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Pharmacognostical evaluation and description of the leaf and root bark of *Citropsis articulata* Swingle & Kellerman

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ABSTRACT

Background: Citropsis articulata Swingle & Kellerman (Family Rutaceae) is a renowned African traditional medicinal plant for the management of erectile dysfunction. The root bark is more commonly used than the leaves. Little has been done to evaluate the pharmacognostical parameters of both parts.

Purpose: The aim of this study was to determine the pharmacognostical parameters of *C. articulata* leaves and root bark.

Methods: The plant parts were collected, washed, dried, and extracted by decoction and soxhlation. The World Health Organisation quality control methods for herbal materials were generally followed for the pharmacognostical evaluation of the root bark and leaf. This involved macroscopic and microscopic analysis, physicochemical analysis, hemolytic activity, and phytochemical analysis.

Results: Macroscopically, the distillate of the root bark showed a fluorescent blue colour under ultra violet light of wavelength 254 nm. Microscopic examination revealed the presence of cellulose, lignified, suberized and cuticular cell walls. Numerous aleurone grains were noted in the root bark. Phytochemical analysis showed the presence of reducing sugars, free amino acids, L-arginine, coumarin, glycosides, phenols, phytosterols, saponins, steroids, tannins, terpenoids, alkaloids and flavonoids. Thin layer and high performance liquid fingerprints were also developed. The *C. articulata* root bark decoction and Soxhlet extracts contained 12.35 ± 0.05, and 110.00 ± 0.03 while the leaf decoction and Soxhlet extracts contained 4.97 ± 0.03 and 39.65 ± 0.00 mg/g diosgenin equivalent saponins respectively. The L-arginine content for the dried root bark decoction and Soxhlet was 2681 ± 7.29, 1660 ± 2.99 μg/g, while that for the leaf decoction and Soxhlet extracts was 135.60 ± 8.28, and 130.20 ± 2.83 μg/g respectively.

Conclusion: This study distinguished the root bark and leaf of *Citropsis articulata* and offered vital information for the quality control and standardization of the plant materials.

Abbreviations: cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; HPLC, high performance liquid chromatography; TLC, Thin Layer Chromatography; UV, Ultraviolet; Vis, Visible; WHO, World Health Organisation.

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1. Introduction

Pharmacognostical evaluation refers to the physical, chemical and biological study of plant materials (Valliammai et al., 2020). It is important for analyzing medicinal plant samples and curbing adulteration. Adulteration of herbal medicines remains a big challenge to their safe and effective use (Sarvananda et al., 2019). This is worsened by the fact that some plant species share vernacular names (Upton et al., 2020). Two or more different plant species could possess varying efficacy and safety levels and should therefore be properly identified and appropriately used (Gakunga et al., 2014, Gakunga et al., 2013).

Citropsis articulata Swingle & Kellerman (family Rutaceae) is one of the popular medicinal plants used in Africa for the management of erectile dysfunction (Ajao et al., 2019; Owaba et al., 2021). It is also used for treating other conditions such as diarrhoea, fatigue, and anorexia especially among people living with acquired immune deficiency syndrome in Uganda (Anywar et al., 2020). In Southwestern Uganda, it is locally referred to as 'Omuboro' in Runyankore and Runyaruguru, and 'Katimboro' in Lutooro, Rukonjo and Luganda languages (Kamatenesi-Mugisha and Oryem-Origa, 2005; Anywar et al., 2020) literally meaning the male sexual reproductive organ/penis. Other plant species including *Mystroxylon aethiopica* (Thunb.) Loes. and *Kigelia africana* (Lam.) Benth., which are also locally used to treat erectile dysfunction are commonly referred to by the same vernacular name in Southwestern Uganda (Gakunga et al., 2014).

Citropsi articulata is commonly known as the African cherry orange, West African cherry orange or Ugandan cherry orange. It grows in the wild, typically in rainforests of tropical and subtropical Africa, as a small tree or shrub up to 30 ft in height (Wangalwa et al., 2021). Its glabrous leaves are about 7 – 35 cm long, with broadly winged rachises and about 3 – 5 leaflets with pointed apices. It has a short tap root system with tiny lateral fibrous roots. It possesses white flowers on a racemose inflorescence, which later bear succulent sub-globose punctate fruits of about 1 – 3 cm diameter, ripening from a green to a bright orange colour. The fruit contains about 3 – 4 seeds, enclosed in a husk, 10 –12 mm long, and 4 – 6.5 mm in diameter (Swingle, 1940).

Traditionally, the root bark of *C. articulata* may be chewed fresh or steeped in hot water or boiled and taken once daily for two to three days to treat impotence and other forms of erectile dysfunction (Tugume et al., 2016). Vudriko et al. (2014) found out that the leaves of this plant also possess tremendous aphrodisiac potential. Use of the root bark predisposes this plant to extinction as it is obtained by uprooting and killing the entire plant (Wangalwa et al., 2021). Using the leaves provides a viable alternative.

L-arginine and saponins are among the most important phytochemicals with aphrodisiac effect (Owaba et al., 2021). Lacroix et al. (2011) isolated coumarins such as rutarin, seselin, suberosin, demethylsuberosin, haploperoside, katimborine and omubioside, and specific alkaloids like hydroxynoracronycine, 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone, trigonelline, and limonoid 7α-obacunyl acetate from the root bark of *C. articulata*. A new orange colored acridone alkaloid Citropsine has also been isolated from the stem bark (Meva'a et al., 2010).

Whereas phytochemical screening has been done to assess some of the different groups of phytochemicals present in *C. articulata* leaf (Gakunga et al., 2014; Vudriko et al. 2014), the biological, physicochemical and biochemical properties of its leaf and root bark have not been comprehensively profiled. The purpose of this study was to evaluate and document the pharmacognostical properties of the leaf and root bark of *C. articulata*

2. Materials and methods

2.1. Plant collection and preparation

The root barks and leaves of *C. articulata* were collected from Kibale Conservation Area, Kanyanchu, Kamwenge District, Southwestern Uganda in July 2020, following permission of Uganda Wildlife Authority under reference COD/96/05. The area is located at Latitude – N 0° 26'8" Longitude – E 30° 23'8", and Altitude – 1252.7 m. A herbarium specimen was deposited at the Makerere University herbarium and given the accession number 50965. The plant name has been checked at https://wfoplantlist.org/plant-list with the identification code – wfo-0000607733.

Fresh root barks and leaves rinsed with running water and air dried for 7 and 11 days respectively at temperature of 22 – 24 °C and relative humidity of 60 – 65 % at Mbarara University Pharmaceutical Chemistry and Analysis Laboratory. Dried plant materials were then pulverized and sieved. Extraction of plant materials was done using decoction and soxhlation. About 500 g of each powdered plant material were placed in a stainless steel vessel and 4 L of distilled water added and boiled at 90 °C for 1 h. Also, 500 g of each powdered plant material were placed in a thimble and extracted with 2.5 L of 70 % ethanol in a Soxhlet extractor (QuickFit®, EX5/105 BS 2071, United Kingdom). The extracts were then filtered, concentrated using a rotary evaporator (IKA® RV 10 digital, Germany), and freeze-dried to obtain dry extracts. The dry extracts were stored in a fridge between 2 – 6 °C and relative humidity 50 – 70 % till needed.

2.2. Organoleptic analysis

2.2.1. Macroscopic analysis

Macroscopic analysis was done through the organoleptic study of the fresh plant materials, dry cut plant materials, powdered plant materials, and their distillates following the WHO recommended procedures (WHO, 2011). The distillates were obtained after boiling 25 g of material in 300 ml of distilled water in a Dean-Stark apparatus until 100 ml were collected. Fluorescence analysis was determined in day light, 254 nm and 365 nm wavelength using a UV lamp for the powdered plant materials and their distillates (Kunle et al., 2012).

2.2.2. Microscopic analysis

Microscopic analysis of fresh and dry powdered plant materials was done following the WHO quality control methods for herbal materials (WHO, 2011). The surface (epidermis) characteristics of the leaf and its stomatal index were also determined. This analysis was done using a light microscope (Olympus® CX21FS1, Japan) with an external microscope digital camera system (AmScope© Version 3.7.10246.20171109, United Kingdom). Histochemical detection of cell walls and contents was also done by applying different reagents to sections of fresh and powdered material (WHO, 2011).

2.3. Physicochemical analysis

Physicochemical parameters such as ash value (total, acid-insoluble and water-soluble ash), extractable matter, swelling index, foaming index, and water and volatile matter were also determined following the WHO quality control methods for herbal materials (WHO, 2011).

2.4. Determination of haemolytic activity

The haemolytic activity was determined using the WHO recommended procedures for quality control methods for herbal materials WHO (2011). About 4.5 ml of fresh blood was obtained from a five-month-old female Wistar albino rat weighing 156. This was done through cardiac puncture following anaesthesia with halothane. As a reference material, the saponin fraction of *Trigonella foenum-graecum* seeds was prepared and used. Studying the haemolytic activity provides a proxy for estimating the relative amount of saponins in the extract. Saponins are known to cause haemolysis of erythrocytes (WHO, 2011; Soltani et al., 2014).

2.5. Phytochemical analysis

2.5.1. Preliminary phytochemical screening

Standard phytochemical tests for primary and secondary metabolites were done following methods earlier described (Evans, 2009; Kumar et al., 2013; Kalita and Sarma, 2014; Vishnu et al., 2019). In a nutshell, for the different phytochemicals, the different tests were done: Benedict's test for reducing sugars, iodine test for starch, translucent spot test for lipids, Biuret test for proteins, ninhydrin test for amino acids, Sakaguchi test for L-arginine, Wagner's test for alkaloids, sodium hydroxide test for coumarins, glacial acetate-ferric chloride test for glycosides, lead acetate test for flavonoids, phloroglucinol-hydrochloric acid test for lignin, lead acetate test for phenols, Liebermann-Burchard test for phytosterols, foaming test for saponins, Salkowski test for steroids, ferric chloride test for tannins, and Salkowski test for terpenoids.

Crude or pure reference material with known presence of the different constituents was used as a positive control while the untreated extract solution or a reaction intermediate was employed as a negative control (WHO, 2011).

2.5.2. Thin layer chromatography (TLC) fingerprinting

The TLC was performed to provide a fingerprint for each plant extract and determine the presence of phytoconstituents especially saponins and L-arginine using different chromatographic conditions (WHO, 2011). Using a couple of mobile phase systems i.e., n-butanol: acetic acid: water (5:3:2) containing 0.4 % ninhydrin (Qiu et al., 2010) and n-butanol: acetic acid: water (12:1:2), chromatograms were developed on precoated silica gel 60 F_{254} aluminium plates. Visualization of the chromatograms was also done by spraying the plates separately with vanillin-sulphuric acid and Liebermann-Burchard reagents (Soltani et al., 2014). The untreated and treated chromatograms were further viewed under UV light at wavelengths of 254 nm and 365 nm. The retardation factor (R_f) values for the different spots were then determined.

2.5.3. High performance liquid chromatography (HPLC) fingerprinting

This analysis was done at Mbarara University Pharmaceutical Chemistry and Analysis Laboratory. All plant extracts were prepared in a similar process by dissolving 10 mg of each plant extract in 10 ml of distilled water, except the Soxhlet extract of *C. articulata* bark (distilled water: methanol – 1:1, due to poor water solubility) to form a concentration of about 1 mg/ml. The solutions were then filtered through 0.45-μ filters and about 0.5 ml loaded into HPLC sample containers. In addition, distillates of each plant material were prepared as earlier described in Section 2.2.1 (macroscopic analysis).

Isocratic reverse phase elution was used to develop the chromatograms using a Shimadzu Model HPLC system (Shimadzu, Tokyo, Japan) consisting of LC-20AD pump, SPD-20A UV/Vis detector, CBM-20A system controller, SIL-20AC HT Autosampler, CTO-20AC Column Oven, and DGU-20A5R degasser. The mobile phase system of methanol: acetonitrile: water/0.01 % trifluoroacetic acid (6:1:3) was used. Ten μl of each prepared sample were injected, and eluted at a flow rate of 1 ml/minute for 20 min, through a C_{18} Column (250 × 4.6 mm × 5 μm) at a temperature of 35 °C. Detection was done at 254 nm.

2.5.4. Determination of total saponin content

Ten milligrams of each plant sample were dissolved in 10 ml of distilled water except the decoction extract of *C. articulata* bark (distilled water: methanol – 1:1) to form a concentration of about 1 mg/ml. Reference standard diosgenin (min 99.9 % w/w, Toronto Research Chemicals, Canada) was prepared by dissolving 10 mg in 16 ml of methanol, followed by the addition of 4 ml of water (Jain and Shrivastava, 2017).

Following the vanillin-sulphuric acid method by Jain and Shrivastava (2017), serial dilutions of standard diosgenin were prepared at concentrations of 100, 200, 500, 700 and 800 μg/ml, and used to generate a standard calibration curve of absorbance against concentration using a UV-Visible Spectrophotometer (Jenway® 6705, United Kingdom) at 544 nm. The absorbance of extract samples was determined five times, and the individual values used to calculate the concentration of total saponins in μg/ml according to the formula derived from the linear regression equation. The average total quantity of saponins was expressed as mg/g diosgenin equivalent.

2.5.5. Determination of L-arginine content

A Shimadzu model HPLC system (Shimadzu, Tokyo, Japan) consisting of LC-20AD pump, SPD-20A UV/Vis detector, CBM-20A system controller, SIL-20AC HT Autosampler, CTO-20AC Column Oven, and DGU-20A5R degasser was used for this determination. All plant extracts including standard L-arginine (min 99.0 % w/w, Loba Chemie, India) were prepared similarly by dissolving 10 mg of each sample in 10 ml of distilled water except the decoction extract of *C. articulata* bark (distilled water: methanol – 1:1) to form a concentration of about 1 mg/ml. The solutions were then filtered through 0.45-micron filters and about 0.5 ml loaded into the HPLC sample containers.

An isocratic reverse phase elution (mobile phase: 0.1 % phosphoric acid, flow rate: 0.5 ml, elution time: 10 min, temperature: 35 °C, detection wavelength: 195 nm) was used. Serial dilutions i.e., 1, 10, 20, 40 and 100 μg/ml of standard L-arginine were run in triplicate to develop a linear calibration curve of area-under-the-curve against concentration. The linear regression equation was then used to calculate the different concentrations of L-arginine in the extracts. The average content of L-arginine was expressed as μg/g of air-dried plant material.

2.6. Data analysis

The quantitative data especially the L-arginine content, Saponin content, was first expressed as mean ± SEM ($n \geq 3$), following descriptive statistics using GraphPad Prism Version 8.0.2.263. It was then presented in form of tables or figures.

3. Results

3.1. Organoleptic analysis

3.1.1. Macroscopic analysis

The decoction method yielded 27.01 – 33.47 % of the dry extracts for root bark and 14.50 – 19.27 % for the leaf while Soxhlation yielded 23.13 – 27.26 % of the dry extracts for root bark and 18.53 – 21.58 % for the leaf. The macroscopic features of the fresh, dry cut, and powdered plant materials, and their distillates are shown in Table 1 and Fig. 1. A unique light blue fluorescence (under UV 254 nm) was noted and reported for the first time for *C. articulata* root bark distillate.

3.1.2. Microscopic analysis

The plant specimens were rich in aleurone grains, cellulose and lignified walled cells as shown in Table 2 and Fig. 2. Powder microscopy revealed presence of cork cells, lignified tissues, aleurone and starch grains in the root bark (Fig. 3). The leaf did not possess any trichomes, but contained needle shaped crystals. The stomatal index of *C. articulata* leaf was determined as 10.71 ± 0.89 for the abaxial and 2.89 ± 0.67 for the adaxial surface. The adaxial surface was noted to possess anomocytic (irregular celled) stomata while the abaxial surface has anisocytic (unequal celled) stomata.

3.2. Physicochemical analysis

While the foaming index was the same for both parts, the swelling index in ml of the leaf (8.67 ± 0.44) and root bark (3.33 ± 0.08) differed. Other parameters like ash value, extractable matter and water/volatile matter are also stated in the Table 3.

Table 1
Macroscopic features of *C. articulata* root bark and leaf.

Nature	Fresh	Dry cut material	Dry Powder	Distillate
C. articulata Root Bark				
1. Colour (day light)	OS (light brown), IS (cream)	Brownish cream	Yellowish brown	Colourless
2. Fluorescence (UV 254 nm)	NA	NA	Pale brown	Light blue
3. Fluorescence (UV 365 nm)	NA	NA	Pale brown	Pale green
4. Odour	Weakly aromatic	Distinctly musty	Distinctly musty	Very distinctly musty
5. Taste	Bitter/ pungent	Bitter/ pungent/ astringent	Bitter/ pungent/ astringent	Bitter/ spicy
6. Shape	Irregular shredded pieces	Irregular shredded pieces	Spherical/ irregular particles	NA
7. Size (length in mm)	29.80 ± 1.85	10.60 ± 1.29	Moderately coarse	NA
8. Size (width in mm)	11.20 ± 0.61	7.70 ± 0.63	Moderately coarse	NA
9. Size (thickness in mm)	2.70 ± 0.30	2.30 ± 0.30	Moderately coarse	NA
10. Texture	Hard	Very hard	Soft	NA
11. Surface characteristics	Rough	Slightly rough	Slightly rough	NA
12. Fracturing characteristics	Brittle	Brittle	NA	NA
13. Fracture plane characteristics	Rough	Slightly fibrous	NA	NA
14. Appearance of the cut surface	Slightly rough	Slightly rough	NA	NA
C. articulata Leaf				
1. Colour (daylight)	LS (light green) US (dark green)	Light green	Green	Colourless
2. Fluorescence (UV 254 nm)	NA	NA	Dark green	Brown
3. Fluorescence (UV 365 nm)	NA	NA	Pale brown	Light brown
4. Odour	Distinctly aromatic	Weakly aromatic	Weakly aromatic	Very weakly aromatic
5. Taste	Slightly bitter/ pungent	Slightly bitter	Slightly bitter	Very slightly bitter
6. Shape	Pointed apex/ ovate/ lanceolate	Ovate/ curled/ irregular pieces	Needle-like/ irregular particles	NA
7. Size (length in mm)	W (212.80 ±6.29), L (93.70 ±6.46)	42.60 ± 5.18	Moderately coarse	NA
8. Size (width in mm)	W (183.10 ±9.57), L (41.20 ±2.74)	12.40 ± 2.00	Moderately coarse	NA
9. Size (thickness in mm)	0.26 ± 0.11	0.16 ± 0.02	Moderately coarse	NA
10. Texture	Soft	Hard	Soft	NA
11. Surface characteristics	Smooth	Slightly rough	Smooth	NA
12. Fracturing characteristics	Flexible	Moderately brittle	NA	NA
13. Fracture plane characteristics	Rough	Rough	NA	NA
14. Appearance of the cut surface	Slightly rough	Slightly rough	NA	NA

OS – Outer surface, IS – Inner surface, NA – Not Applicable, UV – Ultra violet, LS – Lower surface, US – Upper surface, W – whole leaf, L – Leaflet

3.3. Hemolytic activity

The hemolytic activity of extracts of the root bark and leaf was 5.42 ± 0.09 and 5.84 ± 0.27 for the root bark decoction and Soxhlet extracts, and 5.64 ± 0.31 and 500 ± 0.00 for the leaf decoction and Soxhlet extracts respectively. The reference standard material was accorded a hemolytic activity of 1000.

3.4. Phytochemical analysis

3.4.1. Preliminary phytochemical screening

All plant extracts contained various amounts of the different primary and secondary metabolites except proteins, lignin and lipids as shown in the Table 4. The color differences and observations for both reference and test samples are also shown to provide more qualitative deductions.

3.4.2. Thin layer chromatography (TLC) fingerprinting

The different chromatograms developed are shown in Fig. 4. The reference substance L-arginine had a retardation factor of 0.06 – 0.07. The crude saponin fraction of *Trigonella foenum-graecum* seed extract (saponins reference) revealed some saponins that had overlapping R_f values with the extracts. Pure saponins standards could provide more accurate evaluations. The chromatograms show the presence of L-arginine and 7 other amino acids in both the root bark and leaf. *C. articulata* root bark developed diagnostic distinct fluorescent blue colored compounds at retardation factors 0.90 – 0.93 and 0.41 – 0.43, particularly when using the solvent system – n-butanol: acetic acid: water (12:1:2), and viewed under a UV lamp. This solvent system, unlike the other could easily be used to distinguish extracts of the root bark and those of the leaf.

3.4.3. High performance liquid chromatography (HPLC) fingerprinting

Unique HPLC fingerprints for the distillates (Fig. 5) and extracts (Fig. 6) were also developed. Generally, the decoction and Soxhlet extracts produced similar chromatograms, although peaks were more visible and higher or more in number in the latter group. More unique characteristics could be seen in the distillate chromatograms such as the distinct peaks at retention time (in minutes) of 5.582 (root bark) and 10.743 (leaf).

3.4.3. Determination of total saponin content

The total saponin content for *C. articulata* root bark decoction and Soxhlet extracts was 12.35 ± 0.05 and 110.00 ± 0.03 mg/g diosgenin equivalents respectively. The leaf decoction and Soxhlet extracts contained 4.97 ± 0.03, and 39.65 ± 0.00 mg/g diosgenin equivalents respectively. This followed estimation using a diosgenin standard calibration curve with the equation $y = 0.0014x + 0.1184$ and coefficient of determination, $R^2 = 0.9653$.

3.4.4. Determination of L-arginine content

A linear regression equation $y = 26835.38x - 7702.42$ and coefficient of determination $R^2 = 0.9999635$ was obtained from the standard calibration curve of L-arginine. The total amount of L-arginine in the plant samples basing on the different extracts was determined as 2681 ± 7.29, 1660 ± 2.99, 135.60 ± 8.28, and 130.20 ± 2.83 μg/g airdried material for the root bark decoction, root bark Soxhlet, leaf decoction and leaf Soxhlet extracts respectively.

4. Discussion

Citropsis articulata is one of the promising plants for treating erectile dysfunction in Uganda but is highly prone to adulteration. Determining its pharmacognostical parameters provides an avenue for comparison

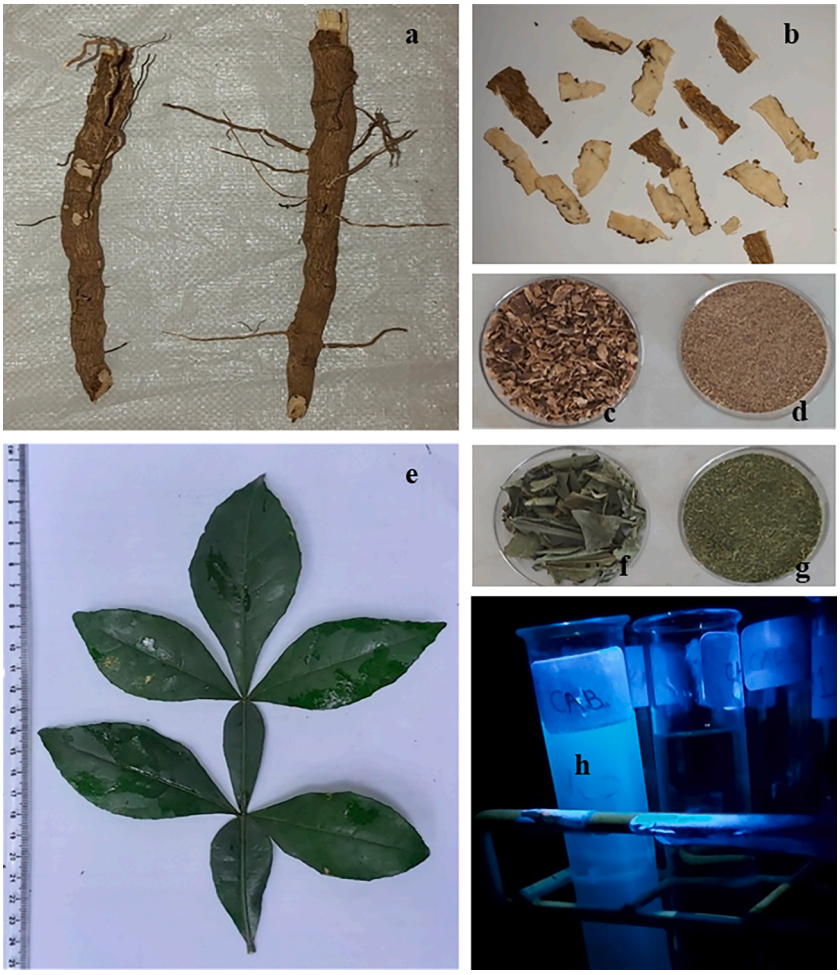


Fig. 1. Macroscopic features of *C. articulata.*.

Table 2
Histochemical detection of cell walls and contents.

Parameter	Root bark		Leaf	
	T/LS	PWD	T/LS	PWD
1. Cellulose cell walls	+++	+++	++	+++
2. Lignified cell walls	++	+++	++	++
3. Suberized/cuticular cell walls	+	+++	+	+
4. Aleurone grains	+++	+++	++	++
5. Calcium carbonate	-	-	-	-
6. Calcium oxalate	+	+	++	++
7. Fats, and fatty oils,	++	+++	+	+
8. Volatile oils and resins	+++	++	-	-
9. Hydroxyanthraquinones	-	-	-	-
10. Inulin	-	+	-	+
11. Mucilage	NA	-	NA	-
12. Starch	+++	+++	-	-
13. Tannin	-	+	+	++

T/LS – Transverse or longitudinal section of fresh plant material; PWD – dry powdered plant material; NA – Not applicable; + mild presence, ++ moderate presence, +++ strong presence, - absent

and contributes towards standardizing its use in herbal medicine (WHO, 2011; Balekundri and Mannur, 2020). One of the first approaches to this is through macroscopic analysis. This current study evaluated the macroscopic and microscopic parameters of the root bark and leaf of *C. articulata*. Macroscopically, the root bark could be distinguished from the leaf or other plant materials using ultra violet detection of the distillate (light blue fluorescence).

Macroscopic analysis alone is often not sufficient for identification and authentication of plant materials. It is usually necessary to perform analysis of samples (fresh and dry powder materials) under a microscope. In this study, microscopic investigation of the root bark and leaf showed the presence of cellulose, lignified, suberized and cuticular cell walls. These enable protection of cellular contents from degradation due to water loss and adverse conditions (Rongpipi et al., 2019; Harman-Ware et al., 2021). The peculiarity of *C. articulata* root bark is due to the presence of numerous aleurone grains, which may explain why it has a lot of amino acids like L-arginine. Aleurone grains are protein-containing cellular inclusions (Antonini et al., 2018). Like most leaves, the stomatal index of the adaxial surface of the leaf of *C. articulata* was lower than the abaxial surface in order to control the rate of transpiration and gaseous exchange (Drake et al., 2019). The generally low values of stomatal index correspond to its known habitat of secondary rainforest which allows only little light penetration for the ground vegetative growth (Ichie et al., 2016).

Furthermore, physicochemical tests were carried out. The water and volatile matter content were not more than 12 % as recommended by the World Health Organisation. Excessive moisture promotes fungal growth and hydrolytic breakdown of phytochemicals (WHO, 2017). *Citropsis articulata* leaf had low amounts of extractable matter (43.70 ± 0.35 mg/g and 36.21 ± 0.44 mg/g) for hot aqueous and cold ethanolic extraction respectively. Generally, hot aqueous extraction was a better extraction method, compared to ethanolic extraction, when extractable matter is considered as the basis for comparison. This could probably explain why herbalists prefer decoction/hot extraction to other extraction methods (Tugume et al., 2016). Extractable matter represents the

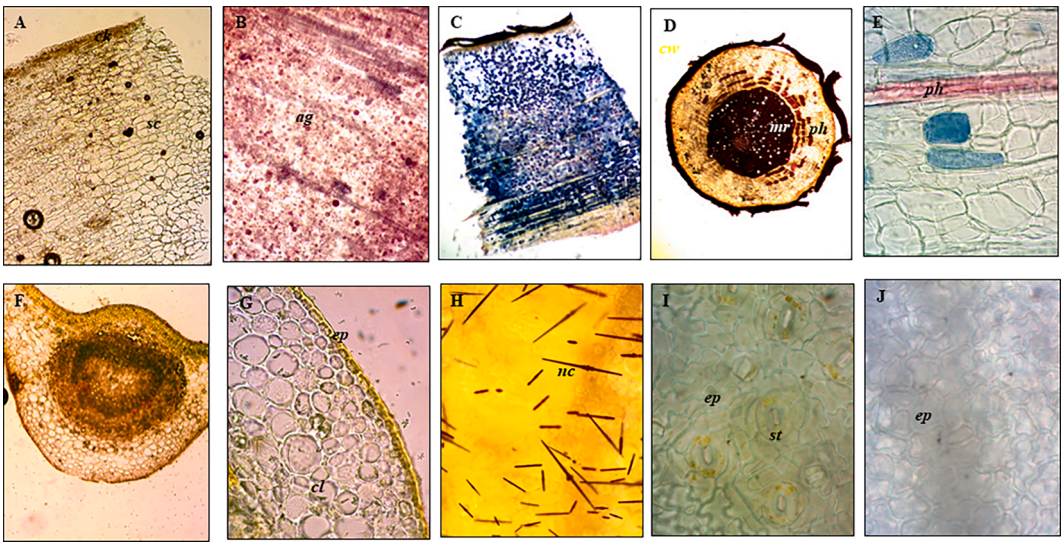


Fig. 2. Microscopic features of *C. articulata* root bark and leaf.

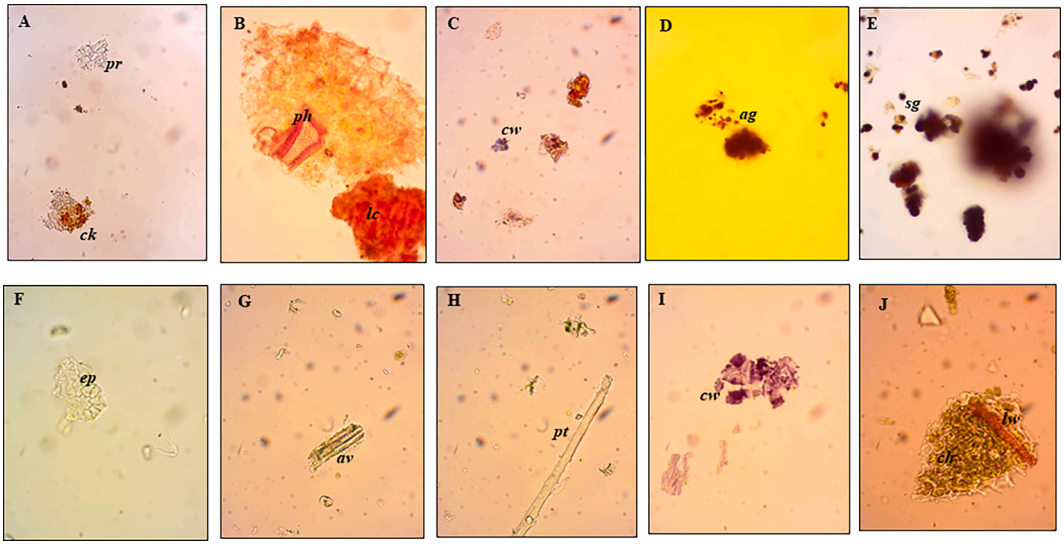


Fig 3. Microscopic features of powdered *C. articulata* root bark and leaf.

Table 3
Physicochemical parameters of *C. articulata* root bark and leaf.

Parameter	Root bark	Leaf	Units
1. Water and volatile matter			
Loss on drying method	9.19 ± 0.16	8.96 ± 0.19	%
Azeotropic method	8.0 ± 0.31	9.20 ± 0.20	%
2. Extractable matter			
Hot extraction (water)	61.53 ± 0.23	43.70 ± 0.35	mg/g
Cold maceration (70 % ethanol)	44.89 ± 1.70	36.21 ± 0.44	mg/g
3. Ash			
Total ash	90.56 ± 1.93	127.90 ± 1.51	mg/g
Acid insoluble ash	6.17 ± 1.45	4.02 ± 0.35	mg/g
Water soluble ash	8.96 ± 0.22	27.02 ± 3.00	mg/g
4. Swelling index	3.33 ± 0.08	8.67 ± 0.44	ml
5. Foaming index	444.4 ± 55.56	444.4 ± 55.56	

relative amount of total phytochemicals available. The high ash values obtained may signify presence of foreign matter including silt, sand, inorganic salts, and organic matter. This can assist in detection of adulterants and contaminants (Chanda, 2014). *Citropsis articulata* leaf had a high swelling index (8.67 ± 0.44 ml) probably due to appreciable amounts of gums, pectin, hemicellulose and other carbohydrate matter (WHO, 2011). The high foaming index in both the root bark and leaf show the presence of saponins (Góral and Wojciechowski, 2020). This was further indicated by the varying haemolytic activity, with the highest value of 500.00 in the leaf Soxhlet ethanolic extract. The Soxhlet ethanolic extracts generally had higher haemolytic activity compared to their aqueous decoction counterparts due to better dissolution of saponins in ethanol compared to water and the exhaustive extraction by soxhlation (Cheok et al., 2014).

Primary metabolites are those directly required and essential for the normal growth, development and reproduction of an organism. They typically include proteins (and their building units i.e., amino acids), carbohydrates (sugars, starch, etc.), lipids (some fats and oils) and nucleic acids. Secondary metabolites are those involved in plant-environment interactions and include phytochemicals like alkaloids, coumarins, saponins, etc. (Erb and Kliebenstein, 2020). Both primary and secondary metabolites have medicinal value with the former having mainly nutritive values (Panchawat and Ameta, 2021). The phytochemical groups detected included reducing sugars, free amino acids, L-arginine, coumarin, glycosides, phenols, phytosterols, saponins,

Table 4
Qualitative phytochemical composition of the plant extracts.

Test	Inference	CABD	CABS	CALD	CALS
1. Reducing Sugars	+++	+	++	++	+++
Glucose	*Reddish-brown ppt*	Green ppt	Orange ppt	Brown ppt	Red ppt
2. Starch	+++	+++	-	-	-
Wheat starch	*Bluish black ppt*	Bluish black ppt	None	None	None
3. Lipids	+++	-	-	-	-
Sunflower seed oil	*Translucent spot*	None	None	None	None
4. Proteins (Biuret Test)	+++	-	-	-	-
Milk	*Purple*	Green	Green	Deep green	Green
5. Amino Acids	+++	+++	+++	++	++
L-arginine	*Dark purple*	Deep blue	Deep blue	Blue	Blue
6. L-Arginine	+++	++	++	+++	++
L-arginine	*Deep red*	Red	Red	Dark red	Red
7. Alkaloids (Wagner's Test)	+++	±	+++	-	++
Quinine HCL	*Reddish brown ppt*	Black ppt	Reddish brown ppt	None	Brown ppt
8. Coumarin	+++	+++	+++	+++	+++
Warfarin	*Yellow*	Yellow	Yellow	Yellow	Yellow
9. Glycosides	+++	+++	+++	+++	+++
Digoxin	*Deep blue*	Deep blue	Deep blue	Deep blue	Deep blue
10. Flavonoids	+++	+++	+++	+	+++
Quercetin	*Yellow ppt*	Yellow ppt	Yellow ppt	Cream ppt	Yellowish green ppt
11. Lignin	+++	-	-	-	-
Bark powder	*Red*	None	None	None	None
12. Phenols	+++	++	++	+++	+++
Phenol	*Bulky white ppt*	White ppt	White ppt	Bulky white ppt	Bulky white ppt
13. Phytosterols	+++	+++	+++	++	++
Beta-sitosterol	*Golden yellow*	Golden yellow	Golden yellow	Deep green	Deep green
14. Saponins	+++	+	+++	++	+++
Crude saponin fraction	*10.0 mm*	0.5 mm foam	11.0 mm foam	1.0 mm foam	7.0 mm foam
15. Steroids	+++	++	+++	++	++
Cholesterol	*Reddish brown ring*	Brown ring	Reddish brown	Brown ring	Brown ring
16. Tannins	+++	+	+	++	++
Tea leaf infusion	*Dark green ppt*	Brown ppt	Brown ppt	Green ppt	Green ppt
17. Terpenoids	+++	++	++	++	++
Artemisia annua infusion	*Reddish brown ring*	Brown ring	Brown ring	Brown ring	Brown ring

Citropsis articulata root bark decoction (CABD) and Soxhlet (CABS), leaf decoction (CALD) and Soxhlet (CALS) extracts. +++ (high), ++ (medium), + (low), ± (undefined), - (absent), ppt – precipitate. Reference materials (italicized) are placed below the name of the test and their observations recorded and noted as the inference. "None" meant that no significant observable change was noted

steroids, tannins, terpenoids, alkaloids and flavonoids. This confirms that the phytochemicals that contribute to aphrodisiac efficacy of *C. articulata* were present (Sharma et al., 2014). The extracts did not contain lipids, lignin, proteins and starch (except the root bark decoction extract). This is in tandem with previous studies by Gakunga et al. (2014) and Vudriko et al. (2014).

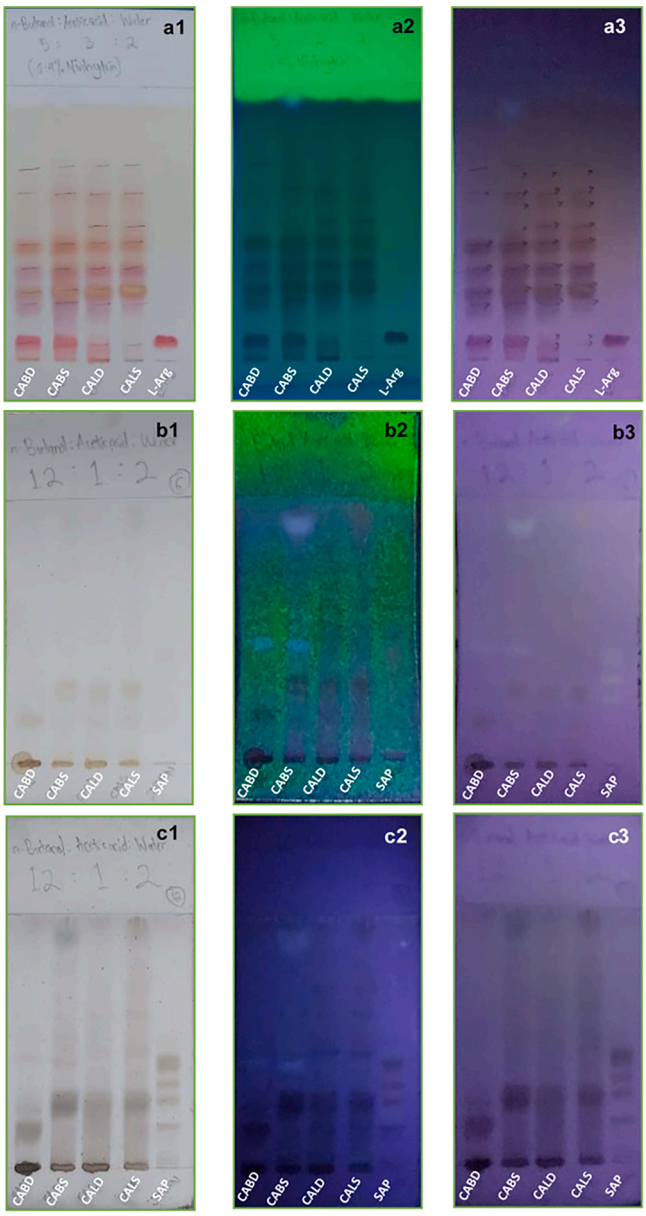


Fig. 4. TLC fingerprints of the plant extracts.

The study developed TLC fingerprints to be used in the quality control of study materials. The Soxhlet extracts of the plants generally showed more distinct components that were separated, hence soxhlation may be a preferable method for TLC analysis of *C. articulata.* The root bark extracts showed distinct fluorescent blue-coloured compounds at retardation factors 0.90 – 0.93 and 0.41 – 0.43, particularly when using the solvent system – n-butanol: acetic acid: water (12:1:2), and viewed under a UV lamp. The amino acid chromatograms were more or less similar but differed in colour intensity of the extracts, especially L-arginine at retardation factor of 0.06 – 0.07 (Qiu et al., 2010). The TLC fingerprints can be used to not only detect presence of phytoconstituents like L-arginine but also give a unique identifier for the plant extract. Nonetheless, there are limitations in derivatization/visualization of developed chromatograms. For instance, ninhydrin can be used to detect amino acids but may cross react with other primary amines. Similarly, the Liebermann-Burchard reagent can be used for visualization of not only steroidal saponins but also other steroidal compounds like phytosterols, some alkaloids, and other phenolic compounds. The HPLC fingerprints developed in the study could serve as important guides for

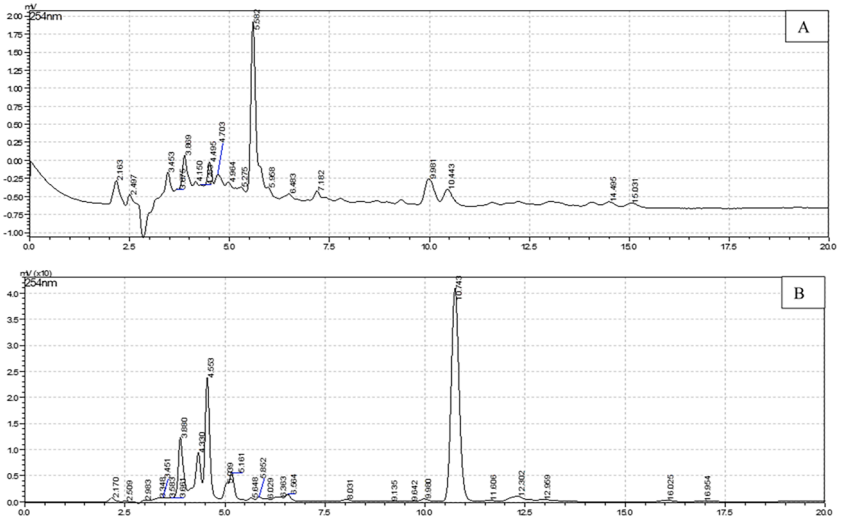


Fig. 5. Chromatograms of the root bark (A) and leaf (B) distillates.

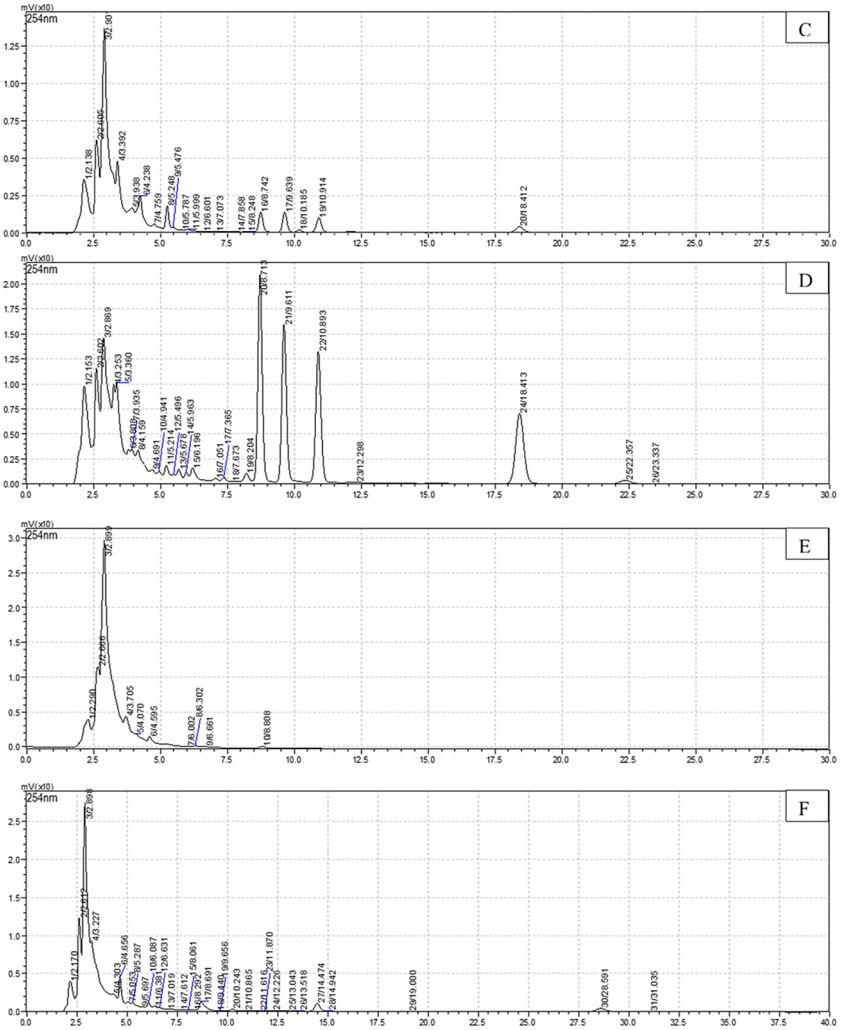


Fig. 6. Chromatograms of root bark decoction (C) and Soxhlet (D) and leaf decoction (E) and Soxhlet (F) extracts.

authentication and identification of the plants particularly using distillates which had clearly resolved fingerprints and distinct peaks (Bārzdiņa et al., 2022). The extract chromatograms could also have better resolution if purification were done. We recommend this for further research studies.

This current determination showed that all plant extracts contained L-arginine, whose role in enhancing sexual function in males has been earlier described (Palmer et al., 1988). The root bark decoction extract

had the highest (2681 ± 7.29 μg/g) levels of L-arginine. This could be explained by its very higher polarity (Ridwan et al., 2018). L-arginine is a known precursor of nitric oxide. Nitric oxide produced activates guanylate cyclase, an enzyme which leads to increase in levels of cyclic guanyl monophosphate (cGMP). The cGMP, through a cascade of phosphorylative action of protein kinases, causes smooth muscle relaxation, responsible for erection (Yafi et al., 2016). These findings are similar to those of Oloro et al., (2016) who established the presence of L-arginine in four medicinal plant species used as aphrodisiacs in Uganda.

Using diosgenin as the reference standard, the total saponin content of saponins of the plant materials in relation to the extraction methods/type of extracts were obtained. The results suggested that the different plant materials contained saponins but at different quantities in the extracts, with the highest (110 ± 0.03 mg/g) values for the root bark Soxhlet extract. Saponins have many pharmaceutical and therapeutic uses including treatment of erectile dysfunction (Gakunga et al., 2014). Saponins improve erectile function by increasing the: 1) expression of endothelial nitric oxide synthase, 2) levels of nitric oxide and 3) levels of cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) and 4) endothelial function in the penile corpus cavernosum (Lin and Gou, 2013). Generally, it was noted that Soxhlet extracts had higher values than the decoctions. This is probably because of the principle of operation of the Soxhlet extraction method (De Castro and Priego-Capote, 2010), which allows exhaustive extraction of phytochemicals including those with relatively low solubility in the extraction solvent. In addition, ethanol extracts more saponins than water (Cheok et al., 2014).

Detecting and quantifying saponins (as diosgenin-equivalents) and L-arginine act as analytical and/or bioactive markers that can be used in the quality control of *Citropsis articulata*. This provides a tool for authentication and standardisation of the plant materials (Kaggwa et al., 2023).

5. Conclusion

The current study determined the pharmacognostical parameters of *C. articulata* root bark and leaf, which is important in standardising herbal medicines containing them. This could be applied in distinguishing plant materials and control adulteration. We developed a simple macroscopic fluorescence technique using herbal distillates, that could be used to distinguish the root bark from the leaf. Several phytochemical compounds were detected. Due to their aphrodisiac pharmacological activity, the most important are saponins and L-arginine. However, other phytochemicals like flavonoids, alkaloids, phytosterols, and reducing sugars also contribute individually and/or collectively to aphrodisiac properties of plants. The decoction extracts had higher levels of L-arginine than their Soxhlet counterparts. Decoction may be a preferable production extraction method for the plant materials due to a high percentage yield. Soxhlation may be useful in qualitative analysis since it provided clear TLC and HPLC chromatograms. However, there is need for further analysis to correlate the different phytochemicals to aphrodisiac activity so as to establish the most relevant one, its relative abundance, and use as a bioactive marker.

CRediT authorship contribution statement

Anyase Ronald Amaza: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Treasure Angie Amutuhaire:** Writing – review & editing, Investigation, Formal analysis. **Jonans Tusiimire:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Vivian Sharon Amito:** Writing – review & editing, Investigation. **Clement Olusoji Ajayi:** Writing – review & editing, Resources, Investigation, Conceptualization. **Jimmy Ronald Angupale:** Writing – review & editing, Resources, Investigation, Conceptualization. **Abdelgadir Alamin Abdelgadir:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Anywar Godwin:** Writing – review & editing, Visualization, Validation. **Patrick Engeu Ogwang:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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