Phytochemical Screening and TLC Profile of *Montricardia arborescens*

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ABSTRACT

Phytochemical screening provides first hand knowledge of a plant's phytochemical constituents. Leaves of *Monticardia arborescens* were ground and subjected to selective extractions using solvents of increasing polarity: n-C_6H_{14}, CH_2Cl_2, CH_3CH_2OH. Phytochemical screening of each solvent type extracts revealed selective presence of natural products. The hexane extract revealed the presence of alkaloids, tannins, carotenoids and cardiac glycosides. The dichloromethane extracts showed positive tests for the presence of steroids , triterpenes, carotenoids, tannins, amino acids, proteins and cardiac glycosides. The ethanol extract revealed the presence of flavone aglycones, amino acids, alkaloids, tannins, anthracenosides, cardiac glycosides and reducing compounds. The selective presence of these natural constituents should provide a guide to their chromatographic separation.

Keywords: *Montricardia arborescens*, selective extractions, alkaloids, anthracenosides, steroids, triterpenes, tannins, flavone aglycones, cardiac glycosides, reducing compounds

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INTRODUCTION

Phytochemical screening is a process of evaluating a plant's phytochemical constituents using standard established tests[1-3] and provides first hand knowledge of a plant's phytochemical constituents where instrumentation such as NMR spectroscopy is lacking. It's a prerequisite for chromatographic separation of plant extracts for known and unknown natural products constituents whose structure would then be elucidated using ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMBC and HMQC spectroscopy[4]. Natural products are secondary metabolites of plants and

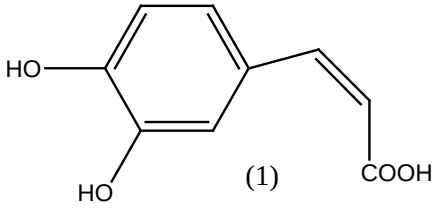
animals which are of restricted occurrence and are classified into major groups such as sterols, triterpenes, flavones aglycones, emodols (anthracenosides aglycones), coumarins, coumarins lactone derivatives, tannins (gallic), reducing compounds, sterols glycosides, cardenolides, saponins and sapogenins [5-6] . Some of these are shown in Fig. 1.0. They vary widely in both type and concentrations in different parts of the plant and are found as minor components of plant tissues. They are synthesized via metabolic pathways [5-6] and can be either novel or known and are isolated from crude plant extracts whose medicinal activity can be compared with that of the isolates [8-17].

Medicinally, isolated natural products after been subjected to clinical trials can be used as drugs for the treatment of cancer[7-8], antimicrobial agent[9], antitumor[10], antiinflammatory[11], antioxidant agents[12-14], Glucosidase Inhibitors[15]. Also, isolated natural products have been the impetus for the design and synthesis of many pharmaceutical drugs to date. For example, *Cyanthiwigin F*, a complex *bis* molecule active against tumours was first isolated from the sea sponge *Myrmekioderma styx*[16,] Fig. 2.0. Crude plant extracts have also been a source of antimicrobial agents [17-24].

Representative examples of some natural products studied are shown in Fig.1.0. Tannins are large polyphenolic compound containing sufficient hydroxyls and other suitable groups, such as carboxyls to form strong complexes with various macromolecules. Flavonoids have similar structures to that of flavones. Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety. Cholesterol have the steroidal nucleus. Sterols have the third carbon of the steroidal nucleus hydroxylated. Sterols may be found either as free sterols, acylated (sterol esters), alkylated, sulfated (sterol sulphate) or linked to a glycoside moiety (steroidal glycosides). Carotenoids are tetraterpenoid (C_{40}) organic pigments. Coumarins are lactones, cyclic esters, Fig. 1.0 and Fig. 2.0. Saponins are amphipathic glycosides noteworthy for their soap like foamimg property. Cardenolides are a special type of steroid. Cardenolide may be linked to a sugar moiety to form a cardenolide glycoside. Alkaloids are a diverse group of plant natural products that contain mostly basic nitrogen atoms. They are usually divided into the true alkaloids, protoalkaloids, polyamine alkaloids, peptide and cyclopeptide alkaloids, pseudoalkaloids.

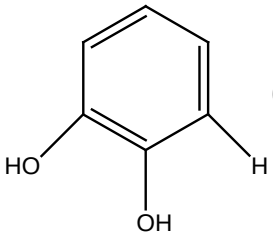
Montrichardia arboesens (moco moco) plant is found in the Araceae family. The plant has a maximum height of 9 feet. It grows along the banks of rivers, creeks and in swamps. The stems have prickles and are swollen at the base, the leaves are arrow-shaped and this is about 2.5 feet long. *Montricardia arborescens* is considered the best food choice for manatees. To date, no natural products have been isolated from the plant.

Simple Phenols and phenolic acids



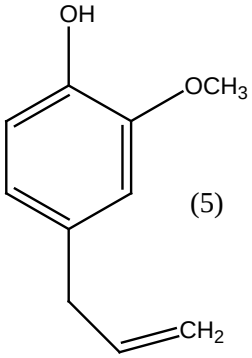
(1)

Caffeic acid



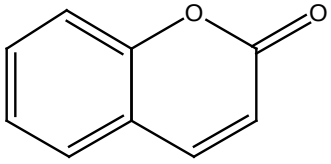
(4)

Catechol



(5)

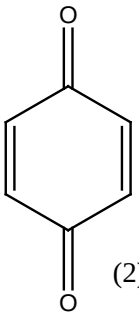
Eugenol



(8)

Coumarin

Quinones

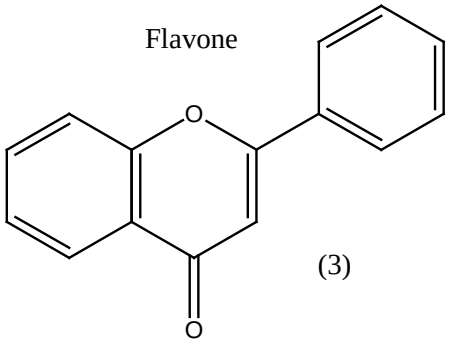


(2)

Quinone

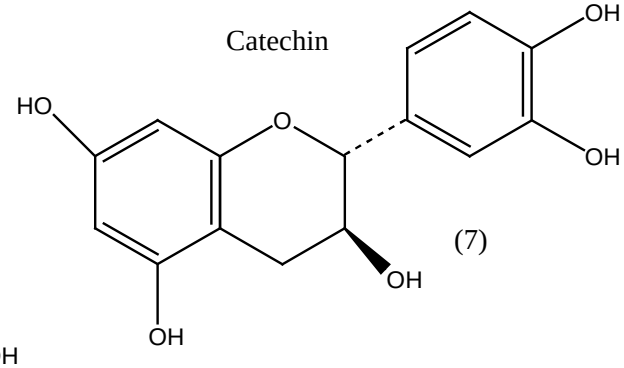
Flavones and Flavonoids

Flavone



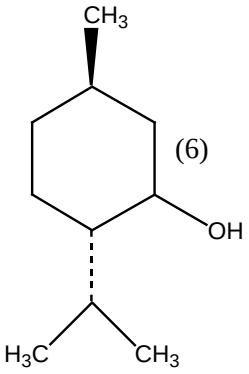
(3)

Catechin



(7)

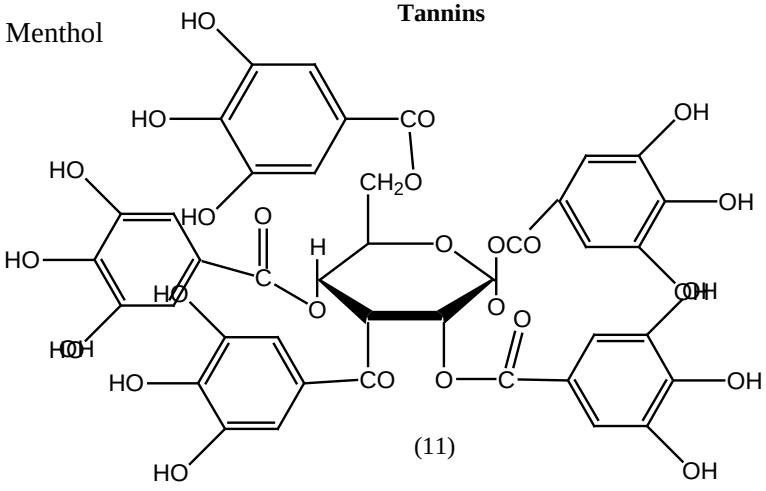
Terpenoids



(6)

Menthol

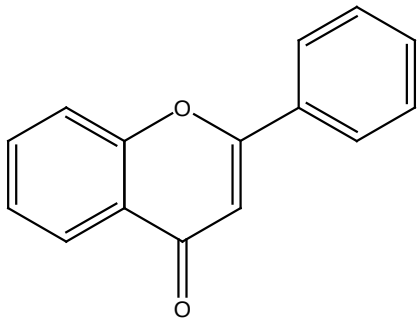
Tannins



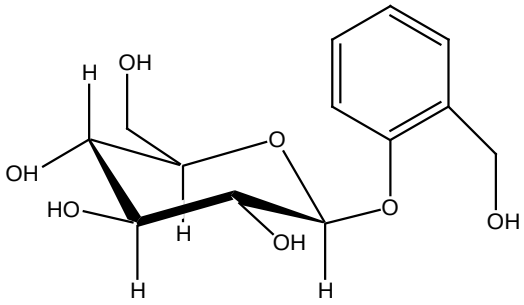
(11)

Pentagalloylglucose (hydrolyzable tannins)

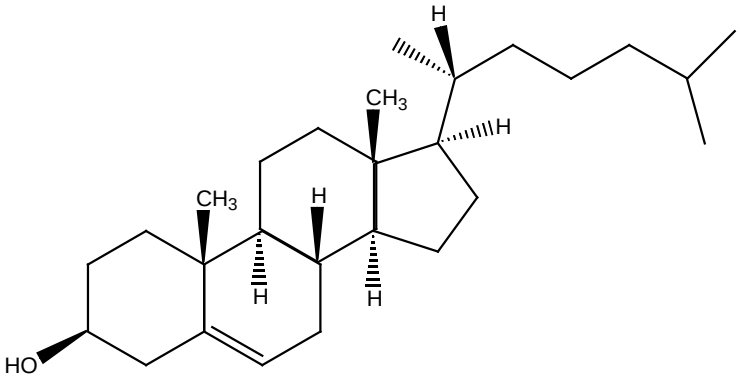
Fig. 1.0. Some types of natural products



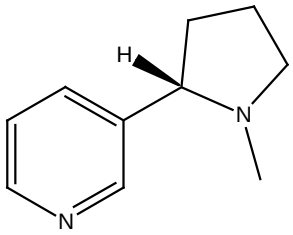
Flavone backbone(2-phenyl-1,4-benzopyrone)



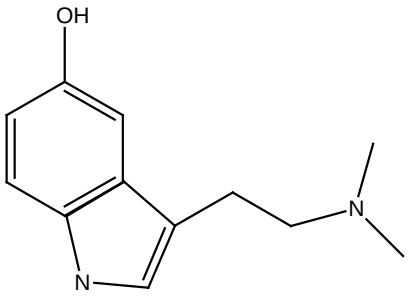
Salicin, a glycoside



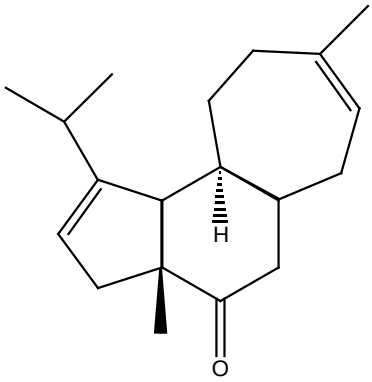
Cholesterol, a sterol



Nicotine, an alkaloid



Bufotenin



Cyanthiwigin F

Fig. 2.0. Some types of Natural Products Continued.

MATERIALS AND METHOD

Leaves of *Montricardia arborescens*, Fig. 3.0. were collected from a pond on the East Coast of Demerara, dried and ground using a grinding mill. Altogether, a total of 400grams of the ground material was obtained and subjected to selective extraction using solvents of increasing polarity: n-C_6H_{14}, CH_2Cl_2 and CH_3CH_2OH. Each solvent type extract was dried over anhydrous sodium sulphate, Na_2SO_4 and solvents removed in *vacuo* to yield extracts of varying hue, weight and yield as shown in Table 1.0. Thin layer chromatographic analyses were done on each extracts using selective eluents and the R_f values are quoted in Table 2.0.

Fig. 3.0. Leaves of *Montricardia arborescens.*

Phytochemical screening for secondary metabolites: On each solvent type extract, test for alkaloids, Saponin glycosides, Cardenolides, Bufadionolides, Flavonoids, Tannins, Polyphenolic compounds, Anthraquinones, Cyanogenic glycosides, Carbohydrates, Fixed oils, Fats, and Volatile oils were carried out using standard Phytochemical methods[4].

Cholesterol: To 2 ml of the extract, 2ml of $CHCl_3$ was added in a dry test tube. This was followed with the addition of 10 drops of acetic anhydride and 2 to 3 drops of conc. H_2SO_4. It is anticipated for a positive test that a red-rose colour should be evident.

Cardiac Glycosides: 5ml of each extract was treated with 2ml of glacial acetic acid, containing one drop of ferric chloride solution. This was underlayered with 1ml of conc. H_2SO_4. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring, whereas the acetic acid layer, a greenish ring might form just gradually throughout the thin layers.

***Glycosides*:** A small amount of alcoholic extract was dissolved in 1ml of water and a few drops of aqueous sodium hydroxide solution were added. A yellow colour was taken to signify the presence of glycosides.

Tannins: About 0.5g of extract was dissolved in 5 to 10ml of distilled water and was filtered. A few drops of a 5% $FeCl_3$ solution were added to the filtrate. A blue, blueblack, green, or blue-green colour or a precipitate was taken as an indication of the presence of tannins

***Flavonoids*:** A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red colour was taken as an indication of the presence of flavonoids.

Sterols and triterpenes:

***Liebermann-Burchard Reaction*:** 10ml of the extract was placed in a test tube and evaporated to dryness on a water bath. The residue was dissolved in 1ml of acetic anhydride and 1ml of chloroform. The solution was then transferred to two clean dry test tubes; one served as the reference tube. 1-2ml of conc. H_2SO_4 was added to the other tube using a teat pipette. A violet ring was formed at the two liquids, with the supernatant becoming violet this indicates the presence of sterols and triterpenoids.

***Carotenoids:* (*Carr-Price Reaction*):** 10ml of the extract was added to a test tube and was evaporated to dryness on a water bath. This was followed with the addition of 2-3 drops of saturated $SbCl_3$ in $CHCl_3$ to the residue. A blue-green colour eventually changing to red indicates the presence of carotenoids.

***Flavone Aglycones: Shibata's Reaction or Cyanidin test*:** 3ml of the extract was evaporated to dryness in a water bath. The residue was then dissolved in 1-2ml of 50% CH_3CH_2OH while heating. A piece of magnesium ribbon and 4-5 drops of concentrated HCl were added. A red or orange colour indicates the presence of Flavone Aglycones.

Emodols (Anthracenoside and Anthracenoside Aglycone): Borntrager's Reaction:

1ml of 25% NH_3 was added to 3ml of the extract in a test tube. The mixture was then shaken well. A red colour indicates the presence of Emodols. To 2ml of the extract in a test tube, 1-2ml of 25% NH_3 was added while it was been shaken. A cherry-red colour indicates the presence of anthracenosides.

Test for Steroids: 2 ml of acetic anhydride was added to 0.5g of the ethanolic extract of each sample with 2ml of H_2SO_4. The colour change from violet to blue or green, indicative of the presence of steroids

Test for Coumarin and derivatives: 5ml of ether extract was evaporated to dryness. The residue was dissolved in 1-2ml of water by heating. It was then divided in two equal portions. To the non-reference tube, 0.5ml of 10% NH_3 was added and then the tubes were viewed under UV light. The presence of blue-green fluorescence indicated the presence of coumarin. 4-5 drops of hydroxylamine hydrochloride solution and alcoholic KOH were added to the non-reference test tube until the pH was approximately 8-9. The resulting solution was evaporated to dryness. The residue was dissolved and the pH was adjusted to 3-4 by adding 10% HCl add 1-2 drops 3% $FeCl_3$ while being observed. A fast disappearing violet colour indicates the presence of coumarin derivatives.

Alkaloids: 10ml of the extract was evaporated to dryness. The residue was then dissolved in 1.5ml of 2% HCl. The solution was divided into two equal portions, one served as a reference. 2-

3 drops of Mayer's reagent was added to the non-reference tube. The development of opalescence or a yellowish white precipitate indicates the presence of alkaloids.

Mayer's reagent: 1.35g of mercuric chloride was dissolved in 60 ml of water, 5g of potassium iodide was added to 10ml of water and diluted to 100 ml. 0.85g of basic bismuth nitrate was dissolved in a mixture of 40 ml of water and 10 ml of acetic acid. 8g of potassium iodide dissolved in 20 ml of water and homogenize was added. The solution obtained was kept in a dark bottle for 2-3 months.

Phlobatinins:

Each plant extract was boiled with 1% aqueous HCl. A red precipitate is expected to be deposited and is taken as evidence for the presence of phlobatinins.

Proteins:

To 2 ml of plant extract, 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet colour indicates the presence of peptide linkage of the molecule.

Amino acids: To 2ml of the sample in a test tube, 2ml of Ninhydrin reagent was added. The test tube was placed in the water bath for 20 minutes.

Non-Hydrolysed CH_3CH_2OH extract

Tannins: 1-2 ml of water was added to 0.5-1.0ml of the extract and then 2-3 drops diluted (3% $FeCl_3$) solution was added to the mixture. A blackish-blue colour indicated the presence of Gallic tannins, while a greenish-black colour indicates the presence of catechol tannins.

Reducing compounds: 1-2ml of water was added to 0.5ml of the extract then 0.5-1.0ml of Fehling solution (1 and 2) were added. The mixture was then heated in a water bath. A brick red precipitate indicates the presence of reducing compound.

Alkaloids salt: 20 ml of the extract was evaporated to dryness and 5-10ml of 10% HCl was added to the residue. 10% NH_3 was then added until pH of 8-9 was achieved. The solution was placed into a separatory funnel and extracted with a polar solvent. The resulting extract was evaporated to dryness. The residue was then dissolved in 1.5ml of 2% HCl and divided into two portions; one was a reference. 2-3 drops of Mayer's reagent was added to the non-reference tube. Development of opalescence or a yellow-white precipitate indicates the presence of alkaloid salts.

Hydrolysed CH_3CH_2OH extract: 2ml of 10% HCl was added to the 25ml extract to hydrolyze the alcohol extract. The solution was refluxed for 30 minutes and then allowed to cool. 10-12ml of ether was extracted three times and the extracts were combined. A spatula full of anhydrous Na_2SO_4, filter was added and left for the following test.

***Cardenolides (Cardiac Glycosides and Aglycones: The Kedee's test*:** 4ml of the ether extract was evaporated to dryness. The residue was dissolved in 1-2 ml methanol. 1-2 ml of alcoholic KOH was added to the mixture. 3-4 drops of 1% alcoholic 3, 5-dinitrobenzene was added and the solution was heated. A disappearing violet colour indicates the presence of Cardenolides.

***Saponins* (foam test):** 2ml of the ether extract was evaporated to dryness. The residue was dissolved in 1ml water and shaken vigorously. The presence of saponins was indicated by persistent foam (1cm in test tube).

Flavanosides: (Shibata's Reaction): 5ml of ether extract was evaporated to dryness. The residue was dissolved by heating in 1-2 ml of 50% methanol. Metallic magnesium was added to the mixture followed with 5-6 drops conc. HCl. The development of an orange colour indicates the presence of flavanones. (Flavanols gave a characteristic red colour).

RESULTS

Table 1.0. Solvent Type Extract's Percentage Yield

Type of Extract	Colour	Weight (g)	Percentage Yield
Hexane	Gummy Black	39.13	9.78
Dichloromethane	Black	10.01	2.5
Ethanol	Black	17.57	4.39

Table 2.0. R_f values for visible components of the various extracts after development with iodine

Type of Extract	R_f values of components in extract
Hexane, n-C_6H_{14}	0.93, 0.78, 0.56, 0.49, 0.41, 0.23, 0.15
Dichloromethane, CH_2Cl_2	0.97, 0.78, 0.32, 0.22, 0.12
Ethanol, CH_3CH_2OH	0.94, 0.78, 0.31, 0.03

Table 3.0: Phytochemically screened data for the n-C_6H_{14}, CH_2Cl_2 and CH_3CH_2OH Extract

Extract	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
n-C_6H_{14}	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
CH_2Cl_2	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	+	+	-
CH_3CH_2OH	+	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	-	+	+

A : Test for Steroids and Triterpenes; B : Test for Carotenoids; C : Test for Flavone Aglycone; D : Test for Emodols; E : Test for Coumarin; F: Test for Alkaloids; G : Test for Tannins; H : Test for Alkaloid salts; I : Test for Coumarin Derivatives; J : Test forAnthracenosides; K : Test for Steroid glycosides; L: Test for Cardenolides; M: Saponins; N: Flavanosides; O: Cholesterol; P: Flavanoids; Q: Amino acids; R: Phlobatinins; S: Proteins; T: cardiac glycosides; U: reducing compounds

DISCUSSION

Phytochemical screening of the stems of *Montricardia arborescens* were conducted using standard phytochemical screening diagnostic tests such as Liebermann-Burchard, Mayer's, Keller-Kiliani, Ammonia, Salkowski, Lieberman's Burchards, Braymer's, Kedde's tests, etc. These tests are colorimetric in nature. Alkaloids were detected using the Mayer's reagents, flavonoids using lead acetate, ferric chloride and sodium hydroxide tests. Tannins were investigated using ferric chloride, Braymer's test. Saponins were detected using the Frothing test. Cardiac glycosides using Kedee's, Liebermann-Burchard, Sakowski and Keller-Keliani's tests. Reducing compounds were detected using Fehling's reagent. Phytochemicals tested for were steroids and triterpenes, carotenoids, flavone aglycone, emodols, coumarin, alkaloids, tannins, alkaloid salts, coumarin derivatives, anthracenosides, steroid glycosides, cardenolides, saponins, flavanosides, cholesterol, flavanoids, amino acids, phlobatinins, proteins, cardiac glycosides and reducing compounds. All these natural products have a basic skeleton and can be functionalised. Some of these are shown in Fig. 1.0 and Fig.2.0. It's the functional group of these compounds that are responsible for the various diagmostic tests.

Phytochemical screening indicated that there are localization of natural products/phytochemicals in the leaves of *Montricardia arborescens*. For example, anthracenosides, steroids and triterpenes are present in the ethanol extract but not in the hexane extract. Likewise steroid glycosides are present in the CH_2Cl_2 extract but not in the n-C_6H_{14} extract. Carotenoids are present in the n-C_6H_{14} and CH_2Cl_2 extract but not in the CH_3CH_2OH extract. Cholesterol, flavanoids, saponins, cardenolides, coumarin, coumarin derivatives, alkaloids salts and emodols showed negative presence in all three solvent type extracts.

The number of phytochemicals present in the C_6H_{14}, CH_2Cl_2 and CH_3CH_2OH extract were four, seven and eight respectively. Those detected in the hexane extract were: carotenoids, alkaloids, tannins and cardiac glycosides. Those showing positive tests in the dichloromethane extract were steroids and triterpenes, carotenoids, tannins, steroid glycosides, amino acids, proteins and cardiac glycosides. The ethanol extract showed positive tests for the presence of steroids and triterpenes, flavone aglycones, alkaloids, tannins, anthracenosides, amino acids, cardiac glycosides and reducing compounds. Alltogether, ten different phytochemicals were detected. These include steroids and triterpenes, carotenoids, flavone aglycones, alkaloids, tannins, anthracenosides, flavanosides, amino acids, cardiac glycosides and reducing compounds. More phytochemicals were seen to be present in the ethanol extract. Some of these may not be discernible in the TLC profile, pending the nature of the staining reagent.

Thin Layer Chromatography, TLC analyses using various solvent system followed by staining with iodine reveals that the hexane, dichloromethane and ethanol extracts contains seven, five and four components respectively. Each spot is presumably due to a pure natural products or phytochemical. TLC analyses of the hexane extract revealed the presence of seven spots: 0.93, 0.78, 0.56, 0.49, 0.41, 0.23, 0.15. TLC analyses of the EtOAc extract revealed the presence of five components with the following R_f values: 0.1, 0.95, 0.8, 0.45, 0.075. TLC analyses of the crude ethanol extract reveal four components with R_f values: 0.94, 0.78, 0.31 and 0.03.

CONCLUSION

The hexane, dichloromethane and ethanol extract of *Montricardia arborescens* were phytochemically screened for natural products. These phytochemicals vary in type in different parts of the plants. The hexane extract revealed the presence of alkaloids, tannins, carotenoids and cardiac glycosides. The dichloromethane extracts showed positive test for the presence of steroids , triterpenes, carotenoids, tannins, amino acids, proteins and cardiac glycosides. The ethanol extract revealed the presence of flavone aglycones, amino acids, alkaloids, tannins, anthracenosides, cardiac glycosides and reducing compounds.

ACKNOWLEDGEMENTS

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