Research Article

A Study of Solvent Optimization and Phytochemical Analysis for Antibacterial Activity of *Nyctanthus arbortristis* and *Ficus bengalensis*

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ABSTRACT

The present study is carried out by evaluation of antimicrobial activity against various pathogens and solvent optimization. In this work the solvent methanol, ethyl acetate, water and acetone were used and 3 bacterial pathogens (*E.coli, S.aureus* and *P.aeruginosa*) were used. The antibiogram analysis was done using agar well diffusion method and best results were obtained for methanolic extract of *Nyctanthus arbortristis* leaves and *Ficus bengalensis* stem. The MIC was done by broth dilution method and the least value obtained 0.0012mg/ml for *Nyctanthus arbortristis*, methanolic leaves extract against *E.coli* and 0.0002 mg/ml for *Ficus bengalensis,* ethylacetate extract stem against *S.aureus*. The phytochemical analysis of plant *Nyctanthus arbortristis* studied showed the presence of reducing sugar, tannins and polyphenol. The phytochemical analysis of plant *Ficus bengalensis* studied showed the presence of reducing sugar, tannins, phlobatanins, and polyphenol.

Keywords: antibiogram analysis, phytochemical analysis and solvent optimization.

INTRODUCTION

Present time the microorganisms have become resistance to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines. So, it has become necessary to find out new antimicrobial agents. Prevention of pathogenic and spoilage microorganisms in food is usually achieved by using chemical preservatives but they are responsible for many carcinogenic and teratogenic attributes as well as residual toxicity and with growing concern of microbial resistance towards conventional preservatives, consumers tend to be suspicious of chemical additives and thus the exploration of naturally occurring antimicrobial for food preservations receives increasing attention. Present time medicinal plants being the effective source of medicines, either it can be modern or traditional medicines, the advantage of medicines are they are useful for health. WHO had given the remark that traditional medicines are safe treatment for the infections originated from microbial and non microbial origin[1] Some antibiotics do not have capability to treat diseases because of drug resistance capacity of pathogens[2]. The uses of herbal treatment are one of the possible ways to treat diseases caused by multi drug resistant bacteria. Though many pharmaceuticals industries have produced a number of antibiotics from several years but in many cases it was observed that the cultures were showing resistance against the medicines[3].

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Nyctanthus arbortristis

Common name

Hindi: Harashringara, English-Coral Jasmine

The leaves are useful in chronic fever and rheumatism. Decoction of leaves prepared over a gentle fire is specific for sciatica. The juice of leaves is used as a laxative for intestinal worms. The powered seeds are used for scaly disease of the scalp. It is informed that Tribal people use flower in stomachic disorder. A decoction of the bark, leaves, roots and flower is given in excessive dieresis and in enlargement of the spleen. The oil from bark is used for pain in the eyes[4].

Ficus bengalensis

Common name

Banyan tree, Hindi- Barh, Sanskrit- Bargad.

The banyan tree is well known all over India. The tree has what is known as the 'aerial roots', its branches drop to the ground, take roots again, and send out more twisting and trailing branches, thus extending the growth of the tree indefinitely. It has smooth, shining, rather stiff and leathery leaves, broadly oval in shape. The flowers and fruits are inconspicuous, very minute, many of them being held together in the fig, which is a sort of pouch that contains hundreds of flowers or fruits[5].

The present study is carried out by solvent optimization and phytochemical analysis of plant sample **(Nyctanthus arbortristis and *Ficus bengalensis*)** for antibacterial activity.

Materials and methods

The plant samples (***Nyctanthus arbortristis*** **and *Ficus bengalensis)*** were collected from, Vishesh khand, Gomtinagar (Lucknow) and the used parts are stem and leaves.

Extract preparation

5 gm of each dried plant leaves was grinded in mortar and pestle. Then grinded sample is mixed with 50ml 0f ethanol, methanol, acetone and water in the ratio 1:10. They were kept in beaker for 3-4 days in dark. These were then filtered through Whatman's filter paper. Add DMSO to filtrate which is plant extract[6].

Antibacterial susceptibility assay

Extracts obtained were evaluated for their potential antibacterial activities by the standard agar well diffusion assay, also called cup plate method **(Kirby Bauer method)[7].** First of all prepare nutrient broth for pathogens *(E.coli, S.aureus and P.aeruginosa).*Prepare 50 ml nutrient agar, pour in petriplates (100mm) and allowed to solidify. Spread 50 µl of inoculum from suspension culture of test organisms and prepare 3 wells of 5mm diameter in a triangular fashion with the help of micro-tips and marked as A (positive control), S (Sample) and D.W (negative control) for antibiotic, crude sample and autoclaved distilled water respectively. Load 50µl (50µg/ml) of Tetracycline, crude extract and autoclaved distilled water in the respective well. Incubate the plates at 37 ^{0}C for 24 hours under strict aseptic conditions to ensure consistency of all findings. Measure the zone of inhibition which was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period.

Assessment of minimum inhibitory concentration

MIC is defined as lowest concentration of the antimicrobial agent that results in inhibition of visible growth. Thus, the lower the MIC values, the higher the antimicrobial activity. Active extracts obtained by agar well diffusion assay were further subjected to determine the minimum inhibitory concentration (MIC) required to inhibit the growth of microorganisms by standard serial dilution broth method. A stock solution of each bio-active extract was serially diluted (1:4) in test tubes with 3 ml nutrient broth. 20 µl of test organisms were added to each test tube. They were then kept at 37°C for an overnight incubation in shaker incubator. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain by taking OD at 620nm by using spectrophotometer[8,9].

Phytochemical Analysis

Phytochemical are the main constituents of any plants sample, which are responsible for secondary metabolites also. The other work of these phytochemical are flavouring, colours etc[10].

Reducing Sugar

Take1 ml or 1 gm of plant sample in a test tube and add 10 ml deionized water then add few drops of fehling solution (1ml fehling solution A and B) heat at 100°C In a water bath. Brick red colour precipitate showed positive result.

Tannins

Crude extract was mixed with 2ml of 2% solution of FeCl3. Ablue-green or black coloration indicated the presence of phenols and tannins.

Phlobatannins

Take 2 ml plant sample in attest tube and add 10 ml deionized water and boil at 100 °C with few drops of 1 % HCl. Deposition of red precipitation gives positive result.

Saponin

Take 5 ml of aq. extract and then add 2ml chloroform followed by addition of 3 ml conc. Sulphuric acid, observed the reddish brown interface for presence of terpenoids.

Flavonoids

Take 1 ml of sample and add 1 % NH_3 solution if yellow colour observed, showed presence of flavonoids then after this take ethanolic or aq. extract and add 10 ml DMSO then heat it followed by adding Mg, add conc. HCl, gives red colour to confirmed flavonoids.

Polyphenol

Take 2 ml ethanolic extract of plants sample and add folin-ciocaltere reagent and 9 ml of distilled water again add sodium carbonate solution,vortex to mix then kept test tube in dark and mix O.Dat 760 nm.

RESULTS

Antibiogram Analysis

Table 1: Antibacterial Activity of *Nyctanthus arbortristis* Leaves

Pathogens	Zone of inhibition (mm)			
	Acetone	Ethyl Acetate	Methanol	D.Water
Escherichia coli	0	0	24	0
Staphylococcus aureus	0	0	0	20
Pseudomonas aeruginosa	23	0	0	0

Table shows that the best activity obtained for methanolic extract of *Nyctanthus arbortristis* Leaves against *E.coli*.

Table 2: Antibacterial Activity of *Ficus bengalensis* stem

Pathogens	Zone of inhibition (mm)			
	Acetone	Ethyl acetate	Methanol	D. Water
Escherichia coli	1	0	0	0
Staphylococcus aureus	0	17	0	0
Pseudomonas aeruginosa	0	1	0	0

Table shows that the best activity obtained for ethyl acetate extract of *Nyctanthus arbortristis* stem against *S.aureus*.

Table 3: MIC (Minimum Inhibitory concentration) for *Nyctanthus arbortristis* Leaves and *Ficus bengalensis* stem

TEST-TUBES	Conc. Of extracts mg/ml	Methanolic leaves extract against *E.coli (OD at 620nm)* (*Nyctanthus arbortristis*)	OD of ethyl acetate extract stem against *S.aureus* (*Ficus bengalensis*)
1	1.66	0.00	0.00
2	0.27	0.00	0.00
3	0.046	0.00	0.22
4	0.0075	0.30	0.63
5	**0.0012**	0.28	**0.07**
6	**0.0002**	**0.22**	0.79

The least value obtained 0.0012mg/ml for *Nyctanthus arbortristis*, Methanolic leaves extract against E.coli and 0.0002 mg/ml for *Ficus bengalensis*, ethyl acetate extract stem against *S.aureus*

Phytochemical Tests

Table 4: Phytochemical tests for *Nyctanthus arbortristis*

Nyctanthus arbortristis		
Compounds	**Leaves**	**Stem**
Reducing sugar	-	+
Tannins	+	-
Phlobatanins	-	-
Saponin	-	+
Flavonoids	-	-
Polyphenol	1.60	1.52

+ and – shows the presence or absence of metabolites

Table 5: Phytochemical tests for *Ficus bengalensis*

Ficus bengalensis		
Compounds	**Leaves**	**Stem**
Reducing sugar	+	+
Tannins	+	+
Phlobatanins	-	+
Saponin	-	-
Flavonoids	-	-
Polyphenol	1.60	1.52

+ and – shows the presence or absence of metabolites

Antibiogram analysis

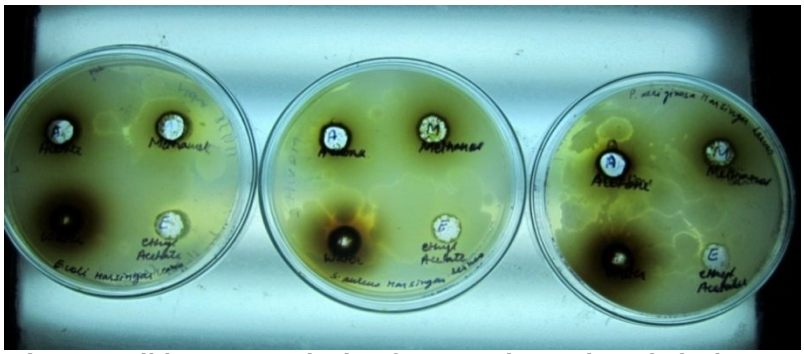

Fig. 1: Antibiogram analysis of *Nyctanthus arbortristis* leaves
Antibiogram analysis shows maximum zone of inhibition in methanolic extract against *S.aureus in Nyctanthus arbortristis*

Fig. 2: Antibacterial Activity of *Ficus bengalensis* stem

Antibiogram analysis shows maximum zone of inhibition in ethyl acetate extract against *S.aureus in Ficus bengalensis* stem

DISCUSSION

Herbal medicines are a valuable and readily available resource for primary health care and complementary health care systems. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered, though large numbers of plants are constantly being screened for their antimicrobial effects. These plants may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary[11].

Plant Extracts were prepared from dried samples of *Nyctanthus arbortristis* and *Ficus bengalensis,* using ethyl acetate, acetone, methanol and water as a solvents. The antibacterial activity of *Nyctanthus arbortristis and Ficus bengalensis* extracted using different solvents showed varying degree of response towards selected pathogens. Extract of *Nyctanthus arbortristis* leaves,were taken for the antibiogram studies using Agar well diffusion method. The maximum zone of inhibition found in methanolic extract of *Nyctanthus arbortristis* leaves against ***E.coli.*** Extract of *Nyctanthus arbortristis* stem were

taken for the antibiogram studies using Agar well diffusion method. But no results were obtained. Extract of *Ficus bengalensis* stems showed maximum zone of inhibition against ***S.aureus*** i.e.,24 mm for ethyl acetate extract. MIC is the least concentration of antibiotics which inhibits the growth of micro organisms. The least value obtained 0.0012mg/ml for *Nyctanthus arbortristis* ,Methanolic leaves extract against *E.coli* and 0.0002 mg/ml for *Ficus bengalensis,* ethylacetate extract stem against *S.aureus*[12,13].

The phytochemical analysis of plant *Nyctanthus arbortristis* studied showed the presence of reducing sugar,tannins,and polyphenol. The phytochemical analysis of plant *Ficus bengalensis* studied showed the presence of reducing sugar, tannins, phlobatanins, and polyphenol[14].

Earlier literature indicate that medicinal plants are the backbone of the traditional medicine and plant based antimicrobials represent vast unused source for medicine and further exploration of plants antimicrobials needs to occur. With extensive use of antibiotics and other antimicrobial agents, more and more of the clinical multidrug- resistant (MDR) pathogens appeared, and the degree of resistance has become progressively serious. At the same time, because of difficulty in developing chemical synthetic drugs and because of their side-effect, scientist are making more efforts to search for new drugs from plants resource to combat MDR microbial infection.

CONCLUSION

The result of Antibacterial susceptibility assay shows promising evidence for the antibacterial effect *Nyctanthus arbortristis* and *Ficus bengalensis* have the wide spectrum of antimicrobial activity on the bacteria. These have various medicinal values and has been used since earliest time as a medicine for curing various diseases.

Future Prospects

Traditional medicinal are now the mainstay of drug recovery, for the treatment of emerging and old diseases. Each part of the plant has some medicinal value and is thus commercially exploitable. It is now and considered as a valuable source of several unique products for the medicines against various disease and also for the development of some industrial products. The present review includes comprehensive information on the chemical constituents, biological activities of important compounds, pharmacological actions and medicinal applications.

However, there is need to ensure that, what is know is made use for financial gain. For improvement of the health of people there is a need to establish the necessary expertise for development of traditional medicines and deliberate efforts should be made to encourage local industrial production of herbal medicines and these herbal products signify the safety in contrast to the synthetics that are regarded as unsafe to human as well for the environment.

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