PRINT ISSN 1119-8362
Electronic ISSN 2659-1499
Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja
J. Appl. Sci. Environ. Manage.
Vol. 28 (10) 3071-3077 October, 2024

Nutraceutical Assessment and Amino Acids Profile of Leaves of Medicinal Plant: *Uvaria chamae* (Bush Banana)

[1]ORHU, A; *[2]KWEKI, GR; [3]KADIRI, HE; [3]ASAGBA, SO

[1]*Department of Science Laboratory Technology, Delta State Maritime Polytechnic, Brutu, Delta State, Nigeria*
*[2]*Department of Medical Biochemistry, Delta State University, P.M.B. 1, Abraka, Delta State, Nigeria*
[3] *Department of Biochemistry, Delta State University, P.M.B. 1, Abraka, Delta State, Nigeria*

**Corresponding Author Email: kweki.godfrey@delsu.edu.ng*
**ORCID: http://orcid.org/0000000177620361*
**Tel: +2347034331346*

Co-Authors Email: orhuanthony1212@gmail.com; soasagba@delsu.edu.ng; kadiri@delsu.edu.ng

ABSTRACT: The availability of good and quality nutrition from plant source still remain one of the key assets in mitigating the global challenge of food insecurity. The readily abundance plants nutrient enable the adequate and sufficient supply of the nutritional needs for human growth and development. This study aimed at exploring essential nutritional composition of *Uvaria chamae* leaves. Evaluation of the proximate composition that depicts the fibre composition was highest, while the least was ash. Micro-nutrients (selenium, iron, manganese and copper) and micro nutrient (calcium potassium, sodium and magnesium) were detected in appreciable concentration. The determination Antioxidant vitamins of A, C and E depicted that vitamin C had the highest concentration. High liquid performance chromatograph Amino acid profiling of the leaves showed the detection of thirteen amino acids with aspartic acid obtaining the highest concentration while the least is histidine. Findings from this study portray the nutraceutical potentials of the leaves of *U. chamae* in providing supplementary nutritional needs for animal and human needs.

DOI: https://dx.doi.org/10.4314/jasem.v28i10.16

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Cite this Article as: ORHU, A; KWEKI, G. R; KADIRI, H. E; ASAGBA, S. O. (2024). Nutraceutical assessment and amino acid profile of leaves of medicinal plant: *Uvaria chamae* (Bush Banana). *J. Appl. Sci. Environ. Manage.* 28 (10) 3071-3077

Dates: Received: 13 August 2024; Revised: 14 September 2024; Accepted: 29 August 2024 Published: 05 October 2024

Keywords: *Uvaria chamae*; Amino acids; Minerals; Vitamins; nutrition

Leaves and foliage provide new frontier that could cater for nutritional deficits and combating global food insecurity. Poor nutrition as arising from food insecurity consequentially bedeviled the society with malnutrition and starvation (Gödecke *et al*., 2018). Nutrition challenges pose a threat to human existence and economy of the world at large, as it a basic necessitate for survival and better health state. *Uvaria chamae* is ascandent shrub and evergreen plant of about 4.5m high evenly across the rainforest of west and central Africa (Olumese *et al*., 2019). It belongs to the family of *Annonaceae*. Roots of the plant are applicable for a variety of purposes around the world, mainly in traditional medicine (Daï *et al*., 2023). The common name bush banana was begotten from the orientation of the fruit carpels which are finger-like cluster and the edible pulp fruits are yellow in nature when ripe (Olumese *et al*., 2019). Plants are economical sources of supplementary food for humans apart from ethnomedicinal relevance. Forest edible plants play a crucial role in the diets, cultural life and economy of communities in sub-Saharan Africa (Kidane and Kejela, 2021). The nutritional facts of plants that are commonly used as “famine foods” when there is severe shortage are scarcely found in literature. There are several food plants that grow spontaneously and are consumed by humans in diverse regions of the world. The plant provides a large

**Corresponding Author Email: kweki.godfrey@delsu.edu.ng*
**ORCID: 0000000177620361*
**Tel: +2347034331346*

number of proteins, vitamins minerals and carbohydrate components of the average diet consumed in most countries (Singh *et al*., 2022). In underdeveloped economies, the accessibility and utilization of plant foodstuffs are insufficient because of flare-ups in population and urbanization. This can lead to food scarcity and consequently, malnutrition among the populace. The challenges can be eased by looking for cheaper sources of nutrients. With growing interest in alternative sources of food, the hidden potentials of underutilized plants that dominate the forest of West African sub-region can be harnessed, but it is still somehow ignored. Recently, forest plants have received attention as new food resources due to their ability to resist adverse environmental conditions and promising satisfying nutritional characteristics. The use of forest plants as nutrients can also be limited by the presence of ant-inutrients and toxic substances (Jikah and Edo, 2023). To assess the nutritional significance of these plants, proximate and critical nutritional experimentation plays a crucial role. Since a lot of food materials are from plant species, it will be beneficial to evaluate their nutritional significance since it will assist in knowing the value of these plant species (Srivastava *et al*., 2023) World Health Organization (WHO) has laid emphasis on the need to assess the proximate vitamins and micronutrients composition of plants that are also used as medicine. (Ibok *et al*., 2023). *U. chamae* is an underutilized shrub plant. It is a notable member of the Annonaceae family common in the Niger Delta region of Nigeria. The plant is widely used in traditional medicine across different cultures globally. *U. chamae*is known in various communities with different names, the Urhobo ethnic call it ebeakpata (Kweki *et al*., 2024). The fruits of the plant are considered a favourable source of food for consumption during extreme famine amongst the Efik/Ibibio, Igbo, Hausa and Yoruba. Given the vast range of uses of *U. chamae* around the world mostly in traditional medicine, there is a need for proper evaluation of the nutritional content of plant leaves as attention and demand for alternative food from plant sources increases. Thus, the aim of this study is to evaluate the nutraceutical potentials of *U. chamae* leaves.

MATERIALS AND METHODS

Plant collection: Fresh leaves of *U. chamae* were collected from the bush of Urhovie Abraka (Latitude: 5° 47'21.9552" N, Longitude: 6° 6' 8.4492" E) in L.G.A in Delta State, Nigeria in the month of February 2022. The botanical identification and authentication were done in the Plant Biology and Biotechnology Department of the University of Benin by Prof. Akinnibosun Henry Adewale. The voucher specimen was assigned specimen number UBH-U353 and deposited at the plant biology and biotechnology department of the University of Benin herbarium.

Preparation of extract: Fresh Leaves of the sample plant, were thoroughly washed in clean water to remove debris and air-dried at 25^0C until a constant weight was achieved. The dried leaves were pulverized to powder using a manual grinder. Cold maceration for 72 hours was used to extract 100g of coarsely crushed leaves with 500ml distilled water. The filtrate was obtained from a double filtration step of using a cheese cloth and then followed by a Whatman No.1 filter paper. The extract was concentrated under a rotary evaporator and residue was kept in dark glass sample bottles for further use.

Proximate analysis: Proximate composition was determined in accordance with AOAC, 1980. Carbohydrates, crude fibre, protein, fat, moisture and ash were determined. Percent (%) carbohydrate was determined by subtracting the obtained fibre, protein, fat, moisture and ash. The percent (%) organic matter was computed by subtracting percent (%) moisture from one hundred (100).

Amino acid profile:

The Salt/Alkaline Extraction Method of Protein: This was carried using the method of Mæhre *et al*. (2018). An alkalis hydrolysis of 0.5g of the sample with 30ml of 0.1M of sodium hydroxide constituted in 3.5w/v% sodium chloride. This was following by an incubation treatment of the homogenate for 90 min at 60^0C. Supernatants via centrifugation of the treated homogenate at 4000× *g* for 30min at low temperature. Further treatment of freezing the supernants at -18^0C ensued before hydrolysis.

Peptide hydrolysation using mixture of trifluoroacetic acid and hydrochloric acid: 40µl of peptide or protein was dissolved in 1,600µl of a mixture of 2 vol conc. HCl and 1 vol. Trifluoroacetic acid (CF_3CO_2H), containing 0.005 % (by volume) freshly distilled phenol (2:1) in a tube. The tube was closed and placed in an oven, the temperature of which is regulated at 170^oC. Hydrolysis time was 60 min. After cooling down, the acid was removed on a steam bath at 65°C. The hydrolysate was dissolved in 2ml mobile phase and filtered with 0.45µm nylon syringe filter into 2ml vial for High-Performance Liquid Chromatography (HPLC) analysis (Tsugita and Scheffler, 1982).

Retinol content analysis: With slight modification the extraction of fat-soluble compounds was carried as described by Melfi *et al*. (2018). 0.5g of the pulverized sample was subjected to a defatting step using 17ml of a mixture of acetone:methanol (2:1 v/v), followed by the addition of 6ml of hexane containing 0.5% BHT (w/v). Under an ice water and a dark condition a sonication a process was carried out for 18mins. To the sonication mixture 16ml of cold 1M NaCl was added and centrifuged at 1600rpm for 10min. Upon centrifugation the supernant was recover, while the residue was constituted in 5mlof the saline solution and subjected to filtration using a a 0.45-µm Teflon membrane. The final filtrate was then subjected to HPLC analysis.

Ascorbic acid content analysis: Five grams (5g) of the leaves blended and 25ml of the extracting solvent (0.3M metaphosphoric acid/ 1.4M acetic acid, 50:50) was added. It was Sonicated for 15min at about 4°C protected from light and centrifuged at 12.000× g for 8min at 4°C.The supernatant was separated and diluted 1ml of it with 5ml of the mobile phase. Which was then Centrifuged and an appropriate amount was placed in the sample vial.

RESULTS AND DISCUSSION

The Proximate analysis of *U. chamae* leaves depict moisture (32.5%), ash (1.61%), crude fibre (30.32%), crude protein (23.30%), crude fat (4.41%) and carbohydrate (7.79%), as shown in Figure 1.The protein, moisture and fibre composition obtained the highest content noticed in the proximate analysis of *U. chamae* leaves. The high fiber content potentiate the biological relevance of the leaves as a good dietary fiber source, useful for digestive health, and can help regulate blood sugar levels (Gill *et al*., 2021). Roughage moderates the bulk and scope of stool it. The dietary fiber intake advised by WHO is 22–23 kg/1000 Kcal of diet. Moisture content indicates the leaves can be easily dried and stored for later use, but a high moisture content candidate the leaves suitable for preservation. Green leaves vegetable are origin of plant-based protein, which are beneficial for overall nutrition and muscle development, while the availability of Proteins in food substance can be impactful in cell replication and restore damaged cells. Consequently, *U. chamae* leaves can also be of nutritional for growth and development in children, teens, and pregnant women (Yang *et al*., 2023). The low carbohydrate value of the leaves, demonstrate it significant as a good source of dietary regulation for carbohydrate disorders since is suitable as low-crab diet (Pavilodu *et al*., 2023). In under developed nations, consuming green leaves vegetables regularly is strongly advised to combat malnutrition brought on by poverty and lower the risk of acquiring chronic ailments (Knez *et al*., 2023; Awuchi *et al*., 2020). Consumption of plant-formulated diets has also shown endless effects on blood pH. They also help our body detoxified unwanted metals and contaminants proceeds in diets (Onyeaka *et al*., 2024).

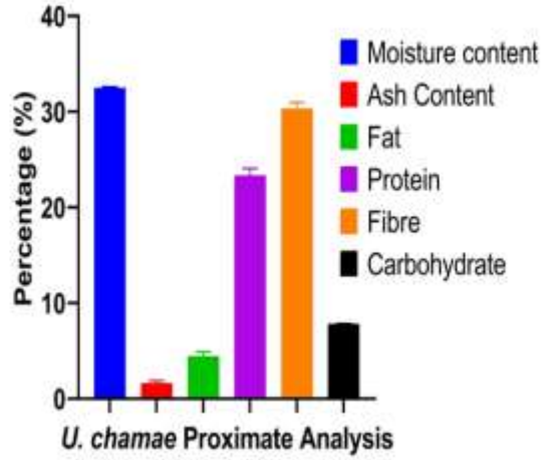

Fig 1: Proximate composition of leaves *U. chamae*

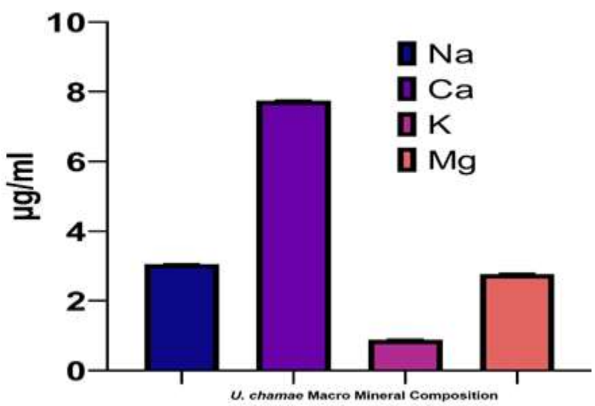

Fig 2: Macro mineral composition of *U. chamae* leaves

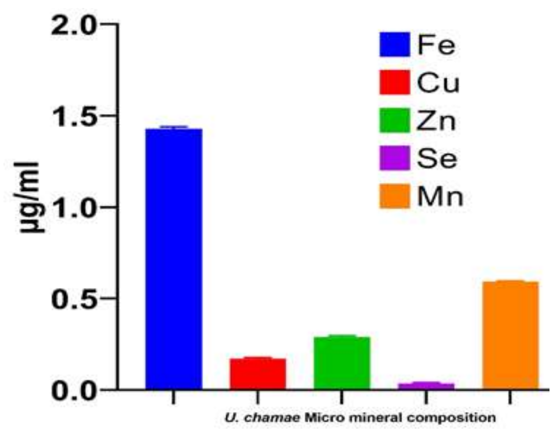

Fig 3: Micro mineral composition of *U. chamae* leaves

However, the minerals concentration in the leaves is presented in fig 2 and 3; sodium, calcium, potassium, magnesium, iron, copper, zinc, selenium, and manganese were observed. Selenium and potassium pose the lowest concentrations while iron and calcium constitute the highest mineral concentration of the leave. Our present study discovered the relevance of *U. chamae* leave as a good dietary source of iron and calcium. The protein molecules of hemoglobin and myoglobin which are essential in oxygen transport in the blood and muscles require iron for their biosynthesis (Günther 2023, Saboor *et al*., 2021) though high iron content may pose a direct threat to human health if consumed in excess. Over consumption of iron dietary sources can lead to gastrointestinal issues, organ damage, and even death in severe cases therefore regulated consumption is usually advised in accordance with quantification on WHO daily iron intake equivalent to facilitate adequate and beneficial health functions and checkmate any possible adverse impact on consumers. Calcium is crucial for bone development and maintenance, as well as cholesterol reduction in humans (Suzuki *et al*., 2020). Calcium supplements lowers LDL cholesterol and total cholesterol levels and potentially reducing the risk of heart disease. This is possible by the binding of calcium to bile acids and fatty acids in the digestive system, preventing absorption and consequently the excretion fatty acids from the body, however, leafy green vegetables are the best sources of calcium (Sharma *et al*., 2021).

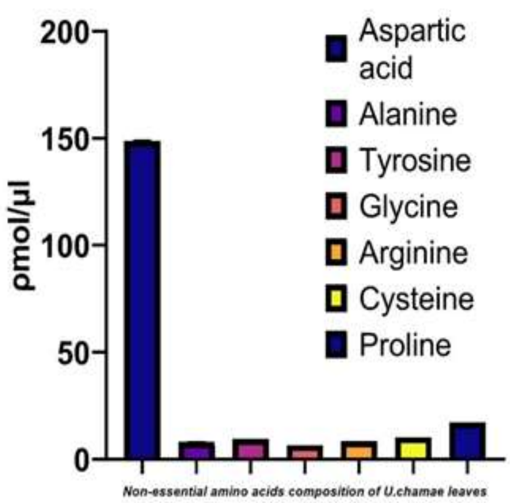

Fig 4: Non-essential amino acids composition of *U. chamae* leaves

The present results indicate that the leaves of *U. chamae* are richer in essential amino acids compared to non-essential amino acids. One nutritional attributes of some amino acids is the inability of the human cell to synthesis them *in-vivo* thus they are classified as essential amino acids. Their enhancement and supply can only be gotten via diet supplementation.

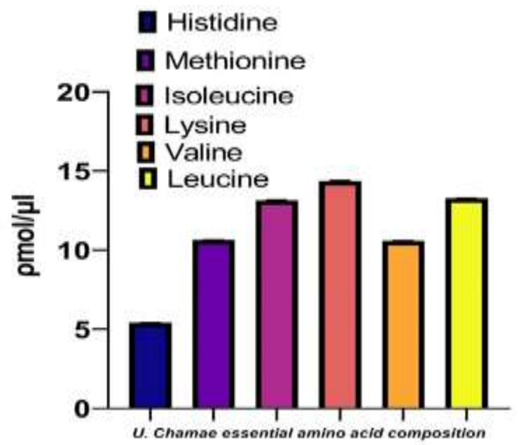

Fig 5: Essential amino acids composition of *U. chamae* leaves

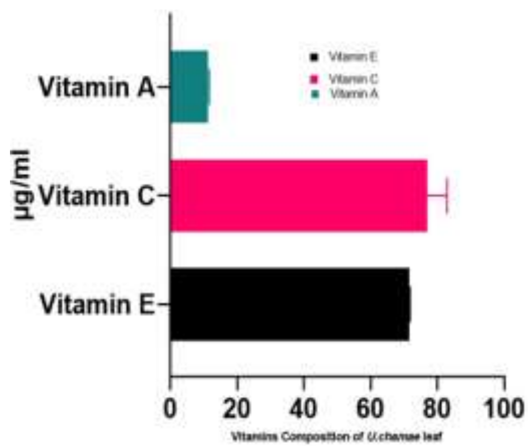

Fig 6: Vitamins Composition of *U. chamae* leaves

They are critical for various physiological processes, such as tissue repair, muscle growth, and immune function. The abundance of these amino acids in *U. chamae* leaves further affirms the nutritional value of the leave for food supplement. (Kelly and Pearce, 2020). The rich therapeutic benefits of essential amino acids presence in *U. chamae* leaves includes anti-hyperprolactinemic activities and neuroprotective effects (Kweki *et al*., 2024). The trace concentration of non-essential amino acids in *U. chamae* leaves suggests its well-balanced amino acid profile, which is imperative for optimal protein utilization and overall nutritional balance, a major perquisite when choosing alternative dietary source (Yakubu and Fayemo, 2021).

The essential amino acids of lysine, leucine and isoleucine and non-essential amino acids of aspartic acids were detected appreciably in high concentrations. These amino acids have important biochemical and nutritional relevance. High concentration of Isoleucine in *U. chamae* suggests it crucial role in glucogenic and ketogenic for energy production, hemoglobin formation, immune function, and regulating blood sugar levels (Liu *et al*., 2021). Aspartic acid is a non-essential amino acid that serves as a precursor for several important biomolecules, like nucleic acids (DNA and RNA), certain neurotransmitters, and various other amino acids. It can also be involved in energy production through the citric acid cycle (Holeček, 2023) while leucine is the only dietary amino acid reported to directly stimulate muscle protein synthesis (Yoshimura *et al*., 2019).

U. chamae, is a plant that is rich in various phytochemicals (Kweki *et al*., 2024), including vitamins C and E. Previous findings of kweki *et al*. 2024 indicate that the leaves of this plant have high antioxidant activity. Water soluble vitamin C posse the antioxidant attributes of annealing the activities reacting oxygen species and maintaining the cell integrity from oxidative crisis (Hastuti and Lukito, 2022). The high vitamin C content *in U. chamae* leaves suggests that consuming this plant as green vegetable could help boost the immune system and prevent bacterial infections, plays a role in collagen synthesis, wound healing, and iron absorption (Irina *et al*., 2024).

Fat soluble vitamin E, whose chemical nature favorably ensures the maintenance of membrane integrity via the inhibition of lipids oxidation at cellular level of organization, the biochemical implications of the high vitamin C and E concentrations in *U. chamae* leaves are related to their ability to scavenge free radicals, reduce oxidative stress, and modulate various cellular processes. This could have potential benefits in the management of diseases related to oxidative stress, such as inflammation, cardiovascular disease, and certain types of cancer (Torquato *et al*., 2020).). Research have shown that the stem and roots of *U. chamae* convey antioxidants that improve bone health (Mangels, 2014), reduce the risk of certain malignancies (Anand *et al*., 2008), treat obesity (Barnard *et al*., 2015), and lessen oxidative and inflammatory-induced diseases (Kweki *et al*., 2024). These good attributes can also be present in leaves as core value but has not be exploit and may be credentials to the adoption of these leaves as vegetables. *U. chamae* is an evergreen shrub documented nobly for antioxidant, antimicrobial, antifungal, antitumor, anti-glycemia anti lipidemia and neuroprotection (Kweki *et al*., 2024).

Human health and nutritional condition are largely determined by diet, which is a fundamental human need. Individual differences in financial status and religious convictions influence dietary choices. Vegetarian cuisine has been a staple of many ethnic and religious communities for many millennia (Chouraqui *et al*., 2021). Vegetables, fruits, whole grains, legumes, nuts, seeds, etc. are all part of a vegetarian diet. Vegetables are plant components that may be eaten raw, cooked, or added to salads. Examples of these parts include flowers, leaves, stems, and roots. They are considered staple foods because of their great range of colour, taste, and texture variations. Veggies are eaten in large quantities for their health advantages as well as their nutritional value (Tachie *et al*., 2024). Plant leaves as an essential component of the human diet, though with lower levels of EPA and DHA (Sanders, 2009). They are cheap sources of nutrients such as protein, vitamins, minerals, fibers, essential amino acids, and certain hormone precursors which are required for physical well-being, growth, metabolism, and overall health (Patil *et al*., 2024, Proshkina *et al*., 2024).

Conclusion: The present research provides information to bridge the gap in the literature on the use and viability of leaves of *U. chamae* as a greenly vegetable. Our findings indicates the leaves of *U. chamae* as a rich source of Protein, fiber, moisture, essential amino acids, vitamins E, C, calcium and potassium making the leaves potent means of macronutrients, minerals, vitamins which are considered to sustain for human health when consumed. Therefore, the study suggests that an adequate intake of *U. chamae* leaves can serve as an alternative source to conventional vegetables due to the rich nutraceutical virtues observed in our study

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