

PHARMACOGNOSTIC AND PHYSICOCHEMICAL EVALUATION OF THE LEAF AND ROOT OF *UVARIA CHAMAE* (P. BEAUV.) ANNONACEAE

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ABSTRACT

The ethnomedicinal use of *Uvaria chamae* (P. Beauv) Annonaceae as an antimalarial has been scientifically justified. This study was carried out to determine the pharmacognostic and physicochemical characters of its leaf and root with a view to establishing pharmacopoeial parameters for their quality control. The fresh leaf and root of *U. chamae*, collected and authenticated in the IFE Herbarium, Obafemi Awolowo University, Nigeria, were preserved in ethanol (50%) while some were oven-dried and powdered. Organoleptic evaluation of the fresh samples and microscopy of the powders were carried out according to standard methods. Transverse section and surface preparation of the fresh leaf as well as radial and tangential longitudinal sections of the root were carried out. Physicochemical properties such as moisture content, ash and extractive values were determined. The methanol extracts of the powders were subjected to HPLC-MS analysis. The transverse section of the leaf revealed a dorsiventral midrib and striated cuticle. On the abaxial surface, paracytic stomata were observed but the adaxial surface lacked stomata while the vascular bundle was of the collateral type. The root of *U. chamae* had prismatic calcium oxalate crystals and tyloses. The physicochemical results were as follow: the total ash (9.6 & 9.7%), loss on drying (8.8 & 8.1%), alcohol-extractive value (3.5 & 0.8%), water-extractive value (4.5 & 1.3%) for the leaf and root, respectively. Flavonoids such as pinocembrin and chamanetin were identified in the root. The study revealed pharmacognostic and chemical profiles that may aid the identity, and therefore, quality control of *U. chamae*.

INTRODUCTION

In recent years, there has been an increase in the use of herbal medicinal products. A major demerit of these medicinal products is the lack of standardisation, both of the raw materials – medicinal plants, and the finished products. Standardization is a necessary step for the establishment of identity, purity and quality which consequently translates to consistent biological activity and positive therapeutic index [1]. Therefore, there is the need to standardise and thus, ensure quality control in the use of the plants for the production of herbal medicinal products.

Uvaria chamae (P. Beauv) Annonaceae, commonly called finger root or bush banana with various local names: *Oko aja*, *Eruju*, *Akisan* (Yoruba), *Mmimi ohia* (Igbo), or *Kas kaifi* by the Hausas in Nigeria, is distributed in savannah and secondary forests in Africa as well as in tropical areas of the world. It is an evergreen fragrant plant, with widely-eaten yellow ripe fruits with sweet pulp. Secondary metabolites that have been isolated from *U. chamae* include acetogenins such as chamuvarinin and cis-bullatencin; essential oils such as cadinene [2]. Others include flavonoids such as uvaretin (chamanetin) and pinocembrin [3] as well as and alkaloids such as isoboldine, arnepavine, thaliporphine, among others [4–7].

Uvaria chamae is reported to have anti-sickling [8], antioxidant [9], antimalarial [10], anti-hyperprolactinaemic [11], antitrypanosomal [12], anti-salmonellal [13], and anti-cancer [14,15] activities.

Despite various studies on the pharmacology and chemistry of this plant, there was limited data on the botanical profile of the plant, although, a recent study highlights the anatomy of *U. chamae* root bark as a component of an anti-anaemic herbal product

[16]. However, no attempt was made as to provide the profile of the chemical constituents of *U. chamae* leaf and root. In addition, there is no monograph of this plant in a herbal pharmacopoeia. Therefore, this study aimed at investigating the pharmacognostic and chemical profiles of the leaf and root of *U. chamae* with a view to contributing to a monograph of the plant.

RESULTS

Macro-morphological characters

The organoleptic properties of the leaf and root of *U. chamae* are highlighted in Table 1. The plant has an alternately-arranged, compound green leaf with aromatic odour. Each leaflet has a lanceolate shape, sinuate margin, apiculate apex, acute base and an entire glabrous surface (Plate 1). The root is dark brown in colour, have cylindrical shape with short rootlets, and an aromatic odour.

Table 1: Organoleptic properties of the leaf and root of *U. chamae*

Organoleptic characters	Leaf	Root
Colour	Abaxial - Light green	Whole root - Dark brown
	Adaxial - Dark green	
	Powder - Dark green	
Odour	Faintly Aromatic	Aromatic
Texture	Granular	Granular

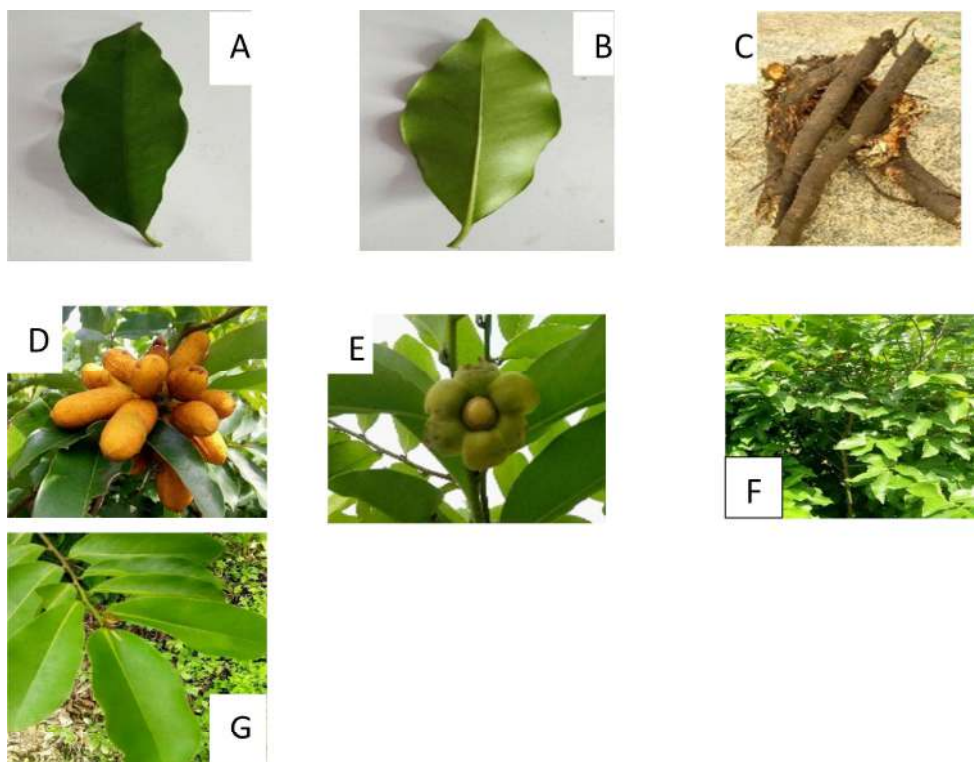


Plate 1: Macro-morphology of *Uvaria chamae* (P. Beauv) Annonaceae; Habitat: Hill Two area, (Savannah habitat) Obafemi Awolowo University Campus, Ile-Ife, Nigeria: A: Adaxial surface of leaflet; B: Abaxial surface of leaflet; C: Whole root; D: Fruit; E: flower; F: Whole shrub G: compound leaf

Microscopical Evaluation of the Powdered Leaf and Root

Starch grains, calcium oxalate crystals, phloem fibre were observed in the powders of the leaf and root. Starch grains were stained blue-black with iodine while fat bodies and lignified tissues were both red and pink, with sudan red and phloroglucinol, respectively (Plates 2-3).

Physicochemical Properties

The moisture content, total ash, sulphated ash, alcohol-extractive and water-extractive values of the leaf and root (in mean percentages $\pm$ standard deviation) are highlighted in Table 2.

Table 2: Physicochemical properties of the powder of the leaf and root of *U. chamae*

Properties (mean % $\pm$ SD)	Leaf	Root
Moisture Content	8.8 ± 0.007	8.1 ± 0.005
Total Ash	9.6 ± 0.003	9.7 ± 0.011
Sulphated Ash	5.5 ± 0.003	5.1 ± 0.009
Alcohol Extractive	3.5 ± 0.020	0.8 ± 0.020
Water Extractive	4.5 ± 0.020	1.3 ± 0.020

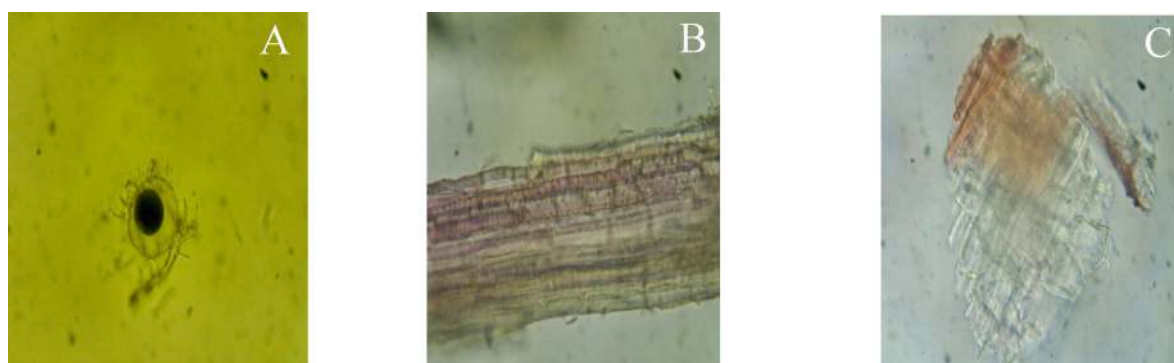


Plate 2: *Uvaria chamae* leaf powder showing A: starch globules, B: lignified tissues, C: fat globules (x400)

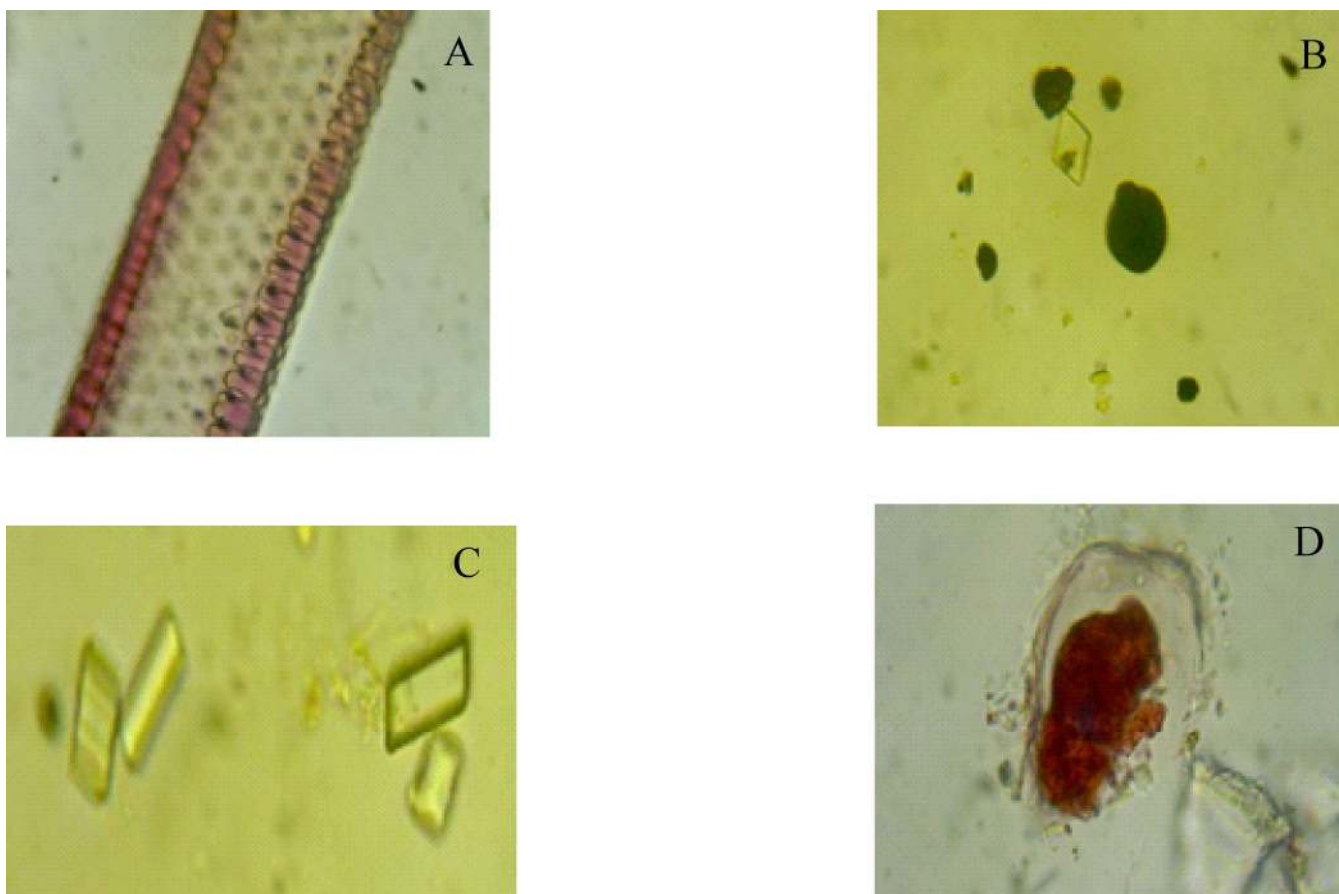


Plate 3: *U. chamae* root powder A: lignified tissues, B: starch globules, C: fat globules, D: prismatic crystals (x400)

Leaf Venation

The venation pattern of the leaf revealed open areoles, polygonal areole shapes, bi-forked and tri-forked vein terminals. The adaxial (upper) surface had no stomata but had distinct polygonal epidermal cells while the abaxial (lower) surface had paracytic stomata, polygonal and rectangular epidermal cells with straight and undulating anticlinal cell wall (Plate 4).

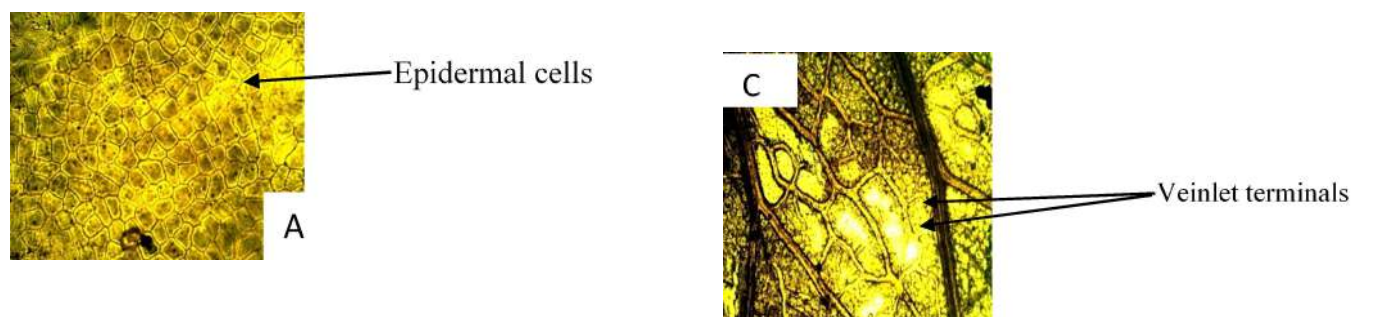


Plate 4: *Uvaria chamae* leaf epidermi: A: adaxial surface, B: abaxial surface; C: Leaf venation pattern (x400 magnification)

Transverse Section of Leaf

The transverse section of *U. chamae* were observed as striated cuticle, circular and oval epidermal cells with the palisade layer

consisting of tightly-packed, elongated, cylindrical-shaped parenchyma cells. The midrib is dorsiventral (Plate 5).

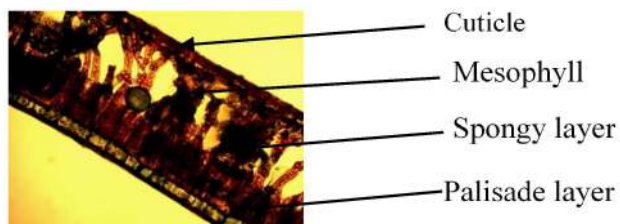
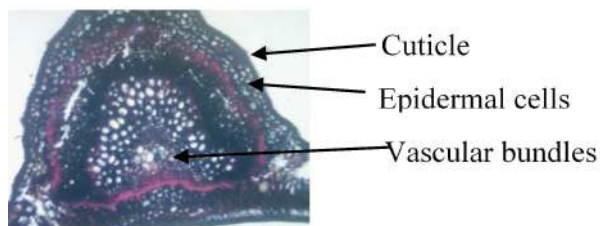


Plate 5: *Uvaria chamae* leaf transverse section. A: Midrib x100, B: Lamina x400

Root Anatomy

The transverse (TS), radial longitudinal (RLS) and tangential longitudinal sections (TLS) of the root are shown in Plate 6. A diffuse pore system and pore multiple, ranging from 2–9 with the presence of tyloses and tannins, were observed in the TS. In the

TLS, uniseriate and multiseriate rays of 2–7 seriate, in addition to the presence of tannins and secretory canals, were seen. Furthermore, procumbent rays, secretory canals, fibres and tannins were observed in the RLS.

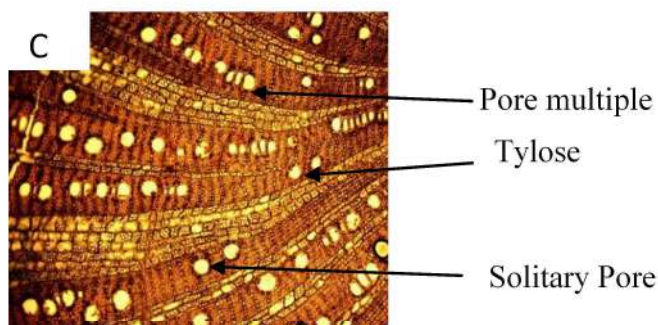
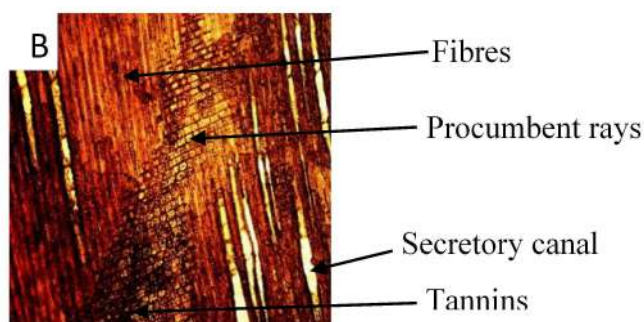
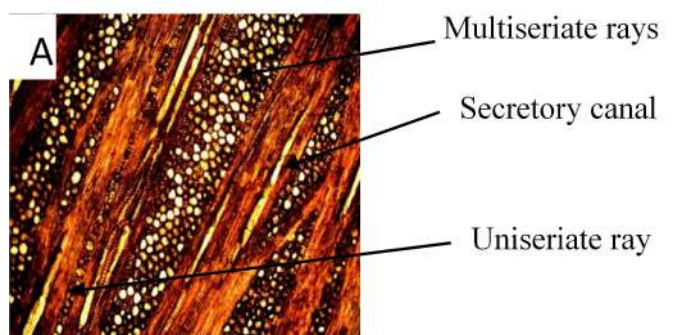


Plate 6: Root sections of *U. chamae*: A: Tangential longitudinal section (TLS). B: Radial longitudinal section (RLS). C: Transverse section (TS) [x400, Magnification]

LC-MS analysis of Leaf and Root of *U. chamae*

Data from high resolution mass spectroscopy (MS) chromatogram of the leaf (Fig 1B) did not match any previously-reported compound in the leaf of the plant. The m/z of identified compounds from the root were listed in Table 3 while some of the structures were shown in Fig. 2.

DISCUSSION

The lack of standardisation is a great challenge to the clinical acceptability of herbal medicines. Standardisation of herbal drugs involves the evaluation of organoleptic characters, macro- and micro-morphological examinations, among others. These

parameters constitute the quality control standards listed in plant monographs. Therefore, this study was carried out to contribute to information on the leaf and root of *U. chamae*, an indigenous antimalarial plant.

Organoleptic evaluation makes use of the sensory perception and this provides the simplest and most rapid feature of identity of a herbal drug. In this study, structures that were observed in the leaf powder included paracytic stomata and fragments of the epidermal layer while the root powder possessed starch granules and calcium oxalate crystals. This observation was similar to an earlier report on *U. narum*, which had abundant starch grains and

few prismatic calcium oxalate crystals in the root [17]. Many commercial herbal drugs are usually presented as powders and therefore, microscopy is an important step in ensuring their identity and purity.

Furthermore, the moisture contents of the leaf (8.8 %) was similar to the value reported for the leaf of *U. narum* (8.1 %)[17]. The presence of moisture in a plant material is important in the determination of the shelf-life of such plant. High moisture content may lead to enzymatic and hydrolytic reactions or promote/accelerate the deterioration of the material by mould and bacteria attack, which may alter the chemical composition and quality of the crude drug. Therefore, in general, the drying of plant materials should be accomplished as rapidly as possible.

The total ash values for the leaf and root (9.6 and 9.7 %) were also consistent. The total ash of the any organic material is composed of their non-volatile inorganic components and it is influenced by adulteration and contamination of plant products. This value varies with different plant species and so should be determined for each plant. The amount of total ash is about <15% as described in some pharmacopoeia [18].

The findings on cytomorphological investigation in this study were similar to the report on *U. narum* which possessed paracytic stomata type found only on the abaxial surface and also, a dorsiventral arrangement in the lamina of the leaf [17]. For the root, a characteristic feature of secondary xylem, tyloses, which are outgrowths of xylem parenchyma cells, were observed in *U. chamae* root. Tyloses have wide occurrence, are induced by the presence of wounds and pathogen infection. When fully grown, starch crystals, resins and gums are formed within them. Their presence and number serve as identification parameters for root drugs. In this study, laterally procumbent rays were observed in addition to the sieve tubes, fibres and axial parenchyma. However, resin ducts and sclereids, reported in [16] were not observed in this study.

The LC-MS analysis of the root revealed the possible presence of jatrorhizine (an aporphine alkaloid), uvaretin (chamanetin), isouvaretin (isochamanetin), diuvaretin and pinocembrin, which were flavonoids reported in the genus *Uvaria* [4,6]. However, none of the abundant peaks of the leaf chromatogram matched previously-reported leaf constituents, such as nantenine, corydine, nornantenine and arnepavine [19]. The structures of identified constituents are shown in Fig 2.

CONCLUSION

The data from the present study would assist in the proper identification and therefore, quality control of *Uvaria chamae*. It would also contribute toward the compilation of a monograph on the plant. It is recommended that sustained research on the botanical, chemical, toxicological and biological standardisation of the plant be carried out in order to provide more information on the quality control of *Uvaria chamae*.

MATERIALS AND METHODS

Plant Collection

Fresh leaf and root of *U. chamae* (P. Beauv) Annonaceae were collected on Hill Two, Obafemi Awolowo University (OAU) campus, Ile-Ife, Nigeria (7.5183°N, 4.5228°E) during the dry season in July 2018. *Uvaria chamae* was identified by Mr. I. Ogunlowo and the voucher specimens were deposited in the Faculty of Pharmacy Herbarium, OAU, with the number FPI 2199. For powder examination, the leaf and root were oven-dried at 40°C and 50°C, respectively, and pulverised separately.

Macroscopical Examinations of Fresh Leaf and Root

The macroscopic features of the fresh leaf and root of *U. chamae* were evaluated for the determination of the shape, size and colour of the fresh whole drugs, surface characteristics, texture and odour using standard protocols [20].

Microscopy of the Powdered Leaf and Root

To the powders (particle size, 250µm) of the leaf and root, a few drops of sodium hypochlorite (3.5 %w/v), were added. In addition, iodine (N10), Sudan III and phloroglucinol-HCl were added to each powder to test for the presence of starch, fat and lignin, respectively. Each powder was mounted with glycerol and viewed under x100 and x400 magnifications. For quantitative microscopy, cellular structures were counted in four replicates at x100 magnification.

Determination of Total Ash:

The powdered leaf and root of *U. chamae* (2g) were separately placed in previously ignited and weighed crucibles. Each powder, in four replicates, was incinerated by gradually increasing the temperature, but not exceeding 450 °C until the drug was free from carbon. It was cooled in a desiccator and weighed. The content in gram of the total ash per 100g of air-dried drug was calculated [21].

Determination of Sulphated Ash

Concentrated sulphuric acid (2mL) was added to each of the leaf and root powders of *U. chamae* (2 g each) and incinerated to about 800 °C. After cooling, the residue was moistened with a few drops of concentrated sulphuric acid and heated gently. The content in gram of sulphated ash per 100g of air-dried drug was calculated [21].

Determination of Ethanol-soluble Extractive

Each of the powdered drug of the leaf and root of *U. chamae* (4 g) was macerated with ethanol (100 mL) in a conical flask. The flask was agitated and filtered after 18 h. About 25mL of the filtrate was evaporated to dryness and the contents of extractives in gram per 100g of the air-dried drug were calculated.

Determination of Water-soluble Extractive

The procedure earlier described for ethanol-soluble extractive was used but water was used instead of ethanol.

Determination of Water and Loss on Drying

This determination was done using the gravimetric method. Two grams each of the powdered leaf and root was oven-dried at 60 °C for 5 h until constant weights were obtained [20].

Leaf Peeling

The middle section of the leaf was cut into 3x3 cm square portions, transferred into glass petri dishes containing concentrated nitric acid and placed under sunlight until swollen. The square portions were removed from the acid and rinsed twice in water. The abaxial and adaxial surfaces were separated with forceps, stained with safranin O for 5 minutes, rinsed twice in water [22]. The peels were mounted with glycerol (25 %), sealed and viewed under x100 magnification (Olympus light microscope) and photo micrographs of the slides were made with a trinocular microscope (ACCU-SCOPE 3001 LED trinocular microscope with 3.2 MP CMOS digital camera).

Leaf Clearing

A portion (1 cm²) of the median part of a matured leaf was boiled in absolute ethanol until the chlorophyll was removed, rinsed twice in water and soaked in sodium hydroxide (5%) for 24 h. After rinsing twice in water, it was steeped in sodium hypochlorite (5%), rinsed in water and stained with safranin O for 5 mins [22]. The stained leaf was viewed under x400 magnification for the determination of the areole type, areole shape, veinlet termination number, vein terminals and vein islets.

Softening and Sectioning

The root of *U. chamae* was cut into cubes (4 cm) and boiled in NaOH (5%) to soften the wood before sectioning.

The TS of the mature leaf and root as well as the TLS and RLS of the root were obtained by cutting with a sledge microtome (Micros

St. Veit, Glan, Austria) to 10 µm thickness. These samples were preserved in ethanol (50%) before use.

Staining

Sections of the root and leaf were stained in safranin O in solid glass wash with lid to exclude dust and prevent evaporation of the stain. Each section was stained for 3 minutes in safranin O, rinsed twice in water, counter-stained for 3 minutes in Alcian blue then rinsed twice in water before washing in ethanol of varying strengths - 50%, 70%, 80%, 90%, and absolute ethanol.

LC-MS Analysis of Leaf and Root

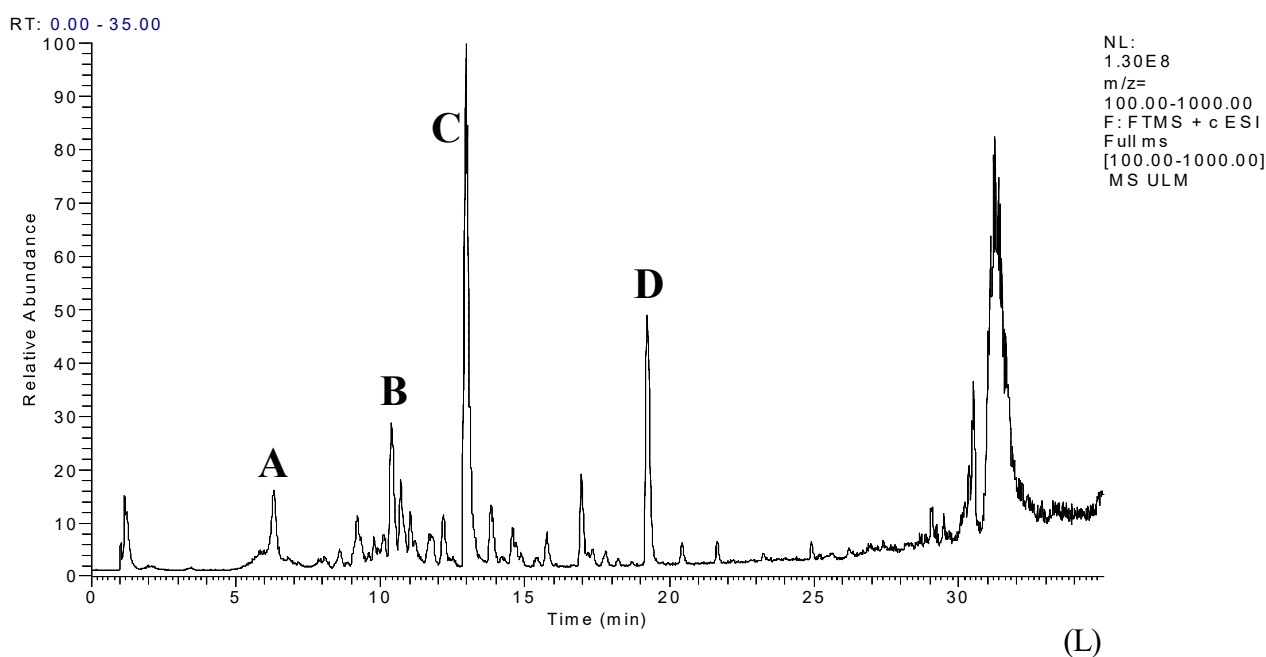
About 2mg of each extract was dissolved in HPLC-grade methanol (0.25mL, 8%w/v) and centrifuged for 5 min (6600 rpm, 2200 g) before LC-MS analysis. High-resolution mass spectra (ESI-HRMS) were acquired on a LTQ Orbitrap spectrometer (Thermo Scientific, USA) equipped with a HESI-II source. The spectrometer was equipped with an Agilent1200 HPLC system (Santa Clara, USA) including pump, PDA detector, column oven (30 °C) and auto-sampler (injection volume: 6µL). The spectrometer was operated in positive mode with a nominal mass resolving power of 60,000 atm/z400 and a scan rate of 1 Hz using the following parameters: spray voltage 6 kV, capillary temperature 300 °C, tube lens 100 V. Argon served as collision gas and N₂ was used as sheath gas (66 arbitrary units) and auxiliary gas at 8 arbitrary units [23].

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Conflicts of interest

The authors declare no conflicts of interest.



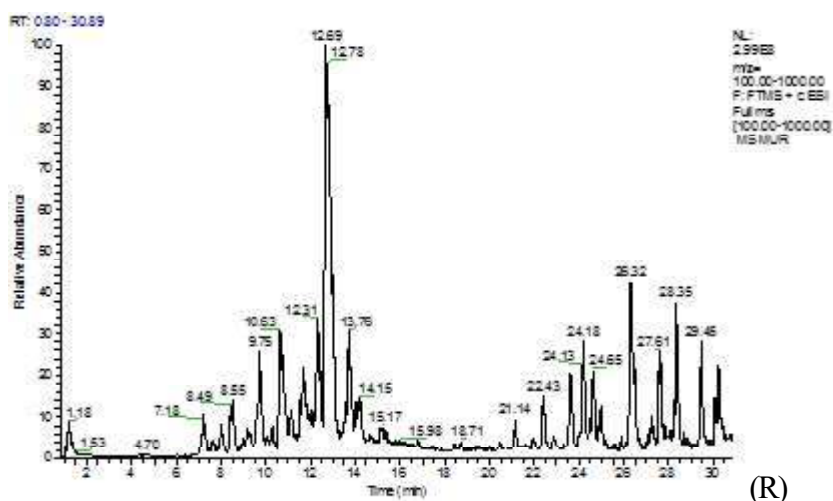


Fig 1. HPLC-MS spectra of the leaf (L) and root (R) of *Uvaria chamae* (Annonaceae)

Table 3: LC-MS identification of compounds in leaf and root of *U. chamae*

Peak	Rt	Mass [M+H ⁺]	Mol formula	Abundance	Proposed compound	Ref
Leaf						
A	6.32	284.1282	C ₁₇ H ₁₈ NO ₃	1.29E7	--	
B	10.39	328.1543	C ₁₈ H ₁₇ O ₄	1.22E7	--	
		297.1121				
C	12.97	339.2068	C ₂₁ H ₂₇ O ₂ N ₂	5.27E7	--	
		307.1805	C ₂₀ H ₂₃ ON ₂			
D	19.22	347.1126	C ₁₈ H ₁₉ O ₇	1.35E7	--	
Root						
	7.24	327.2067	C ₂₀ H ₂₇ O ₂ N ₂	1.59E7	--	
	8.02	315.2067	C ₁₉ H ₂₁ N ₂	1.44E7	--	
	8.52	329.2224	C ₂₀ H ₂₉ O ₂ N ₂	1.85E7	--	
	9.72	339.2069	C ₂₁ H ₂₇ O ₂ N ₂	2.45E7	Jatrorrhizine	
	10.53	313.2277	C ₂₀ H ₉ ON ₂	5.27E7	--	
	11.15	299.2119	C ₁₉ H ₂₇ ON ₂	1.68E7	--	
	11.74	297.1963	C ₁₉ H ₂₅ ON ₂	2.76E7	--	
	12.35	517.2189	C ₂₆ H ₃₃ O ₉ N ₂	3.69E7	--	
	12.73	397.2125	C ₂₃ H ₂₉ O ₄ N ₂	1.44E8	--	
	13.76	413.2073	C ₂₃ H ₂₉ O ₅ N ₂	3.97E7	--	
	14.18	311.2118	C ₂₀ H ₂₇ ON ₂	1.83E7	--	
	21.14	257.0808	C ₁₅ H ₁₃ O ₄	1.34E7	Pinocembrin	[6]
	22.43	377.1386	C ₂₃ H ₂₁ O ₅	1.44E7	--	--
	23.65	363.1228	C ₂₂ H ₁₉ O ₅	3.26E7	Chamanetin	[5]
					(uvaretin)/Isochamanetin	
					(Diuvaretin)	
	24.66	379.1545	C ₂₃ H ₂₃ O ₅	1.69E7	Uvaretin/isouvaretin	[6]
	25.0	483.1805	C ₃₀ H ₂₇ O ₆	1.22E7	--	--
	26.32	379.1541	C ₂₃ H ₂₃ O ₅	3.94E7	Uvaretin/isouvaretin	--
	27.59	485.1953	C ₃₀ H ₂₉ O ₆	2.70E7	Diuvaretin	[6]
	28.35	645.4702		2.45E7	--	--
	29.45	585.2016	C ₃₆ H ₂₉ O ₆ N ₂	2.01E7	--	--

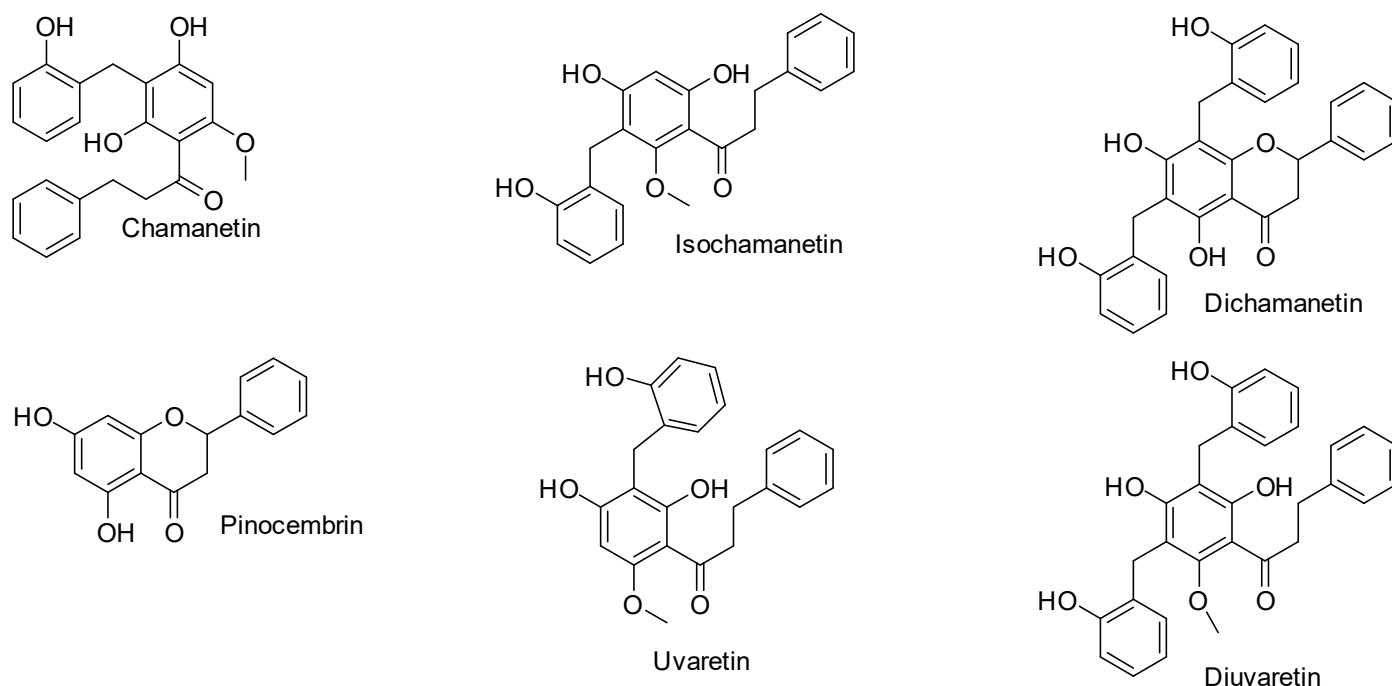


Fig 2: Some constituents identified in *Uvaria chamae* (Apocynaceae) root

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