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ANTIMICROBIAL STUDIES AND PHYTOCHEMICAL SCREENING OF THE LEAVES IN *TILIACORA ACUMINATA* (LAM.) HOOK. & THOMSON AND *DIPLOCLISIA GLAUCESCENS* (BLUME) DIELS. (MENISPERMACEAE)

M. Immanuel Sagayaraj*, S. John Britto, M. Thamacin Arulappan, J. Krishnakumar, Sinjumol Thomas and Mariat George.

The Rapinat Herbarium and the Centre for the Molecular Systematics, St. Joseph's College (*Autonompous*), Tiruchirappalli, Tamilnadu, India.

ABSTRACT

Leaves of *Tiliacora acuminata* (Lam.) Hook & Thomson and *Diploclisia glaucescens* (Blume) Diels**.** are most commonly used to cure various human ailments. Antimicrobial and qualitative phytochemical studies have been carried out in them using chloroform, ethanol and aqueous extracts. Studies showed that leaf ethanolic extracts were active against *E.coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*. Phytochemical studies reveal the presence of tannins, alkaloids and, proteins and other biocompounds.

Key words: Antimicrobial, Phytochemical, Biocompounds.

INTRODUCTION

Plants have been used for therapeutic purposes long before recorded history. Ancient Chinese and Egyptian Papyrus writings describe medicinal uses of plants from as early as 3000 B.C. Indigenous cultures such as African and Native American tended to use herbs in their healing rituals, while others developed traditional medicinal systems such as Ayurveda and Traditional Chinese Medicine in which herbal therapies were used [1]. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as *Ayurveda*, *Unani* and *Siddha*. In India, it is reported that traditional healers use about 2500 plant species and among them100 species of plants as regular sources of medicine. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use [2].

Plants have played an important role in the discovery of novel and useful drugs used in modern medicine. There are a number of drugs of plant origin which are useful, with lifesaving capacity and providing immediate therapeutic benefit [3]. Among the estimated 250,000 – 500,000 plants species, only a small number has been screened phytochemicaly and their fraction submitted for biological (or) pharmacological screening. Over 2000 plant species are found to have medicinal value and are used for medicinal purpose in different forms [4]. The most important of these bio active compounds of plant origin are alkaloids, glycosides, essential oil, sponins, tannins, steroids, terpenoids, flavonoids, protenis, and others [5].

MATERIALS AND METHOD

Collection of Plant materials

The matured plant leaf part of *Tiliacora acuminata* (Lam.) Hook. & Thomson (Menispermaceae), *Diploclisia glaucescens* (Blume) Diels (Menispermaceae) were collected from two different places *T. acuminata* from Cauvery river banks while, *D. glaucescens* from the Palni hills. Taxonomic identification of these plants was carried out by Dr. S John Britto, Director at the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. Voucher specimens (*D. glaucescens* RHT 56221, *T. acuminata* RHT 56238) have been deposited at the Rapinat Herbarium.

Corresponding Author :- **M. Immanuel Sagayaraj** Email:- immanuelraj108@gmail.com

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***Diploclisia Glaucescens* (Blume) Diels.**

A dioecious large woody, climber with stems up to 20 cm diam. Leaves usually coriaceous, 4-10 cm long, broadly ovate to suborbicular, apex rounded to acute, margin broadly and shallowly crenate. Inflorescences cauliflorous, about 50 cm long. Flowers very small, yellowish. Drupes yellow to orange, 14-22 ´ 8-13 mm, slightly curved.

Ethnomedicinal Uses:

Diplocilsia glaucescens plant leaves, gingelly oil and coconut (*Cocosnucifera*) oil 100 ml each is heated and applied externally for sprain.

Tiliacora acuminate (Lam.) Hook & Thomson

Dioecious woody straggler. Stems striate, sparsely puberulous or glabrous. Leaves alternate, ovate or lanceolate, truncate, cordate, rarely acute at base, acuminate at apex, 8-14 x 3.5- 8 cm, chartaceous, glabrous, 3-5 nerved at base; petiole 1.5-3 cm long, sulcate, glabrous. Inflorescences axillary, panicled, 3.5-10 cm long, pubescent. Male flowers 2-7 at apex of inflorescence, yellow; sepals 6 in 2 rows; inner ones broadly elliptic, glabrous; petals 6, obovate, glabrous; stamens 6, cylindric. Female with sepals and petals as in male ones; carpels 8-12, glabrous. Drupes, oblong to obovoid, 10-15 x 6-7 mm, glabrous, red when ripe; endocarp reticulate.

Ethnomedicinal Uses:

Tiliacora acuminata plant is used as an antidote for snake bite. The leaf paste is applied on the bitten area soon after snake bite.

Preparation of extracts

Leaves of two plants of *Tiliacora acuminata* and *Diploclasia glaucescens* were shade dried and then powdered with the help of waring blender. 25g of shade-dried powder was filled in the thimble and extracted successively with ethanol, chloroform, and aqueous in soxhlet extractor for 48 h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Antimicrobial studies

Bacterial isolates and Bioassay

The extracts of chloroform, ethanol and aqueous from the selected plant tissues were screened against 10 bacterials strains of the test organisms, *Escherichia coli*,(MTCC # 119) *Pseudomonas aeruginosa* (MTCC # 2474), *Salmonella paratyphi* (MTCC # 734), *Vibrio cholerae* (ATCC # 14104), *Streptococcus pneumonia* (ATCC # 7066), *Bacillus subtilis* (MTCC # 441), *Bacillus cereus* (ATCC # 4342), *Proteus vulgaris* (MTCC # 1771), *Proteus mirabilis* MTCC # 1429), *Serratia marcescens* (MTCC # 2645).

Preparation of inoculums

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to the test tubes of Mueller Hinton broth (MHB) for bacteria and were incubated without agitation for 24h at 37°C.

Phytochemical screenings

Preliminary Phytochemical Analysis

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids and terpenoids were carried out for all the extracts by the method described by Mukherjee [6].

Test for Phenol (*Ferric chloride test*): To 1ml of the leaf extract and 1ml of tuber extract 2ml of distilled water was added followed by few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for Sterols* (Liebermann-Burchard test*): To the test solution, 3-4 drops of acetic anhydride was added, the solution was boiled cooled and conc. Sulphuric acid (3 drops) was added. A brown ring appears at the junction of the two layers. The upper layer turns green showing the presence of steroids.

Test for Tannins:

***(a)* Gelatin test:** To 2ml test solution, 1% Gelatin solution containing 10% sodium chloride was added to obtain a white precipitate.

Test for Flavanoids:

(a)* Zinc chloride reduction test*): To 2ml test solution, a mixture of zinc dust (Merck, India) and conc. HCl (Qualigens, India) was added. A red colour was obtained after few minutes.

***(b)* Alkaline reagent test:** To 2ml test solution, sodium hydroxide (Qualigens, India) solution was added to give a yellow or red colour.

Test for Alkaloids:

***(a)* Mayer's test:** To 2ml test solution, 2N HCl was added. The aqueous layer formed was decanted and Mayer's reagent (Qualigens, India) was added to it. A cream coloured precipitate indicated the presence of alkaloids.

Test for fats and fixed oils:

(a) Stain test: Small amount of the extract was pressed between two filter papers; the stain on the filter paper indicates the presence of fixed oils.

(b) Saponification test: Few drops of 0.5N alcoholic potassium hydroxide was added in small quantity to the extract solution with a drop of phenolphthalein and heated on a water bath for 1-2h. The formation of soap or partial

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neutralization for the alkali indicated the presence of fats and fixed oils.

Test for Glycosides: To 2ml test solution, equal quantity of Fehling's solution A and B was added and solution was heated. A brick red precipitate indicated the presence of glycosides.

Test for proteins and amino acids:

(a) Millon's test*:* To 2ml test solution, Millon's reagent was added which gave a white precipitate, which on heating changed to red.

(b) Ninhydrin test: To 2ml test solution, Ninhydrin solution was added and the solution was boiled. Amino acids and proteins when boiled with 0.2% Ninhydrin reagent showed violet colour.

Table 1. Antibacterial activity of *Tiliacora acuminata* and *Diploclisia glaucescens* Leaf extract (25/µl) and control Antibiotic (15/µl)

S. No.	Microorganisms	Zone of inhibition (mm)			
		Tiliocora acuminata		*Diploclisia glaucescens*	
		Ethanol	Chloroform	Ethanol	Chloroform
1	*Escherichia coli*	25	-	7	-
2	*Pseudomonas aeruginosa*	19	-	7	-
3	*Salmonella paratyphi*	13	-	8	-
4	*Vibrio cholerae*	-	15	-	12
5	*Streptococcus pneumoniae*	7	-	7	-
6	*Bacillus subtilis*	-	-	-	-
7	*Bacillus cereus*	16	-	7	-
8	*Proteus vulgaris*	-	20	-	-
9	*Proteus mirabilis*	-	-	7	-
10	*Serratia marcescens*	-	-	7	-

Standard Disk = 6 mm

Table 2. Phytochemical test in the Matured Leaf of Ethanolic, Chloroform and aqueous extracts of *Tiliocora acuminata*

S.No.	Phytoconstituents	A	E	C
1	Phenol	-	+	-
2	Steroids	-	-	-
3	Tannins	+	+	-
4	Flavonoids	+	+	-
5	Alkaloids	+	+	-
6	Saponins	+	-	-
7	Glycosides	-	+	+
8	Proteins	+	+	+
9	Aminoacids	+	+	+

A= Aqueous, E= Ethnolic, C= Chloroform; (+) Present, (-) Absent).

Table 3. Phytochemical test in the Matured Leaf of Ethanolic, Chloroform and aqueous extracts of *Diploclisia glaucescens*

S.No.	Phytoconstituents	A	E	C
1	Phenol	+	+	+
2	Steriods	-	-	-
3	Tannins	+	+	-
4	Flavonoids	+	+	+
5	Alkaloids	+	-	-
6	Saponins	+	-	-
7	Glycosides	-	+	+
8	Proteins	+	+	+
9	Aminoacids	+	+	+

A= Aqueous, E= Ethnolic, C= Chloroform; (+) Present, (-) Absent).

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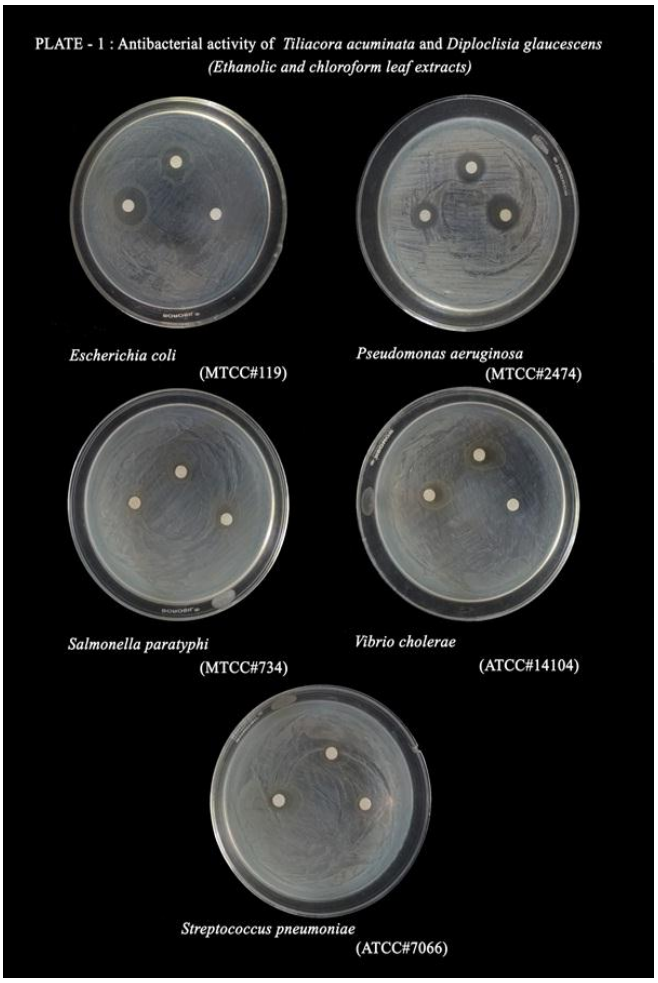

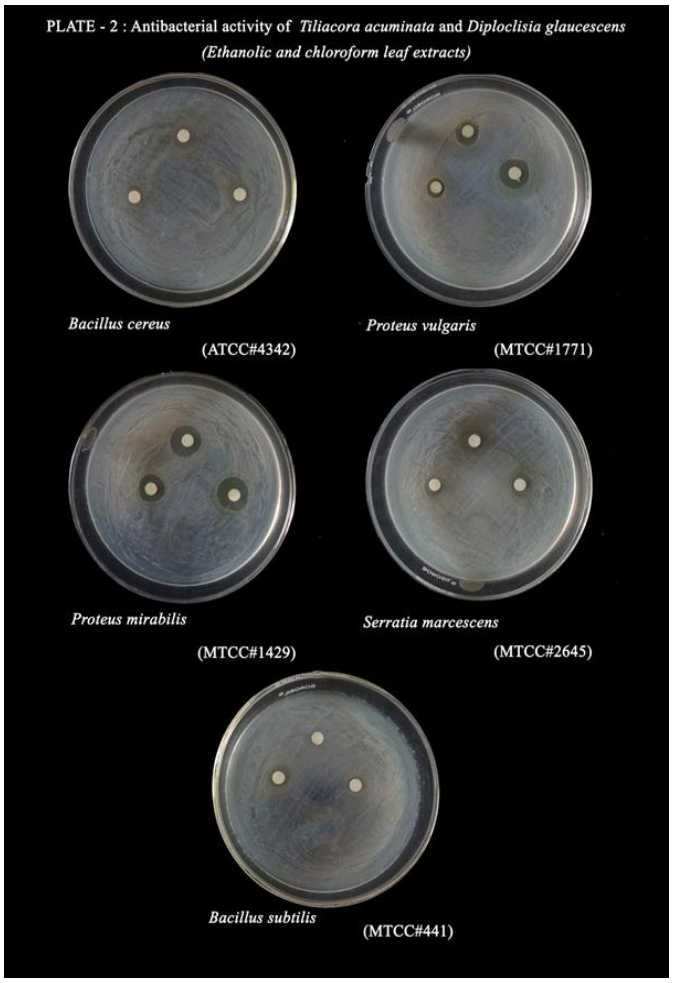

RESULTS AND DISCUSSION

Antibacterial studies

The ethanolic extract of *Tiliocora acuminata* showed various microbial activities in microorganisms like, *E.coli* (21mm), *Pseduomonas aerogenosa* (19mm), *Salmonella paratyphi* (13mm) and *Bacillus cereus* (16mm). It is observed that *E.coli* showed high microbial activity followed by *P.aerogenous*, *S.paratyphi* and *B.cereus*. While, the chloroform extract of the same plant shows microbial activities only in two microorganisms, i.e. *Vibrio choleare* (15mm) and *Preteus vulgaris* (20mm). *Preteus vulgaris* shows high activity as compared to *Vibrio cholerae*.

The ethanolic extract of *Diploclisia glaucescens* shows *E.coli* (7mm), *Pseudomonas aerogenosa* (7mm), *Salmonella paratyphi* (8mm), *Streptococcus pneumoniae* (7mm), *Bacillus cereus* (7mm), *Proteus mirabilis* (7mm) and *Serratia marscescens* (7mm). *Salmonella paratyphi* showed the highest microbial activity as compared to other microorganism, while the chloroform extract of the same plant showed the activity of *Vibrio cholerae* (12mm) only.

Phytochemical Studies

The triphytochemical screening (aqueous, ethanolic and chloroform) of the extracts of leaves in *Tiliocora acuminata* (leaf) revealed that amino acids and proteins were present in all extracts. Glycosides were observed in ethnolic and chloroform extracts and it was absent in the aqueous extract. Tannins, flavanoids and alkaloids were present in aqueous and ethnolic extracts and while chloroform showed absence of them. Phytoconstituents like phenol and saponins were observed from one extract only. The former was present in ethnolic extract and the latter was observed in aqueous extract. The triphytochemical screening was conducted on the leaf extracts of *Diploclisia glucescens.* Phenols, flavanoids, proteins and amino acids were observed in all the extracts (aqueous, ethnanolic and chloroform). Ethnolic and chloroform extracts showed the presence of only one phytochemical constituent, glycosides. Tannins were present in aqueous and ethanolic extracts and while chloroform showed its absence. The aqueous extract alone showed the presence of alkaloids and saponins.

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CONCLUSION

This study supports that *Tilacora acuminata* and *Diplocilisa glaucescens* showed has potential antimicrobial activity against different bacterial strains. This antibacterial activity was associated with the variety of phytochemicals found in these plants. The above species has potential to be harnessed for further study in drug discovery.

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