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

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Abstract
The medicinal plants are the key source in the life of human beings. The use of herbal drugs for the prevention and treatment of various health ailments has been in practice from time immemorial. A large number of medicinal plants are explored from flora for production of commercial drugs. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests. Canthium parviflorum is an important medicinal plant used in indigenous system of medicine in India and abroad. Plants are the richest source of medicinal drugs. India is one of the richest Bio Source nations in the world. In India, infectious diseases are still a challenging health problem. To isolate and characterize biologically active molecules, many medicinal plants were screened. Though the medicinal importance of this plant is known, but the potential source of this plant for biologically active molecules is not known. So, the present review on Canthium parviflorum is opens a gateway to find out useful and novel drugs.

Keywords: Canthium parviflorum, extraction, phytochemicals, antibacterial.

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DOI: 10.48047/ecb/2023.12.si5a.0515

Introduction

The traditional medicine all over the world is now a days revalue by an extensive activity of research on different plant species and their therapeutic principles. The increasing cost, non-availability of modern drugs, and limited access to adequate health care have compelled about 80% world population to use traditional pharmacopeia for primary health care especially in the tropical and sub-tropical regions (Sabannar et al.,2017). Plants have been used by the human beings since time immemorial. Plants are significant sources of medicines that are used in the treatment of various categories of human diseases. Traditional drugs derived from herbal plants are used by about 60% of the world‟s population. India is a home to a variety of traditional medicine system that relies largely on native plant species for the raw drug material and holds a credibility of diverse social, cultural and medical heritage with an unbroken tradition coming down across millennia(Ncube et al.,2008).

Herbal medicine has been practiced worldwide and it is recognized by World Health Organization (WHO) as essential building blocks for primary health care. WHO has estimated that up to 80% of people still rely on traditional remedies which are 21,000 plants around the world, among them 2500 species are in India, out of these 150 species are commercially used. Phytocompounds are synthesized by primary or rather secondary metabolism of living organism. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. The overall activity of herbal medicines depends on the active constituents present in them (Mangai et al.,2018). The herb Canthium parviflorum belongs to the family Rubiaceae. It is a thorny shrub. The biological type for the genus consists of specimens originally described by Jean-Baptiste Lamarck as Canthium parviflorum(Bridson et al., 1992).

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 antivenom 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, woundhealing, anticancer, antibacterial and antioxidant (Elayaraja et al.,2007; Sathish kumar 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 Kollam district, 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 2 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 solvent Petroleum ether 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.

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. H2SO4 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.

Test 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. H2SO4 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
Plant sample from Kollam were extracted and assessed in Well diffusion method to check the Minimum Inhibition Concentration (MIC) against pathogens.

Procedure
Sample preparation: 10mg of the sample extract of Petroleum ether was dissolved in 1 mL DMSO (Dimethyl sulfoxide) respectively and sample was prepared 100µg, 200µg, 300µg, and 400µg by pipetting 10µL, 20µL, 30µL,40µL and the final

volume was made upto 50µL by adding deionized water.

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) agar (tryptone 10g, sodium chloride 10g, yeast extract 6g, agar 20g and distilled water 1000mL) was prepared and autoclaved at 121°C for 15mins.

Plate preparation: Approximately 25mL of the media was poured into the sterilized petriplates and allowed it to solidify, later 24hrs cultured 100µL inoculum of *Bacillus cereus, Staphylococcus aureus, Streptococcus mutans, E-faecalis, Staphylococcus epidermis, Pseudomonas aeruginosa, E- coli, Salmonella typhi, Klebsiella* and *Serratia marcescens* added into the respective plates and spreaded throughout the plate using plate spreader.

Five wells are made using well borer and the sample containing 100µg, 200µg, 300µg, and 400µg are loaded into the respective wells and 50µL of deionized water loaded in the center well as control blank and incubated at 37°C for 24hrs.

RESULTS

Table – 1 Phytochemicals in petroleum ether extract

Name of phytochemicals	Petroleum ether extract
Alkaloids	Present
Carbohydrates	Present
Cardiac glycosides	Present
Flavonoids	Present
Phenols	Absent
Phlobatannins	Absent
Amino acids & proteins	Present
Saponoins	Present
Sterols	Present
tannins	Present
Terpenoids	Present
Quinones	Present
Oxalates	Present

Table- 2 Zone of inhibition in anbacterial activity.

Organism	**Zone of Inhibition in Mm for Petroleum ether extract in µg**											
	100 µg			**200 µg**			**300 µg**			**400 µg**		
Bacillus cereus	-	-	-	12	11	12	13	13	13	14	14	14
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-
Streptococcus mutans	13	12	12	13	13	13	14	14	15	16	17	17
E-faecalis	15	15	16	17	17	17	18	18	17	19	19	20
Staphylococcus epidermidis	-	-	-	-	-	-	11	12	12	14	14	14
P.aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-
E-coli	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-	11	13	12	14	15	14
K. pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-
S. marcescens	-	-	-	-	-	-	-	-	-	-	-	-

Plate – 1 Bacillus cereus

Plate- 2 Staphylococcus aureus

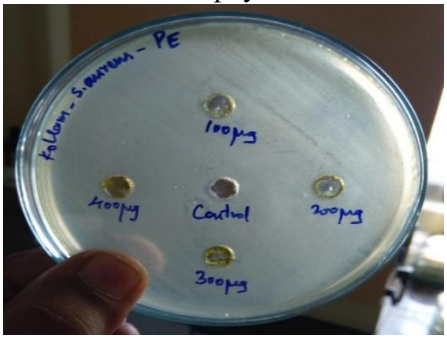


Plate-3 Streptococcus mutans

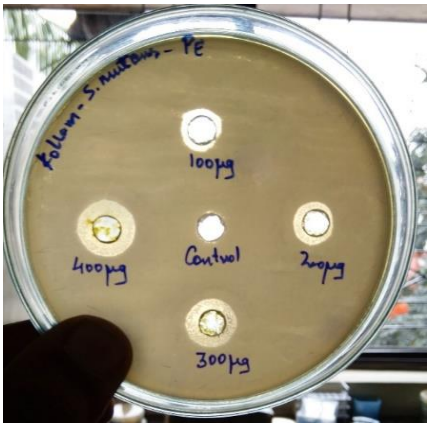


Plate – 4 E. faecalis

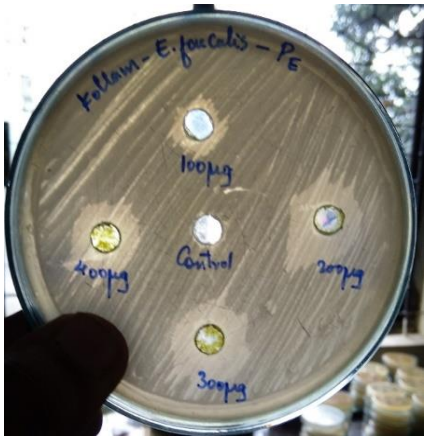


Plate- 5 Staphylococcus epidermidis

Plate- 6 E.coli

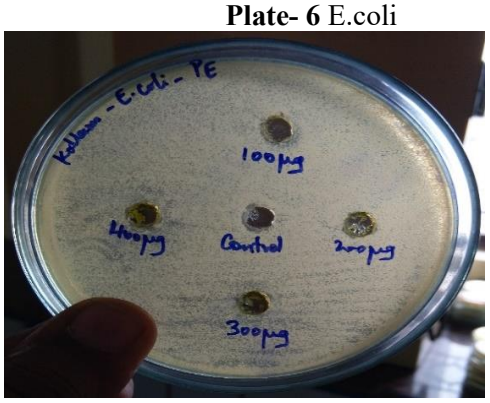


Plate – 7 Salmonella typhi

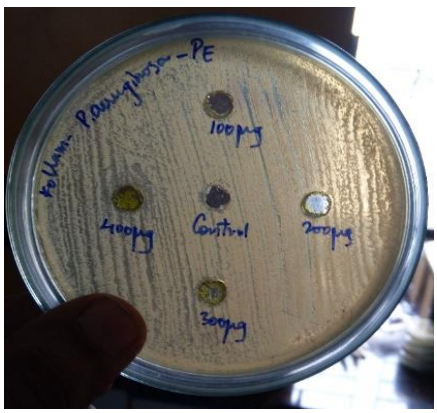


Plate- 8 Pseudomonas aeroginosa

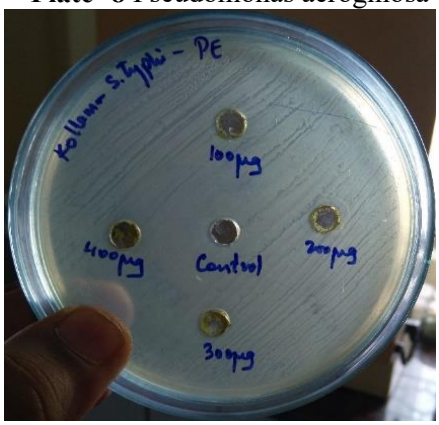
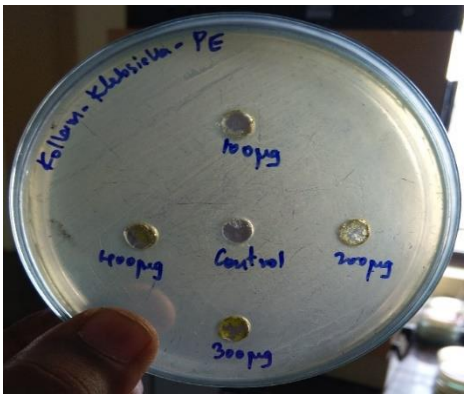


Plate- 9 *Klebsiella*

Plate- 10 *S. marcescens*



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 was determined by measuring the diameter of the zone of inhibition. The extracts of the plant Canthium parviflorum leaves were found to have maximum antibacterial activity. The results of the antibacterial activity of different extracts against some bacterial strains are depicted in (Table 1). Petroleum ether extracts of Canthium parviflorum showed significant activity against *E- Faecalis, Staphylococcus epidermidis* and no activity against Staphylococcus aureus, *Bacillus cereus, Streptococcus mutans, P.aeruginosa, E-coli, Salmonella typhi, K. pneumoniae, S. marcescens.* The zone of inhibition increases as the concentration of the extract increases. So it is observed that the extract shows good activity against gram positive bacteria.

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