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Study on Phytochemical and Thin layer chromatography of *Canthium coromandelicum* (Burm.f.) Alston leaves

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Abstract : In ethno medicine, *Canthium coromandelicum* (Burm.f.) Alston leaves are recommended to treat different ailments including wound, cholesterol, diabetes. *Canthium* sp. laid under Rubiaceae family, this study aim was to screen the bioactive compounds which present in methanolic extract obtained from*Canthium coromandelicum* (Burm.f.) Alston leaves. According to the present preliminary analysis results, alkaloids, cardiac glycosides, quinones, flavonoids, steroids, saponins, phenol compounds, leucoanthocyanidines gum and mucilage, protein and carbohydrate were detected and chromatographic results showed 10 spots, 13 spots, 1 and 2 spots in thin layer chromatograms of acetone, hexane, ethyl acetate and methanol extracts respectively. Overall, it is postulated that *Canthium coromandelicum* (Burm.f.) Alston has medicinal and nutritional potentials.
Keywords : *Canthium coromandelicum* (Burm.f.) Alston, Flavonoids, Steroids, Saponins.

Introduction

Canthium sp. is widely recommended indigenous medicinal practitioners for the treatment of different ailments. Recently, the plants which could be used to formulate drugs are gaining interest field for the researcher. Therefore, many researches had been conducted to identified the true medicinal potentials of this *Canthium coromandelicum* (Burm.f.) Alston plant. Vernacular name of this plant (*Canthium coromandelicum* (Burm.f.) Alston) is Kara in Sinhala. This *Canthium* genus comprises 230 spices which grow as shrubs or small trees found in Southeast Asia including Sri Lanka and India[1]and native to India, Sri Lanka and East Africa[2]. Edible parts of the plant are fruits and leaves that leaves are consumed as raw form with coconut scrapes called Sambal and cooked form called Mallum[3] and in Northern province of Sri Lanka, leaves are used in curries[4].Several researches study on *Canthium coromandelicum* have been reported its antimicrobial, antidiabetics, wound healing, diuretic activities[5].

Preliminary screening of bio active compounds is used to identify the chemical composition of the sample in order to isolate the beneficial compounds to formulate drugs. Presence of the phytochemicals such as

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alkaloids, cardiac glycosides, quinones, flavonoids, steroids, saponins, phenol compounds, leucoanthocyanidines gum and mucilage, tannins and phlobatannins show different medicinal and nutritional values which chromatographic techniques are used to further identification of these compounds. Among them, thin layer chromatogram is used to separate and quantify the compounds[6]. It is a simple, reliable method which retention factor of each separated band is used to identify the components and using reference compounds, quantification can be done[7].

Materials and Methods

Collection of plant materials

Fresh fully matured healthy*Canthium coromandelicum (Burm.f.)Alston* leaves were collected from Kadawatha, Gampaha district during 8-10 in the morning in July.

Authentication of sample

Herbarium specimen was prepared with use of collected leaves then plant material was identified and authenticated by a taxonomist of National herbarium of Peradeniya, Sri Lanka.

Preparation of extraction for preliminary screening

Selected fresh healthy leaves were washed using chlorinated water, secondly with distilled water to remove any debris and then kept in a shade room to dry for 10 days. Well dried leaves were pulverized into coarse powder. 20g of coarse powder was added into a thimble and it was extracted using 250 ml of 80% methanol/water in a Soxhlet apparatus for 6 hours. Excess coarse powder was stored at 4 ^{0}C in an airtight container. Extracted solution and coarse powder were used to carry out the preliminary screening.

Phytochemical analysis

Phenolic compounds[8]

Lead acetate test was conducted to screen phenolic compounds. 1ml of methanolic extract and 3 drops of 1% lead acetate were mixed in a dry tube. Presence of phenolic compounds was signified as the formation of white colour.

Tannins[8]

Gelatin test and ferric chloride test were carried out to identify the presence of tannins. To conduct gelatin test, 5 ml of distilled water and 3 ml 10% of sodium chloride were added to 2 ml of 1% gelation solution contained 50mg of extracted sample. Positive result was signified as formation of white precipitate. For ferric chloride test, 0.5 ml of 5% $FeCl_3$ and1ml of extract were mixed in a dry tube and green color precipitate signified the positive test for tannins.

Phlobatannins[8]

Presence of phlobatannins was tested with adding 2ml of extract to 2ml of 1% aqueous HCl then heated the sample until it boiled. Positive result was signified as formation of red color precipitate.

Alkaloids[9]

Mayer's test and Wagner's test were carried out to identify the presence of alkaloids. 3 drops of Mayer's reagent were added to 1 ml of extract and formation of yellow colour precipitate was signified as presence of alkaloids in Mayer's test. In Wagner's test, 3 drops of Wagner's reagent were mixed with 1ml of extract in a dry tube. Yellow colour precipitate was observed as the presence of alkaloids.

Cardiac glycosides[10]

Keller-Killiani test was conducted by adding 1ml of glacial acetic acid, 0.5ml of conc. $H_2SO_4$and 2 drops of $FeCl_3$ to 0.2g of coarse. Formation brown colour ring at the interface was observed as presence of cardenolids.

Quinones[10]

Presence of quinones was screened by adding 1ml of alcoholic KOH to 2ml of methanolic extract and reddish blue colour was showed for the positive result.

Steroids[11]

Pre-weighed 0.2g of coarse powder was added to 2ml of acetic anhydride and 2ml of conc. $H_2SO_4$and blue colour was revealed as the positive for steroids.

Leucoanthocynidines[10]

Leucoanthocynidine was determined by adding 2ml of extract to 1ml of conc. HCl and then heated until it boiled. Formation of reddish color was indicated as positive for leucoanthocynidine.

Saponins[12]

Frothing test was carried out by adding 20 ml of distilled water to 100mg of powder and then shaken for 30 min. positive result was observed as formation of persistence frothing.

Flavonoids[12]

Ferric chloride test

0.5ml of ferric chloride and 1ml of extract were mixed and woody brown colour indicate the presence of flavonoids

Gum and Mucilage[10]

Presences of gum and mucilage was identified as follows;100 mg of dry powder was mixed with 10 ml of distilled water and 2ml of absolute alcohol and then stirred constantly. White or cloudy precipitate was showed as positive for this test

Carbohydrates[10]

Fehling's test and Bradford's test were carried out to determine carbohydrates. 2ml of each Fehling's A and B was mixed with 2ml of extract and 2ml of distilled water followed by heating up until it boiled. Brick red colour precipitate was signified as positive results for Fehling's test. For Bradford's test, 2ml of methanol extract was added into dry test tube and then 4 drops of Bradford's reagent were added to it. Blue colour precipitate was signified for presence of reducing sugar.

Protein[10]

Millon's test was carried out to determine the presence of protein. 2ml of extract sample was mixed with 6 drops of Millon's reagent and formation of white colour precipitate was signified the presence of protein.

Thin layer chromatographic analysis

Extracted samples of various solvents such as acetone, methanol, hexane and ethyl acetate were prepared by maceration of 3g of coarse powder with 100ml of each solvent in a shaker for 3 days followed by sonicating for 20min and then solution was filtered and evaporated to obtain dry extract with use of a rotary evaporator at 40^0C. Extracted samples were dissolved by adding few drops of acetone. Then pre-coated silica plate was cut into 6X7.5 cm. The solvent end was marked and spots were applied with use of capillary tubes. Chromatographic tanks were prepared using four different solvent systems; (I) hexane: ethyl acetate (4:1), (II)

hexane: ethyl acetate: methanol: water (5:3:1:1), (III)hexane: ethyl acetate: methanol (3:1:1) (IV)ethyl acetate: methanol (1:1). Spotted plates were kept in tanks until it developed then solvent front was marked and kept drying. Day light, 254nm and 365nm of ultraviolet light and an iodine chamber were used to visualize the developed plates. For separated each band, retention factor was calculated using below equation;

$$R_f = \frac{Distance\ moved\ by\ the\ solute/compound}{Distanced\ moved\ by\ the\ solvent} \quad (1)$$

Results and Discussion

Phytochemical analysis

Obtained results of phytochemical analysiswere showed in table 01 given below. *Canthium coromandelicum* (Burm.f.)Alston leaves contained alkaloids, cardiac glycosides, quinones, flavonoids, steroids, saponins, phenol compounds, leucoanthocyanidines gum and mucilage, protein and carbohydrate while without tannins and phlobatannins. The previous study on this plant showed both tannins and phlobatannins[1].

Table 01: Results obtained from phytochemical analysis

No.	Bioactive compound	Test/s	Colour change/colour of precipitate	Methanol extract
1	Phenol compounds	Lead acetate test	White colour	+
2	Tannins	Ferric chloride test Gelatin test	Green color precipitate White precipitate	- -
3	Phlobatannins		Red color precipitate	-
4	Alkaloids	Mayer's test Wagner's test	Yellow colour precipitate Yellow colour precipitate	+ +
5	Cardiac glycosides	Keller-Killiani test	Brown colour ring	+
6	Quinones		Reddish blue colour	+
7	Steroids		Blue colour	+
8	Leucoanthocyanidines		Reddish color	+
9	Saponins	Frothing test	Persistence frothing	+
10	Flavonoids	Ferric chloride test	Woody brown colour	+
11	Gum and mucilage		White/cloudy precipitate	+
12	Carbohydrates	Fehling's test Bradford's test	Brick red colour precipitate Blue colour precipitate	+ +
13	Protein	Millan's test	White precipitate	+

(+) Presence; (-) Absence

Saponins, polyphenols, phytosterols, quinones, flavonoids have anti-cancer activities[13-17] while saponins, polyphenols and cardiac glycosides also have anti-cardiovascular activity[13-14,18]. Study on alkaloids and quinones have been reported for their antibacterial activity[17,19]and antifungal, anti-parasitic activities[20] of alkaloids meanwhile, anti-HIV activity for quinones[17]. Polyphenols and flavonoids are natural antioxidant[21] and flavonoids have been reported for their anti-allergic[22], anti-thrombotic[23], anti-diabetes[24] and anti-atherosclerotic activities[25]. Disintegration properties of property of mucilage has been reported in several studies[26]and carbohydrates play a significant role as energy source[27] and protein as structural component of the cell[28].

Thin layer chromatographic studies

Thin layer chromatographic analysis results revealed that 26 spots were separated in four different solvent systems which extracted in four solvents which were shown in figure 01. Ten spots were detected in acetone extract while 13, 1, 2 for ethyl acetate, hexane and methanol extracts respectively. Obtained retention factors for 26 spots were shown in table 02.

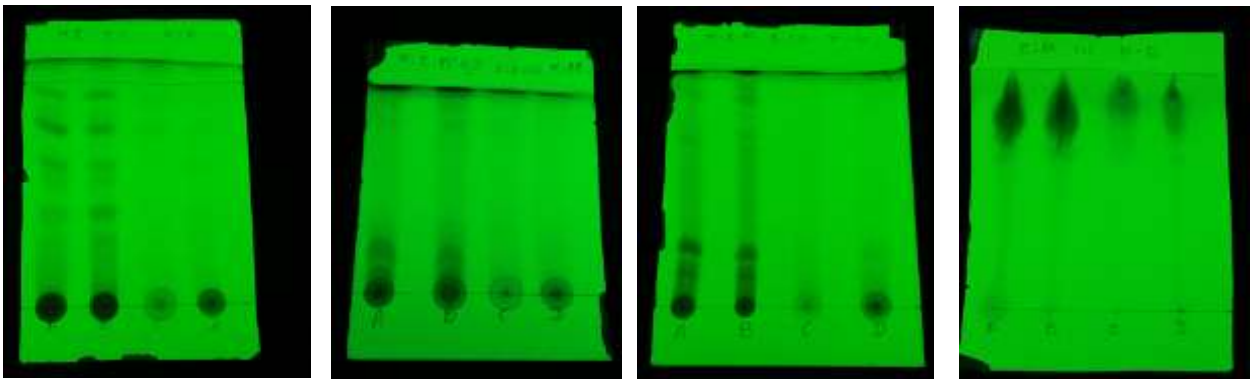

Figure 01: TLC of 1. Solvent System I; 2. Solvent System II; 3. Solvent System III; 4. Solvent System IV

Table 2: Results of TLC for four solvent systems

Extract	**Solvent system I**		**Solvent system II**		**Solvent system III**		**Solvent system IV**	
	No. Of spots	R_f value	No. Of spots	R_f value	No. Of spots	R_f value	No. of spots	R_f value
Acetone	5	0.40 0.55 0.63 0.79 0.96	2	0.10 0.20	2	0.11 0.23	1	0.81
Ethyl acetate	6	0.42 0.57 0.63 0.79 0.96 0.98	2	0.16 0.94	4	0.11 0.22 0.84 0.89	1	0.83
Hexane	0	0.00	0	0.00	0	0.00	1	0.94
Methanol	0	0.00	0	0.00	1	0.22	1	0.92

Different retention factors reveal the natural of polarity of each separated bans. In order to further separation of these compounds using advanced chromatographic techniques, polarity of compounds can be used for better isolation.

Conclusion

Based on the results, it can be concluded that *Canthium coromandelicum* (Burm.f.) Alston leaves contained alkaloids, cardiac glycosides, quinones, flavonoids, steroids, saponins, phenol compounds, leucoanthocyanidines gum and mucilage, protein and carbohydrate which have medicinal activities and further isolation and purification of these compounds can be used to formulate drugs in pharmaceutical industry.

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