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QUALITATIVE PHYTOCHEMICAL AND FTIR ANALYSIS OF ROOT EXTRACTS OF *CANTHIUM PARVIFLORUM LAM*

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ABSTRACT

Canthium parviflorum commonly known as karai was one of the member of the family Rubiaceae. Qualitative phytochemical screening of *Canthium parviflorum* root was studied. Five solvents viz; methanol, ethyl acetate, water, hexane and acetone were used to obtain extracts from powdered plant part. The extracts were subjected to qualitative phytochemical screening using standard procedures. Results showed that 13 of 23 phytochemicals screened were present in various solvents of root extract. They are betacyanin, acids, quinones, alkaloids, carbohydrates, terpenoids, fixed oils and fats, flavanoids, gums and mucilages tanins, reducing sugars and volatile oils. However, coumarins, proteins, aminoacids, resins, phlobatannins, phenols, steroids, cardiac glycosides, anthroquinones, emodols and starch were completely absent in all the five solvents of root extract. The results also shows that more separation of compound is extracted by aqueous root extract of *Canthium parviflorum*. Less separation of compound is recorded from hexane, ethyl acetate, acetone and methanolic root extracts. The diversity of phytochemicals found present suggests that *Canthium parviflorum* root could serve as a source of useful drugs. Further FTIR analysis showed the presence of the alkene (C=C) and ester (C=O) group.

KEYWORDS: *Canthium parviflorum*, Phytochemicals, root extracts.

1. INTRODUCTION

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites and they are naturally synthesized in all parts of the plant body, bark, leaves, stem, root, flower, fruits, seeds etc (ie) any part of the plant body contain active components (Criagg and David; Tiwari, 2011). The medicinal value of a plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants include alkaloids, tannins, carbohydrates, terpenoids, steroids and flavanoids (Edeoga, 2005). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources (Lena, 2010). Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Tiwari, 2011). This therefore underscores the need to try as much solvents as possible in screening plant parts for phytochemicals.

Canthium parviflorum (var name Eng: Carrai cheddie, Hin: Kirma and Kadbar, Tel: Balusu) is a thorny subscandent shrub with branches distributed throughout India in scrub forests and dry plants. It occurs in peninsular India, coramandal coast and in dry plains. This plant is distributed from Konkan southwards to Ceylon ascending up to 4000 feet. The plant is gregarious and useful for hedges. Its leaves and roots are medicinally important and belongs to the family Rubiaceae. The leaves and roots are astringent, sweet, thermogenic, diuretic, febrifuge, constipating, anthelminthic, and these are used in vitiated conditions of kapha, diarrhea, strangury, fever, leucorrhoea, intestinal worms, and general debility (Warrier, 1996). Decoction of leaves is used for wound healing in animals. It is traditionally used for snake bites (Mahishi, 2005). Significant antioxidant and diuretic activity was exhibited by extracts of leaves (Mohideen, 2003). Leaf paste is externally applied twice a day to treat scabies and the ring worm infection (Anitha, 2008). The fruits and leaves are edible. The stem yields a fibre. The wood is hard and suitable for turning. Canthium as herbal medicine is used for the treatment of diabetes among major tribal groups in South Tamilnadu (Ayyanar, 2008). Though the ethno-medicinal importance of this plant is known but the phytochemical basis for such kind of medicinal property is not known. Hence, the present investigation is carried out to find out the qualitative phytochemicals present in various solvent extracts of root of *Canthium parviflorum* using standard procedures.

2. MATERIAIS AND METHODS

2.1. Collection and Identification of Plant Materials: *Canthium parviflorum*, commonly known as Karai was one of the member of the family Rubiaceae. *Canthium parviflorum* was selected for the present study. Fresh part such as root of *Canthium parviflorum* was collected from Marthandam, Kanyakumari District during the month of October- December in the year 2013 and identified by the botany department, Sree Ayyappa College for Women, Chunkankadai, TN. The plant material was transported in polythene bags to the research laboratory, CMST, Rajakkamangalam, where the study was carried out.

2.2. Preparation of powder from Plant Parts: The plant parts such as root of *Canthium parviflorum* were collected freshly and transported to the laboratory, they were then washed thoroughly in running tape water and then with distilled water. The whole plant parts were cut into small bits to facilitate shade drying and the drying process was continued to decrease the moisture content. After drying, the plant root was ground well using mechanical blender into fine powder. Then the powder was stored in airtight container with proper labelling and kept in refrigerator for further use.

2.3. Preparation of plant extract (Percolation process): For the percolation process, the macerated plant root powder was soaked in solvents such as Methanol, Ethyl acetate, Acetone, Aqueous and Hexane individually. Extraction was done by soaking one part of plant powder to three parts of liquid solvent (1:3) and kept for percolation process for 3-5 days. Then the crude extracts were filtered using whatman No.1 filter paper, evaporated and concentrated into solid extracts under room temperature.

2.4. Phytochemical Analysis: The root extracts of each solvent was used to analyse the presence of different phytochemical constituents. The method employed to analyse the phytochemicals are described below.

2.4.1. Test for Carbohydrates Molisch's Test (Sofowara, 1993): The extracts were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml conc.H_2SO_4 was added carefully along the sides of the test tube. Formation of dull violet/red ring at the interphase indicates the presence of carbohydrates.

2.4.2. Test for Acids: To 1 ml of extract I ml of sodium bicarbonate solution was added. Formation of effervescence indicates the presence of acids.

2.4.3. Test for Betacyanins (Harborne, 1973): To 2 ml of plant extract, 1 ml of 2N NaOH was added and heated for 5 minutes at 100^0 C. Formation of yellow colour indicated the presence of betacyanin.

2.4.4. Test for Quinones (Evans, 1996): To 1 ml of extract, 1 ml of Conc.H_2SO_4 was added. Formation of red colour indicated the presence of quinones.

2.4.5. Test for Coumarins: A few drops of ammonia were added on a filter paper. To this, a drop of the extract was added and the paper was observed for fluorescence.

2.4.6. Test for Alkaloids - Mayer's Test (Evans, 1997): The extracts were treated with Mayer's reagent (1.36 g mercuric chloride and 5 gms of potassium iodide was dissolved in 100 ml distilled H_2O). The formation of a yellow cream precipitate indicates the presence of alkaloids.

2.4.7. Test for Aminoacids Ninhydrin Test (Yasuma and Ichikawa, 1953): To the extract 0.25% Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acids.

2.4.8. Test for Proteins (Brain and Turner, 2006) Biuret Test: Extracts were treated with 1 ml of 10% NaOH solution & heated. To this a drop of 0.7% $CuSO_4$ solution was added.Formation of purplish violet colour indicates the presence of proteins.

2.4.9. Test for Reducing sugars - Benedict's test (Tiwari, 2011; Sofowara, 1993): The extracts were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.

2.4.10. Test for Fixed oils and Fats - Stain Test: Small quantities of the extracts were pressed between 2 filter papers. Formation of an oily stain on the filter paper indicates the presence of fixed oils and fats.

2.4.11. Test for Flavanoids - Ferric Chloride Test (Raman, 2006): The extract was treated with a few drops of $FeCl_3$ solution. Formation of a blackish red colour indicates the presence of flavanoids

2.4.12. Test for Gums and Mucilages (Whistler and Bemiller, 1993): About 5 ml of the extract was slowly added to 5 ml of absolute alcohol under constant stirring. The appearance of precipitation indicates the presence of gums and mucilages.

2.4.13. Test for Steroids (Kokate, 1994): 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. Change in colour from violet to blue or green indicates the presence of steroids

2.4.14. Test for Tannins (Trease and Evans, 1989): To 1 ml of the solvent extract, few drops of 1% $FeCl_3$ solution were added. The appearance of a blue, black, green or blue green precipitate indicated the presence of tannins.

2.4.15. Test for Resins - Acetone-H_2O Test: The Extracts were treated with acetone. A small amount of water was then added and shaken. Appearance of turbidity indicates the presence of resins.

2.4.16. Test for Phlobatannins (Harborne, 1973): About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatannins.

2.4.17. Test for Terpenoids - Salkowski Test (Evans, 1997): To 1 ml of the solvent extract, 2 ml of chloroform was added. Then 3 ml of conc.H_2SO_4 was added carefully to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

2.4.18. Test for Phenols - Ferric Chloride Test (Mace, 1963): To 1 ml of solvent extracts, 3 ml of distilled H_2O was added. To this, a few drops of neutral 5% $FeCl_3$ solution was added. Formation of a dark green colour indicated the presence of phenolics.

2.4.19. Test for Saponins - Foam Test (Kumar, 2009): About 2 ml of distilled H_2O and 1 ml of solvent extract were mixed and shaken vigorously. Formation of a stable persistent froth indicated the presence of saponins.

2.4.20. Test for Cardiac glycosides - Keller-Killani Test (Sofowara, 1984): The **e**xtract was dissolved in glacial acetic acid containing traces of $FeCl_3$. The tube was then held at an angle of 45^0 and 1 ml of Conc.H_2SO_4 was added along the sides of the tube. Formation of a purple ring at the interface indicates the presence of cardiac glycosides.

2.4.21. Test for anthroquinones - Borntrager's Test (Sofowara, 1993; Harborne, 1998): Small portion of the extract was shook well with 10 ml benzene and filtered. 5 ml of 10% ammonia solution was added to the filtrate and stirred. The production of a pink red or violet colour indicates the presence of free anthroquinones.

2.4.22. Test for volatile oils (Trease and Evans, 1989): To 1 ml of the extract, 1 ml of 90% ethanol was added, followed by the addition of a few drops of $FeCl_3$ solution. Formation of a green colour indicated the presence of volatile oils in the given sample

2.4.23. Test for Emodols: The dry extract was added to 25% ammonia solution. The formation of a cherry-red solution indicated the presence of emodols.

2.4.24. Test for starch (Harborne, 1998): To 1 ml of the extract 10 ml of saturated NaCl solution was added. It was then heated. After heating, starch reagent was added. Formation of a blue-purplish/pink colour is a positive test for the presence of starch.

2.4.25. Test for fatty Acids (Ayoola, 2008): 0.5 ml of extract was mixed with 5 ml of ether. This mixture was allowed to evaporate on the filter paper and then the filter paper was dried. The appearance of transparence areas on filter paper indicates the presence of fatty acids.

2.5. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis: FTIR is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). Dried powder of *Canthium parviflorum* was considered for instrumental analysis. For the FTIR study dried powder of *Canthium parviflorum* was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample was treated for FTIR spectroscopy, Scan range: from 400 to 4000 cm-1 with a resolution of 4 cm^{-1}.

3. RESULTS AND DISCUSSION

Phytochemical analysis was carried out on the plant *Canthium parviflorum* which revealed the presence of medicinally important bioactive compounds. The presence of phytochemical compounds in the plant *Canthium parviflorum* was evaluated in root using different solvents such as methanol, ethyl acetate, water, hexane and acetone. Result obtained for qualitative screening of phytochemical root extracts of *Canthium parviflorum* in five different solvents are presented in Table 1. In the present study, the preliminary phytochemical screening of root extracts of *Canthium parviflorum* Results showed the presence of 13 of 23 phytochemicals screened such as betacyanin, acids, quinones, alkaloids, carbohydrates, terpenoids, fixed oils and fats, flavanoids, gums and mucilages tanins, reducing sugars and volatile oils in various solvents of root extract. There is no source of coumarins, proteins, aminoacids, resins, phlobatannins, phenols, steroids, cardiac glucosides, anthroquinones, emodols and starch in any solvents of root extract.

The results also shows that more separation of compound is extracted by aqueous root extract of *Canthium parviflorum*. Less separation of compound is recorded from hexane, ethyl acetate, acetone and methanolic root extracts. This was correlated with the work done by Harold Peter, 2011, reported that the whole plant extracts of *Canthium parviflorum* revealed the presence of phytochemicals such as alkaloids, oils, flavanoids, gums, phenols, saponins, steroids, tannins and terpenoids. More phytochemicals ie, 7 of 11 were found to be present in acetone root extract of *Canthium parviflorum*, so the result indicates that *Canthium parviflorum* root hold promises as source of pharmaceutically important phytochemicals.

Flavonoids possess anti-allergic, anti-inflammatory, antiviral and antioxidant activities (Bbosa, 2010). Flavoanoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjori, 1996) .Glycosides is known to lower the blood pressure according to many reports (Nyarko and Addy, 1990). The presence of this type of phytochemical compounds in the screened medical plants has a wide range of applications and could be certainly used for a variety of applications (Lena, 2010). The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents of the plant *Canthium parviflorum* is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Table 1: Qualitative Phytochemical Analysis of *Canthium parviflorum* Root Extracts

No.	Tests	Aq	Ac	EtAc	MOH	Hx
1	Acids	+	-	-	-	-
2	Betacyanin	-	-	+	+	-
3	Quinones	+	+	-	+	-
4	Coumarins	-	-	-	-	-
5	Carbohydrates	-	-	-	-	+
6	Alkaloids	+	+	-	+	-
7	Proteins (Biuret test)	-	-	-	-	-
8	Aminoacids (Ninhydrin test)	-	-	-	-	-
9	Resins	-	-	-	-	-
10	Phlobatannins	-	-	-	-	-
11	Terpenoids	+	-	-	+	-
12	Phenols	-	-	-	-	-
13	Saponins	+	-	-	-	+
14	Fixed oils and fats	+	-	-	-	-
15	Flavanoids (Ferric chloride test)	+	-	-	-	-
16	Flavanoids (Alkaline reagent test)	-	+	-	+	+
17	Gums and mucilages	+	-	-	-	-
18	Steroids	-	-	-	-	-
19	Tannins	+	-	-	-	-
20	Reducing sugars (Benedict's test)	+	+	-	+	-
21	Reducing sugars (Fehling's test)	-	+	+	+	-
22	Cardiac glycosides	-	-	+	-	-
23	Anthroquinones	-	-	-	-	-
24	Volatile oils	+	-	-	-	-
25	Emodols	-	-	-	-	-
26	Starch	-	-	-	-	-

Aq=Aqueous, Ac=Acetone, EtAc=Ethyl Acetate, MOH=Methanol, Hx=Hexane.
+ indicates presence of the phytochemical and – indicates absence of the phytochemical.

Table: 2 FTIR Absorption frequencies of functional groups

Peak	Characteristic observation	Intensity	Possible functional group
1629.74	1620-1680	variable	Alkene (C=C)
1456.16	1400-1600	Medium-weak	Aromatic (C=C)
1402.15	1400-1600	Medium-weak	Aromatic (C=C)
1112.85	1000-1300	Strong	Ether (C-O)

The FTIR (Fourier Transform Infrared Spectrometer) was performed from the plant extract of *Canthium parviflorum* to analyse the functional group. In *Canthium parviflorum* extract IR spectrum shows strong absorption peaks at 1666.38 cm^{-1}, 1456.16cm^{-1}, 1402.15cm^{-1}, 1112.85cm^{-1}, which corresponds to alkene (C=C) and ether (C-O) groups (Table.2 and Fig 1).

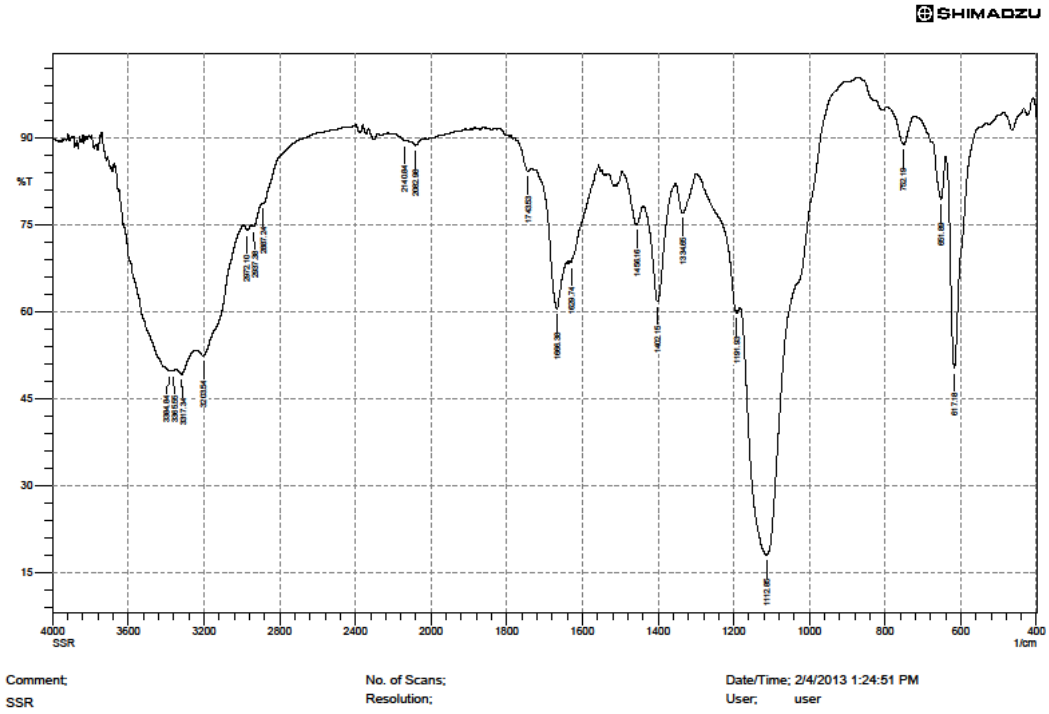

Fig: 1 Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

Thus the results revealed the presence of medicinally important constituents in the plant studied. Thus overall, the phytochemicals found present in root extracts of *Canthium parviflorum* indicates their potential as a good source of novel useful drugs. The present study suggests that the extracted pyhochemicals are very valuable. Further studies are therefore suggested to ascertain their antimicrobial activities. Furthermore, isolation, purification and characterization of the phytochemicals will make interesting studies. Further investigations are planned to conduct the pharmacological studies to know the potency of these extracts.

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