

## Pharmacognostic Standards for *Uvaria chamae* P. Beauv. Leaves (Annonaceae)

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

### Abstract

#### Background

Standardisation as a tool in quality control ensures purity, safety and efficacy of herbal medicines. *Uvaria chamae* from the family Annonaceae has wide medicinal application which includes its use as a febrifuge and as an anti-inflammatory.

**Objective:** The study was aimed at developing some Pharmacognostic standards for the leaves of *U. chamae*.

**Material and Methods:** *Uvaria chamae* plant was collected, identified and authenticated. Fresh leaves were examined macroscopically and microscopically based on standard procedures. Fluorescence behavior was assessed by adding various reagents and viewing at daylight, 254 nm and 365 nm. Chemomicroscopy and physicochemical analysis of powdered samples were done using standard procedures. Phytochemical and Thin layer chromatographic analysis of the extracts were also carried out.

**Results:** Leaves of *U. chamae* are alternately arranged and elliptic in shape. The abaxial surface has straight polygonal epidermal cells with paracytic stomata. Vascular bundles are arranged in fan shape from the transverse section of the leaf. Chemomicroscopic evaluation revealed the presence of lignin, starch and oil. Alcohol and water soluble extractive values were  $4.00 \pm 0.00$  and  $5.6 \pm 0.67$ , respectively. The membrane stabilization activity of the extracts at 0-1 mg exceeded that of the standard drug diclofenac. The ethyl acetate fraction had the highest percentage of inhibition of haemolysis at 0.4 mg.

**Conclusion:** The features established in this study may contribute in the identification and quality control of *U. chamae*.

**Keywords:** *Uvaria chamae*, standardisation, herbal medicine, chemomicroscopy, medicinal plants

### INTRODUCTION

Medicinal plants have been a major source of treatment for human diseases since time immemorial. It has also gained a reputation of being used both in the developed and developing countries as remedies for treating different ailments. They are also a valuable source of raw materials for the pharmaceutical industry (WHO, 1998; Kunle *et al.*, 2012) and account for a significant portion of the worldwide drug market; this necessitates prioritization of the safety and quality of medicinal components and final products (Patel *et*

*al.*, 2011). Limitations to the development and use of medicinal plants include among other factors, lack of standardisation of the plants used (Okigbo *et al.*, 2006). Standardisation of plants can be achieved through stepwise pharmacognostic studies with macroscopic and microscopic descriptions of the plant material preceding other tests in establishing the identity and purity (Sonibare *et al.*, 2015).

*Uvaria chamae* (P. Beauv), a highly aromatic plant commonly known as finger root or bush banana,

belongs to the family *Annonaceae* and it is called *Mmimi ohia* by the Igbo people, *Okoko aja* or *Eruju* by the Yorubas and *Kas Kaiji* by the Hausas in Nigeria. It is found in the savannah and rain forest regions of Africa and other tropical areas of the world. It is an evergreen tree which grows up to 4m (Omale et al., 2013; Olumese et al., 2016).

The plant has many uses in ethnomedicine, including anti-inflammatory, antimalarial, analgesic, and treatment of jaundice, epilepsy, and microbiological infections (Suleiman et al., 2017). Among other things, the root is said to be beneficial as a febrifuge, purgative, stomachic, and vermifuge (Burkil 1985;

Olumese et al., 2016). Studies have shown that *U. chamae* plant parts possess anticonvulsant, anti-snake venom, anti-inflammatory, uterine contraction, antimalarial, antitrypanosomal and antioxidant activities (Okwu et al., 2009; Omale et al., 2013; Adelodun et al., 2013; Ita et al., 2017; Suleiman et al., 2017).

Despite the extensive use of *U. chamae* in traditional medicine, there is paucity of information on its standardisation. This study therefore aims to establish some pharmacognostic standards for *U. chamae* for proper identification, standardisation and quality control of the plant drug.

## METHODOLOGY

### Plant collection and Authentication

Dirt free sample of *Uvaria chamae* was collected from Wasinmi (6.9945° N, 3.2269° E) in Osun State in September, 2017. The plant was identified and authenticated by Mr. Odewo S.A at the Forest herbarium Ibadan (FHI) of the Forestry Institute of Nigeria, Ibadan, Nigeria. Voucher specimen was prepared and deposited at the FHI with voucher specimen number FHI.111423.

### Macroscopic study

The macroscopic evaluation of the plant involved the assessment of organoleptic parameters such as Length, Width, Shape, Apex, Margin, Base, Petiole, Surface, Colour, Odour, Taste and leaf arrangement according to standard methods (Trease and Evans, 2002)

### Microscopic study

Free hand sectioning was used to prepare the transverse sections of the leaf and petiole of *Uvaria chamae*. Thin sections were placed in 2% sodium hypochlorite for 2 min until the sample was completely cleared. The leaf epidermis was carefully scraped to obtain a transparent layer using a new safety razor blade. The scrapping of the upper surface of the leaf yielded the abaxial surface while scrapping the lower surface of the leaf yielded the adaxial layer. Transparent samples were then bleached using 2% sodium hypochlorite for about 2 min. The sections were rinsed 2-3 times in distilled water and stained with Safranin O dye after which they were dehydrated serially in 50%, 70% and 90% ethanol and then mounted with glycerin on a microscope slide and viewed under the microscope at low and high-power objectives (WHO, 1998).

### Palisade ratio determination

Palisade ratio was determined following the procedure described by Khan et al. (2016). Pieces of *U. chamae* leaves were cleared by boiling in 200% chloral hydrate

solution, this was mounted and viewed under the microscope with a X4 objective. Groups of four epidermal cells were first focused. Using the fine adjustment, the underlying palisade cells within the four epidermal cells were focused and counted to include palisade cells which are more than half covered by the epidermal cells. The palisade ratio was obtained by dividing the number of palisade cells by 4. This was repeated for up to five readings and the average obtained.

### Epidermal cell size determination

The size of epidermal cell was measured quantitatively using calibrated light microscope with the aid of eye piece and stage micrometer as described by Omitola et al. (2019). Briefly, measurements were taken in a total of twenty fields of view from five different slides.

### Fluorescence study

Fluorescence analysis of the powdered plant was studied following standard procedure (Sonibare et al., 2015). Samples of *U. chamae* powder were treated with different acids and alkalis, (50% H<sub>2</sub>SO<sub>4</sub>, 50% HCl, 50% HNO<sub>3</sub>, 1N NaOH, 5% KOH, Acetic acid) gently mixed and then viewed/observed in the visible/day light and ultra violet light at 254nm and 365nm wavelength.

### Physicochemical screening

The powdered leaves were subjected to various physicochemical parameters such as moisture content, alcohol soluble extractive value, water-soluble extractive values and crude fibre determination according to standard methods (Kshisagar et al., 2017).

### Chemomicroscopy

Chemomicroscopic study of the plant was done by mixing the plant powder with suitable reagents on a microscopic slide and viewing under the microscope at low and high-power objectives. Reagents used include concentrated

H<sub>2</sub>SO<sub>4</sub>, conc. HCl, Iodine, Sudan III and Phloroglucinol (Trease and Evans, 2002; Sonibare and Adebodun, 2018).

### Plant extraction

Coarsely powdered sample of *U. chamae* was macerated in 100% methanol for 72 h with frequent agitation for the first 6 h. The same process of extraction was repeated using the same solvent for 48 and 72 h, respectively for effective extraction. The mixture was filtered and the filtrate evaporated *in vacuo* using a Rotary evaporator (Buchi, rotavapour R-210, Switzerland).

Final solvent elimination was done by air - drying. The crude extracts were stored in sterile airtight bottles. The crude extract of *U. chamae* (93.43 g) was partitioned using the solvent-solvent partitioning method. The extracts were first dissolved in 100 mL of methanol: water (3:1) and poured into a separating funnel. The partitioning was done beginning with *n*-hexane, dichloromethane and ethyl acetate, the residual aqueous fraction was also obtained. The fractions were air-dried and stored in air tight containers.

### Thin Layer Chromatography

Thin layer chromatography (TLC) of the crude extract and partitioned fractions of *U. chamae* was done for the assessment of different classes of bioactive secondary metabolites using pre-coated TLC plates (Silica gel G60F<sub>254</sub> sheets 20 × 20 cm, 0.5 mm thickness, Merck). The crude extract, *n*-hexane and dichloromethane (DCM) fractions of *U. chamae* were spotted on TLC plates and subsequently developed in suitable solvent systems. The crude extract was developed in hexane and ethyl acetate, in ratio 3: 2; the *n*-Hexane fraction was developed in hexane and ethyl acetate in ratio 4:1, while the dichloromethane fraction was developed in hexane, dichloromethane and ethyl acetate in ratio 1.5:2:1.5. The developed plates were dried and visualized in daylight and ultraviolet (UV) lamp at 254 and 365 nm. They were also sprayed with visualizing reagents such as 1 g Aluminum chloride (AlCl<sub>3</sub>) in 100 mL 95% ethanol to detect flavonoids and vanillin in sulphuric acid (WHO, 1998).

### Phytochemical screening

Phytochemical screening of medicinal plants is useful in revealing the various phytochemical groups present in the

## RESULTS AND DISCUSSION

Macro-morphological evaluation shows extensive branching and solitary flowers. Some of the branches have special adaptive features for climbing. Its leaves are alternately arranged, elliptic in shape with acute apex and entire margin. The leaf surface is green in colour, glabrous and smooth to touch. The leaf has a short petiole (0.74 cm) while stipules are absent. The

plant material which may be useful in its quality assurance and may be responsible for the plant's therapeutic properties (Mondal and Roy, 2022). The crude extract of *U. chamae* leaves was screened for the presence of secondary metabolites according to standard procedures (Trease and Evans, 2002).

### Assay of Membrane Stabilizing Activity

#### Preparation of red blood cell (RBC)

The bovine red blood cell was prepared according to the method of Oyedapo *et al.* (2004). Fresh bovine blood was collected into anti-coagulant (3.8% tri-sodium citrate) in a clean bottle in ratio 2:1 (blood: anti-coagulant) and mixed gently by inversion. The blood sample was centrifuged at 3000 rpm on a Bench centrifuge Model 90-2 for 10 min at room temperature. The supernatant (plasma and leucocytes) was carefully removed, while the packed red blood cell was washed again in fresh normal saline (0.85% w/v NaCl). The washing and centrifugation were repeated severally until the supernatant became clear. The bovine erythrocyte (2% v/v) was prepared by diluting 2 mL of packed cell with normal saline to 100 mL and kept undisturbed at 4 °C in the refrigerator.

#### RBC membrane stabilization assay

The membrane stabilizing activity assay was done based on the procedure described by Aina and Oyedapo (2013). The assay mixture consisted of hyposaline (1 mL), 0.1 M phosphate buffer, pH 7.4 (0.5 mL), varying concentrations of *U. chamae* extracts and fractions (0.2 – 1mg/mL) and 0.5 mL of 2% (v/v) erythrocyte suspension in a total volume of 3 mL. The control was prepared as above without the drug while the drug control (3 mL) lacked erythrocyte suspension. Diclofenac was used as the standard drug. The reaction mixtures were incubated at 56 °C for 30 min. The absorbance of the released haemoglobin was read at 560 nm against reagent blank. The percentage membrane stability was estimated using the expression:

$$\text{percentage membrane stability} = 100 - \frac{\text{Abs}_{\text{test}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{blood}}} \times 100$$

Where Abs refers to Absorbance.

The blood control represented 100% lysis.

average leaf length and width is 10.78 cm and 3.85 cm, respectively. There is presence of prominent midrib and reticulate venation pattern. The macro-morphological features obtained can be used as diagnostic parameters. According to the Plant resources of South East Asia, the absence of stipules is a characteristic of the genus *Uvaria* (PROSEA, 2016).

Organoleptic characters revealed that *U.chamae* is smooth to touch and glossy in appearance, dark green in colour (figure 1) and tasteless with strong aromatic odour. Microscopically, the abaxial surface (Figure 2) revealed the presence of polygonal epidermal cells with straight anticlinal walls, paracytic stomata and calcium oxalate crystals. The foliar epidermal features of the adaxial surface as observed in figure 3a, was characterised with absence of stomata, presence of crystals and polygonal epidermal cells with straight anticlinal walls. Palisade cells were present with a palisade ratio of 2.8 - 6.0. Table 1 gives a summary of the foliar epidermal features of the adaxial and abaxial surfaces of *Uvaria chamae*. Reticulate pattern of venation is shown in figure 3b. The leaf transverse sections of *U. chamae* (Figure 4a) showed the presence of well-developed collenchyma cells beneath

the epidermal cells of the abaxial surface and the vascular bundles (phloem and xylem). The vascular bundle of the leaf is fan shaped with the phloem appearing as rings (figure 4a). Petiole transverse section showed distinct fan shaped vascular bundles appearing in three bands surrounding the pith (figure 4b). This could be useful as a distinguishing feature of the plant.

Microscopical evaluation alone is not sufficient in establishing the standardization profiles of crude drugs but when combined with other analytical parameters could give full evidence of the evaluation parameters that could be used for proper plant identification (Dinesh *et al*, 2011).



Figure 1: Leaves of *Uvaria chamae*

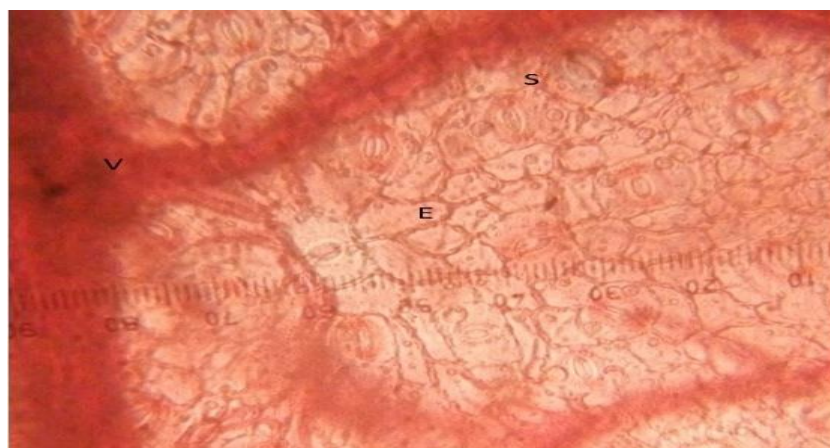


Figure 2: Abaxial surface showing paracytic stomata (S- Stomata, E- epidermal cells and V- veins)

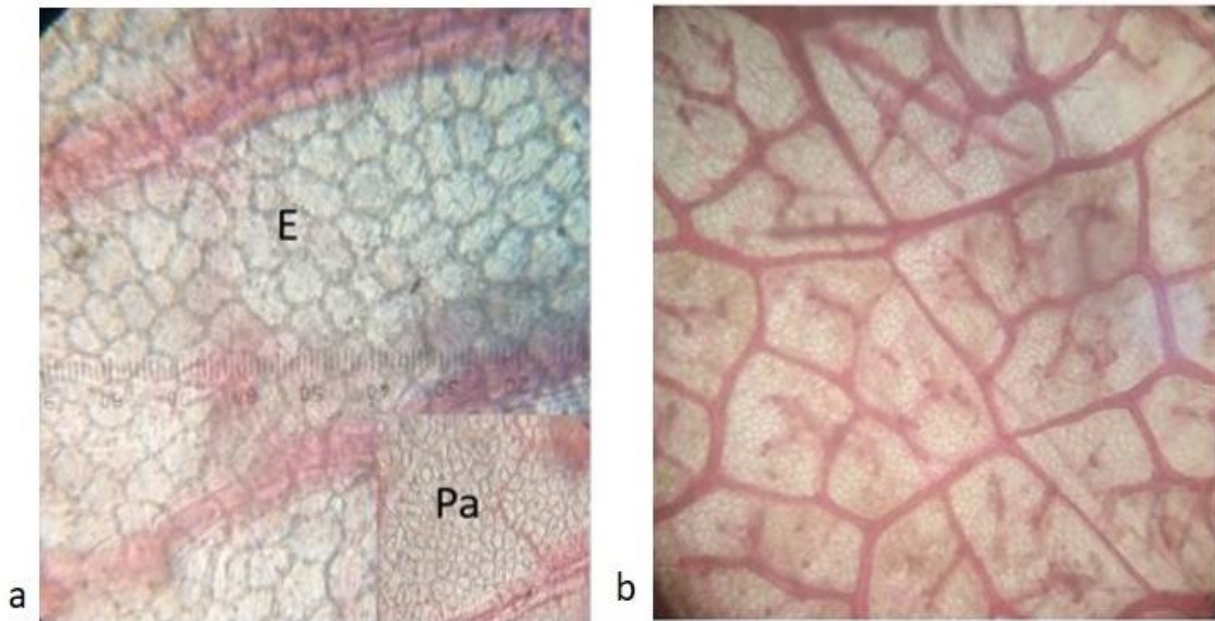


Figure 3: (a) Adaxial epidermis of *Uvaria chamae* x400 showing E: epidermal cells, Pa: palisade cells (b) Reticulate venation pattern of *Uvaria chamae*

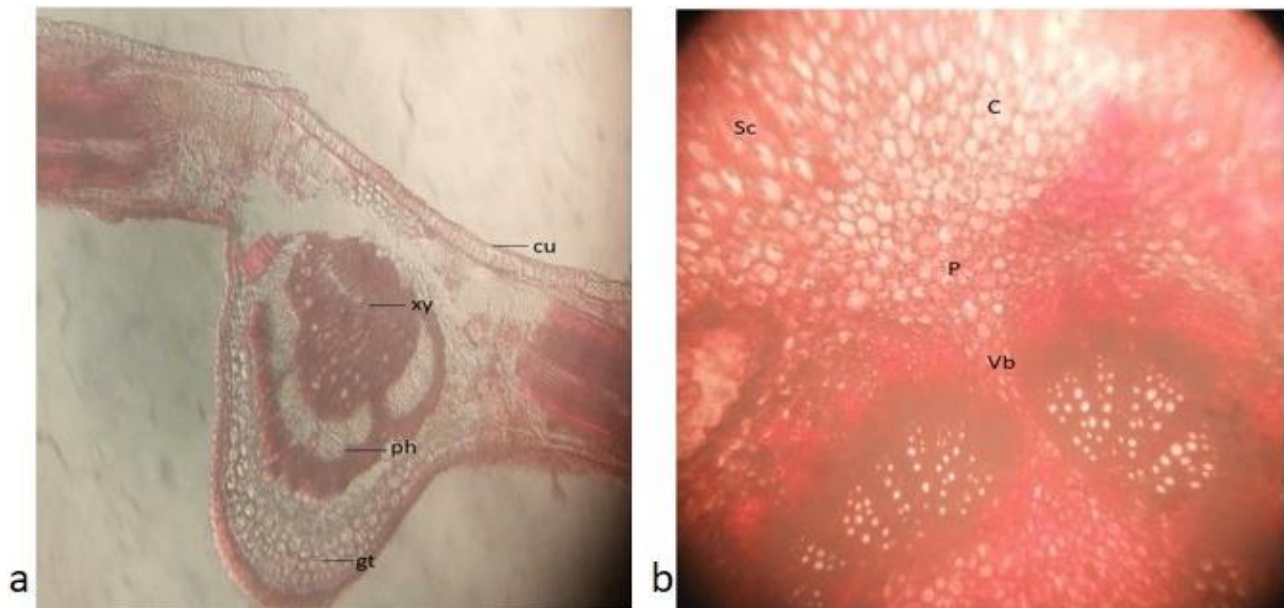


Figure 4: (a) Leaf Transverse section of *Uvaria chamae* x100 (b) Petiole transverse section x100 (S: stomata, E: epidermal cells, V: veins, cu: cuticle, xy: xylem, ph: phloem, gt: ground tissue, Vb: vascular bundle, P: parenchyma, C:collenchyma, Sc:sclerenchyma)

**Table 1: Foliar epidermal features of the adaxial and abaxial surfaces of *Uvaria chamae***

Character	adaxial surface	abaxial surface
Epidermal cell shape	Polygonal	Polygonal
Anticlinal wall	Straight	Straight
Stomata	Absent	Present
Stomata type	---	Paracytic
Trichome type	None	None
Crystal type	Calcium oxalate	Calcium oxalate
Palisade cells	Present	Absent
Palisade ratio	2.8 – 6.0	---
Epidermal cell size range (micrometer)	----	92 – 246

Fluorescence analysis is an important parameter in crude drug identification (Sumitra *et al.*, 2014). It is a valuable analytical tool appraised for its simplicity and rapidity. It's useful in the identification of authentic samples as well as the detection of adulterants

(Sonibare *et al.*, 2015). The fluorescent behaviours of different samples differ when treated with different chemicals and viewed at different wavelengths. The result of fluorescence analysis of *U. chamae* is shown in Table 2.

**Table 2: Summary of fluorescence studies of powdered sample of *Uvaria chamae***

Reagents	Daylight	254 nm	365 nm
Sample + Picric acid	Green	Bright Green	Green
Sample + 50% HCl	Green	Dull Green	Green
Sample + 50% H <sub>2</sub> SO <sub>4</sub>	Green	Green	Green
Sample + FeCl <sub>3</sub>	Green	Dark Green	Green
Sample + 50% HNO <sub>3</sub>	Reddish brown	Green	Green
Sample + Acetic acid	Green	Green	Green

Physicochemical parameters are useful in determining the purity, quantity and quality of herbal medicines. Moisture content determination serves as a guide to processing and storage of the crude drug (Annan *et al.*, 2013). Low moisture content leads to longer shelf life of drugs. The moisture content of *U. chamae* ( $7.59 \pm 1.24$ ) is indicative of low moisture content. This is slightly below limit for water content (8 to 14%) for

vegetable drugs (African Pharmacopoeia, 1985; Fatokun *et al.*, 2017). Extractive values are used to determine the number of active constituents in a defined amount of plant material when extracted with a particular solvent. It can also be used to determine exhausted drugs (Sumitra 2014). Table 3 shows the summary of the physicochemical properties of the plant.

**Table 3: Summary of Physicochemical properties of *Uvaria chamae***

Physicochemical property	<i>Uvaria chamae</i>
Water soluble extractive	5.67 ± 0.67
Alcohol soluble extractive	4.00 ± 0.00
Moisture content	7.59 ± 1.24
Crude fibre	8.86 ± 0.49

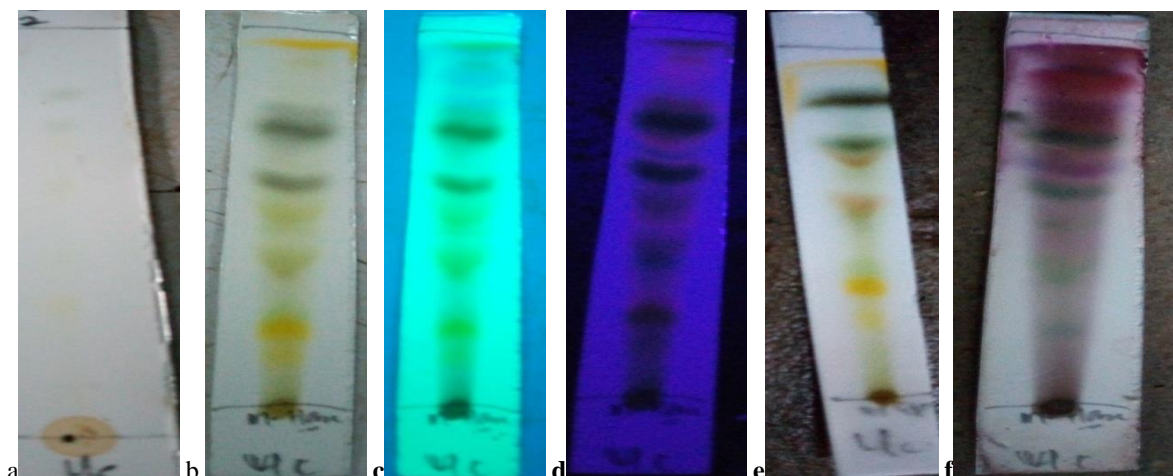
Chemomicroscopy of powdered samples of *U. chamae* showed the presence of starch, oil, lignin and tannins. Calcium oxalate crystals were present while cellulose was absent. Thin layer chromatography is very useful in the separation of compounds. Analytical TLC of the extracts on silica gel revealed the presence of certain prominent compounds, which could be used in the identification of the plant. The *n*-hexane fraction of *U. chamae* revealed up to eleven spots while the dichloromethane fraction showed six spots (Table 4).

These spots are indications of numerous compounds contained in the plant material, which can be linked to their pharmacological activity. The bright yellow colour suggests the presence of flavonoids in the plants when sprayed with AlCl<sub>3</sub> (Figure 5e). The *n*-hexane fraction in Table 4 showed green, purple, mauve and red colours when treated with vanillin in sulphuric acid which is suggestive of the presence of steroids, phenols, carbohydrates etc (Jork *et al.*, 1990).

**Table 4: R<sub>f</sub> values for the Dichloromethane fractions**

Spot position (cm)	R <sub>f</sub> value	Daylight	254nm	365 nm	AlCl <sub>3</sub>	Vanillin in sulphuric acid
2.0	0.40	LG	LeG	Ash	LG	---
2.4	0.48	AG	Grey	Grey	AG	---
2.7	0.54	---	LB	---	---	---
3.4	0.68	---	LB	---	---	---
3.7	0.74	Y	GY	---	Y	---
3.9	0.78	---	B	Grey	---	---

**Key:** UC *U.chamae*, R<sub>f</sub> retardation factor, --- not detected, A- Ash, GY greenish yellow, Y yellow, AG- army green, B blue, LG light green, LeG –lemon green, LB – Light blue.



**Figure 5:** (a) TLC profile of *U. chamae* crude extract in daylight, solvent system Hexane : Ethyl acetate in ratio 3:2. (b-f) TLC profile of *U. chamae* n-Hexane fraction. Solvent system Hexane : Ethyl acetate in ratio 4:1 ( b- Daylight, c- 254 nm, d- 365 nm, e-  $\text{AlCl}_3$ , f- Vanillin in  $\text{H}_2\text{SO}_4$  )

Preliminary phytochemical screening shows that *Uvaria chamae* contains a wide array of secondary metabolites as also observed in the TLC profile. Phenols and tannins, glycosides, terpenoids, coumarins, saponins, alkaloids and flavonoids were in abundance, Cardiac glycosides and Anthraquinones were moderately present, while steroids were

sparingly present. The presence of these phytochemicals is in line with that obtained by Borokini and Omotayo 2016. These phytoconstituents may be responsible for the biological activities of the plant. (Olumese *et al.*, 2016; Udoh *et al.*, 2019). The phytoconstituents observed in the leaves of *Uvaria chamae* are as shown in Table 5.

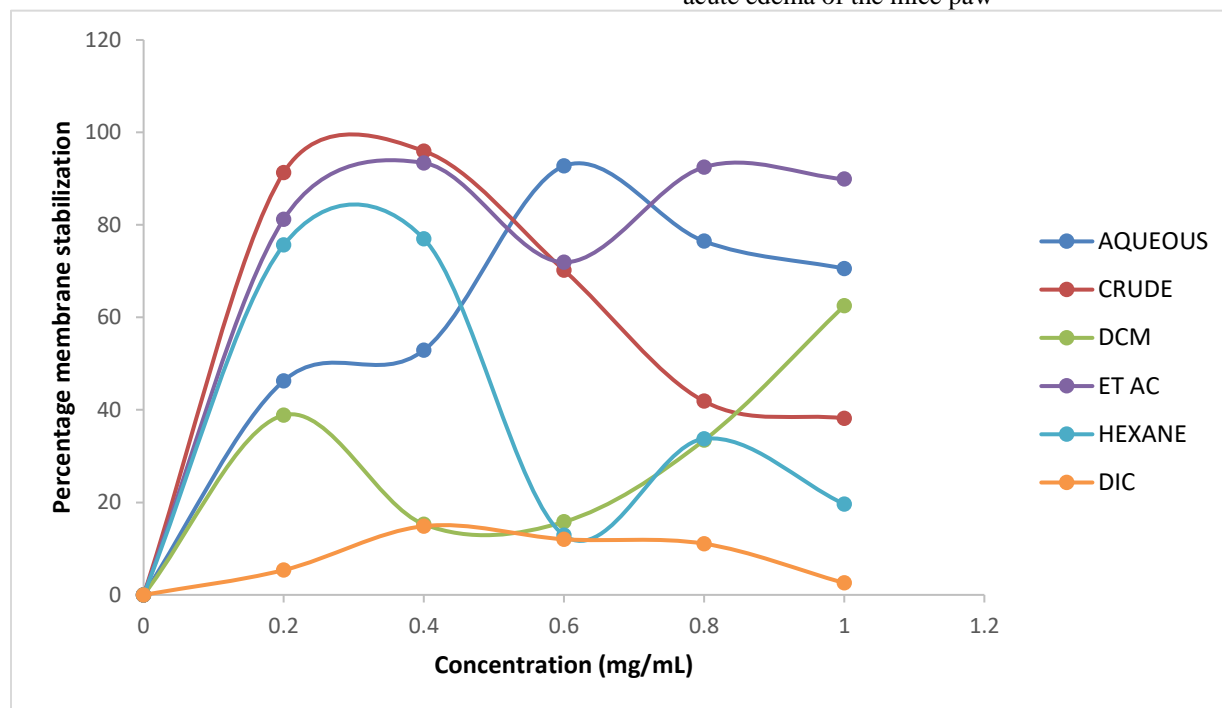
**Table 5: Phytochemical screening result of *Uvaria chamae* methanolic extracts.**

Test	Observation	Inference
Coumarins	Yellow colour	Present
Sterols/ steroids		
Salkowski's test	Red upper and yellow lower layer	Present
Liebermann-Burchard test	Deep green colour	Present
Terpenoids	Deep red colour	Present
Glycosides	Yellow colour	Present
Saponins ( foam test)	Frothing	Present
Cardiac glycosides		
Keller Killani test	Brown ring, bluish-green layer	Present
Kedde test	Violet colour	Present
Anthraquinones glucosides	Delicate rose – pink	Present
Flavonoids	Dark green colour	Present
Anthocyanins	Pinkish red	Absent
Phenols/ Tannins		
Ferric chloride test	Intense blue-black	Present
Alkaloids		
Mayer's test	Cream precipitate	Present
Dragendorff's test	Reddish- brown ppt.	Present
Wagner's test	Yellow precipitate	Present



Different models are employed for screening for the anti-inflammatory properties of drugs as drugs having anti-inflammatory activities exhibit their effects through different modes of action (Aina and Oyedapo, 2013). Membrane stabilization activity of red blood cells has been used by many researchers for the study of interaction of drugs with membranes (Oyedapo *et al.*, 2004; Sonibare *et al.*, 2015). It is a useful *in vitro* method for assessing the anti-inflammatory activity of different plants. The results in figure 6 shows that the extracts of the plant exhibited membrane stabilization effect in a non- dose dependent manner.

The membrane stabilization activity of the extracts at the concentration assessed in the study exceeded that of the standard drug, diclofenac. It is observed that the membrane stabilization effect for most of the extracts reduced at 1mg/mL concentration with the exception of the DCM fraction which had its maximum activity at that concentration (62.51%). The Ethyl acetate fraction showed good activity for all the doses used. The anti-inflammatory effects obtained is in line with the findings of Adelodun *et al.*, 2016 in which the leaves of *Uvaria chamae* was found to possess anti-inflammatory properties as the administration of the extracts profoundly suppressed the development of acute edema of the mice paw



**Figure 6: Plot of percentage membrane stabilization against different concentrations (mg/mL) of extracts of *U. chamae* (DCM- dichloromethane, EtOAC – ethyl acetate and DIC- diclofenac)**

**CONCLUSION**

This study presents useful diagnostic features of *Uvaria chamae* which can be incorporated into the plant's monograph.

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