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ANTIMICROBIAL ACTIVITY OF *COLEUS FORSKOHLII* ROOT EXTRACT AGAINST HUMAN PATHOGENS

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ABSTRACT

Medicinal plants are the wealthy source of antibacterial agents and curatives. *Coleus forskohlii* are commonly practiced medicinal plants in the villages of Salem District, Tamilnadu (India). Plants grown in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular. Hence, In vitro antibacterial activity of crude root extracts of the plants was tested by disc diffusion method against human pathogenic bacteria *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia Staphylococcus aureus, Staphylococcus epidermidis*. Gram-negative bacterial strains were more susceptible to the crude extracts as compare to gram-positive. However, this study revealed maximum growth inhibition and effectiveness was remarkably observed in the extracts of *Coleus forskohlii*. These results indicate that leaves have a potential broad spectrum antibacterial activity.

Key words: *Coleus forskohlii*, Antibacterial activity, Human pathogens and Disc diffusion.

INTRODUCTION

Plants are the first medicines for mankind and hundreds of plant species are harvested for their medicinal properties all over the world. In spite of modern development of sophisticated pharmaceutical chemicals to treat illnesses, medicinal plants remain an important tool for treating illness. In some regions, traditional medicines made from local plants are the only available and affordable source for treating various ailments. World Health Organization (2003) estimates that 80% of the world's population depends on traditional medicine for their health needs. In many developed countries, traditional herbal remedies are making a comeback as alternatives to modern medicine.

The existence of traditional medicine depends on plant diversity and the related knowledge of their use as herbal medicine. India is one of the twelve mega diversity hot spot regions of the world and one fifth of all plants found in India are used for medicinal purpose (Schippmann *et al.,* 2002). Nearly 25,000 effective plant based formulations are used in folk medicine by rural communities in India (Ramakrishnappa *et al.,* 2002). Both plant species and traditional knowledge are important to the herbal medicine trade and the pharmaceutical industry, whereby plants provide raw materials and the traditional knowledge prerequisite information (Tabuti *et al.,* 2003). Encompassing concepts and methods for the protection and restoration of health, traditional medicine has served as source of alternative medicine, new pharmaceuticals and healthcare products. Medicinal plants are important for pharmacological research and drug development, not only when constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Mukherjee *et al.,* 2003). The world market for plant derived chemicals viz., pharmaceuticals, fragrances, flavours and colour ingredients exceeds several billion dollars per year. Classic examples of phytochemicals in biology and medicine include taxol, vincristine, vinblastine, colchicine as well as the Chinese antimalarial - artemisinin and the Indian ayurvedic drug - forskolin.

C. forskohlii Briq. is a member of the mint family, Lamiaceae. It is indigenous to India and is recorded in Ayurvedic Materia Medica under the

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Sanskrit name 'Makandi' and 'Mayani" (Shah *et al.,* 1996).

The genus Coleus was first described by Loureiro in 1790 and the generic name was derived from the Greek word 'COLEOS' meaning sheath. All the species of Coleus have four didynamous, dedinate stamens, and the filaments of the stamens unite at their base to form a sheath around the style. The species name forskohlii was given to commemorate the Finnish botanist, Forskel. The genus Coleus consists of 150 species and the following species viz., *C. amboinicus, C. forskohlii, C. spicatus and C. malabaricus* occur naturally.

The therapeutic properties of forskolin, the main diterpene constituent of this plant contributed to the emergence of *C. forskohlii* as a taxon of importance in modern medicine. Forskolin is used for the treatment of eczema, asthma, psoriasis, cardiovascular disorders and hypertension, where decreased intracellular cAMP level is believed to be a major factor in the development of the disease process (Rupp et al., 1986). The presence of yellowish to reddish brown cytoplasmic vesicles in cork cells of *C. forskohlii* tubers is unique character of this plant and these vesicles store secondary metabolites including forskolin (Abraham *et al.,* 1988).

The experimental plant of the present study *coleus forskohlii* root is mint family has long been cultivated in India, Thailand and parts of south East Asia. The plant was known for their medicinal properties and extensively used in Indian medicinal system. The present study was aimed to elucidate the medicinal property of the plant through their antimicrobial feature. The plant contains their active alkaloid components in roots system. The leaf of the plant was found to have more active substances and in the root. The antimicrobial effect of coleus root against pathogens, *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus epidermidis*. Disc diffusion method has been adopted in this study and petridishes containing nutrient agar medium seeded with the test pathogens was used for antimicrobial assay. Test materials diffuse from the disc to the surrounding medium of the plate. Then the plates were incubated at 37^0C for 24hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition in terms of millimetre. The antibacterial screening showed that the root extract of coleus forskohlii showed antimicrobial activity against test pathogens used in this study depending on the nature of their active alkaloid compounds in the extract and capacity of diffusion into the agar medium. Among the test pathogens.

A survey of World Health Organization (WHO) indicates that about 70-80% of the world population in the developing countries depends on herbal sources as their primary healthcare system. Phytoconstituents such as flavonoids, alkaloids, tannins, and triterpenoids are rich source of many medicinal plants challenges the modern medicine and stimulating opportunity for the expansion of modern chemotherapies against wide range of microorganisms. Due to the increasing failure of chemotherapeutics and rapid development of multi resistant bacterial strains of clinically important medical pathogens acquired the interest of scientist to develop newer broad spectrum antimicrobial agents. The less availability and unaffordable cost of new generation antibiotics initiated to look for alternative phyto medicine to discover plant derived constituents with claimed antimicrobial activity. The extractable bioactive compounds in medicinal plants are a significant alternative approach to synthetic antibiotics, which could be used as valuables in human disease management. The plant has ethno medicinal importance for the treatment of arterial hypotension, hypoglycemia and diabetes. The leaves contain active alkaloids such as Tecomine and Tecostanine. Many other diterpenoids as deacetyl forskolin, 9-deoxyforskolin, 1,9-deoxyforskolin, 1,9-dideoxy-7-deacetylforskolin have been isolated. Other minor phytochemicals are Allylroyleanone, Barbatusin, Plectrin, Plectirinon A, Acetoxycoleosol, Coleol, Coleonone, Coleosol, Deoxycoleonol, Crocetin dialehyde, apthopyrones.

MATERIALS AND METHOD

Plant Sample collection

Coleus forskohlii root sample was collected from horticultural research centre, Salem city using sterilized bag and was transported to the laboratory within 6 hours after collection. The sample were immediate analyses were not possible, the samples were preserved at 4°C (Pavendan *et al.,* 2011).

Plant Identification

The *Coleus forskohlii* plant was identified with the help of published regional flora (Gamble *et al.,* 1935; Matthew *et al.,* 1983) and the identified were verified with the help of botanical survey of India, Salem.

Preparation of plant extracts

The Roots of *Coleus forskohlii* were collected and were shade dried, powdered, and extracted in soxhlet apparatus successively with methanol, ethanol, chloroform, and water respectively due to their nature of polarity. After extraction, the hexane and aqueous extracts were filtered through Whatman No.1 filter paper and stored for further use.

From the stock solution different concentrations 25 % (0.5 ml of extract + 1.5 ml of distilled water) 50 % (1.0 ml of extract + 1 ml of distilled water), 75 % (1.5ml of extract + 0.5 ml of distilled water) and 100 % (2 ml of

extract only) of the extracts were prepared four extracts were prepared namely, Methanol, Ethanol, Chloroform and Aqueous using distilled water.

Phytochemical Screening

The leaf extracts of *Coleus forskohlii* were analyzed for the presence of Trepenoids, Flavonoids, Steroids, Anthroquione, Glycosides, Sugars, Alkaloid, Quinones, Phenols, Tannins, Saponins, Protein, lipids and Coumarin according to standard methods (Sai Ramesh *et al.,* 2010; Harbone *et al.,* 1973; Trease and Evans *et al.,* 1989).

Sterilization

All the glass wares used were washed, dried and sterilized in hot air oven at a temperature of 160°C for 1 h according to the method described by Adibe and Eze (2004); Eze et al., 2011. Culture media used were sterilized in an autoclave at a temperature of 121°C for 15 min. The wire loop was also sterilized using spirit lamp.

ISOLATION AND IDENTIFICATION OF BACTERIA

Sample Collection

Wound from diabetic patients samples was collected from various regions around Trichy city using swab disinfected container and was transported to the laboratory within an hour after collection. When immediate analyses were not possible, the samples were preserved at 4^0C (Pavendan *et al.,* 2011).

Microbial Screening

The samples were serially diluted, and spread on to the Sterile Nutrient agar, EMB agar, Mannitol salt agar, Macconkey agar, and Pseudomonas isolation agar plate. All the plates were incubated at 37^0C for the 24- 48 hrs. The isolated bacterial colonies were identified by using their morphological characteristic, cell shape by Grams staining, motility, and Based on their living cell with standard procedure (Holt *et al.,* 1994).

Preparation of the compounds (Powder)

The *Coleus forskohlii* plant root extracts and makes them to 500 gms of pieces. The forskolin compounds were separated from that plant root, after that root pieces transfer to a mixture of acetone 1ml of water 2ml (1:2) (v/v) and then this set up is allowed for stirring 50-60^{o}c for 3hours, and then it is filtered.

Finally the filtrate was separated using extraction flask and adds 2 litre acetone and again allows it for stirring 50-60^{o}C for 2 hours. This procedure Repeated up to 5 extractions respectively. From this extraction TDS assay were done.

Disc diffusion method using root compound extracts (Powder)

Antimicrobial activity of the root extracts was tested using the disc diffusion method (Aida *et al.,* 2001; Peach and Tracey *et al.,* 1950). Sterile nutrient agar plates were prepared for bacterial strains and inoculated by a spread plate method under aseptic conditions. The filter paper disc of 6 mm diameter (Whitman's No. 1 filter paper) was prepared and sterilized.

The root compound extracts to be tested were prepared various concentrations of 25 %, 50 %, 75 % and 100 % and were added to each disc of holding capacity 10 micro litre. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Ciprofloxacin disc was used as positive control. All the plates were incubated at 37^0C for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured (Doughari *et al.,* 2006).

Disc diffusion method using root extracts (Liquid)

The root of *coleus forskohlii* were collected and were air dried, powdered using a mortar and pestle and were further the powder was transferred into closed containers. Each of the powdered air-dried plant root material was extracted with water, methanol and ethanol. 25g of each powdered sample was mixed in a conical flask with 100ml of deionised distilled water or organic solvent, plugged, then shaken at 120 rpm for 30 minutes and kept for 24 h. After 24 h, each of the extracts was filtered rapidly through four layers of gauge and then by a more delicate filtration through Whatman No1 filter paper.

The resulting filtrates were then concentrated in a rotary evaporator and subsequently lyophilized to dryness. The yield of extract were prepared various concentrations of 25 %, 50 %, 75 % and 100 % and were added to each disc of holding capacity 10 micro litre.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extracts was estimated for each of the test organisms in triplicates. To 0.5ml of varying concentrations of the extracts (25 %, 50 %, 75 % and 100 %), 2ml of nutrient broth was added and then a loop full of the test organism previously diluted to 0.5 McFarland turbidity standard for (bacterial isolates) was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37^{o}C for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity.

To determine the MBC, for each set of test tubes in the MIC determination, a loop full of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar by streaking. Nutrient agar were streaked with the test organisms respectively to serve as control. Plates inoculated with bacteria were then incubated at 37^{o}C for 24 hours. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration.

RESULTS AND DISCUSSION

Natural products have been shown to be a tremendous and consistent resource for the development of new drugs (Kalaivani *et al.,* 2010). Plants are known to have beneficial therapeutic effects document in traditional Indian System of Medicine (Desai Nivas *et al.,* 2010). Much work has been done on ethnomedicinal plants in India (Vinoth Raja *et al.,* 2009). It has been suggested that phytochemical extracts from plants holds promises to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents (Nair *et al.,* 2005). Sometimes plant derived natural compounds have gained attention because of their potential to act as cytotoxic and chemopreventive activity (Kalaivani *et al.,* 2010). Various plants have already been proved to possess high antioxidant property containing high amounts of phenolics and flavonoids (Kalaivani and Mathew *et al.,* 2009).

The results of the antibacterial activity of *Coleus forskohlii* crude extracts, assayed in vitro by the disc diffusion method. The growth inhibitory effect of *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Klebsiella pneumoniae* are presented in Table – 1. All the tested pathogens are highly susceptible to the crude extracts. However, our study revealed a remarkable antibacterial activity against gram-negative bacterial strains than gram-positive. The most effective activity was proven by *Coleus forskohlii* with maximum zone of inhibition ranging from 15mm against *Salmonella typhi* and 14mm with *Staphylococcus aureus*. *Coleus forskohlii* inhibited the growth of *Staphylococcus epidermidis* with 15mm. All the 3 extracts inhibited *Klebsiella pneumoniae* with 8mm and 10mm respectively.

Though all the 3 extracts were found effective, the highest zone of inhibition and the effectiveness is the major consideration in the case of antibacterial activity. In comparison, the maximum growth inhibition was observed in the extracts *of Coleus forskohli*. These results indicate that leaves have a potential broad spectrum antibacterial activity. In future, these extracts can be combined as a formulation to treat the infectious diseases caused by the test organisms.

Table 1. Biochemical analysis

S.No	Biochemical Reaction	*E. coli*	*Klebsiella sp.*	*Pseudomonas* sp.	*Salmonella typhi*	*Staphylococcus aureus*	*Staphylococcus epidermis*
1	Indole	Positive	Negative	Negative	Negative	Negative	Negative
2	Methyl red	Positive	Negative	Negative	Positive	Positive	Positive
3	Voges proskauer	Negative	Positive	Negative	Negative	Positive	Positive
4	Citrate	Negative	Positive	Positive	Positive	Negative	Negative
5	Urease	Negative	Positive	Negative	Negative	Negative	Negative
6	Oxidase	Negative	Negative	Positive	Negative	Negative	Negative
7	Catalase	Positive	Negative	positive	Negative	Positive	Positive

Table 2. Characterization of isolated pathogen morphology

S.No	Character	*E. coli*	*Klebsiella* sp.	*Pseudomonas* sp.	*Salmonella typhi*	*Staphylococcus aureus*	*Staphylococcus Epidermidis*
1	Gram reaction	Negative	Negative	Negative	Negative	Positive	Positive
2	Morphology	Rod	Rod	Rod	Rod	Cocci	Cocci
3	Motility	Motile	Motile	Motile	Motile	Non-motile	Non-motile
4	Colony character	Metallic sheen on macconkey	Metallic sheen on macconkey	Produce yellow pigment	Red pink colonies	Ferment on blood agar	Ferment on blood agar

Table 3. Qualitative analysis of Secondary Metabolites on *coleus forskohlii* extracts

S.No	Extracts	Analysis														
		Trepenoids	Flavonoids		Steroids	Anthroquione	Glycosides	Sugars	Alkaloid		Quinones	Phenols	Tannins	Saponins	Protein	Lipids	Coumarin
			T_1	T_2					T_1	T_2							
1	Aqueous	-	-	-	-	-	+	+	-	+	-	-	+	-	-	-	+
2	Ethanol	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	+
3	Methanol	-	-	-	-	-	+	+	-	-	+	+	+	-	-	-	-

Table 4. Antibacterial activity of *coleus forskohlii* extracts

S.No	Plant extracts	Zone of inhibition (mm) ***E. coli***				
		Positive Control	25 µl	30 µl	35 µl	40 µl
1	Aqueous	17	0	0	05	07
2	Ethanol	17	0	0	6	11
3	Methanol	17	0	0	06	07
		Zone of inhibition (mm) *Klebsiella* sp.				
1	Aqueous	22	0	01	03	04
2	Ethanol	22	04	06	11	15
3	Methanol	22	01	02	05	07
		Zone of inhibition (mm) *Pseudomonas* sp.				
1	Aqueous	20	0	01	02	07
2	Ethanol	20	6	7	8	11
3	Methanol	20	0	6	7	08
		Zone of inhibition (mm) *Salmonella typhi*				
1	Aqueous	22	0	0	01	08
2	Ethanol	22	04	06	07	12
3	Methanol	22	04	06	07	08
		Zone of inhibition (mm) *Staphylococcus aureus*				
1	Aqueous	22	02	05	06	08
2	Ethanol	22	04	09	12	16
3	Methanol	22	01	03	06	09
		Zone of inhibition (mm) *Staphylococcus epidermis*				
1	Aqueous	22	02	05	09	12
2	Ethanol	22	03	06	10	16
3	Methanol	22	04	09	13	19

Table 5. Minimal inhibitory concentration of *Coleus forskohlii* extracts

S.No	Sample	5 µl	10 µl	15 µl	20 µl	µl
		Absorbance at 620nm *E. coli*				
1	Positive Control	0.064	0.051	0.048	0.043	0.040
2	Aqueous Extract	0.765	0.758	0.734	0.721	0.712
3	Ethanol	0.655	0.581	0.545	0.528	0.450
4	Methanol	0.963	0.864	0.750	0.702	0.687
		Absorbance at 620nm *Klebsiella* sp.				
1	Positive Control	0.043	0.038	0.034	0.032	0.028
2	Aqueous Extract	0.921	0.860	0.839	0.810	0.767
3	Ethanol	0.530	0.465	0.465	0.397	0.360
4	Methanol	0.890	0.846	0.831	0.763	0.700

		Absorbance at 620nm *Pseudomonas* sp.				
1	Positive Control	0.067	0.052	0.048	0.046	0.037
2	Aqueous Extract	0.843	0.832	0.820	0.796	0.755
3	Ethanol	0.420	0.370	0.354	0.327	0.271
4	Methanol	0.810	0.787	0.770	0.690	0.655
		Absorbance at 620nm *Salmonella typhii*				
1	Positive Control	0.049	0.040	0.032	0.028	0.022
2	Aqueous Extract	0.743	0.670	0.654	0.648	0.635
3	Ethanol	0.620	0.596	0.570	0.543	0.531
4	Methanol	0.910	0.890	0.875	0.856	0.841
		Absorbance at 620nm *Staphylococcus aureus*				
1	Positive Control	0.068	0.057	0.049	0.043	0.039
2	Aqueous Extract	0.743	0.732	0.721	0.710	0.698
3	Ethanol	0.460	0.452	0.439	0.430	0.421
4	Methanol	0.810	0.803	0.790	0.785	0.765
		Absorbance at 620nm *Staphylococcus epidermis*				
1	Positive Control	0.056	0.050	0.043	0.035	0.030
2	Aqueous Extract	0.743	0.729	0.714	0.701	0.690
3	Ethanol	0.460	0.451	0.439	0.425	0.410
4	Methanol	0.810	0.799	0.787	0.768	0.750

Table 6. Qualitative Phytochemical tests

S. No	Experiment	Observation	Inference
1	Test solution + minimum amount of chloroform + 3 drops of conc. H2 So^4 (Libermann Burchard test)	Purple colour changing to blue or green	Presence of Steroids
2	Test solution + piece of tin + 3 drops of Thionyl chloride	Violet or purple colour	Presence of Triterpenoids
3	Test solution + Molish's reagent	Purple colour	Presence of reducing sugars
4	Test solution +10% NaOH solution and heated/	Solution turned brown on heating	Presence of Carbohydrates
5	Test solution shaken with 2N HCL. Aqueous layers formed decanted to Mayers reagent are added	White turbidity or precipitate	Presence of Alkaloid
6	Alcoholic solution of test solution+ one drop of Ferric chloride	Intense colour	Presence of Compounds
7	Test solution + water, shaken well	Foamy lather	Presence of Saponins
8	Test solution + conc.HNO_3 excess Ammonia	Reddish orange precipitate	Presence of Xantho proteins
9	Water solution portion of the extracts treated with basic lead acetate solution	White precipitate	Presence of Tannins
10	Test solution+ Magnesium powder and treated with conc. HCL and heated .cool the test tube under the running water	Orange colour	Presence of Flavonoids

In the recent years many research work reported on the antibacterial activity of plant extracts on human pathogenic bacteria. The present study also revealed the antibacterial potential and ethno medicinal claims for *Coleus forskohlii*. The traditional practice of crude extracts of these plants holds active constituents with antimicrobial properties and suggested as antimicrobial therapeutic agents against infectious diseases caused by the tested pathogens of this study. The growth media also seem to plays a vital role in the evaluation of the antibacterial activity. Visible zone formation, bacterial growth inhibition and bacterial lawn or growth pattern of cells with equally distributed characteristics is the major reason to define a good culture media. Our study included two different media explicitly nutrient agar media and Muller-Hinton agar.

All these plant extracts showed bacterial growth inhibition in both the media with maximum inhibition values. Besides, the ZOI obtained in Muller-Hinton agar by the extracts was not observed in nutrient agar.

Consequently, the ZOI noted in nutrient agar lacked in Muller-Hinton agar. These differences in susceptibility patterns may be due to the less diffusibility of the crude extracts in the agar. In comparison, Muller-Hinton agar is the best medium showed visible zone formation and growth inhibition, to be used to determine the antibacterial potential. A previous study also reported that Muller-Hinton agar appears to be the best medium to describe the antibacterial activity. Not only crude extracts, use of different solvent extracts is also a matter of concern to isolate higher active compounds from the plants.

Many studies suggested that different solvent extracts of various plants has tremendous biological activity. Such an effective extracts can be subjected to isolation of the therapeutic compounds and antimicrobials for further Pharmacological studies. Ethno botanical approach is one of the universal practices applied in choosing the plants for pharmacological study. Although, these plants declared the antibacterial activity against 5 medically important human pathogens, to support this claim on the basis of scientific origin, the rate and extent of bacterial killing (kill kinetics) - Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs) are the matters under study. This study is extendable with other major pathogenic bacteria to develop a novel broad spectrum antibacterial formulation in future. Now, our research will be focused to develop a broad spectrum antibacterial combined herbal formulation with these plants.

CONCLUSION

The studies reveal that the Ethyl acetate is better than that of Chloroform and Aqueous extracts of *Coleus forskohlii* in respect to their anti - allergic, anti-inflammatory, eczema, asthma, psoriasis, cardiovascular.

The qualitative analysis of phytochemical screening of *Coleus forskohlii* is shown in Table 03 and 06:- Aqueous extract of leaf Glycosides, Sugars, Alkaloid, Tannins, Saponins and Coumarin was present. Ethyl acetate extract of leaf Trepenoids, Flavonoids, Steroids, Sugars, Quinones, Phenols, Saponins, and Coumarin was present. Chloroform extract of leaf Glycosides, Sugars, Quinones, Phenols, and Tannins was present.

Larger quantities in core-wood in comparison with that of bark. Hexane extract of core wood showed the presence of triterpenoids while bark extract did not show any trace of triterpenoids. Both the extracts were negative for cardiac glycosides, acids, alkaloids, sugars and proteins. The data obtained was consistent with Dymock *et al.,* 1891, Row *et al.,* 1970 and Sharma *et al.*, 1982, who had mentioned the presence of tannins, flavonoids, and terpenoids in the bark.

Terpenoids, Flavonoids, Steroids, Anthroquione, Glycosides, Sugars, Alkaloid, Quinones, Phenols, Tannins, Saponins, Coumarin.

Varying concentration of all extracts ranging from 10 micro grams to 1000 micro grams was tested for antimicrobial activity. None of the extracts showed marked activity. Ramya *et al.,* 2008 had reported that aqueous extract of sensitive towards *E. coli, S. aureus and P. aeruginosa*.

Phytochemical analysis confirms the presence of Trepenoids, Flavonoids, Steroids, Anthroquione, Glycosides, Sugars, Alkaloid, Quinones, Phenols, Tannins, Saponins, Coumarin. We recommend further research on this plant for possible isolation characterization of the various chemical active substances.

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