



ISSN (E): 2320-3862

ISSN (P): 2394-0530

NAAS Rating: 3.53

www.plantsjournal.com

JMPS 2021; 9(3): 195-200

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Received: 01-03-2021

Accepted: 03-04-2021

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Comparative study on the levels of phytochemicals present in n-hexane, ethanol, aqueous, ethylacetate and methanol extracts of *Uvaria chamae* stem

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Abstract

A comparative phytochemical analysis was conducted to determine the levels of the phytochemicals present in n-hexane, ethanol, aqueous, ethylacetate and methanol extracts of *Uvaria chamae* stem. The qualitative phytochemical screening showed the presence of phenols, alkaloids, flavonoids, tannins, saponins, steroids, and glycosides in *U. chamae* stem. The result of the quantitative estimations of the concentrations of the various phytochemicals in the different solvent extracts recorded variations, with some showing higher concentration than others. Ethanolic extract of the plant showed significantly ($p < 0.05$) higher concentrations of saponins, ($602.00 \pm 1.00 \mu\text{g/ml}$) and phenols ($82.00 \pm 2.00 \mu\text{g/ml}$) than in other solvent extracts. The concentrations of alkaloids in ethanolic and aqueous extracts ($708.00 \pm 1.00 \mu\text{g/ml}$) were higher than in other extracts. Also, aqueous extracts showed significantly ($p < 0.05$) higher concentrations in flavonoids, ($0.82 \pm 0.02 \text{ mg/ml}$), glycosides ($10.30 \pm 0.10 \mu\text{M}$) and tannins ($0.19.00 \pm 0.15 \text{ mg/ml}$) than in other extracts. The concentrations of steroids were found to be significantly ($p < 0.05$) higher in the methanolic extract ($107.00 \pm 2.00 \mu\text{g/ml}$) than in other extracts. Therefore, the study showed that *Uvaria chamae* stem contains appreciable amounts of medicinally important phytochemicals, with solubilities (extraction powers) differing in different solvents. Hence, the various solvents with higher yield can be exploited to get higher concentrations of the required phytochemical.

Keywords: *Uvaria chamae*, phenols, alkaloids, flavonoids, tannins, saponins, steroids, glycosides

Introduction

Many plants have been scientifically analyzed to ascertain their possible medicinal importance. The therapeutic value of plants lies in some phytochemical constituents present in them that may be useful for healing of human diseases (Pradeepa *et al.*, 2014) [25]. Phytochemicals are secondary metabolites, which are naturally occurring in the leaves, vegetables, and roots that have defense mechanism and protect from various diseases. Primary metabolites are proteins, carbohydrates, chlorophyll, lipids and common sugars, which are synthesized during photosynthesis, and these organic compounds are essential for plant life, growth and development (Wadood *et al.*, 2013) [32]. Secondary metabolites are tannins, flavonoids, phenolics, saponins and alkaloids, which are synthesized by the plant during development and are time, tissue and organ specific (Linga Rao *et al.*, 2012) [15].

Screening of these phytoconstituents can be covered by nitrogenous compounds, acetogenins compounds, and isoprenoids compounds. These organic compounds allow easy transport across the cell membrane to induce different biological activities and physiological on the human body (Loizzo *et al.*, 2007) [16]. Nitrogenous compounds like alkaloids and amino acids which are critical to life, and have many functions in metabolism. These are commonly used in food technology and industry (Senthilkumar, 2013) [28].

Uvaria chamae belongs to the family Annonaceae (Irvine, 1961). It is a small tree that grows up to about 4.5 m high. It is commonly found in the savanna and rain forest regions of Nigeria and other African countries. It is called "Mmimi ohia", "Kas kaifi" and "Akisan" amongst the Ibos, Hausas and Yorubas respectively (Adetunji, 1999) [1]. The fruits are yellow when ripe and have a sweet pulp which is widely eaten. The fruit carpels are in finger-like cluster. The root barks, stem barks and leaves have a wide spread medicinal use. In Nigeria a decoction of the stem is used in the treatment of diarrhea (Igoli *et al.*, 2005) [10]. Personal interaction with traditional medicinal practitioners indicated that they use the various parts of the plant in the

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treatment of cough, various stomach problems and urinary tract infections (Okoko, 2011) [20].

Pharmaceutical research has found antifungal, bacteriostatic, antimalarial, and especially cytostatic capability of some chemical constituents of the leaves and bark of *Uvaria chamae* (Irobuchi, 2008). In folk medicine, extracts of the roots, barks and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, sore throats, inflamed gums and a number of other ailments (Okwu, 2007) [23].

Researches has proven that *U. chamae* is one of the medicinal plants used in the treatment of diseases due to the phytochemicals present in it. Since the stem of *U. chamae* contains some bioactive compounds or phytochemicals, there is need to determine the solvents that extract more of the individual phytochemical, which is necessary for commercial or research purposes.

Materials and Methods

Materials

Some of the materials used are: spectrophotometer, water bath, beakers, electronic weighing balance, mechanical grinder, filter paper, measuring cylinder, stop watch, brush, test tubes, separating funnel, conical flask, spatula, nose mask, foil paper.

Chemicals/Reagents

All chemicals used in this work were of analytical grade. They include: distilled water, sodium chloride (NaCl), Wagner's reagent, Mayer' reagent, Folin-Ciocalteu's phenol reagent, ferric chloride (FeCl₃), hydrochloric acid (HCl), sulphuric acid (H₂SO₄), ethanol, chloroform.

Collection and preparation of plant material

Stems of *Uvaria chamae* were collected from Umuka-Okposi Ohaozara L. G. A., Eboyi State, and was authentication by a botanist. It was dried under laboratory temperature, after which the dry stems were ground with a mechanical grinder, sieved and then stored in an air-tight container.

Methods

Sample preparation

The preparation of *Uvaria chamae* extracts was carried out using different solvents - ethanol, methanol, ethyl acetate, n-hexane and water. Exactly 20 grams of the powdered stem of *Uvaria chamae* in a beaker was soaked successively with 200 ml of each of the solvents (ethanol, ethyl acetate, methanol, n-hexane and water). Each of them was mixed well, covered with aluminum foil and allowed to stand for 48 hours at room temperature. The mixture was filtered using Whatman No. 1 filter paper to remove extractable substances. The liquid extracts (filtrates) were collected and stored at room temperature in air-tight sterile container. The filtrate was concentrated in rotary evaporator (45 °C - 50 °C) to about 1/10th of the original volume, after which the concentrate was allowed to evaporate to complete dryness in a water bath (45 - 50 °C) and used for phytochemical analysis.

The preliminary phytochemical for the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins, terpenoids and steroids were carried out on the extracts from the different solvents.

Qualitative phytochemical screening

Test for alkaloids

This was carried out by the method of Trease and Evans

(1989) [31].

Procedure: Exactly 2 ml of each extract was collected using syringe and dispensed into different labelled test tubes. The test tubes were heated for 2 minutes and 5 ml of hydrochloric acid (HCl) was added into each tube, mixed and heated again and allowed to cool. The mixture was divided into two groups, A and B in two different test tubes for each. To A, 2 drops of Meyer's reagent was added and white precipitate was observed which showed the presence of alkaloids. To B, 2 drops of Dragendroff' s reagent was added and the formation of red precipitate was observed which confirmed the presence of alkaloids.

Test for cardiac glycosides

This was carried out by the method of Harborne (1973) [9].

Procedure: Two milliliters (2 ml) of each extract was collected into test tubes and 5 ml of glacial acetic acid was added, followed by 2 ml of FeCl₃ and 2 ml of concentrated ferric acid. A brown ring formation at interphase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

Test for flavonoids

This was carried out by the method of Harborne (1973) [9].

Procedure: Five milliliters (5 ml) of the extracts was collected using syringe and dispensed into a test tube. Exactly 10 ml of distilled water, 5 ml of dilute ammonium (NH₄OH) and few drops of tetraoxosulphate (IV) acid (H₂SO₄) were added in the test tube. A yellow colouration was observed which showed the presence of flavonoids.

Test for phenols

This was carried out using the method of Harborne (1973) [9].

Procedure: Exactly 5 ml of each extract was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

Test for saponins

This was carried out by the method of Harborne (1973) [9].

Procedures: (a) Frothing test: To 3 ml of each extract was diluted with 2 ml of distilled water in a test tube. The mixture was shaken vigorously for about 5 minutes and was allowed to stand. A persistent frothing movement was observed which indicate the presence of saponins.

(b) Emulsion test: To 3 ml of each extract was added 5 drops of olive oil in a test tube and the mixture was vigorously shaken and allowed to stand for about 30 minutes. Observation of emulsification indicated the presence of saponins.

Test for tannins

This was carried out by the method of Harborne (1973) [9].

Procedure: One milliliter (1 ml) of each extract was collected using syringe and dispensed into a test tube. Then, 1 ml of (10 %) ferric chloride (FeCl₃) was added to the test tube. Dirty green precipitate was observed which showed the presence of tannins.

Test for terpenoids

This was carried out using the method of Harborne (1973) [9].

Procedure: To 2 ml of each extract in a test tube were added 2 ml of acetic anhydride and 2 ml of concentrated H₂SO₄. A change in color from pink to violet (blue - green ring) shows the presence of terpenoids.

Test for steroids

This was carried out using the method of Trease and Evans (1989) [31].

Procedure: In 0.2 ml of each extract, 2 ml of acetic acid was added, and the solution was cooled well in ice followed by the addition of concentrated H₂SO₄ carefully. Color development from violet to blue or bluish green indicated the presence of a steroidal ring, i.e., aglycone portion of cardiac glycoside.

Quantitative phytochemical analysis**Estimation of alkaloids**

A method used by Madhu *et al.* (2016) [17] was used to quantify the alkaloid. To 1ml of each extract, 5 ml pH 4.7 phosphate buffer was added and 5 ml BCG solution and the mixture shaken with 4 ml of chloroform. The extract was collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with atropine equivalents.

Estimation of glycosides content

Into a test tube was added 1 ml of each extract and 2 ml of DNS reagent (1 g DNS dissolved in 100 ml distilled water). The test tube was put in a beaker of boiling water and boiled for 5 minutes. The test tube was cooled in cold water and 10 ml of distilled water was added. The absorbance was read at 540 nm using a spectrophotometer.

Estimation of total flavonoids content by Aluminium chloride method

Total flavonoids content (TFC) was estimated spectrophotometrically as proposed by Zhishen *et al.* (1999) [33] with slight modification. To 0.1 ml of each extract, distilled water was added to make the volume up to 5 ml. To this was added 0.3 ml of 5% NaNO₂ and 3 ml of 10% AlCl₃, after 5 minutes later. After 6 minutes, 2 ml of 1 M NaOH was added and the absorbance was measured at 510 nm. Rutin was used as standard for constructing a calibration curve.

Estimation of total phenolic content (TPC) by Folin-Ciocalteu method

A method proposed by Singleton and Rossi (1965) [29] was

adopted to determine the total phenolic content (TPC). To 0.1 ml of each extract was mixed with 0.5 ml of Folin-Ciocalteu reagent in a test tube and incubated at room temperature for 3 minutes. Into the tube was added 2 ml of 20% sodium carbonate and kept in a boiling water bath for 1 minute. The blue colour formed was read at 650 nm. Gallic acid was used as a standard for constructing a calibration curve.

Estimation of saponins

A method used by Madhu *et al.* (2016) [17] was used to quantify saponins. Exactly 1 mg of each extract was dissolved in 10 ml of 80% methanol, 2 ml of Vanilin in ethanol was added, and mixed well. Then 2 ml of 72% sulphuric acid solution was added, mixed well and heated in a water bath at 60 °C for 10 minutes. The absorbance was measured at 544 nm against reagent blank with a spectrophotometer. Diosgenin was used as the standard absorbance curve.

Estimation of tannins content by modified prussian blue method

A method proposed by Graham (1992) [6] was used to quantify the tannins. To about 0.1 ml of each extract, 6.9 ml of distilled water was added, followed by 1 ml of 0.008 M potassium ferric cyanide, 1 ml of 0.2 M ferric chloride in 0.1 M HCl, and mixed well. The blue colour formed was read at 700 nm. Tannic acid was used as a standard for constructing a calibration curve.

Estimation of sterols by Liebermann-Burchard method

Total sterol content was measured spectrophotometrically by Liebermann-Burchard method. To 1.0 ml of each extract, chloroform was added to make the volume up to 5 ml in a test tube. To this, 2 ml of Liberman-Burchard reagent (0.5 ml of concentrated sulphuric acid in 10 ml acetic anhydride) was added and mixed well. The green colour complex formed was measured spectrophotometrically at 640 nm. Cholesterol was used as standard for constructing a calibration curve.

Statistical analysis

The data obtained was analyzed by one-way ANOVA using the SPSS Statistical package version 20.0. All data was expressed as mean ± SD of triplicates (n = 3) and hypothesis was tested at 95% level of significance.

Results**Qualitative phytochemical analyses**

The results of the qualitative phytochemical analyses of different extracts *Uvaria chamae* stem are presented in Tables 1. The result showed that each extract of *Uvaria chamae* stem contains alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins and steroids, while terpenoids were absent.

Table 1: Result of qualitative phytochemical analysis of *Uvaria chamae* stem

Phytochemicals	n-Hexane	Ethanol	Water	Ethylacetate	Ethanol
Alkaloids	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Flavonoids	+	+	+	+	+
Phenols	+	+	+	+	+
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Steroids	+	+	+	+	+
Terpenoids	-	-	-	-	-

Key: + = Present, - = Absent

Quantitative phytochemical analyses

Table 2 shows the results of the quantitative analyses of different extracts of *Uvaria chamae* stem.

Alkaloids

There was a significant difference ($P < 0.05$) in the concentrations of alkaloids in different solvent extracts of *Uvaria chamae* stem with highest concentrations in ethanolic and aqueous extracts ($708.00 \pm 1.00 \mu\text{g/ml}$). Next were ethylacetate and methanolic extracts ($700.00 \pm 1.00 \mu\text{g/ml}$), while n-hexane extract ($670.00 \pm 1.00 \mu\text{g/ml}$) had the lowest concentration.

Saponins

Ethanolic extract had the highest concentration of saponins ($602.00 \pm 1.00 \mu\text{g/ml}$), followed by ethyl acetate and methanol extracts. Next was the aqueous extract, while n-hexane extract had the lowest concentration.

Tannins

The concentration of tannins was found to be highest in the aqueous extract of *Uvaria chamae* stem ($0.19.00 \pm 0.15 \text{ mg/ml}$), followed by ethanolic and methanolic extracts; while n-hexane and ethylacetate had the lowest concentrations respectively.

Phenols

There was highest concentration of phenols in the ethanolic extract ($82.00 \pm 2.00 \mu\text{g/ml}$), followed by the methanolic, ethylacetate, n-hexane and aqueous extracts respectively.

Flavonoids

Flavonoids concentrations in different solvent extracts of *Uvaria chamae* stem showed highest concentration in the aqueous extract ($0.82 \pm 0.02 \text{ mg/ml}$), followed by n-hexane and ethanolic extracts that showed no significant difference ($P < 0.05$); whereas methanol and ethylacetate had lower concentrations. However, all the solvents had high extraction power for flavonoids.

Glycosides

Aqueous extract of *Uvaria chamae* stem contained the highest level of glycosides ($10.30 \pm 0.10 \mu\text{M}$), followed by ethylacetate, ethanol, n-hexane and methanol respectively.

Steroids

The highest concentrations of steroids were found in the methanolic ($107.00 \pm 2.00 \mu\text{g/ml}$) and aqueous ($102.67 \pm 2.00 \mu\text{g/ml}$) extracts, followed by ethanolic, n-hexane and ethylacetate extracts of *Uvaria chamae* stem.

Table 2: Result of quantitative phytochemical analysis of *Uvaria chamae* stem

Phytochemical	n-Hexane	Ethanol	Water	Ethylacetate	Methanol
Alkaloids ($\mu\text{g/ml}$)	670.00 ± 1.00	708.00 ± 1.00	708.00 ± 1.00	700.00 ± 1.00	700.00 ± 1.00
Saponins ($\mu\text{g/ml}$)	103.33 ± 1.00	602.00 ± 1.00	406.00 ± 1.00	508.00 ± 1.00	508.00 ± 1.00
Tannins (mg/ml)	0.02 ± 0.10	0.05 ± 0.10	0.19 ± 0.15	0.02 ± 0.10	0.04 ± 0.10
Phenols ($\mu\text{g/ml}$)	57.33 ± 1.00	82.00 ± 2.00	37.00 ± 1.00	64.00 ± 1.00	70.00 ± 2.00
Flavonoids (mg/ml)	0.78 ± 0.02	0.78 ± 0.02	0.82 ± 0.02	0.72 ± 0.01	0.75 ± 0.02
Glycosides (μM)	8.23 ± 0.10	9.13 ± 0.10	10.30 ± 0.10	9.73 ± 0.10	4.37 ± 0.10
Steroids ($\mu\text{g/ml}$)	54.00 ± 1.00	66.00 ± 1.00	102.67 ± 2.00	54.00 ± 1.00	107.00 ± 2.00

Discussion

The results of the qualitative phytochemical screening on the n-hexane, ethanol, aqueous, ethylacetate and methanol extracts of the stem of *Uvaria chamae*, revealed the presence of medicinally active secondary metabolites (phytochemicals) such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides and sterols. These phytochemical constituents have been reported to have varying degrees of pharmacological activities (Badam *et al.*, 2002; Gupta and Tandon, 2004) [2, 8]. The antifungal and antibacterial inhibitory properties of *U. chamae* have been reported (Irvine, 1961).

The quantitative estimation of the phytochemical composition of *U. chamae* on the different solvent extracts showed disparities in the concentrations of saponins, tannins, alkaloids, flavonoids, phenols, glycosides and sterols. The alkaloids content of *Uvaria chamae* was extracted most by water and ethanol. Hence, the aqueous and ethanolic extracts contain the highest concentration of alkaloids compared to other extracts. Alkaloids are known to exhibit marked physiological activity when administered to animals (Okwu, 2004) [21]. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for analgesic, antispasmodic and bactericidal effects (Stray, 1998) [30].

Also, the concentration of flavonoids in *U. chamae* stem in the different solvent extracts was found to be highest in the aqueous extract, followed by ethanol and n-hexane. The presence of flavonoids in the stem of *U. chamae* indicates that the plant has medicinal value. Flavonoids are antioxidants, which are free radical scavengers that prevent oxidative cell

damage. They have strong anticancer activity and protect the cells against all stages of carcinogenesis (Okwu, 2004) [21]. Flavonoids in intestinal tract lower the risk of heart disease (Okwu, 2005) [22]. Flavonoids reduce the risk of estrogen-induced cancers by interfering with the enzyme that binds estrogen to receptors in several organs. Flavonoids significantly inhibit lysosomal enzyme secretion and arachidonic acid release from membrane by inhibiting lipoyxygenase, cyclooxygenase and phospholipase A₂ (Del-rio *et al.*, 1977) [4].

It was observed that the concentration of phenols was highest in the ethanol extract. It showed that ethanol has higher affinity for phenols than other solvents used. The presence of phenolic compounds in the stem of *U. chamae* indicated that the plant may have antimicrobial activity. Phenols and phenolic compounds are extensively used in disinfection and remain the standard with which other bactericides are compared (Osugwu *et al.*, 2007) [24].

The tannins concentration was found to be highest in the aqueous extract. This showed that water has higher affinity for tannin in *U. chamae* compared to other solvents used. Tannins have been reported to possess astringent properties, hasten the healing of wound and inflamed mucous membranes (Okwu, 2004) [21]. Tannins are also used in the treatment of wounds emanating from varicose ulcer and haemorrhoids (Nguyi, 1988) [18] and to stop bleeding during circumcision (Joshua, 2006) [14]. In addition, tannins have soothing relief, help to regenerate the skin, possess anti-inflammatory and anti-diuretic properties (Okwu and Okwu, 2004) [21]. Tannins

are complex phenolic polymers which can bind to proteins and carbohydrates resulting in reduction in digestibility of these macromolecules and thus inhibition of microbial growth (Nwogu *et al.*, 2008) ^[19]. The oxidation inhibiting activities of tannins have been known for a long time and it is assumed to be due to the presence of gallic and diagallic acids (Ihekoronye and Ngoddy, 1985) ^[11]. Tannins from the part of the plant bark, root, stem and other parts of many plants especially *Euphorbiaceae* are used to treat cells that have gone neoplastic (Duke and Wain, 1981) ^[5].

The saponins concentration was found to be highest in the ethanol extract; showing that ethanol has higher affinity for saponins than the other solvents used in this study. Saponins are usually detected by their ability to foam persistently and to hemolyze blood cells (Trease and Evans, 1989) ^[31]. It has been discovered that saponins and other flavonoid compounds at low concentrations inhibited the growth of micro-organisms and they can also act as bactericidal agents at higher concentrations by coagulating protoplasm of the organism, and researches have recently shown that consumption of saponins by human beings may be beneficial in reducing heart disease (by binding of saponins with plasma membrane and cholesterol). Saponins in *Quillaga saponaria* extract are used as foaming agent in carbonated beverages and cosmetics, as emulsification in preparations containing lipophilic colour or flavours, as preservatives, and for removal of dietary cholesterol (Guclu-Ustundag and Mazza, 2007; San Martin and Briones, 1999) ^[7, 27]. Likewise, Licorice saponin extract are used as flavor modifiers in baked foods, chewing gum, beverages, candies, herbs, seasonings and dietary supplements (Guclu-Ustundag and Mazza, 2007) ^[7].

Steroids showed highest level in the methanol extract, indicating that methanol extracted more steroids than other solvents. Steroids have been reported as the main treatment of inflammatory conditions, such as systemic vasculitis (inflammation of blood vessels) and myositis (inflammation of muscle) (Rhen and Cidlowski, 2005) ^[26]. It is a known fact that steroids are components of cell membrane which influence membrane fluidity. Some steroids function as signaling molecules.

The concentration of glycosides was found to be highest in aqueous extract among the different solvent extracts of the *U. chamae* stem. Hence, glycosides are more soluble in water than other solvents used in this study. Brian *et al.* (1985) ^[3] reported that the clinical effects of glycosides in cases of congestive heart failure are to increase the force of myocardial contraction. They exert their hypotensive effect by inhibiting Na⁺-K⁺ ATPase.

Conclusion

The study showed that *Uvaria chamae* stem contained appreciable amounts of different phytochemicals with varying concentrations in different solvent extracts. Amongst the solvents used, aqueous, ethanolic and methanolic extracts showed higher concentrations of the phytochemicals, indicating their higher affinity and better extraction of the phytochemicals. Also, our study affirms that *Uvaria chamae* stem could serve medicinal purposes due to the presence of these phytochemicals in it.

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