PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF *CANTHIUM PARVIFLORUM*, TAMILNADU, INDIA.

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Abstract

The bioactive compounds present in the plant are responsible for the medicinal properties of the plant. The present investigation is aimed in screening the bioactive compounds present in leaf *of Canthium parviflorum*, an important ethanomedicinal plant. The phytochemical analysis revealed the presence of alkaloids, carbohydrate, cardiac glycosides, flavonoids, phenols, phlobatannins, aminoacids & proteins, saponins, sterols, tannins, terpenoids, quinones and oxalates. The antibacterial activity was carried out against gram positive and gram negative bacteria by microtiter well plate method. Since the plant contains high quantities of bioactive compounds, it is reliable to possess large number of properties like antioxidants, antifungal, antibacterial, anti-inflammatory and are being employed for the treatment of different ailments in the indigenous system of medicine.

Key words: *Canthium parviflorum*, extraction, phytochemicals, antibacterial.

Introduction

Canthium parviflorum of Rubiaceae family is commonly called as Mullukaarai in Tamilnadu. It is a thorny shrub grows up to 3 meters height with spreading branches distributed throughout India. In Ayurveda system of medicine, *Canthium parviflorum* used as a laxative and also to cure gout. (Hosagouda and Archana.,et al 2009). Based on the previous reports, this plant material is used for its pharmacological importance as an anthelmintic, antidysentric, antispasmodic and as a diuretic (Srigiri Chandra kala, 2015). Traditionally the roots and leaves are used to cure vitiated conditions of kapha in fever and constipation (kritikar KR et al., 2001). Since *Canthium parviflorum* leaf is used as an astringent, it is presumed that the leaf shows wound healing property (Mohideen S et al., 2006). Leaf paste is externally applied twice a day to treat scabies and the ringworm infection (Anitha Roy et al.,2011). The *Canthium parviflorum* as herbal medicine is used for the treatment of diabetes among major tribal groups in Tamilnadu (Ayyanar M et al., 2008). The leaves and roots are used in conditions of kapha, diarrhoea, strangury, fever, leucorrhoea, intestinal worms and general deability (Warrier et al., 1994). Tribes of Orissa state in India use fruit of this plant to treat a headache. It is traditional medicine used for snake bites (Parinitha mahishi et al., 2005). Methanol extract of root of *Canthium parviflorum* shows anthelminthic activity (Krishna et al., 2014). The root and leaf paste of *Canthium parviflorum* are very useful for diuretic (Salai Senthilkumar et al.,2014). The personal survey on anti-venom plants in western ghats region of Karnataka (India) and careful literature study reveals *Canthium parviflorum* has been known to treat snake bites (Gomes et al., 2010). At first *Canthium parviflorum*, an important medicinal plant belonging to Rubiaceae family has been widely used for fever, leucorrhoea, intestinal worms, general disability, snake bite, wound-healing, anticancer, antibacterial and antioxidant (Elayaraja et al.,2007; Sathishkumar et al., 2008; Hiremath et al., 2010; Purushoth Prabhu., 2011; Purushoth Prabhu et al ., 2013; Deepashree., 2013).

Materials and methodology

Collection of plant sample

The plant were collected from the location in Kanyakumari District, Tamilnadu, India. The plant samples were dried in shade at 25 to 35 degree celsius for 10-15 days , then crushed to coarse powder using grinder and stored for further analysis.

Preparation of extracts

Soxhlet extraction

The powdered samples were subjected for the sequential extraction of secondary metabolites with a series of solvents. 100g of powdered sample was filled in a Whatmann filter paper and placed inside timble. 200-250mL of the solvent was added in

timble. The timble was fit into a round bottom flask containing 700mL of the solvent and run for 6-8 hours at the temperature based on the boiling point of the respective solvent (Petroleum ether, Ethyl acetate, Chloroform and Methanol) using soxhlet apparatus. Later the extract was subjected for the distillation for 2-3 hours. These extracts were placed in hot air oven at 40ºC for drying. The dried extracts thus obtained were used for various analysis.To obtain aqueous extract, 10g of the powdered samples were dissolved in 100mL distilled water and incubated at 37°C overnight. The next day, samples were filtered and the filtrate was evaporated to dryness at 40°C. The dried extract was further used for the analysis.

Preliminary phytochemical screening

All the extracted samples were screened for the presence of different secondary metabolites according to the protocol mentioned below. For this purpose, a little amount of each samples (around 10-15mg) dissolved in the respective solvents was used.

Test for Alkaloids (Wagner's reagent**)**

A fraction of extract was treated with 3-5drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

Test for Carbohydrates (Molisch's test**)**

Few drops of Molisch's reagent were added to 2ml portion of the various extracts. This was followed by addition of 2ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Test for Cardiac glycosides (Keller Kelliani's test**)**

5ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

T**est for Flavonoids (**Alkaline reagent test**)**

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Phenols (Ferric chloride test**)**

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for deep blue or black colour.

Test for Phlobatannins (Precipitate test)

Deposition of a red precipitate when 2ml of extract was boiled with 1ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Amino acids and Proteins (1% ninhydrin solution in acetone).

2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Test for Saponins (Foam test)

To 2mls of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Sterols (Liebermann-Burchard test)

1ml of extract was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of dark pink or red colour.

Test for Tannins (Braymer's test)

2mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Test for Terpenoids (Salkowki's test)

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow precipitate (or colouration).

Test for Oxalate

To 3ml portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates presence of oxalates.

Antibacterial activity

Extracted plant sample were assessed in Micro titer plate method (96 wells) to check the Minimum Inhibition Concentration (MIC) against 5 Gram positive and 5 Gram negative bacteria. Procedure

Sample preparation: 10mg of the sample extracts (Petroleum ether, Ethyl acetate, Chloroform, Methanol and Water extract) dissolved in 1 mL DMSO (Dimethyl sulfoxide) respectively.

Test organism: 24hr cultured Gram positive bacteria- *Bacillus cereus, Staphylococcus aureus, Streptococcus mutans, E-faecalis and Staphylococcus epidermis. Gram negative bacteria- Pseudomonas aeruginosa, E- coli, Salmonella typhi, Klebsiella and Serratia marcescens.*

Media preparation: Luria Bertani (LB) broth (tryptone 10g, sodium chloride 10g, yeast extract 6g and distilled water 1000mL) 250mL of LB broth was prepared and autoclaved at 121°C for 15mins.

Plate preparation: 300µL of deionized water was added in the border wells of the micro titer plate (A1 to A12, B12 to H12, H11 to H1, and G1 to B1) to prevent the sample from drying. 100µL of sterilized LB broth was added to all the remaining testing wells. 100µL of 0.1% of resazurin was added to the wells B2 to F2 in respective plates and named as colour blank. In wells B3 to G3 test organism and 100µL of 0.1% of resazurin was added in respective plates as culture control. 100µL of the sample extract (Petroleum ether, Ethyl acetate, Chloroform, Methanol and Water extract) was added to respective plates from wells B4 to F4 and serially diluted by transferring 100µL of the sample to subsequent wells upto 11th well and 100µL of the excess sample was discarded from 11th well, 100µL of respective organisms and 100µL of 0.1% of resazurin dye was added to the diluted samples. The plates were incubated at 37° C for 24h.

Presence of blue colour indicates no growth of the organism whereas appearance of pink colour indicates growth.

Result

Preliminary phytochemical analysis of Petroleum ether, Ethyl acetate, Chloroform, Methanol and aqueous extracts of leaf of *Canthium parviflorum* were carried out. The results are given in table.1

Screening of the dried leaf extracts in Petroleum ether, Ethyl acetate, Chloroform, Methanol and aqueous extracts was performed for antibacterial activity by microtiter plate method against gram positive bacteria *Bacillus cereus, Staphylococcus aureus, Streptococcus mutans, E-faecalis and Staphylococcus epidermis and gram negative bacteria Pseudomonas aeruginosa, E- coli, Salmonella typhi, Klebsiella and Serratia marcescens*. The minimum inhibition concentration of leaf samples were given in table.2 and figure 1-5.

Discussion

The preliminary phytochemical investigations in *Canthium parviflorum* leaf was observed that alkaloids, carbohydrate, cardiac glycosides, flavonoids, tannins, saponins, quinones, oxalates were present in petroleum ether extract but it showed negative for phenols, phlobatannins, aminoacids & proteins, terpenoids and sterols. Ethyl acetate of leaf extract showed positive for alkaloids, carbohydrate, cardiac glycosides, flavonoids, phenols, phlobatannins, tannins, terpenoids, oxalates except amino acids & proteins, saponins, sterols and quinones compared to presence of terpenoids, saponins, steroids, tannins, quinones and gum in *Canthium parviflorum* leaves identified by Ramanathan et al.,2013 . The phytochemicals present in chloroform extract were alkaloids, carbohydrate, cardiac glycosides, flavonoids, saponin, tannin, terpenoids, quinones and oxalates remaining shows negative. Alkaloids, carbohydrate, flavonoids, phenols, aminoacids & proteins, saponin, tannin and quinones shows positive in methanol extract whereas cardiac glycosides, phlobatannins, sterols, terpenoids and oxalates shows negative. Aqueous extract revealed the presence of phytochemicals alkaloids, carbohydrate, cardiac glycosides, flavonoids, aminoacids & proteins, saponin and quinones but it showed negative for phenols, phlobatannins, sterols, tannin, terpenoids and oxalates. Pasumarthi et al.,2011 reported that the *Canthium parviflorum* leaves with aqueous and methanol extracts revealed the presence of tannins, alkaloids, flavonoids, saponin, steroids, anthraquinones and reducing sugars.

The antibacterial activity of *Canthium parviflorum* leaf extracts was determined by resazurin dye reduction method. The Minimum Inhibition Concentration(MIC) values also greatly incoherence with the activity observed in screening test. The MIC values ranged from 15.6 µg/ml to 1000 µg/ml for different solvent extracts of *Canthium parviflorum* leaf against test bacteria. The antibacterial activity was not observed in gram positive bacteria such as *Bacillus cereus, Staphylococcus aureus and E-faecalis* and shows activity against *Streptococcus mutans, Staphylococcus epidermis, Pseudomonas aeruginosa, E- coli, Salmonella typhi, Klebsiella and Serratia marcescens* in petroleum ether extract. The chloroform extract of leaf showed antibacterial activity against gram positive bacteria such as *Streptococcus mutans* and gram negative bacteria *Pseudomonas aeruginosa, E- coli, Salmonella typhi, Klebsiella* and showed negative for *Bacillus cereus, Staphylococcus aureus, E-faecalis* gram positive bacteria and *Serratia marcescens* gram negative bacteria. The ethyl acetate extract showed positive for both gram positive and gran negative bacteria except *E-faecalis*. The methanol extract

shows positive for gram positive bacteria such as *Streptococcus mutans, Staphylococcus epidermis* and all gram negative bacteria. Aqueous extract showed the inhibition property against gram positive bacteria such as *Streptococcus mutans, E-faecalis, Staphylococcus epidermis* where it showed negative for all gram positive bacteria. The least MIC in petroleum ether extract of sample was found to be 1000µg/ml against both gram positive and gram negative bacteria. While least MIC of ethyl acetate extract shows 15.6µg/ml against gram positive bacteria *Streptococcus mutans*. The chloroform extract showed the least MIC as 62.5µg/ml in gram positive bacteria *Streptococcus mutans*. In methanol extract of leaf sample the least MIC was found as 62.5µg/ml in gram positive bacteria *Streptococcus mutans*. The aqueous extract showed the least MIC in gram positive bacteria *Streptococcus mutans* as 31.25µg/ml.

Conclusion

Vast wealth of medicinal sources still has to use for curing a number of diseases. In order to find new sources of plant drugs, number of plants have been screened for various biological activities in various research institutions. *Canthium parviflorum* is an important medicinal plant used in indigenous system of medicine in india. So, based on these medicinal attributes concluded that *Canthium parviflorum* is most economically and medicinally valuable plant.

Table -1 Showed preliminary phytochemical screening of Petroleum ether, Ethyl acetate, chloroform and methanol and aqueous extract of leaf of *Canthium parviflorum*

Name of phytochemicals	*Canthium parviflorum* leaf				
	Petroleum ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	Present	Present	Present	Present	Present
Carbohydrates	Present	Present	Absent	Present	Present
Cardiac glycosides	Present	Present	Present	Absent	Present
Flavonoids	Present	Present	Present	Present	Present
Phenols	Absent	Present	Present	Present	Absent
Phlobatannins	Absent	Present	Absent	Absent	Absent
Amino acids & proteins	Absent	Absent	Absent	Present	Present
Saponoins	Present	Absent	Present	Present	Present
Sterols	Absent	Absent	Absent	Absent	Absent
tannins	Present	Present	Present	Present	Absent
Terpenoids	Absent	Present	Present	Absent	Absent
Quinones	Present	Absent	Present	Present	Present
Oxalates	Present	Present	Present	Absent	Absent

Table-2 showed the presence or absence of antibacterial activity of petroleum ether, Ethyl acetate, chloroform and methanol and aqueous extract of leaf of *Canthium parviflorum* against gram positive and gram negative bacteria

Name of pathogens	Petroleum ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract	Aqueous extract
Bacillus cereus	nil	Present	nil	nil	nil
Staphylococcus aureus	nil	Present	nil	nil	nil
Streptococcus mutans	Present	Present	Present	Present	Present
E-faecalis	nil	nil	nil	nil	Present
Staphylococcus epidermis	Present	Present	nil	Present	Present
Pseudomonas aeruginosa	Present	Present	Present	Present	nil
E- coli	Present	Present	Present	Present	nil
Salmonella typhi	Present	Present	Present	Present	nil
Klebsiella	Present	Present	Present	Present	nil
Serratia marcescens	Present	Present	Present	Present	nil

Figure.1 Antibacterial activity of Petroleum ether(PE) leaf extract against gram positive gram negative bacteria
Bacillus cereus- Bc, *Staphylococcus aureus*- Sa, *Streptococcus mutans*- St, *E-faecalis*- Ef, *Staphylococcus epidermis*- Se, *Pseudomonas aeruginosa*- Ps, *E- coli*- Ec, *Salmonella typhi*- Sal, *Klebsiella*- Kl, *Serratia marcescens*- Sm.

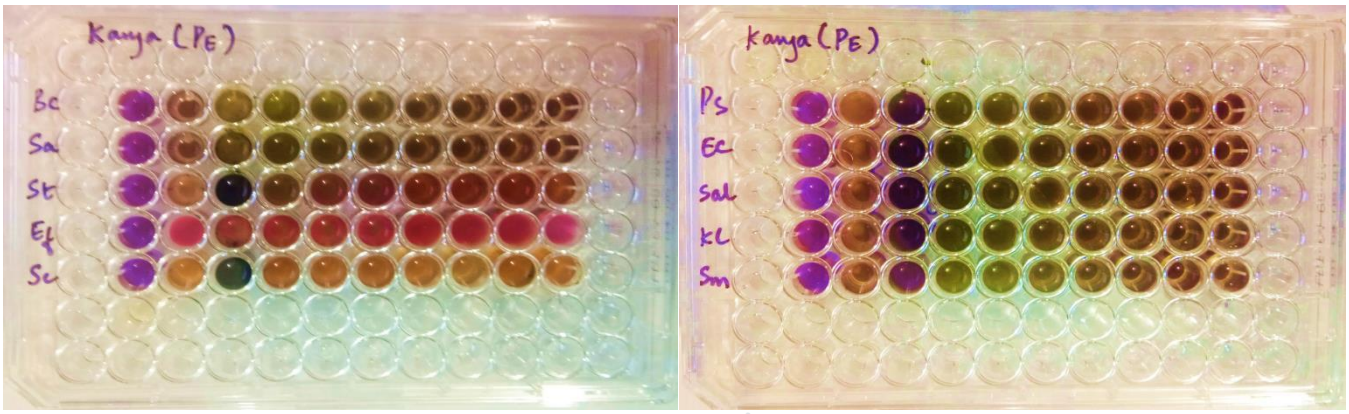


Figure .2 Antibacterial activity of Ethyl acetate(EA) leaf extract against gram positive and gram negative bacteria

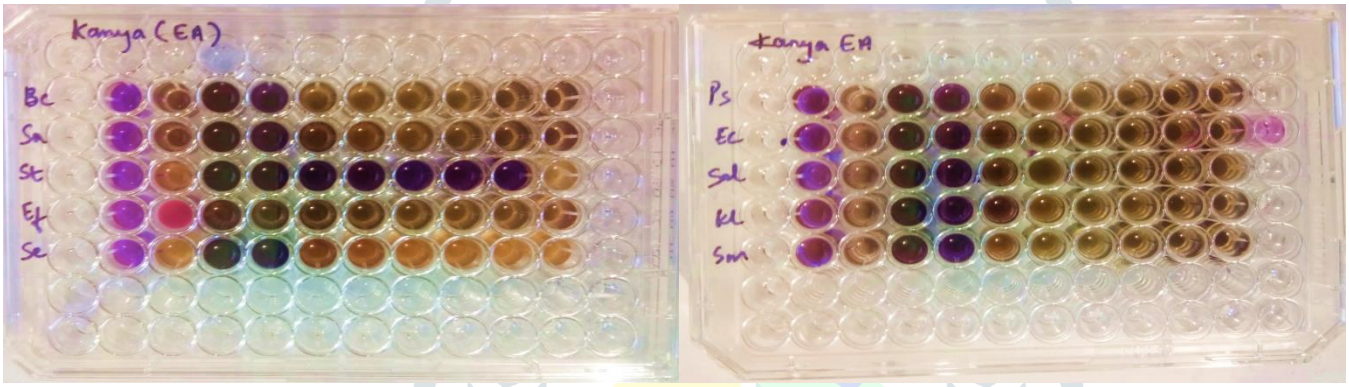


Figure.3 Antibacterial activity of Chloroform(Chloro) leaf extract against gram negative and gram positive bacteria

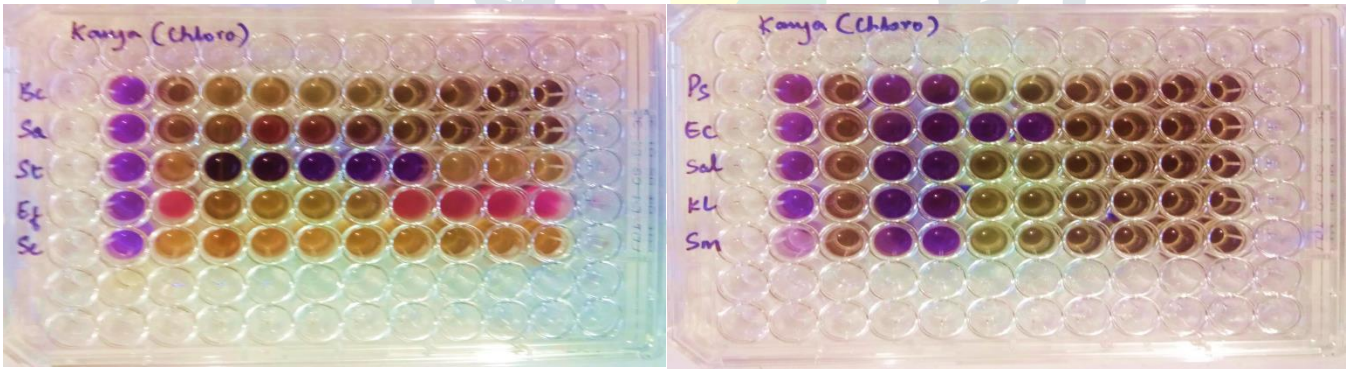


Figure.4 Antibacterial activity of Methanol leaf extract against gram positive and gram negative bacteria

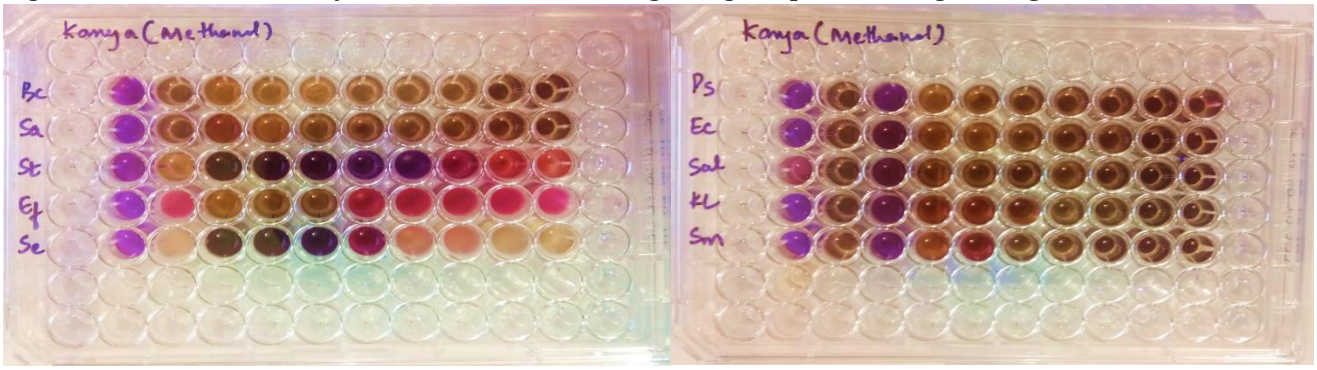
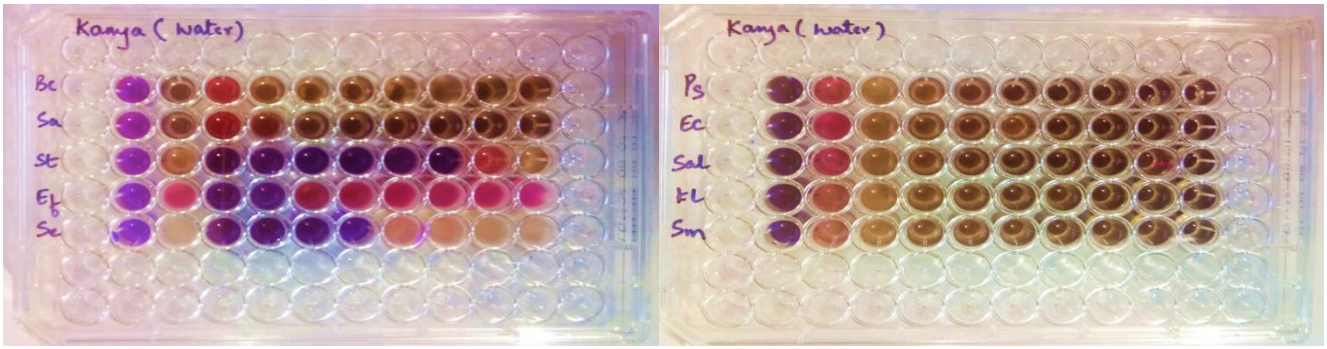


Figure.5 Antibacterial activity of aqueous(Water) leaf extract against gram positive and gram negative bacteria



Reference

1. Anitha Roy, Geetha, R.V. Lakshmi T. 2011. Averrhoa bilimbi Linnature's drug store a pharmacological review. Int J Drug Dev Res 3:101-6.
2. Ayyanar, M. Sankarasivaraman, K. lgnacimuthu, S. 2008. Traditional herbal medicines used for the treatment of diabetes among two major tribal groups in south Tamil Nadu, India. Ethanobotanical Leaflets 12: 276-80 .
3. Deepashree, C.L and Shubha Gopal. 2013. Free radical scavenging potential of *Canthium parviflorum* extracts. J Herbs Spices Med Plants Sci, 3:166.
4. Elayaraja, A. Kathirvelu, S and Mohideen S Rao. 2007. Comparative evaluation on diuretic efficacy of aqueous and ethanolic extracts of *Canthium parviflorum* leaves. Indian J Nat Prod 23:21.
5. Gomes, A. Das, R. Sarkhel S, Mishra R, Mukherjee S, Bhattacharya, S and A Gomes. 2010. Herbs and herbal constituents active against snake bite. Indian J Exp Bio, 48:865.
6. Hiremath, V.T and Taranath, T.C.2010. Traditional phytotherapy for snake bites by tribes of Chitradurga district, Karnataka, India. Ethnobotanical leaflets, 14:120.
7. Hosagoudar, V.B and Archana, G.R.2009. Host range of meliolaceous fungai in India. J Threatened taxa 1: 269-282.
8. Krishna, B. Sanjeeva Kumar, A. Praneetha, R. Rathnakar, E. Venkata Ramana Murthy, B. Pavan Kumar, G. 2014 Phytochemical screening and anthelminthic activity of *Canthium parviflorum* roots. World J Pharm Pharm Sci 3: 566-70
9. Kritikar, K.R. Basu, B.D and Lalit Mohan Basu. 2001. Indian medicinal plants 1: 35-45.
10. Mohideen, S. Illavarasan, R. Hemalatha, A.N. Sasikala, E. 2006. Wound healing and diuretic activities of *Canthium parviflorum* Lam. KR Soc Pharmacognosy 5: 23-5.
11. Parinitha Mahishi. Srinivasa, B.H. Shivanna, M.B.2005. Medicinal plant wealth of local communities in some villages in Shimoga District of Karnataka, Ihdia. J Ethanopharmacol 98: 307-12.
12. Purushoth Prabhu, T. Paneerselvam, P, Selvakumari, S and Sivaraman, D. 2011. Invitro and invivo anticancer activity of ethanolic extract of *Canthium parviflorum* Lam on DLA and Hela cell lines. IJDDR, 3:280.
13. Purushoth Prabhu, T. Paneerselvam, P. Suresh, R. Clement Atlee Wand and Balasubramanian, S.2013. GC-MS analysis of ethanolic extract of *Canthium parviflorum* Lamk Leaf. J App Pharm Sci, 3: 166.
14. Ramanathan, R. Bhuvaneswari, R. Dhandapani, R. 2013. Antimicrobial activity of *Canthium parviflorum* Lam and Pergularia daemia(Forsk). Int J Comprehensive Pharm 9:34-8.
15. Salai Senthilkumar, M.S. Vaidyanathan, D. Sivakumar, D. Ghouse Basha, M. 2014. The diversity of ethnomedical plants used by malayali tribes in yelagiri hills of Eastern ghats, Tamilnadu, India. Asian J Plant Sci Res 4: 69-80.
16. Sathishkumar, Shanmugam, T.S. Palvannan, V.M and Bharathikumar.2008. Evaluation of antioxidant properties of *Canthium parviflorum* Lam. Leaves. Nat Prod Rad, 122.
17. Sathish Kumar, T. Shamugam. Palvannan, S. Bharathi Kumar, V.M.2008. Evaluation of antioxidant properties of *Canthium parviflorum* Lam. Leaves. Indian J Nat Prod Resour, 7:122-6.
18. Sasidhar Pasumathi, Murali Krishna Chimata, Chellu, S Chetty, Suresh Challa. 2011. Screening of phytochemical compounds in selected medicinal plants of Deccan Plateau and their viability effects on Caco-2 cells. J Medicinal Plants Res, 5:6955-62.
19. Sirigiri Chandra Kala. 2015. A short review on callus studies of *Canthium parviflorum* Lam. Int J Institutional Pharm Life Sci, 5:76- 80.
20. Warrier, P.K. Nambia, V.P.K. Ramankutty, R.C and Vasudevan Nair, R. 1996. Indian medicinal plants, Orient Blackswan publishers, New Delhi, 336.