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Phytochemical, Vitamins, Amino Acid and Mineral Composition of Hydro-Methanol Extract of *Ficus Auriculata* Leaves

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Abstract
Ficus auriculata is a fig belonging to the family *Moraceae* and is commonly known as Elephant ear fig. Different parts of the plant have being widely used in traditional medicine for treatment of diarrhoea and dysentery, cuts and wounds, mumps, cholera and vomiting. It has been found to have antihepatotoxic, antibacterial, antioxidant, anti-inflammatory, antiproliferative, and antidiabetic activity. This study aimed to evaluate the phytochemical, vitamin, amino acid, and mineral constituents of the extract of *Ficus auriculata* leaves. Extraction was performed by cold maceration using 80 % ethanol. High performance liquid chromatography (HPLC) and Atomic Absorption Spectroscopy (AAS) were used for determining the phytochemical, vitamin, amino acids and mineral content. Alkaloids (9.04 %), Saponins (2.18 %), Flavonoids (4.55 %), Phenols (11.58 %) Tannins (9.74 %) and Sterols (0.74 %) were present in the extract. Among the Fat-soluble vitamins, the extract showed only the presence of vitamin A (7.223 mg/mol), none of the water-soluble vitamins were present in the extract. Result of amino acid assay showed the presence of Asparagine, Lysine, Leucine, Glycine. The extract was found to be rich in potassium, Sodium and calcium, while other minerals and heavy metals such as magnesium, iron, zinc, copper, Lead, cadmium, and chromium were present in little amounts. The results of this study reveal the component of *Ficus auriculata* leaves which could be responsible for the pharmacological activity documented, as well as its nutritive value.
Keywords: Amino acids, Vitamins, Minerals, Ficus Auriculata, Phytochemicals

Introduction

The *Ficus* genus of the *Moraceae* family (trees, shrubs, climbers, and some herbs) grow mainly in the tropic, subtropic and temperate regions [1], Most members of this family produce a milky latex sap and compound fruits [2]. *Ficus* is distinguished by its distinctive inflorescence, known as figs or syconia. This has a wide range of physiological and medicinal benefits, including antioxidant properties [3-5], antihepatotoxic effects [3], antimicrobial activity [6-8], anticancer potential [9, 10], antidiabetic properties [11], antipyretic qualities [12], and anti-inflammatory attributes [13].

The genus has over 800 species and 2000 varieties and they serve various purposes for humans, including food, health, and cultural significance across their global range [14]. Some Notable species in this genus include the sacred fig (*Ficus religiosa L.*) revered by Hindus, Buddhists, and others worldwide [15], and the sycamore fig or fig-mulberry (*Ficus sycomorus L.*), known as the "tree of life" in Egypt [14]. The Indian rubber tree, a potential source of natural rubber [16]; the cluster fig, revered as sacred to the god Dattaguru in India [17]; the banyan tree, held in high regard by Hindus and Buddhists; the common fig, a widely cultivated species for food and medicine for over 11,000 years [18]; and the roxburgh fig and white fig, commonly consumed in tropical regions, particularly their young leaves and leaf buds [19].

Ficus auriculata, is a globally distributed and recognized for its large, circular leaves. It is originally found in the Himalayan region of India, as well as Bhutan, Nepal, Sikkim, Myanmar, Thailand, Vietnam, and various parts of South China, including South Guangdong, Guangxi, South West Guizhou, Hainan, South West Sichuan, and Yunnan. This species has also been introduced and cultivated in other regions including Nigeria [20]. It is also known as Broad leaf fig, Rox fig, Coconut – Strawberry fig, Giant Indian fig, Himalayan fig tree, Ara, Anjir, Kelebok Nangtan [20]. *Ficus auriculata* fruit has 87.1 % moisture and 7.5 % total soluble solids. It contains 6.12 % reducing sugars, 0.03 % non-reducing sugars, and 0.48 % pectin. The vitamin C content in 100g of the fruit is only 3.35 mg, and its protein content is 0.59 %. When fully ripe, the fruit contains 1.068 % total minerals, with phosphorus, potassium, calcium, magnesium, and iron making up 0.039 %, 0.331 %, 0.039 %, 0.045 %, and 0.003 % respectively [20]. Traditionally, crushed leaves of *F. auriculata* are applied to wounds and are also used in treating diarrhoea and dysentery. People lop the leaves for use as fodder. The juice from the stem bark is effective for diarrhoea, cuts, and wounds, [21]. The fruits are edible and can be used to make jams and curries. Roasted figs are consumed to alleviate diarrhoea and dysentery. The latex from the roots is utilized for mumps, cholera, diarrhoea, and vomiting. In North-Eastern India, particularly in Manipur, various tribes traditionally utilize the leaves of *Ficus auriculata* for managing diabetes, [22, 23]. Root latex is used to treat diarrhoea, cholera, measles, and vomiting [24]. When someone has jaundice, a mixture of root powder from *Ficus auriculata* and bark from *Oroxylum indicum* is taken [25].

Phytochemical analysis of *F. auriculata* leaf and fruit extracts have been reported by Saklani *et al.* [26] to contain phenols, flavonoids, glycosides, resins, and tannins. Also, the leaf extracts contained carbohydrates, terpenoids, and alkaloids. In chloroform extract of the leaf, glycosides and saponins were absent[27]. The leaves of *Ficus auriculata* when analyzed quantitatively, revealing a total phenolic content of 21.404 mg GAE/mg dry weight and a total flavonoid content of 50.83 ± 1.32 mg GAE/mg dry weight [22]. Quercetin and epigallocatechin were found in *F. auriculata* or *F. roxburghii* at concentrations of 3.79 and 4.64 mg/100g, [28]. Phytochemical examination of the stem bark extracts from *Ficus auriculata* has been reported to contain fatty acids, alkaloids, carbohydrates, saponins, glycosides, phytosterols, resins, phenols, tannins, diterpenes, flavonoids, proteins, and amino acids [29]. Some compounds have also been isolated, which included betulinic acid, lupeol, stigmasterol, bergapten, scopoletin, β-sterol-3-O-β-D-glucopyranoside, myricetin, and quercetin-3-O-β-D-glucopyranoside from the leaves and fruits of *F. auriculata* [30]. The GC-MS analysis of *F. auriculata* fruit oil, showed the presence of stearic acid, palmitic acid, oleic acid, linoleic acid, linolenic acid, 3-hydroxy lauric acid, and vaccenic acid. The primary components of the oil was namely; unsaturated fatty acids like linolenic acid and linoleic acid, possess significant therapeutic and preventive health properties. Additionally, saturated fatty acids such as palmitic acid and stearic acid found in the oils have substantial industrial applications [29].

A wide range of pharmacological activities have been credited to *F. auriculata.* Antibacterial and antifungal activities of ethanolic extract of *F. auriculata* fruits was demonstrated by Saklani *et al.* [26]. It has also been found to have anti-inflammatory effect in carrageenan-induced paw edema model by [30]. Antioxidant activity of the leaves and fruits of *F. auriculata* in-vitro has also been documented [30, 31]. Other pharmacological properties of *F. auriculata* include hepatoprotective activity [30], antidiabetic and hypolipidemic effects [32], antiproliferative activities against A549 cells [33] and Anti-ulcer activity [34].

The fact that *F. auriculata* exhibit a wide range of pharmacological activities suggest the presence of valuable phytochemical and nutritional factors that contributes to the biological activities so far reported. It is also imperative to evaluate the nutritional composition of the plant to validate its use as fodder for animal and food for humans. This study focused on evaluating the phytochemical, vitamin, amino acids and mineral composition of hydro-ethanolic leaf extract of *Ficus auriculata lour.*

Materials and Methods

Collection and identification of plant material

The fresh leaves of *F. auriculata* were collected at 72 Army Barracks, North Bank Makurdi Benue State and sent to and authenticated at the Department of Forest Production and Products, Joseph Sarwuan Tarka University, Makurdi Benue State. The plant sample was deposited at the department's herbarium for further reference (Voucher No: UAM/FH/0446).

Preparation and extraction of the plant material

The leaves of *F. auriculata* were thoroughly rinsed with clean running water and allowed to air-dry in a shaded area at ambient temperature. Once dried, the leaves were pound into a fine powder using a mortar and pestle. Subsequently, 300 g of the powdered plant material was measured with an electronic scale and macerated with 3000 mL of 80 % aqueous ethanol for 48 hours at room temperature, with intermittent stirring. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a water bath set at 40 °C. The resulting colloidal extract was weighed, transferred into vials, and stored in a refrigerator at room temperature until the commencement of the study. The percentage yield of the plant extract was calculated using the formula provided below:

$$\% \, Yield = \frac{Final \, weight \, of \, extract}{total \, weight \, of \, ground \, plant} \times 100$$

Determination of phytochemical, Vitamin and amino acid composition of hydro-ethanol extract of Ficus auriculata leaves

Determination of phytochemical, vitamin and amino acid content of hydro-methanol extract of *Ficus auriculata* leaves using HPLC. The extract was dissolved using the same solvent used for extraction, 2 mL of the sample solution was then filtered with 0.45 µm syringe filter into a vial. The mobile phase solvent was filtered using 0.45 µm filter in a vacuum flask. The vial was labeled appropriately with the sample ID. The vial was placed in the auto sampler of the HPLC machine (Sykam HPLC Equipment Sykam, S 3250 UV/Vis detector S 5300 Sample Injector S 1130 Pump system) and the vial number noted. The phytochemical, vitamin and amino acid analysis methods stored in the System Application were called up as well as their calibration files stored in the system application. The analysis sequence was then created and the wavelengths of 214nm, 228nm and 230nm for phytochemicals, vitamins and amino acid analysis respectively, were set and ran for 10 minutes (vitamin and Amino acids) while phytochemical analysis was run for 30 minutes, all using the Reprosil 100, C8, 5µm, 4.6 x 150mm column. Each sequence was run and observe for retention time and peaks in the chromatograph. The chromatogram was then printed out for Phytochemical, vitamins and amino acids.

Determination of mineral composition of hydro-methanol extract of ficus auriculata leaves

Mineral element content of the extract was determined by the use of atomic absorption spectroscopy (AAS). The sample solution for the determination of minerals was prepared according to AOAC, 1990 [35]. A homogenized sample was first dried in a hot air oven at 100 ^{O}C till sample became moisture free and constant weight was obtained. The sample was then grounded to fine powder with the help of mortar and pestle. The sample (2 g) was heated in a crucible at about 550 ± 20 ^{O}C for 5 h. Heating was continued for another 30 min, for complete white ash to be formed and process was repeated till complete grey ash was obtained. This was transferred completely to 100 mL volumetric flask and was dissolved in 25 mL 10 % HCl solution with stirring. The volume was made up to the mark with the addition of deionized water. Finally, the solution was filtered using Whatman filter paper no 41. This sample solution was submitted for AAS analysis. The concentrations of the following elements were determined; Ca, K, Na, Pb, Cd, Zn, Cu, Se, Fe, Cr and Mg.

Results and Discussion

Percentage yield

The pulverized leaves of *Ficus auriculata* (300 g) in 80 % aqueous ethanol yielded 39.09 g of dried extract, giving a percentage yield of 12.36%. The phytochemical composition of hydro-ethanol leaf *Ficus auriculata* was evaluated in this study,

alongside its mineral, vitamin and amino acid content. *Ficus auriculata* have been widely used in folk medicine as remedy for a wide range of disease condition including dysentry, diarrhea, wounds, cuts mumps, cholera, jaundice, fever, etc [36, 27]. The leaves are edible, they are also used to reduce peroxidation of palm oil during its preparation probably due to the its antioxidant properties [37, 38]. This study revealed that leaves of *F. auriculata* contain a variety of phytochemical and nutritional constituents. These phytochemicals have been said to be responsible for the variety of pharmacological activities reported, supporting its traditional use.

Qualitative and quantitative phytochemical constituent of hydro-methanol extract of *ficus auriculata* leaves.

The phytochemical constituents of hyro-ethenol leave extract of *F. auriculata* is presented in table 1. The results agreewith the findings of Kumari et al. [27] using methanol and chloroform, except saponins and sterols which were absent. Also, the report of Gaire et al. [39] reveals similar phytochemical content in the stem bark extract of *F. auriculata lour* using methanol as the solvent. The antioxidant activity of *F. auriculata* leaves reported by Kumari et al. [27] is buttressed by the presence of Phenols and flavonoids which are known to be anti-oxidative in nature [40]. Alkaloids and flavonoids and tannins are also known to demonstrate antimicrobial activity [41, 42]. Flavonoids posse other pharmacological activities such as anti-inflammatory, cytotoxic or anticancer activity which have been reported for F. auriculata leaves [43, 44]. Saponins and sterols have the ability to inhibit inflammatory processes and have been reported by El-Fishawy et al. [30].

Table 1: Qualitative and quantitative phytochemical constituent of hydro-methanol extract of *ficus auriculata* leaves

Phytochemicals	Presence	Quantity (%)
Alkaloids	+	9.05
Saponins	+	2.18
Flavonoids	+	4.55
Phenols	+	11.58
Tannins	+	9.74
Sterols	+	0.74
Glycosides	-	0
Terpenoids	-	0

Vitamin composition of hydro-methanol extract of Ficus auriculata leaves

The presence of fat-soluble and water-soluble vitamins in hydro-ethanol leave extract of *F. auriculata* was evaluated and results presented in table 2 and 3 as well as figure 1 and 2. In determining the vitamin content of *F. auriculata*, Vitamin A was found amongst the lipid-soluble vitamins with a concentration of 7.223 mg/mol. None of the water-soluble vitamin was found. Vitamin A occurs in plants as pro-vitamin carotenoids, which may include α-carotene, β-carotene and β-cryptoxanthin and are found in all plant parts except α-carotene which is rare in flowers, tubers and buibs [45]. Vitamin A essential in plant and animal development and has diverse functions including maintenance of vision, reproduction and immune modulation [45-47]. Vitamin A also has antioxidative properties [45] which also validate the antioxidant properties of this plant. Vitamin C, a water-soluble vitamin has been reported in the fruits of *F. auriculata lour* [48, 25], whereas in the leaves, water-soluble vitamins are absent.

Table 2: Fat-soluble vitamin composition of hydro-methanol extract of Ficus auriculata leaves

S/N	Retention Time (Min.)	Response	Amount (mg/mol)	Amount (%)	Compound Name
1	2.700	356.058	7.223	100.0	Vitamin A
2	3.853	2491.094	0.000	0.000	NVM
4	7.943	31.665	0.000	0.000	NVM
	Total		7.223	100.0	

Note: NVM - No vitamin match.

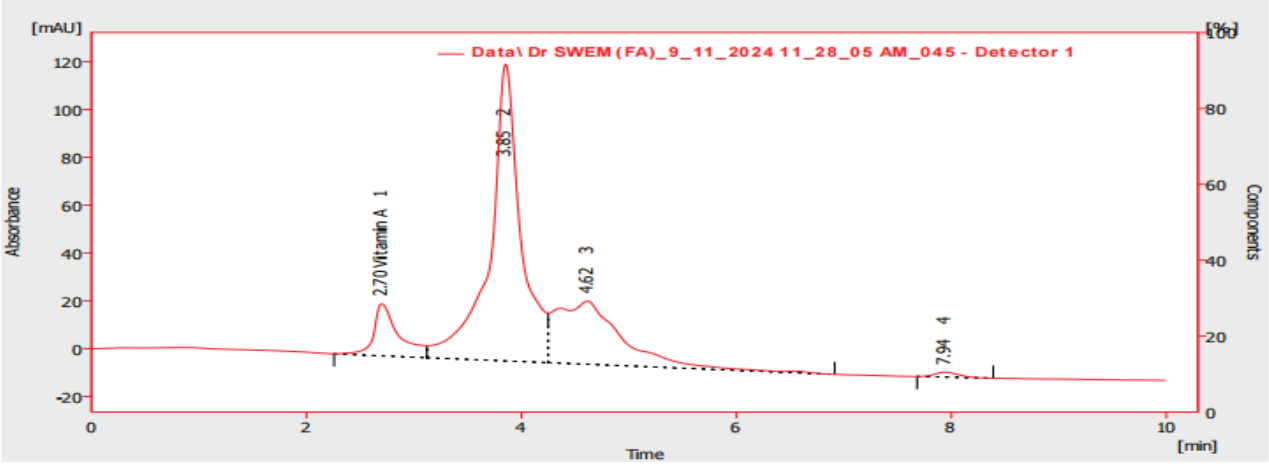

Figure 1: Chromatogram for the analysis of fat-soluble vitamin composition of hydro-methanol extract of Ficus auriculata leaves.

Table 3: Water-soluble vitamin composition of hydro-methanol extract of Ficus auriculata leaves

S/N	Retention Time (Min.)	Area (mAU.s)	Height (mAU)	Area (%)	Height (%)	W05 (Min.)	Compound Name
1	0.380	9.587	0.641	2.3	3.9	0.28	NVM
2	3.903	149.390	7.346	36.1	44.2	0.37	NVM
3	4.418	254.440	8.633	61.5	51.9	0.66	NVM
	Total	413.417	16.620	100.0	100.0		

Note: NVM - No vitamin match.

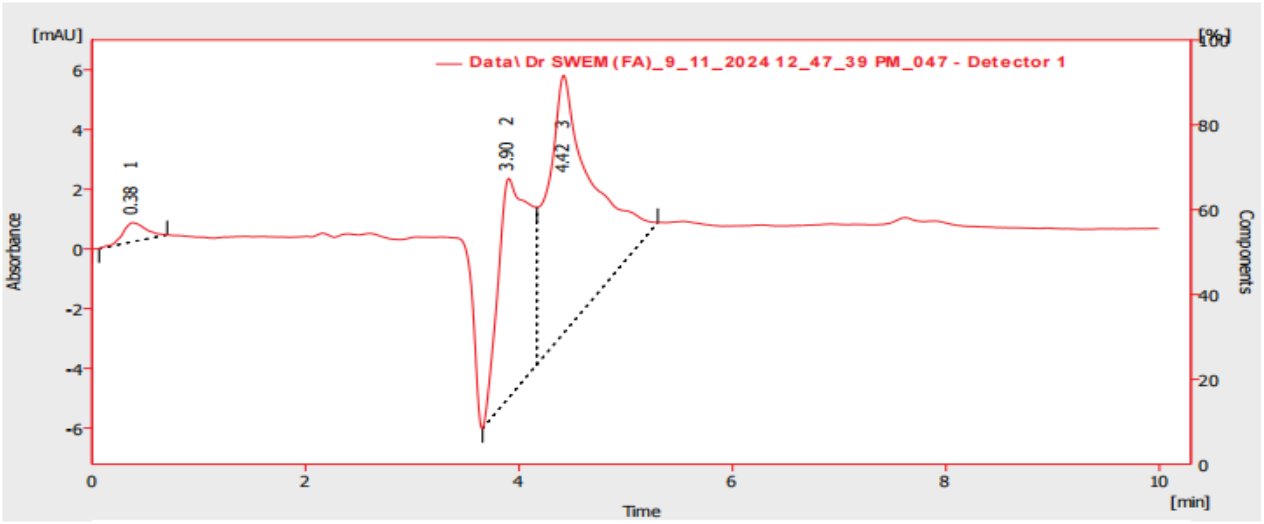

Figure 2: Chromatogram for the analysis of water-solubele vitamin composition of hydro-methanol extract of Ficus auriculata leaves.

Amino acid composition of hydro-methanol extract of *Ficus auriculata* leaves

Amino acid composition and their concentration is presented in table 4 and figure 3. the extract was found to contain four amino acids namely; Asparagine 3.058 ul/mol (0.4 %), Lysine 61.322 ul/mol (8.0 %), leucine 453.545 ul/mol (59.5 %), Glycine 244.842ul/mol (32.1 %).

Amino acids found in the extract include asparagine, leucine, lysine and glycine. Lysine and leucine are essential amino acids while asparagine and glycine are none essential amino acids. A study by Ahmed et al. [49] evaluated the presence of free and protein amino acids in leaves and stem of *Ficus cordata* thunb. Subsp. *Salicifolia* (VAHL), a species of ficus and found an array of amino acids, both essential and nonessential. The presence of Lysine, leucine and glycine in the leaves of *F. auriculata* agrees with the findings of Ahmed *et al.* [49] in both leave and stem of *Ficus cordata* thunb. Subsp. *Salicifolia* (VAHL). Amino acids are important biomolecules that both serve as building blocks of proteins and are intermediates in various metabolic pathways [49]. Synthesis of a wide range of important substances in living cells including; nucleotides, peptide hormones, and neurotransmitters require amino acids as precursors [49]. Cell

signaling, regulation of gene expression and protein phosphorylation cascade [50], nutrient transport and metabolism in animal cells [51], innate and cell-mediated immune responses are all dependent on amino acids [49]. Aspartic acid plays an important role in the citric acid cycle, or Krebs cycle, during which other amino acids and biochemicals (such as asparagine, arginine, lysine, threonine and isoleucine) are synthesized [52]. Quantitative and qualitative changes in the synthesis of protein of the plants may be due to response to water deficient [53].

Table 4: Amino acid composition of hydro-methanol extract of Ficus auriculata leaves

S/N	Retention Time (Min.)	Response	Amount (µl/mol)	Amount (%)	Compound Name
1	0.408	6.904	3.058	0.4	Asparagine
2	2.648	241.897	61.322	8.0	Lysine
3	3.847	1517.488	453.545	59.5	Leucine
4	4.752	421.808	244.842	32.1	Glycine
	Total		762.748	100.0	

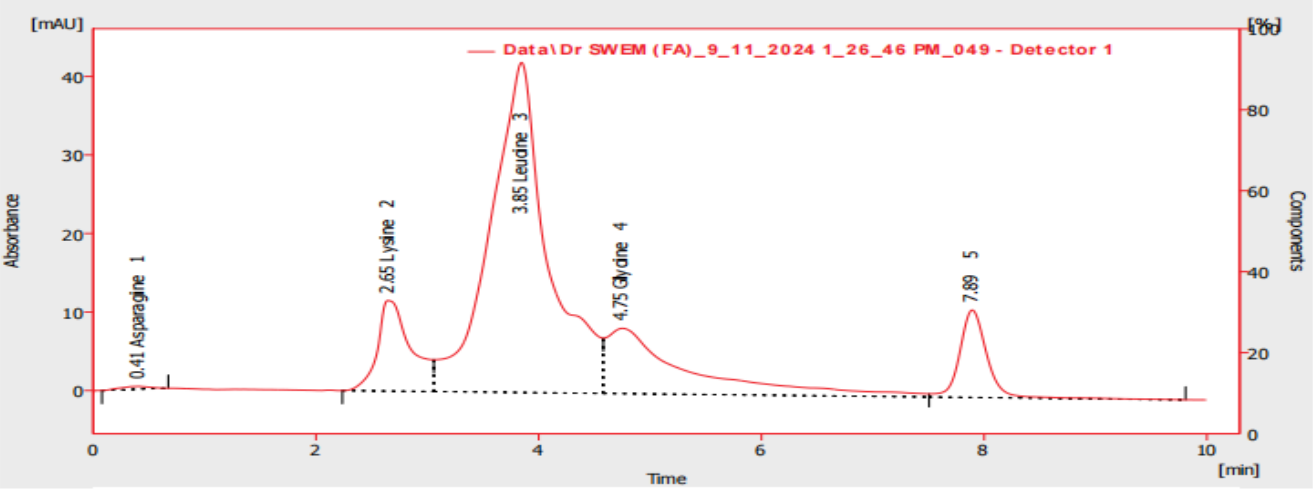

Figure 3: Chromatogram for the analysis of amino acid composition of hydro-methanol extract of Ficus auriculata leaves

Mineral element composition of hydro-ethanol leaf extract of ficus auriculata

Mineral composition of hydro-ethanol leaf extract of *Ficus auriculata* is presented on Table 5. Previous study by Khatun *et al.,* [48] and Shrestha *et al.* [54] on the fruits extract showed similar mineral composition of *Ficus auriculata* leaf. The fruit contained calcium, magnesium, potassium sodium and phosphorous in previous studies. Ash content is an indication of the inorganic (mineral elements) composition of the plant part under investigation [55]. The ash composed of inorganic material of the plant sample [56]. Increase in ash contents signifies high concentrations of various mineral elements [57]. Different ficus species show different ash content with *Ficus carica* having the highest ash content compared to others Ficus. Proximate analysis of fruits of *F. auriculata lour* have been studied by Saklani *et al.* [26], Rusmadi *et al.* [55] and Shrestha *et al.* [54], revealing ash content of 3.9 %, 15.22 % and 1.45 % respectively. differences in nutritional content can be due to various factors such as environmental conditions, geographical location, and analytical methods used. Because of the variety of mineral content in the leaf, it can be opined that *Ficus auriculata* leaves are a good source of essential minerals, particularly potassium, magnesium, calcium, sodium and iron. These minerals have essential roles to play in various physiological activities, such as muscle contraction, nerve impulse transmission, and bone health. They also are vital in metabolic processes, acting as co-factors of enzymes catalyzing important metabolic reaction, thereby improving the growth and development of both animals and humans [57, 55, 54]. Leaves of *F. auriculata* are used as fodder for feeding livestock especially ruminants [58], Therefore, *Ficus auriculata* leaves appears to be of great value in diet of animals of human beings, especially in cases of mineral deficiencies.

Table 5: Some mineral constituents of hydro-methanol extract of Ficus auriculata leaves

Mineral	Concentration (ppm)
Lead (Pb)	0.247±0.002
Copper (Cu)	0.100±0.002
Magnesium (Mg)	0.080±0.001
Potassium (K)	11.510±0.002
Calcium (Ca)	3.640±0.002
Cadmium (Cd)	0.004±0.001
Chromium (Cr)	0.022±0.001
Iron (Fe)	0.200±0.001
Zinc (Zn)	0.400±0.002
Sodium (Na)	4.710±0.001

Conclusion

The amino acids, vitamin A and mineral composition of Ficus auriculata leaves reported in the study underscores its nutritional value, therefore, F. Auriculata can be a good supplement in treatment hypoproteinemia, mineral deficiency diseases and hypovitaminosis A in both animals and humans. The phytochemical constituents found further supports the pharmacological activities documented in literature. Further studies are need to evaluate its *in-vivo* nutritional value with the view of developing a supplement that will enhance the well-being of humans and animals.

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