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SCREENING AND PHYTOCHEMICAL ANALYSIS OF PHARMACOLOGICALLY ACTIVE COMPOUNDS FROM *Abutilon Indicum* and *Phyllanthus Niruri* AND ASSESSING THEIR IN VITRO ANTI MICROBIAL ACTIVITY AGAINST PATHOGENS

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ABSTRACT

In the present investigation, the evaluation of anti-bacterial activity of *Abutilon indicum* and *Phylanthus niruri* has been carried out against human pathogenic microbes by disc diffusion agar method. The shade dried and powdered leaves were used for the extraction with different solvents. Thin Layer Chromatography profiles and phyto chemical screening were carried out for these extracts. The antagonistic activities of the extracts were tested against human pathogens. The results were tabulated and discussed with the related studies reported earlier. It is seen that the ethyl acetate extract of the leaves showed significant (0.7cm) anti bacterial activity when compared to the standard tetracycline against selected gram positive and gram negative bacteria.

Keywords: *Abutilon indicum; Phylanthus niruri*; Thin Layer Chromatography; Phyto chemical analysis; Anti-bacterial activity; Disc diffusion method.

[I] INTRODUCTION

Nature has best owned on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the World. There has been an increasing interest worldwide on therapeutic values of natural products. It is generally considered that the cure to any debilitating human ailments and diseases may be found among the world's flora in nature's pharmacy. Throughout the ages, humans have relied on nature for their basic needs for shelter, clothing, fertilizers, flavors and fragrances, and above all for food and medicinal remedies. Natural products have played an important role in treating and preventing human ailments. It is estimated that over 50 per cent of all drugs (and their derivatives and analogs) in clinical use, are higher plant-derived natural products.

According to the World Health Organization (WHO), about 80 per cent of the people in developing countries still rely on traditional medicine for their primary health care, and about 85 per cent of such medicines involve the use of plant extracts. These natural products have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates[1]. More recently, many compounds of pharmaceutical importance are commercially produced by microbes. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The plants having medicinal values are a source of great economic value all over the world . The earliest mention of medicinal use of plants in Hindu culture is founds in ―Rigveda, which is said to have been written between 4500-1600 B.C. This script is considered to be the oldest repository of human knowledge. Ayurveda, which is considered the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing.[2].

In recent years due to going reorganization of natural products and process in sustaining human and environment health the economic environment and importance of medicinal plants resources have increased tremendously[3]. The demand of natural food addicts is increased considerably, so the herbs and species can be a better option for the replacement of synthetic anti microbial agent. In general the medicinal plant containing essential oil, alkaline, flavanoids, and phenol contents may passes strong anti microbial properties.

Abutilon indicum (Malvacae) and *Phylanthus niruri* (Euphorbiaceae) known commonly as thuti and keezhanelli are distributed throughout India. These leaves have been used in siddha and ayurvedic system of medicine as remedy for jaundice, skin diseases, anti multi drug resistance, ulcer, asthma and chronic bronchial infection.[4,5]. The present study is carried out to determine the anti-bacterial activity of two different extracts of leaves and treated against gram positive and gram negative bacteria using the disc diffusion agar method. To best our knowledge there are no reports regarding the combined anti-microbial activity of these two species. Hence the present study is an attempt to screen the phytochemical analysis and investigate the combined antimicrobial activity of *Abutilon indicum and Phylanthus niruri.*

[II]MATERIALS AND METHODS

2.1.Plant material:

The leaves of *Abutilon indicum* and *Phyllanthus niruri* were collected from the Cauvery river bank, Lakkapuram, Erode, Tamil Nadu, India during July-August. Again *Abutilon indicum* and *Phyllanthus niruri* leaves and their nodes were collected from the campus of the K.S.R College of the Tiruchengode. The stock specimens were authenticated at the Rajsinat herbarium, Thiruchirapalli, Tamil Nadu, India.

2.1.1.Preparation of crude extract:

10 grams each of the cold dried and coarsely powdered plant material were exhaustively extracted for 2 hours with different solvents like petroleum ether (60-80°C), ethanol (78.4°C), ethyl acetate (77°C), and acetone (56.5°C) in soxhlet apparatus. The extracts were dissolved DMSO to make final concentrations which was kept in refrigerator till used. For the antibacterial activities, the positive control used was Tetracycline standards for gram positive and gram negative bacteria respectively [6]. 0.2 ml of the samples was added to the well. The zones of the inhibition produced by the extracts were compared with standards disc.

2.1.2 Thin Layer Chromatography (TLC)

TLC analysis was carried out for the plant extracts dissolved in different solvent. For the analysis the silica gel sheet was used, fresh leaf extracts and the cold dried leaves extracts were analyzed using TLC. The sheets are kept

in TLC Chamber for one hour, depending on the polarity of the eluted fractions to be analyzed. The sheets were treated with 1% ninhydrin diluted to acetone and heated in an oven at 50°C for 30 seconds.

2.2. Phytochemical screening

Phyto chemical screening was performed using standard procedures [7]

2.2.1. Test for Reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction.

2.2.2 Test for Anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

2.2.3. Test for Terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

2.2.4. Test for Flavonoids

Three methods were used to test for flavonoids. First, dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution.

In all the cases, a yellow colorations indicating the presence of flavonoids was observed.

2.2.5 Test for Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and the mixture is observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.2.6 Test for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.2.7 Test for Cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

[III] Results

The Phyto chemical screening of *Abutilon indicum and Phyllanthus niruri* showed positive results in the tests like Reducing Sugar, Anthraquinone, Terpenoids, Flavonoids, Saponins, Tannins, and Cardiac glycosides of Abutilon indicum. (Figure 1) But Cardiac glycosides test for *Phyllanthus niruri* showed negative result. (Table 1)

The extract of the leaves of the *Abutilon indicum and Phyllanthus niruri* were subjected to different solvents like Acetone, Ethanol, Petroleum ether and Ethylacetate were subjected to preliminary screening for antibacterial activity against five standard bacteria *Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Klebsiella, Bacillus subtilis.* The petroleum ether and aqueous extract of *Abutilon indicum and Phyllanthus niruri* were found to be inactive against all organisms tested and there was no zone of inhibition (Figure 2) Acetone extract of *Abutilon indicum and Phyllanthus niruri* showed low activity against *Bacillus substilis* (9mm),

pronounced activity against *Klebsiella* (19mm) and inactive against the rest of the organism. (Figure 3 & Figure 4) The Ethylacetate extract of *Abutilon indicum* and *Phyllanthus niruri* exhibited pronounced activity against gram positive *Staphylococcus* (18mm), then *Bacillus subtilis* (9mm), then gram negative *E.coli*(12mm), *Klebsiella sp* (9mm) and low activity against *Pseudomonas aeruginosa*(3mm) (Figure5 & Figure6) Ethanol extracts of *Abutilon indicum* and *Phyllanthus niruri* exhibited pronounced activity against gram negative bacteria like *E.coli* (3mm), *Klebsiella* (14mm), inactive against *Pseudomonas* and gram positive bacteria like *Bacillus substili* (15mm) and staphylococcus (10mm) (Figure 7 & Figure 8)

[IV] Discussion

Every medicinal plants have their own medicinal compounds and its effectively fought against different organisms. Single plant activity is very less compared to combined plant activity. Phytochemical screening of the plants revealed some differences in the constituents of the plants tested. In this study the combined antimicrobial activity of these two plants showed very high activity. *Abutilon indicum and Phyllanthus niruri* showed positive results for phytochemical tests like Sugars, Terpenoids, Flavanoids but *Phyllanthus niruri* showed negative results for cardiac glycosides.

The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity [8]

Steroids and Flavanoids were found to be present in all the plants. It has been observed that these investigated plants might contain steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones [9]

Flavonoids have been reported to be synthesized by plants in response to microbial infection and have been shown to have antibacterial activities [10]. Tannins were also reported have demonstrated activity against bacteria. [11]. In the antimicrobial activity, the predominant activity of combined plant extract was clearly observed with Ethyl Acetate on *Staphylococcus* (18 mm) and *E.coli* (12 mm). 19mm zone of inhibition was observed in *Klebsiella* culture by acetone solvent (Table 2). Apart from the Ethyl acetate and Acetone the rest of the solvents also showed less zone of inhibition than the normal antibiotic disc like tetracycline (Table 3). The phytochemical and Antimicrobial investigations suggest that that the presence of flavanoids and triteraphenoids, phenolic steroids, ketones, and tetra triterpenoids compounds[12] in *Abutilon indicum and Phyllanthus niruri* extracts. Traditionally *A.indicum* is used in inflammation, piles, gonorrhea treatment and as an immune stimulant. Root and bark are used as aphrodisiac, anti diabetic, nervine tonic, and diuretic. Seeds are used as aphrodisiac and in urinary disorders.[13]

The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. According to Dhanalaksmi *et al.* [14] stated that that the presence of flavonoids and triterpenoids was presented in the ethanolic extract of *A. indicum*. Phytochemicals or secondary metabolites usually occur in complex mixtures that differ among plant organs and stages of development. [15]. Understanding of the phytochemical constituents is important for exploration of the authentic effectiveness of the plant. It is not surprising that there are differences in the antibacterial activities of the extracts of the different extracts of tested plants.[16].

The phytochemical analysis of *Abutilon indicum* and *Phylanthus niruri* revealed the presence of alkaloids saponins glycosides, tannins, steroids, flavonoids and carbohydrates. This confirms its therapeutic potentials as claimed by ethnobotanical users.

[V] CONCLUSION

Results had indicated that the phenolics, saponins and flavonoids, are present in the tested species. Also, these species record a good antagonistic activity against human pathogenic microbes. It is also possible to prospect on these plant species and identify the allelopathic effects on the other organisms and ecotypes.

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Table 1: Screening of Phyto chemical analysis

S.NO	Test	*Abutilon indicum*	*Phylanthus niruri*
1.	Reducing Sugar	+	+
2.	Anthraquinone	+	+
3.	Terpenoids	+	+
4.	Flavonoids	+	+
5.	Saponins	+	+
6.	Tannins	+	+
7.	Cardiac glycosides	+	-

Table 2: Preliminary screening for antimicrobial activity against standard organisms

	Acetone	Petroleum ether	Ethyl acetate	Ethanol
E.Coli	**-**	**-**	**12**	**12**
Klebsiella.sp	**19**	**-**	**9**	**14**
Pseudomonas.sp	**-**	**-**	**3**	**-**
Bacillus subtilis	**9**	**-**	**-**	**9**
Staphylococcus aureus	**-**	**-**	**18**	**15**

Values represented above are the mean diameter inhibition zone in mm of the combined extracts of *Abutilon indicum and Phylanthus niruri*

Table 3: Screening of Antibacterial activity of tetracycline against standard organisms

Drug	Concentration (µg/ml)	Mean diameter Inhibition Zone (mm)				
		Klebsiella sp	*Pseudomonas sp*	*Bacillus substilus*	*E.coli*	*Staphylococcus*
Tetracycline	100	27	22	13	31	24
	40	25	18	14	30	25
	20	24	21	12	26	23
	10	20	17	13	22	20

Values represented above are the mean diameter inhibition zone in mm of the combined extracts of *Abutilon indicum and Phylanthus niruri*

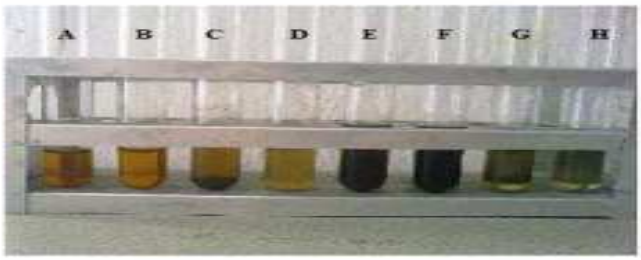

Figure 1: Phytochemical Analysis

A-Fehalings test for A.indicum, B-Fehilings test for P.niruri
C-[Flavonoids test for A.indicum, D-Flavonoids test for P.niruri
E-Tannis test for A.indicum, F-Tannis test for P.niruri
G-Cardiac glycosides test for A.idicum, H-Cardiac glycosides test for P.niruri

Figure 2: Petroleum ether extract shows negative result.

Figure 3: Acetone Extract's Zone of Inhibition on *B.subtilis*

Figure 4: Acetone Extract's Zone of Inhibition on *Klebshiella*

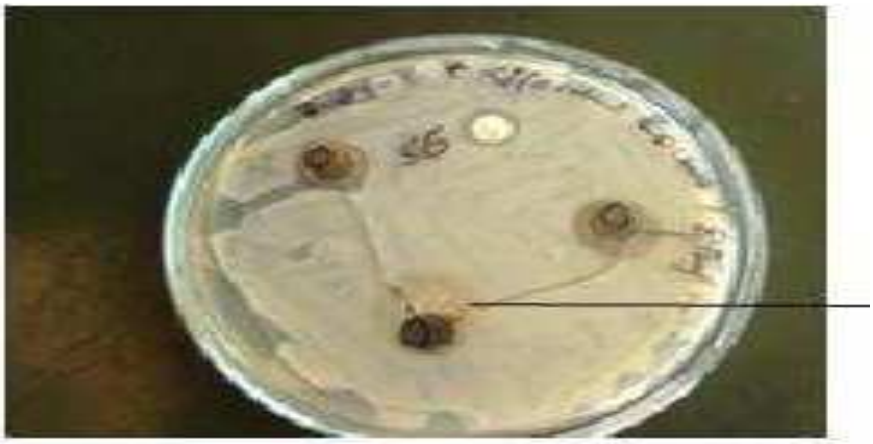

Figure 5: Ethylacetate's Extract's Zone of Inhibition on *Sthaphylococcus sp.*

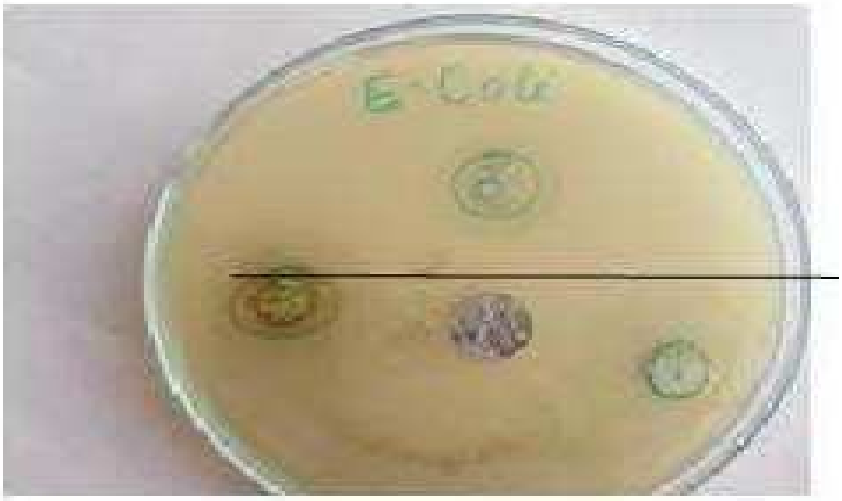

Figure 6: Ethyl Acetate Extract's Zone of Inhibition on *E.coli*

Figure 7: Ethanol's Extract's Zone of Inhibition on *E.coli*

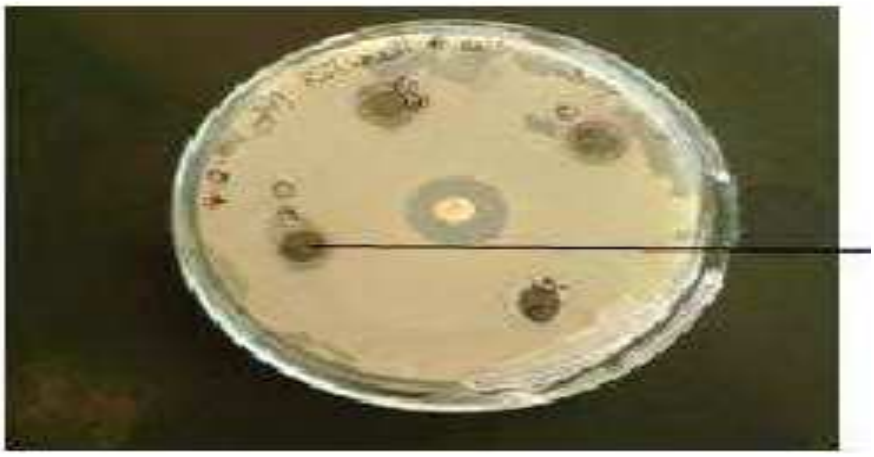

Figure 8: Ethanol's Extract's Zone of Inhibition on *Staphylococcus sp.*