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Research Article

QUALITATIVE PHYTOCHEMICAL CHARACTERIZATION OF THORN 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* thorn 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 show that 11 of 23 phytochemicals screened were present in various solvents of thorn extract. They are betacyanin, quinones, coumarins, carbohydrates, aminoacids, terpenoids, fixed oils and fats, flavanoids, cardiac glycosides, volatile oils and starch. However, acids, alkaloids, resins, phenols, saponins, gums and mucilages, steroids, tannins, anthroquinones, emodols, proteins, phlobatannins and reducing sugars were completely absent in all the five solvents of thorn extract. The results also shows that betacyanin, quinones, coumarins, terpenoids, fixed oils and fats, flavanoids, and volatile oils were more readily extracted by acetone thorn extract of *Canthium parviflorum*. Less separation of compound is recorded from hexane, ethyl acetate, aqueous and methanolic thorn extracts. The diversity of phytochemicals found present suggests that *Canthium parviflorum* thorn could serve as a source of useful drugs.
Key words: *Canthium parviflorum*, Phytochemicals, thorn extracts

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 [1,2]. 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 [3]. 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 [4]. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [2]. 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 [5]. Decoction of leaves is used for wound healing in animals. It is traditionally used for snake bites [6]. Significant antioxidant and diuretic activity was exhibited by extracts of leaves [7]. Leaf paste is externally applied twice a day to treat scabies and the ring worm infection [8]. 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 [9].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 thorn of *Canthium parviflorum* using standard procedures.

MATERIALS AND METHODS

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 thorn 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.

Preparation of powder from Plant Parts:

The plant parts such as root, leaf, bark, stem, spine and seed 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 materials were ground well using mechanical blender into fine powder. Then the powder was stored in airtight containers with proper labeling and kept in refrigerator for further use.

Preparation of plant extract: (Percolation process)

For the percolation process, the macerated plant powders were 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.

Phytochemical Analysis:

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

Test for Carbohydrates

Molisch's Test [10]

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.

Test for Acids

To 1 ml of extract I ml of sodium bicarbonate solution was added. Formation of effervescence indicates the presence of acids.

Test for Betacyanins [11]

To 2 ml of plant extract, 1 ml of 2N NaOH was added and heated for 5 minutes at 100° C. Formation of yellow colour indicated the presence of betacyanin.

Test for Quinones [12]

To 1 ml of extract, 1 ml of Conc.H_2SO_4 was added. Formation of red colour indicated the presence of quinones.

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.

Test for Alkaloids

Mayer's Test [13]

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.

Test for Aminoacids

Ninhydrin Test [14]

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.

Test for Proteins [15]

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.

Test for Reducing sugars

Benedict's test [2, 10]

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.

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.

Test for Flavanoids

Ferric Chloride Test [16]

The extract was treated with a few drops of $FeCl_3$ solution. Formation of a blackish red colour indicates the presence of flavanoids

Test for Gums and Mucilages [17]

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.

Test for Steroids [18]

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

Test for Tannins [19]

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.

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.

Test for Phlobatannins [11]

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.

Test for Terpenoids

Salkowski Test [13]

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.

Test for Phenols

Ferric Chloride Test [20]

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.

Test for Saponins

Foam Test [21]

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.

Test for Cardiac glycosides

Keller-Killani Test [22]

The **e**xtract was dissolved in glacial acetic acid containing traces of $FeCl_3$. The tube was then held at an angle of 45° 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.

Test for anthroquinones

Borntrager's Test [10, 23]

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.

Test for volatile oils [19]

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

Test for Emodols

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

Test for starch [24]

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.

Test for fatty Acids [25]

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.

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* were evaluated in thorn using different solvents such as methanol, ethyl acetate, water, hexane and acetone. Result obtained for qualitative screening of phytochemical thorn extracts of *Canthium parviflorum* in five different solvents are presented in **Table 1.** In the present study, the preliminary phytochemical screening of thorn extracts of *Canthium parviflorum* showed the presence of 11 of 23 phytochemicals such as betacyanin, quinones, coumarins, carbohydrates, aminoacids, terpenoids, fixed oils and fats, flavonoids, cardiac glycosides, volatile oils and starch were present in various solvents of thorn extract. There is no source of acids, alkaloids, resins, phenols, saponins, gums and mucilages, steroids, tannins, anthroquinones, emodols, proteins, phlobatannins and reducing sugars in any solvents of thorn extract. Less separation of compound is recorded from aqueous and methanolic thorn extracts and very less separation of compound is recorded from hexane and ethyl acetate thorn extracts. This was correlated with the work done by Harold Peter *et al.*, 2011 [26] 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 thorn extract of *Canthium parviflorum*, so the result indicates that *Canthium parviflorum* thorn hold promises as source of pharmaceutically important phytochemicals.

Flavonoids possess anti allergic, anti-inflammatory, antiviral and antioxidant activities [27]. 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 [28] .Glycosides is known to lower the blood pressure according to many reports [29]. 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 [4]. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents and this plant (*Canthium parviflorum)* is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. This suggests that the thorn of *Canthium parviflorum* offers a wide array of phytochemicals than the other parts of *Canthium parviflorum.*

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

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

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

CONCLUSION

Thus the results revealed the presence of medicinally important constituents in the plant studied. Thus overall, the phytochemicals found present in thorn 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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