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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL STUDIES OF *STROBILANTHES CONSANGUINEUS* (NEES) CLARKE

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ABSTRACT

The aim of the present study was to investigate the antimicrobial and preliminary phytochemical properties of *Strobilanthes Consanguineus* (Nees) Clarke. The organic solvents (Petroleum ether, Chloroform and Methanol) and aqueous extracts from the leaf of *Strobilanthes Consanguineus* (Nees) Clarke (Acanthaceae) were tested against *Salmonella typhi, Klebsiella pneumonia, E. coli, Staphylococcus aureus* and *Candida albicans* by agar well diffusion method. In antibacterial activity, Chloroform and methanol extracts show marked activity and zone of inhibition were closest value to the standard drug (Ciprofloxacin) than the aqueous extract. The methanolic extract showed good activity of antifungal screening and zone of inhibition is closest value to the standard drug (Ketoconazole). Preliminary phytochemical analysis of extracts revealed the presence of phytochemicals such as alkaloids, tannins, carbohydrates, phenolic compounds, steroids, phytosterols, flavonoids, saponins, fats and fixed oils. The result of this study validates to the isolation of antibacterial agents from the leaf extracts of *Strobilanthes Consanguineus* (Nees) Clarke.

KEYWORDS: *Strobilanthes Consanguineus* (Nees) Clarke, Phytochemical analysis, Antimicrobial activity, well diffusion method.

INTRODUCTION

The importance of plants in traditional medicine and as raw materials in pharmaceutical industries cannot therefore be over emphasized. The use of herbs to treat diseases is almost universal among non-industrialized societies. Many of the pharmaceuticals currently

available to physicians have a long history of use as herbal remedies, including opium, aspirin, quinine, and digitalis. The use of medicinal plants is increasing worldwide, in view of the tremendous expansion of traditional medicine and a growing interest in herbal treatments. Plants are used in medicine to maintain and augment health-physically, mentally, and spiritually as well as to treat specific conditions and ailments. Chemically prepared drugs may act quickly, but they have side effects which affect human body negatively in the long run, whereas, medicinal plants work in an integrated or pro-biotic with little or no adverse effects on the body.

The true power of herbs lies in their wealth of protective polyphenols-plant compounds with potent antioxidant and anti-inflammatory effects. Piles of studies show that polyphenols in herbs help combat such diseases as cancer, heart disease, Alzheimer's, diabetes and more. Polyphenols are anti-microbial, so they can help protect us from harmful bacteria. Herbal medicine has been used as a reputable method of healing for aeons.[1]

Recently, increasing public concern about hygiene has been driving many studies to investigate antimicrobial and antiviral agents. However, the use of any antimicrobial agents must be limited due to their possible toxic or harmful effects. In recent years, due to previous antibiotics' lesser side effects, the use of herbal materials instead of synthetic or chemical drugs is increasing. Herbal materials are found in medicines. Herbs can be used in the form of plant extracts or as their active components. Furthermore, most of the world's populations used herbal materials due to their strong antimicrobial properties and primary healthcare benefits. For example, herbs are an excellent material to replace nanosilver as an antibiotic and antiviral agent.

Natural material has become ideal for treatment of microbial infections due to the possible toxic or harmful effects of many chemical antimicrobial agents. The antimicrobial mechanisms of chemical antimicrobial agents against herbal antimicrobial agents. The application of herbal materials would be an ideal alternative and can also open up a new chance for producing new anticancer, antimicrobial, and antiviral drugs with lower side effects.[1]

Strobilanthes consanguineus, often known as common madder or Indian madder, is a species of flowering plant in the coffee family, Acanthaceae. The species is found throughout the hilly subtropical to sub-temperate regions of India, between 300 m and 2000 m altitudes 57-

65. The plant's roots contain an organic compound called Alizarin that gives its red colour to a textile dye known as Rose madder. It was also used as a colourant, especially for paint, that is referred to as madder lake.

The genus of *Strobilanthe*s is used in traditional medicine for the treatment of several ailments. The species are commonly used in rheumatic complaints, sprain of the ankle, and hernia.[2] The leaves of *Strobilanthes* are usually boiled in water and used in folk medicine with blood-pressure lowering, antidiabetes, anticancer and diuretic properties and have been scientifically proven to have high antioxidant activity.[3] The *Srobilanthes* genus have reported to contain substantial amounts of effective phenolic compounds such as caffeic acid, quercetin, rutin, and catechin.[4] Phytochemical investigations have revealed that the *Strobilanthes crispus* polyphenols, flavonoids, alkaloids, caffeine,[5] steroids, saponin,[6], indole alkaloids (IAs), quinolone alkaloids, phenylethanoid glycosides, lignan glycosides, triterpenoids, amino acids[7], phenols, tannins.[8] The phytochemical analysis of the *Strobilanthes heyneanus* extract showed the presence of glycosides, carbohydrates[8], terpenoids.[9] The research objectives were to investigate the antimicrobial activity of the extracts from *Strobilanthes consanguineus* against standard and multi drug resistant gram positive and gram negative bacteria.

MATERIALS AND METHODS

Plant Material

Leaf of *Strobilanthes consanguineous (Nees*) *Clarke* were collected from Puttady-Vandanmedu Idukki in the month April 2023.The plant was authenticated by Prof. Paul V Karamthanan, Head of the department, St. Thomas college Pala, Kottayam. A voucher specimen of *Strobilanthes consanguineous (Nees*) *Clarke* (SJCPSR/4/23) was deposited in the department of pharmacognosy in St. John's College of Pharmaceutical Science and Research, Kattappana for future reference. The plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh and stored in an air tight and light resistant container for future use.

Preparation of Extract

The coarsely powdered plant material was first defatted with petroleum ether using Soxhlet apparatus. The extract was concentrated by using rotary evaporator to get solid residue. The marc the central compartment was removed, dried successively extracted with a series of

solvent of increasing polarity with Soxhlet extractor was done. Solvent used with increasing polarity are chloroform, methanol and water.

Preliminary phytochemical screening

The prepared extracts were subjected to routine phytochemical analysis[10] to identify the presence of various phytochemicals such as Alkaloids, Tannins, Carbohydrates, Flavonoids, Saponins, Phenolic compounds, Steroids and triterpenoids, Fats and fixed oils and Phytosterols according to the standard procedures. These results are depicted in Table 1.

Alkaloids

Dragendorff's test: The extract is treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Mayer's test: To a few ml of plant sample extract, 2 drops of Mayer's reagent (Potassium mercuric iodide solution) are added along the sides of the test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

Flavonoids

Alkaline reagent test: Extract is combined with a few drops of sodium hydroxide solution. The appearance of intense yellow colour, which turns colourless on addition of dilute acid, signifies the presence of flavonoids.

Shinoda test: 1ml of extract is added with 0.5ml of hydrochloric acid and magnesium metal. The presence of flavonoids is confirmed by reddish colouration.

Tannins

Bromine water test: 0.5 g of plant extract is treated with 10ml of bromine water. Decolouration of bromine water indicates the presence of tannins.

Braymer's test: 1ml of extract is added with 3ml of distilled water and 3 drops of 10% ferric chloride solution. A blue green colouration indicates the presence of tannins.

Glycosides

Legal test: 50 mg of extract is dissolved in pyridine and then sodium nitroprusside solution is added. The solution is made alkaline using 10% sodium hydroxide. Pink colour means glycosides are detected.

Borntrager's test: 2 ml of the plant extract is treated with 3 ml of chloroform and shake well: the chloroform layer is separated and add 10% ammonia solution. Presence of glycosides can be confirmed by the presence of a pink coloured solution.

Cardiac glycosides

Keller-killiani test: To 1l of filtrate add 1.5ml glacial acetic acid, 1 drop of 5% ferric chloride and concentrated sulphuric acid (along the side of the test tube). A blue coloured solution (in acetic acid layer) indicates the presence of cardiac glycosides.

Bromine water test: Plant extract is treated with few ml of bromine water. A yellow precipitate indicates the presence of cardiac glycoside.

Anthraquinones test

Borntrager's test: To 10 ml of 10% ammonia solution add few ml filtrates and shake vigorously for 30 seconds. A pink or violent or red colour solution indicates the presence of Anthraquinone's.

Carbohydrates

Molisch's test: To 2 ml of plant sample extract, 2 drops of alcoholic solution of alpha naphthol are added. The mixture is shaken well and few drops of con. Sulphuric acid is added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

Barfoed's test: 1 ml plant extract is added with 1 ml Barfoed's reagent and heated for 2 minutes. A red precipitate indicates the presence of carbohydrates (monosaccharides).

Protein and Amino acids

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman no.1 filter paper and the filtrate are subjected to test for protein and aminoacids.

Million's test: To 2 ml of filtrate few drop of million's reagent is added. A white precipitate indicates the presence of water.

Biuret test: 2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution to this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein.

Test for saponins

Foam test: The extract (50 mg) is diluted with diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

Test for phenolic compounds

Iodine test: 1ml extract is treated with few drops of dilute iodine solution. Appearance of a transient red colour indicates the presence of phenolic compounds.

Ferric chloride test: Extract aqueous solution is treated with few drops of 5% ferric chloride solution. Appearance of dark green/bluish-black colour indicates the presence of phenolic compound.

Test for steroids and triterpenoids

Salkowski's test: Extract is treated with chloroform and then filtered and added with a few drops of conc. Sulphuric acid. It is then shaken and allowed to stand. The appearance of golden yellow colour or red brown colour indicates the presence of triterpenes.

Liberman-Burchard reaction: Mix 2 ml of extract with chloroform and 1 - 2 ml acetic anhydride and 2 drops of conc. Sulphuric acid from the side of test tube. First red then blue and finally green colour indicates the presence of steroids.

Test for fats and oils

Spot test: A small quantity of extract is pressed between 2 filter papers. Oil stain on the paper indicates the presence of fixed oils.

Saponification test: A few drops of 0.5 N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on a water bath for 2 hours. Formation of soap for partial neutralisation of alkaline indicates the presence of fixed oils and fats.

Test for phytosterols

Salkowski's test: Extract is treated with chloroform and then filtered and added with a few drops of con. Sulphuric acid. It is then shaken and allowed to stand. The appearance of red colour in lower layer or brown ring indicates the presence of phytosterols.

Libermann's-Burchard test: 50g extract is dissolved in 2ml acetic anhydride, add 1-2 drops of con. Sulphuric acid (along the side of test tube). An array of colour change indicates the presence of phytosterols.

Test for gums and mucilage

Alcohol test: The extract (100mg) is dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilage.

Antibacterial Activity

Well diffusion assay

The petroleum ether, chloroform, methanol and aqueous extract were subjected to antibacterial activity. Agar diffusion assay is used widely to determine the antibacterial activity of crude extract. The technique works well with defined inhibitors. However, when examining extract containing unknown components, there are problems leading to false positive and false negative.[11] Muller Hinton Agar prepared and was poured in the petri dish. 24 hour growing culture (*Salmonella typhi, Klebsiella pneumonia, E. coli and Staphylococcus aureus)* were swabbed on it. The wells (10mm diameter) were made by using cork borer. The concentration (100 microgram) of the crude extract were loaded in the wells. The plates were then incubated at 37°C for 24 hours. The inhibition diameter was measured.[12]

Antifungal Activity

Well diffusion assay

The petroleum ether, chloroform, methanol and queous extracts were subjected to anti-fungal activity. Solution containing Muller Hinton Agar, 2% glucose and 0.5 mg/ml. Methylene blue dye were prepared and was poured in the Petri dish. 24 hours growing culture (*Candidia albicans)* was swabbed on it. The wells (10mm diameter) were made by using cork borer. The concentration (100µg) of the crude extracts were loaded in the wells. The plates were then incubated at 37°C for 24 hours. The inhibition diameter was measured.[12]

RESULTS AND DISCUSSION

As the bioactivity of extracts from *Strobilanthes consanguineus (Nees) Clarke* depends on the presence of various phytoconstituents present in them individual extracts their subjected to phytochemical test to detect the presence of alkaloids, flavonoids, tannins, carbohydrates,

saponins, phenolic compounds, steroids and triterpenes, fats and oils, phytosterols and gums and mucilage.

Table 1: Preliminary phytochemical screening of Petroleum ether, Chloroform, Methanol and Water extracts.

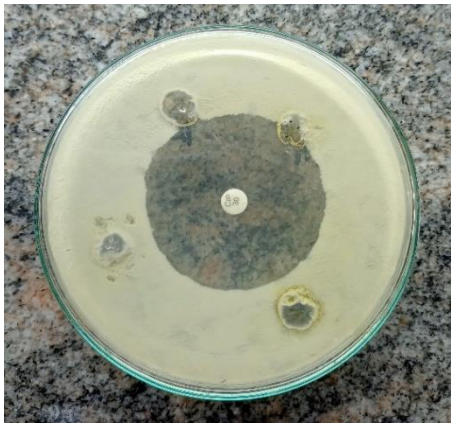
Sl. No	Phytochemicals	Petroleum ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
1	Alkaloids	+	+++	+	+++
2	Flavonoids	-	+	+	-
3	Tannins	+	+	-	-
4	Carbohydrates	+	++	++	++
5	Glycosides	-	-	-	-
6	Cardiac glycosides	-	-	-	-
7	Protein	-	-	-	-
8	Amino acids	-	-	-	-
9	Saponins	-	-	+	-
10	Phenolic compounds	+++	+++	+	-
11	Steroids and triterpenoids	+	+++	-	
12	Fats and fixed oils	++	-	+	+
13	Phytosterols	++	+++	++	-
14	Gums and mucilage	++	-	-	-

(-) Not detected; (+) Slightly positive reaction; (++) Positive reaction and (+++) Strong positive reaction.

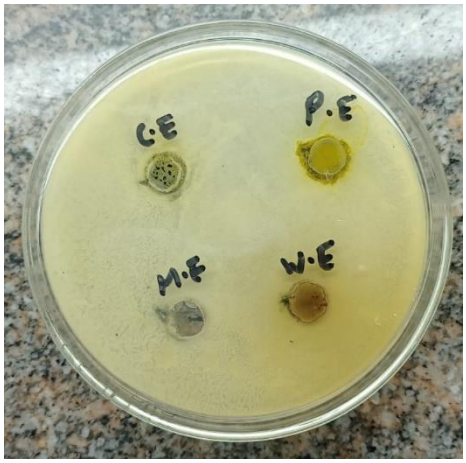
The antimicrobial activity of Petroleum ether, Chloroform, Methanol and Aqueous extracts of *Strobilanthes consanguineus (Nees) Clarke* on different bacterial and fungal organisms have been shown Table 2 and 3. Antibacterial screening for the crude extracts were performed by agar well diffusion assay method against gram-positive organisms *Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus* and gram-negative organism *Escherichia coli.* The zone of inhibition result was compared with standard drug Ciprofloxacin. Antifungal screening was carried out using the organism *Candida albicans* by agar well diffusion assay method and activity was compared with the standard Ketoconazole.

Table 2: Effect of Petroleum ether, Chloroform, Methanol and Aqueous extract on Antibacterial activity.

Sl No	Name of the extract	*S.typhi (100µg)*	*S.aureus (100µg)*	*K.pneumoniae (100µg)*	*E. coli (100µg)*
1	Standard	39mm	32mm	30mm	31mm
2	Petroleum ether extract	16mm	16mm	14mm	15mm
3	Chloroform extract	16mm	-	14mm	-
4	Methanol extract	13mm	15mm	15mm	16mm
5	Aqueous extract	-	-	13mm	14mm

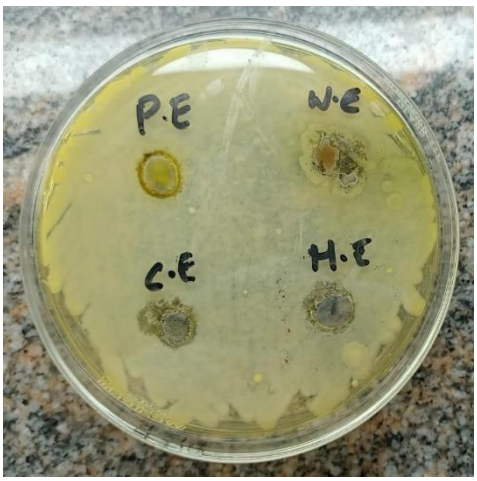


Standard (Ciprofloxacin)

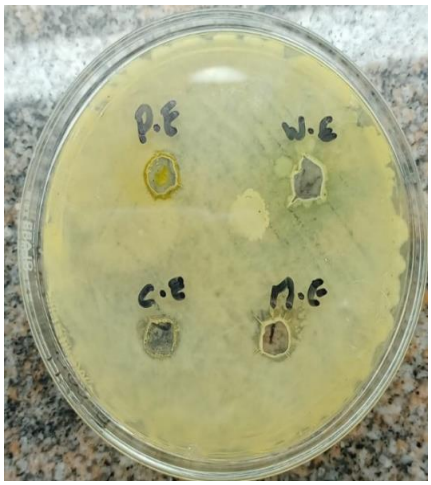


S. typhi (100µg)

S.aureus(100µg)



K.pneumoniae(100µg)

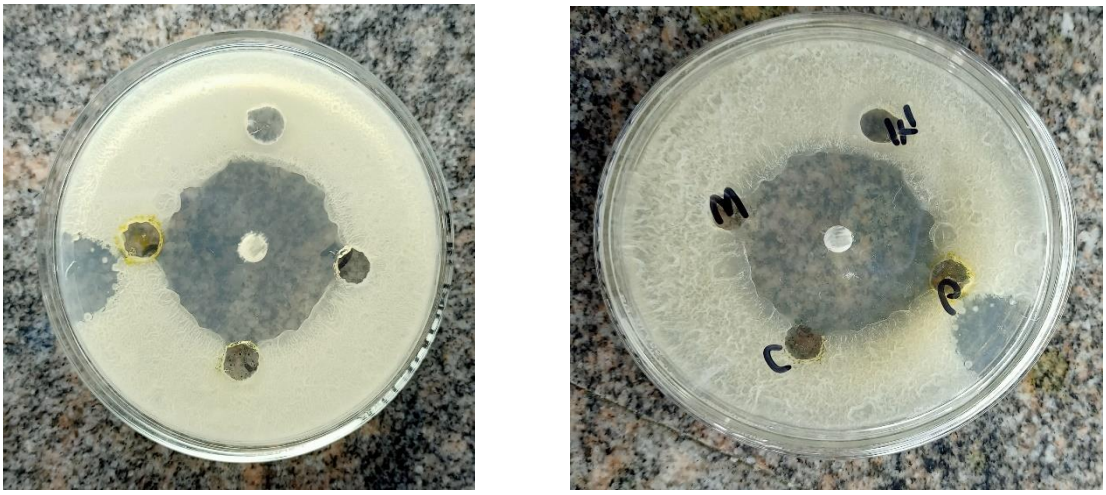


E.coli(100µg)

Figure 1: Zone of inhibition of standard drug and tested organisms with extracts.

Table 3: Effect of Petroleum ether, Chloroform, Methanol and Aqueous extract on Antifungal activity.

Sl. No	Name of the extract	*C.albicans*
1	Standard	31mm
2	Petroleum ether extract	11mm
3	Chloroform extract	12mm
4	Methanol extract	14mm
5	Aqueous extract	-



C. albicans (Standard) *C. albicans(100µg)*

Figure 2: Zone of inhibition of standard drug and tested organisms with extracts.

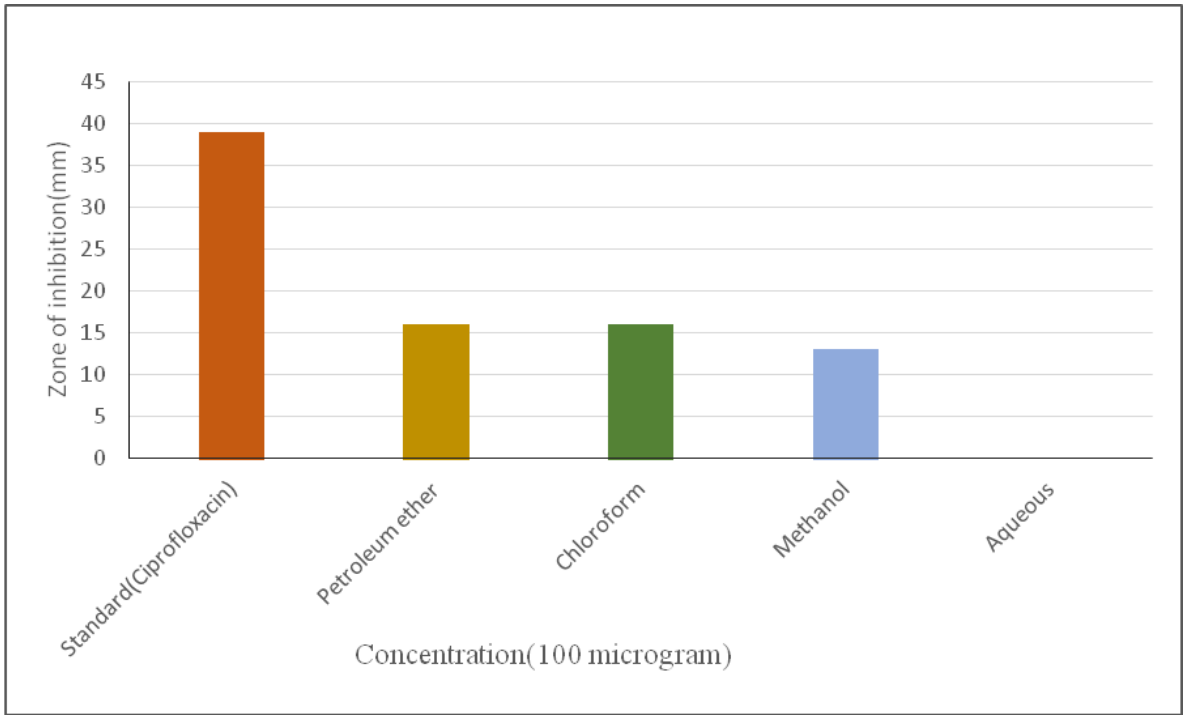


Figure 3: Effect of Petroleum ether, Chloroform, Methanol and Aqueous extract on antibacterial activity-*S. typhi.*

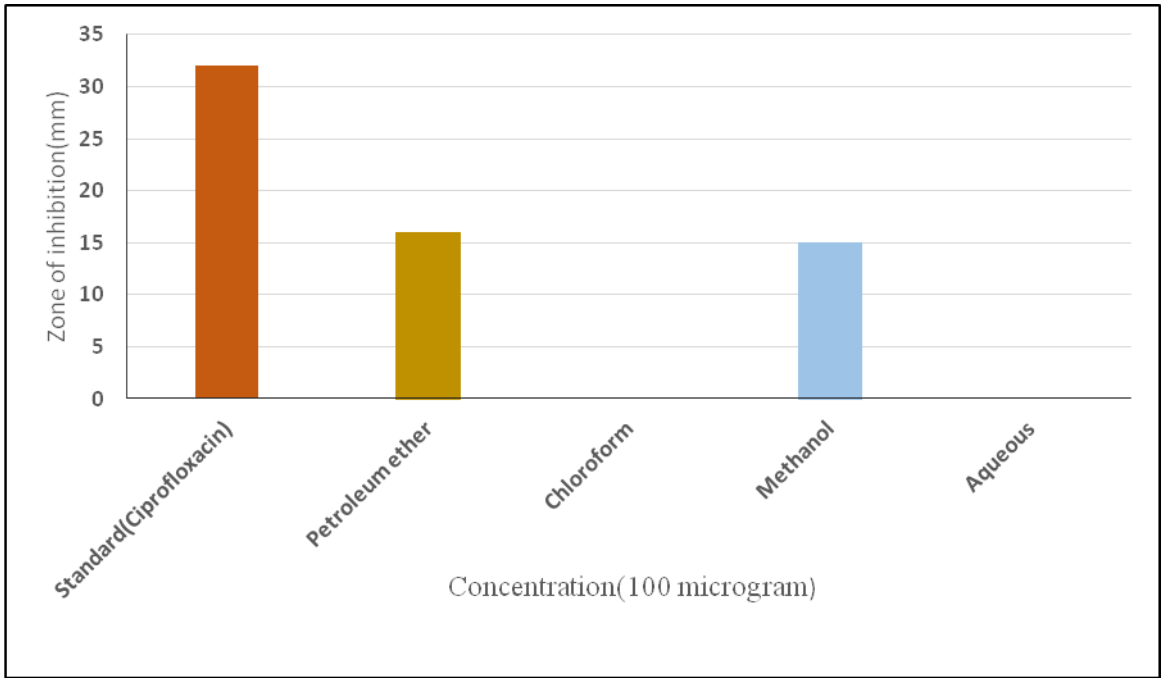


Figure 4: Effect of Petroleum ether, Chloroform, Methanol and Aqueous extract on antibacterial activity-*S. aureus.*

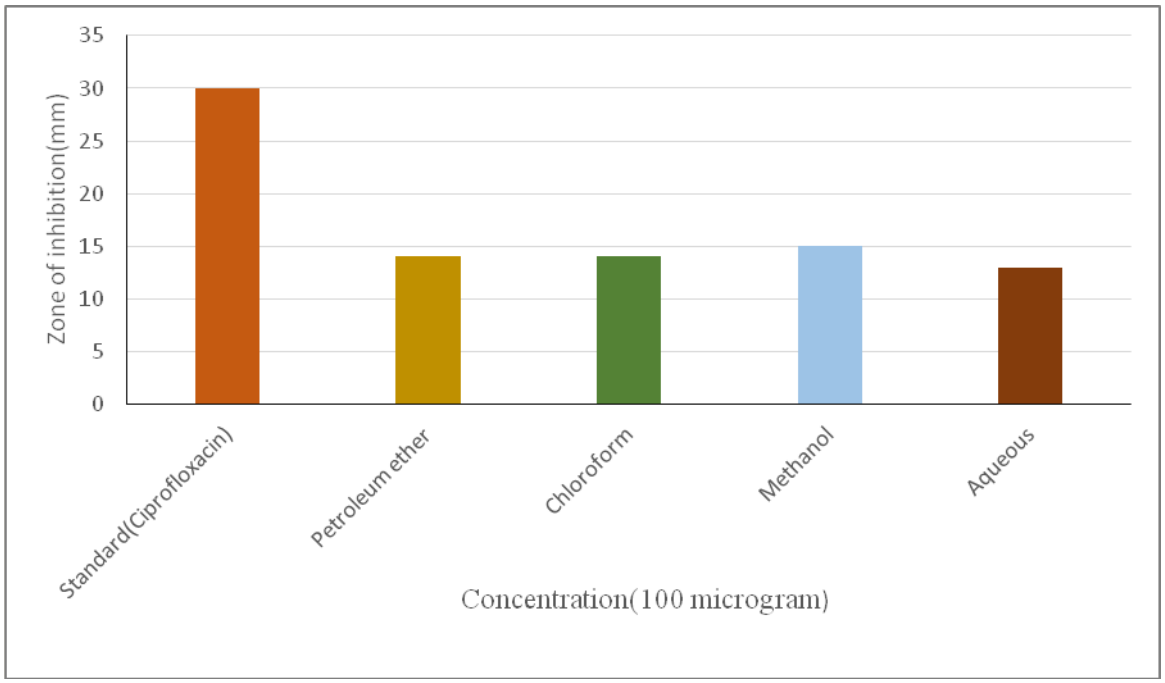


Figure 5: Effect of Petroleum ether, Chloroform, Methanol and Aqueous extract on antibacterial activity-*K. pneumoniae.*

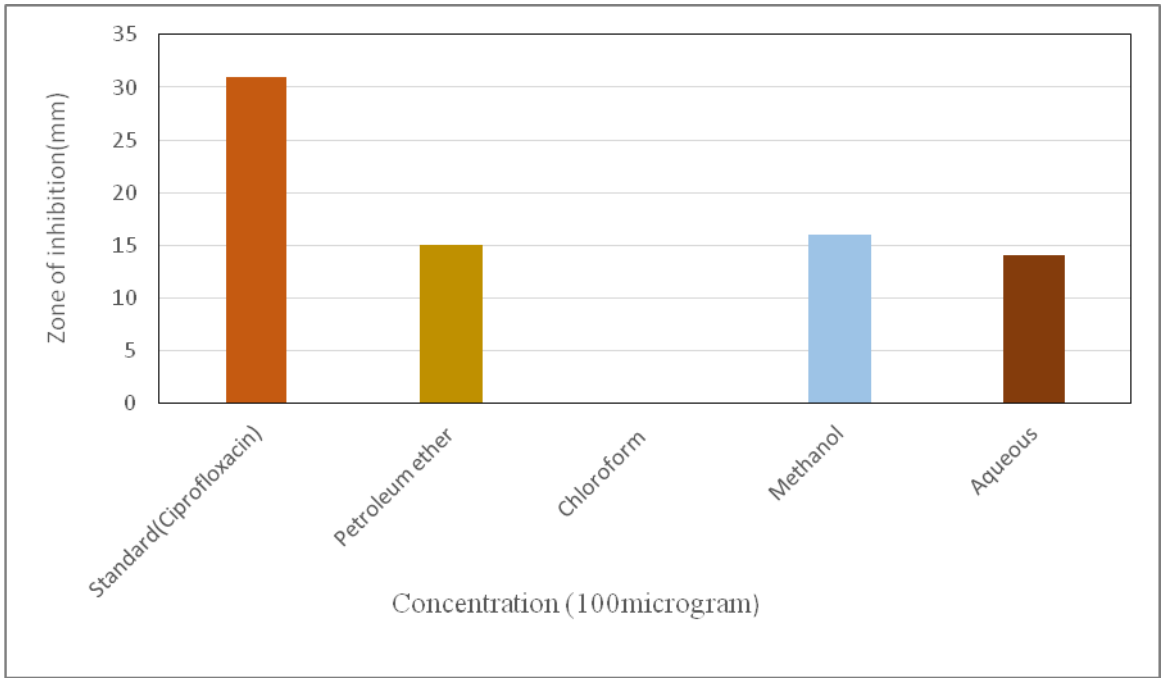


Figure 6: Effect of Petroleum ether, Chloroform, Methanol and Aqueous extract on antibacterial activity-*E. coli.*

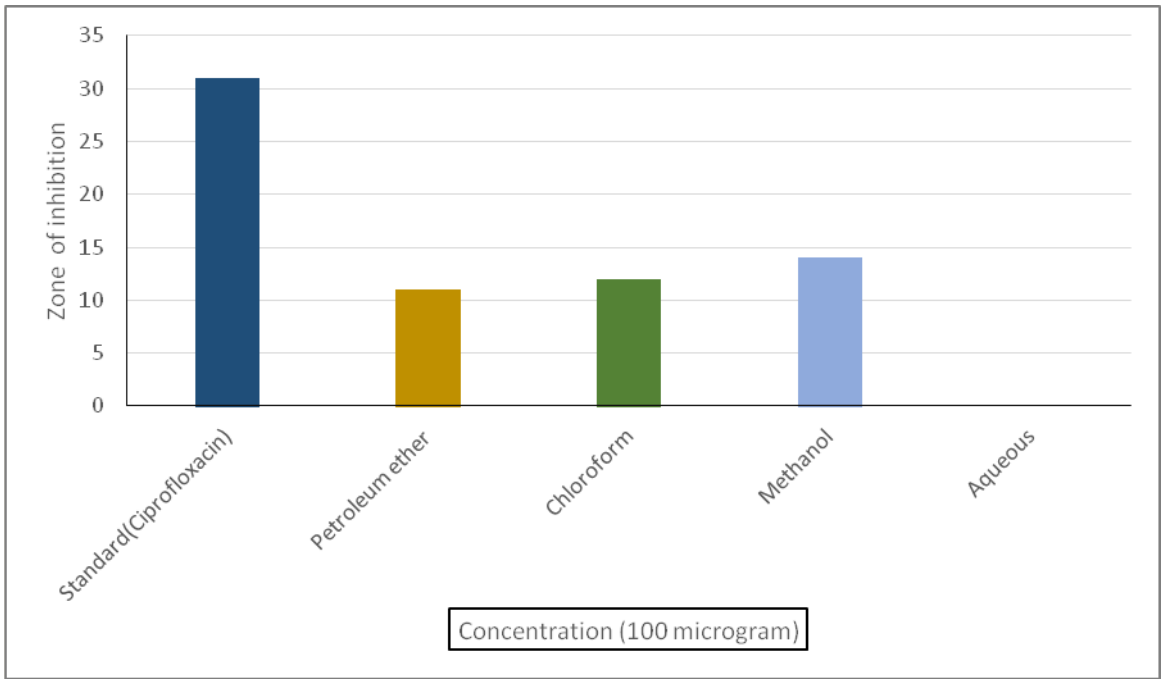


Figure 7: Effect of Petroleum ether, Chloroform, Methanol and Aqueous extract on antifungal activity-*C. albicans.*

In antimicrobial screening the petroleum ether, methanol and chloroform extracts exhibited marked activity and zone of inhibition were closest value to the standard than the aqueous extract. The present study revealed that, *S. typhi* and *S. aureus* was highest susceptible

bacteria with zone of inhibition 16mm followed by E. coli with zone of inhibition 15mm and *K. pneumonia* with zone of inhibition 14mm.All the observation results are depicted in table 2. In antifungal screening, the methanol extract has also produced good antifungal activity with zone of inhibition 14mm. The observation results are depicted in table 3.

Among the four extracts screen for antibacterial activity, the petroleum ether, methanol, chloroform extracts exhibits marked antibacterial activity against *S. typhi, K. pneumonia, S. aureus* and *E. coli*. Then the methanolic extract marked antifungal activity against *C. albicans*.

CONCLUSION

The present investigation reported that the infections due to bacterial and fungal species. This study is to report that the medicinal plant *Strobilanthes consanguineous* used in Indian herbal medicine may possess antimicrobial activity against *S.typhi, K.pneumonia, S.aureus, E.coli and C.albicans*. Among the petroleum ether, chloroform, methanol and aqueous extracts being subjected to antimicrobial activity by well diffusion method. Results was observed that the methanolic extract exhibited better antimicrobial activity than other three extracts. This suggests the presence of phytochemical constituents with better antimicrobial activity in methanol extract when compared with other extracts. So the methanol extract can be subjected to further isolation to identify the potent phytochemical constituents responsible for exhibiting marked antimicrobial activity. These findings can form the basis for further studies to toxicity testing, isolate the active compounds, elucidate the structures and also evaluate the antimicrobial study of the isolated compounds.

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REFERENCES

1. Shokoh Parham, Anousheh Zargar Kharazi, Hamid Reza Bakhsheshi-Rad, Hadi Nur, Ahmad Fauzi Ismail, Safian Sharif, Seeram RamaKrishna. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. Antioxidants (Basel), 2020; 9(12): 1309.
2. Vijayakumar Sundaram, Selvaraju Sadhasivam, Sivaraj Chandrasekaran, Raman Nanjian & Arjun Pandian. *Strobilanthes heyneanus* root extract as a potential source for

antioxidant and antimicrobial activity. Future Journal of Pharmaceutical Sciences, 2021; 7 Article No 91.

3. Xiao-Ling Zhu, Yang Xu, De-Juan Sun, Hua Li, Li-Xia Chen. TMR Modern Herbal Medicine homepage: The genus *Strobilanthes*: phytochemistry and pharmacology, 2022.
4. Ali Ghasemzadeh, Hawa ZE Jaafar & Asmah Rahmat. Phytochemical constituents and biological activities of different extracts of *Strobilanthes crispus* (L.) Bremek leaves grown in different locations of Malaysia. 2015; 15 Article No 422.
5. Nurraihana H, Norfarizan-Hanoon NA. Phytochemistry, pharmacology and toxicology properties of *Strobilanthes crispus*. International Food Research Journal, 2013; 20(5): 2045-56.
6. Prabakaran R, Kirutheka. GCMS, phytochemicals and antioxidant activities of *in vitro* callus extracts of *Strobilanthes kunthiana* (*Nees)*T.Anderson ex Benth: An endemic plant of Acanthaceae. Brazilian Journal of Biological Sciences, 2018; 5(10): 359-72.
7. Yongle Hu, Dongna Ma, Shuju Ning, Qi Ye, Xuanxuan Zhao, Qiansu Ding. High-Quality Genome of the Medicinal Plant *Strobilanthes cusia* Provides Insights into the Biosynthesis of Indole Alkaloids. Front Plant Sci, 2021; 12(2): 742-50.
8. Ming Guan Ng, Chew Hee Ng, Khuen Yen Ng, Soi Moi Chye, Anna Pick Kiong Ling and Rhun Yian Koh. Anticancer Properties of *Strobilanthes crispus*: A Review, 2021; 9: 1370.
9. Vika Ramadhani, Rusdi, Zikra Azizah, Harrizul Rivai. Overview of Phytochemicals and Pharmacological Activity of Keji Beling Plant (*Strobilanthes crispus* Bl.) 2021; 6(7): 25-29.
10. Vishnu Balamurugan, Sheerin Fatima MA, Sreenithi Velurajan. A guide to Phytochemical Analysis. IJARIIE, 2019; 5(1): 2395-4396.
11. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Plant Medica, 1998; 64: 711-13.
12. Fazeli F, Ghorbalni M, Niknam V. Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. Biologia Plantarum, 2007; 51: 98-103.