evaluation are good. The pharmacological evaluation is required for the treatment of Strep throat infection. Phytochemical analysis showed the presence of typical plant constituents such as alkaloids, steroids, tannins, flavonoids, reducing sugars, saponins, and terpenoids. Previous study showed that leaves of N. arbortristis

supposed to have anti-asthmatic activity due to the presence of β-sitosterol. Other chemical constituents isolated in the leaves of this plant are iridoid glucosides; main alkaloid nyctanthin; arbortristosides A, B, C; nyctanthic acid; tannic acid; d-mannitol; methyl salicylate; volatile oil; carotene; terpenoid; and cardiac glucosides. These activities of the leaves of N. arbortristis connect with the traditional use of this plant in treatment of Strep throat

5. SUMMARY AND CONCLUSION

Nyctanthes arbor-tristis is among the wonder of medicinal plants as it has a wide a variety of medicinal properties. The leaves of *Nyctanthes arbor-tristis* were found to have significant activity against *Streptococcus pyogenes*. Phytochemical activity implies that the leaves of *Nyctanthes arbortristis* have actually flavonoids, terpenoids, saponins, and glycosides within the extracts Anthraquinones are somewhat contained in some extracts. These metabolites work well against the *Streptococcus pyogenes.* All the pharmacological studies done so far are of a preliminary kind.

Further research focuses on identification and characterization of the bioactive compound and their molecular mechanism towards immunostimulatory, immunomodulatory, antipyretic, analgesic, anti-arthritic, hepatoprotective, antistress, anxiolytic, antiulcerogenic, hypoglycemic, and hypolipidemic activities should be checked for its toxicity level and finally for human intervention.

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