PRELIMINARY PHYTOCHEMICAL SCREENING AND ELEMENTAL ANALYYSIS OF EXTRACTS OF FICUS POLITA AND FICUS PLATYPHYLLA.

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ABSTRACT

This study was conducted to determine the elemental composition and phytochemical composition of leaves and stem bark of *Ficus polita* and *Ficus platyphylla*. The result of the study showed that considerable amount of phytochemicals (saponins, glycosides, flavonoids, alkaloids and terpenoids) were found in both the leaves and stem bark of both plant parts extract. This suggests that the plant parts can be used as a source of medicine. Elemental analysis revealed the presence of some essential and trace elements in the leaves and stem bark. Heavy metals analyzed in the study were either not detected or found in traces (within maximum residue limit recommended by FAO and WHO). The result of this study is an important result as human health is directly affected by ingestion of plant parts.

Key Words: *Ficus polita*, *Ficus platyphylla,* phytochemicals, elemental analysis.

1.0 INTRODUCTION

Plants play a very important role in health care system both in rural and urban areas. In rural areas, the use of modern health facilities is very expensive beyond the reach of the rural habitants. Therefore, the use of plants based medicines are considered due to its low cost, availability and sufficiency. It is therefore important to establish the concentration of phytochemicals and elements that play important roles in drug development (Manoharachary and Nagaraju, 2016).

Ficus constituted one of the largest genera of medicinal plants with about 750 species of woody plants, trees and shrubs primarily occurring in subtropical and tropical reginos throughout the world. The genus is remarkable for the large variation in the habit of its species (Jander and Machado, 2008). The most important species of Ficus are *F. bengalensis,* F. Carica, *F. racemose* and *F. elastic*. *Ficus caricais* commonly referred to as 'fig' various parts of the plant like bark, leaves, tender shoots, fruits, seed and latex are medicinally important. The fig is a very nourishing food and used in industrial products. It is rich in vitamins, minerals, elements, water and fats. Figs are one of the highest plant sources of calcium and fiber. According to USDA data for the mission variety, dried figs are riches in fiber, copper, manganese, magnesium, potassium, calcium and vitamin k, relative to human needs (Vinson and Joe, 2000). Literature survey indicated that figs have been cultivated over 1100 years and these are among the earliest cultivated plants for human use (Chang *et al*., 2005).

1.1 *Ficus Polita*

Ficus polita is a tropical African evergreen shrub or small tree belonging to the family Moracease, and usually growing up to 15 meters tall, and sometimes to 40 meters tall. The leaves are occasionally harvested from the wild for food. Like most other Ficus species, the fruits are sometimes eaten as aphrodisiac and stimulant. The plant is commonly called heart-leaved fig, polish fig, rubber plant, wild rubber fig tree. Locally, it is called 'durumi' in Hausa. Traditionally the fruit and young leaves are chewed for dyspepsia (Kuete *et al.*, 2011). The young leaves are also edible and the bark and roots infusions are used in the treatment of infectious disease, abdominal pain, dyspepsia and diarrhea like many of the species or the moraceae family (Kamga *et al*, 2010; Kuete *et al.*, 2011). It has been reported that *F. polita* has good nutrient profile and can be used as feed in combination with *Panicum maximum* to as high as 60 % *F. polita* inclusion level for optimal performance of West African dwarf goats without any adverse effect on intake and growth rates of animals most especially when supplemented with approximately 10 % cassava peel (Abegunde and Akinsoyimu, 2011). The extracts of the plant such as water, methanol and dichloromethane had been reported to exhibit anti-inflammatory activities (Egharevba, Ibrahim and Kunle, 2015).

1.2 *Ficus Platyphylla*

Ficus platypylla is an evergreen plant locally known as 'gamji' in *Hausa* and widely distributed throughout the savannah region of West African Coast. The common name is Gutta percha tree. In Northern Nigeria, the plant is used by herbalist for treating several diseases such as insomnia, psychosis, depression, and as analgesic (Chindo *et al*., 2003). In Sokoto state, the decoction of the stem bark, leaves and seeds of this plant are used in combination as a medicine to promote fertility. Previous studies revealed that the stem bark of the plant possesses anti-nociceptive, anti-inflammatory and gastrointestinal activities (Amos *et al.*, 2001). Preliminary phytochemical analysis of the stem barks revealed presence of flavonoids, tannins and saponins (Amos *et al*., 2001). The central nervous system (CNS) activity of *F. platyphylla* has also been evaluated for the scientific basis for the use of this plant in traditional medicine for the treatment of CNS disorders .The stem bark of *F. platyphylla* is used traditionally to treat malaria in Africa and in treating tuberculosis (Chindo *et al.,* 2003).

This present research focuses on the investigation and study of the chemical constituents of *Ficus polita* and *Ficus platypylla* in Mubi North Local Government Area.

2.0 Materials and Methods

All materials used are of analytical grade.

2.1 Sample Collection and Treatment

The two samples of the selected medicinal plants (*Ficus platypylla* and *Ficus polita*) were collected from Mubi North Local Government Area of Adamawa State, Nigeria and identified by Mr. Timon David of the Department of Botany Adamawa State University. Fresh leaves and bark of the plants were washed and air dried. The dried samples were pulverized using mortar and pestle.

2.2 Extraction

2.2.2 Aqueous Extraction (maceration)

50 g of the powdered sample was mixed with 250 mL distilled water and allowed to stand at room temperature for 5 hours. The solution therefore, was filtered using Whatman filter paper No 1 and the residue was discarded. The filtrate was concentrated by direct exposure to air. The

extract was weighed and stored in air-tight container for further analysis (Mohammed *et al.*, 2015).

2.2.3 Ethanol Extraction (Soxhlet Extraction)

50 g of the powdered samples was weighed and mounted on a Soxhlet apparatus and extracted with 250 ml of ethanol for 12 hours. The resulting solution was evaporated to dryness on a water bath and the percentage yield was determined. The extract was poured into a petri dish and stored for further analysis (Gusthinnadura *et al.*, 2017).

2.3 Sample Preparation for Elemental Analysis

Samples were accurately weighed (0.5 g each) and placed in a 100 ml beaker. To the sample, 5 mL of 65 % HNO_3 was added, and then the mixture was boiled gently over a water bath (90 °C) for 1 hour until a clear solution was obtained. Later, 2.5 ml of 65 % HNO_3 was added, followed by further heating until total digestion (Uddin *et al.*, 2016).

2.3.1 Elemental Determination

Few drops of distilled water was added to the prepared samples and allowed to cool. The samples were transferred into 100 mL volumetric flask by adding distilled water in them. The extract was filtered with filter paper and the filtrate was collected on labeled plastic bottle. The solutions were analyzed for the element of interest utilizing AAS ((Buck Scientific model. 210 VGP) with suitable hollow cathode lamp (Muhammad *et al.*, 2015).

2.4 Phytochemical Analysis

The aqueous and ethanol extracts of the samples was subjected to qualitative phytochemicals analysis to test for the presence of secondary metabolites following the procedure of Sofowora (1993).

Test for Flavonoids

1 mL each of the extract was added to 1 mL of 10 % lead acetate solution. The formation of a yellow precipitate indicates the presence of flavonoids.

Test for Alkaloids

3 mL of each of the extract was stirred with 3 mL of 1 % HCl on a steam bath. Wagner's reagent was then added to the mixture. Turbidity of the precipitate indicates the presence of alkaloids.

Test for Terpenoids

2 mL of each of the extracts was dissolved in 2 mL of chloroform and evaporated to dryness. 2 mL of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated presence of terpenoids.

Test for Saponins

3 mL of each of the extract was shaken vigorously with 5 mL of distilled water in a test tube and warmed. The formation of stable form indicates the presence of Saponins.

Test for Glycosides

Liebermann's test: 2 mL of each of the extract was dissolved in 2 mL of chloroform and 2 mL of the acetic acid was added. The solution was cooled well in ice. Sulfuric acid was then added carefully. A color change from violet to blue to green indicates the presence of a glycone portion of glycoside.

2.5 Quantitative Estimation of Phytochemical Constituents Using HPLC

A known amount of the sample was weighed and dissolved with hexane in a 1.0 mL. The prepared sample was injected into a buck scientific (USA) BLC10/11 High Performance Liquid Chromatography (HPLC) system with fluorescence (excitation at 295 nm and emission at 325 nm) and an analytical silica column (25 cm x 4.6 mm ID stainless steel, 5 µm) was used to analyze phytochemicals. The mobile phase used was hexane: tetrahydrofuran:isopropanol (250:15:1 v/v/v) at a flow rate of 1.0 mL/min. standard sample was prepared using similar method. Concentration of phytochemicals in samples was calibrated using authentic standards. The result obtained was used to calculate the concentration of phytochemicals in the sample, using the formula below;

$$\text{PHYTO} = \frac{[\text{A SAMPLE} \times [\text{STD}](\text{ppm}) \times \text{VHEX}(\text{ml})]}{[\text{A STD} \times \text{Wt SAMPLES}(\text{g})]}$$

Where;

[PHYTO] = concerntration of phytochemicals in ppm

[STD] = concerntration of standard

A SAMPLE = area of sample

A STD = area of standard

VHEX = volume of hexane

Wt SAMPLE = weight of sample (AOAC, 2000).

Statistical Analysis

The results obtained was presented as a mean ± standard deviation and analyzed as simple percentage.

3.0 RESULTS AND DISCUSSION

Qualitative Phytochemical Screening of *Ficus polita*

The result of the phytochemical screening of aqueous and ethanolic extract of *Ficus polita* and *Ficus platyphylla*, are presented on Tables 1 and 2.

Table 1: Qualitative phytochemical screening of aqueous and ethanol extracts of *Ficus polita* Leaves and Stem bark.

S/n	Phytochemicals	A.E Leaves	E.E Leaves	A.E Stem bark	E.E Stem bark
1	Saponins	+	+	+	+
2	Flavonoids	+	+	+	+
3	Alkaloids	+	+	+	+
4	Steroids	+	+	+	+
5	Glycosides	+	+	+	+

Keys, - = negative, + = positive
A.E = Aqueous extract
E.E = Ethanol extract.

Table 2: Qualitative phytochemical screening of aqueous and ethanol extracts of *Ficus platyphylla* Leaves and Stem bark.

S/n	Phytochemicals	A.E Leaves	E.E Leaves	A.E Stem bark	E.E Stem bark
1	Saponins	+	+	+	+
2	Flavonoids	+	+	+	+
3	Alkaloids	+	+	+	+
4	Steroids	+	+	+	+
5	Glycosides	+	+	+	+

Keys, - = negative, + = positive
A.E = Aqueous extract
E.E = Ethanol extract.

Quantitative phytochemical concentration of aqueous and ethanol extracts of *Ficus platyphylla* Leaves and Stem bark

Saponins are the most abundant phytochemical in both the aqueous and ethanol leaves and stem bark extract of *Ficus platyphylla* while terpenoids are the least most abundant in the aqueous and ethanol leaves extract. Terpenoids and flavonoids are the least most abundant in the aqueous and ethanol stem bark extracts respectively.

Table 3: Quantitative phytochemical concentration of aqueous and ethanol extracts of *Ficus platyphylla* Leaves and Stem bark

Phytochemicals	A.E Leaves	E.E Leaves	A.E Stem bark	E.E Stem bark
Saponins	6.16 ± 0.05^{a}	8.12 ± 0.01^{c}	7.16 ± 0.02^{b}	9.06 ± 0.01^{d}
Flavonoids	1.20 ± 0.00^{c}	1.77 ± 0.03^{d}	0.55 ± 0.00^{a}	0.97 ± 0.02^{b}

Alkaloids	5.11 ± 0.05^{b}	6.38 ± 0.02^{c}	4.77 ± 0.00^{a}	7.82 ± 0.01^{d}
Terpenoids	0.37 ± 0.01^{a}	0.85 ± 0.00^{b}	0.46 ± 0.02^{a}	1.15 ± 0.01^{c}
Glycosides	4.16 ± 0.01^{c}	6.75 ± 0.03^{d}	1.86 ± 0.01^{a}	2.96 ± 0.02^{b}

values are mean ± SD of triplicate determinations. Means with thesame superscript letter in thesame row are not significantly different at $p < 0.05$.
Keys, A.E = Aqueous extract
E.E = Ethanol extract.

Elemental Composition of Aqueous and Ethanolic Extracts of the Leaves and Stem bark of and *Ficus platyphylla*

The results of the elemental composition of aqueous and ethanolic extract of *Ficus polita* and *Ficus platyphylla* leaves and stem bark are presented in Table 3. Calcium was the most abundant in the aqueous leaves extract of *Ficus polita* while, Manganese was the least. Iron was higher in the ethanol leaves extract and copper was least. Calcium was the most abundant in the aqueous stem bark extract of *Ficus polita* while, Iron and Manganese were the least (Table 4). Iron was the most abundant element in the ethanol stem bark extract of *Ficus polita* while, Copper was the least in ethanol stem bark extract.

Table 4: Elemental Concentration in the Aqueous and Ethanol Extracts of *Ficus polita* Leaves and Stem Bark.

Phytochemicals	**A.E Leaves**	**E.E Leaves**	**A.E Stem bark**	**E.E Stem bark**
Fe	0.188	0.741	0.002	0.323
Ca	1.673	0.166	0.260	0.238
Cu	0.032	0.025	0.027	0.037
Mn	0.010	0.116	0.002	0.104
Zn	0.188	0.093	0.073	0.119

Keys, A.E = Aqueous extract
E.E = Ethanol extract.

Table 5: Elemental Composition of the Aqueous and Ethanol Extracts of Ficus platyphylla Leaves and Stem bark

Phytochemicals	A.E Leaves	E.E Leaves	A.E Stem bark	E.E Stem bark
Fe	0.059	0.010	0.004	0.762
Ca	1.630	0.123	1.311	0.207
Cu	0.034	0.014	0.010	0.015
Mn	0.047	B.D	0.004	0.106
Zn	0.805	0.096	0.080	0.137

Keys, A.E = Aqueous extract
E.E = Ethanol extract.
B.D =Below Detection limit

Calcium was the most abundant in both the aqueous and ethanol leaves extract of *Ficus platyphylla* while, copper and iron were the least. In the aqueous stem bark extract of *Ficus platyphylla*, calcium was the most abundant while iron and manganese were the least in the aqueous stem bark extract. Iron was the most abundant element in the ethanol stem bark extract of *Ficus platyphylla* while, copper was the least in the ethanol stem bark extract of *Ficus platyphylla*

Qualitative Phytochemical Screening

Various secondary metabolites which constitute an important source of the pharmaceutical drugs have been isolated from different parts of plants. Some of these compounds have been reported to be present in the Ficus species such as tannins, saponins, flavonoids, steroids, anthraquinone glycosides and reducing sugars (Hassan, 2005; Sandabe *et al*., 2006).

The result of the preliminary phytochemical screening of aqueous and ethamol extract of leaves and stem bark of *Ficus polita* and *Ficus platyphylla* was found to contain saponins, flavonoids, alkaloids, glycosides and terpenoids. This finding is in line with the findings of Solomon-Wisdom *et al.* (2011), who detected saponnins, steroids and tannins but no alkaloids, triterpenoids and glycosides.

Mudi and Dauda (2011) in another study tested for the presence of secondary metabolite in *Ficus platyphylla* which showed the presence of alkaloid, reducing sugar, saponins, tannins, resins and flavonoids in ethanol soluble fraction. Another study by Ugwah-Oguejifor *et al*.

(2011), revealed that the extract contains saponnins, tannins, flavonoids volatile oils, glycosides and steroids but no alkaloids and cardiac glycosides.

The variation in the presence of the phytochemicals could be due to some environmental factors such as climate and human activities. It is therefore important to examine several specimens of the same species, if possible specimens grown under different conditions (Egbe *et al*., 2018). According to Egbe *et al*. (2018), tissues such as the heartwood of trees, usually show a more constant chemical composition than living organs, since they are much less subjected to the influence of environmental factors.

Quantitative Phytochemical Analysis

The quantitative test revealed that the concentration of phytochemicals in these plants ranged from 0.23 mg/100g to 8.16 mg/100g (Table 3) for *Ficus polita* leaves and stem bark extract. The highest quantitative yield of flavonoid was obtained in both the ethanol extract of the leaves and aqueous extract of the stem bark (2.12 mg/100g), followed by the aqueous leave extract which gave a quantitative yield of 1.34 mg/100g, the least is flavonoid in ethanol extract of the stem bark (0.85mg/100g). Aqueous extract of stem bark recorded the highest quantitative yield of alkaloid (7.16 mg/100g), however there was no significant difference in the amount of alkaloid in the aqueous leave extract, ethanol leaves extract and ethanol stem bark extract of *Ficus polita*. There was significant difference at $p < 0.05$ in the yield of saponin across the leaves and stem bark extract of *Ficus polita*. This value is higher than those reported by Anukworji *et al*., (2012). This may be attributed to difference in soil type and solvent used in extraction.

The quantitative test revealed that the concentration of phytochemicals in these plants ranged from 0.37 mg/100g to 9.06 mg/100g (Table 4) for *Ficus platyphylla* leaves and stem bark extracts. The highest quantitative yield of flavonoid was obtained in both the ethanol extract of the leaves (1.77 mg/100g), followed by the aqueous leave extract which gave a quantitative yield of 1.20mg/100g, the least in flavonoid was aqueous extract of the stem bark (0.55mg/100g). Ethanol extract of the leaves recorded the highest quantitative yield of glycosides (6.75 mg/100g, however there was significant difference in the amount of alkaloid, saponins and terpenoids in the aqueous leave extract, ethanol leaves extract and ethanol stem bark extract of *Ficus platyphylla*.

Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. Unlike pharmaceutical, they do not have any side effects. Since the phytochemicals cure diseases without any harm to human beings these can also be considered as 'man-friendly medicines' (Sahira *et al.*, 2015). This shows that these plants can be used as crude drug to cure different ailments.

Elemental Concentration

From the table above (Table 4 and 5), low amount of iron was recorded in the ethanol and aqueous extract of the leaves and stem bark of the samples investigated with highest amount in the ethanolic leave extract of *Ficus polita* (0.741 mg/100g). Iron is one of the chief extracellular ions in the body. It involves in the production of energy, transport of amino acids and glucose into the body cells and its deficiency results in hyponatremia (Norman and Joseph, 1996). Excessive accumulation of Iron in the liver, pancrease, heart lungs and other tissues cause haemosiderosis.

The mean values for manganese recorded in the aqueous and ethanol extracts of the leaves and stem bark of the samples were between the range of 0.002 to 0.116, which was lower than the amounts obtained by Kubmarawa *et al*., (2007). However, manganese was not detected in the ethanolic leave extract of *Ficus platyphylla*. Manganese plays important role in maintaining electrical potential in nerves and membranes. It improves insulin sensitivity, protect against diabetes and its complications and also reduce blood pressure (Brenna and Shelly, 1999). Mn in excess levels increases the risk for tendon and ligament tears and pneumonia.

The aqueous and ethanol extract of the leaves and stem bark of *F. polita* and *F. platyphylla* was found to contain calcium between the range of 0.123 to 1.673 mg/100g which were comparatively lower than the results (3.17 mg/100g) reported by Kubmarawa *et al*., (2007). The results obtained are within the range of permissible limit as prescribed by WHO (Kiran *et al*., 2004). Calcium is needed for muscles development, heart and digestive System. It is also essential for the normal development and maintenance of bones (Afshin and Masoud, 2011). Excess level of Calcium in the blood causes renal insufficiency, vascular and soft tissue calcification.

The leaves and stem bark extracts of *F. polita* and *F. platyphylla* recorded minimal presence of copper in all the samples. Copper plays important role in treatment of chest wound and prevent inflammation arthritis and similar diseases. It is also essential for the formation haemoglobin of the red blood cells. It is required by trace quantity by humans. Excess consumption of Cu may cause its accumulation in the liver with decrease in blood haemoglobin concentration.

The aqueous extract of both species contained more amounts of zinc than the ethanol extracts of both species with a range value of 0.073 to 0.805 mg/100g which was lower than 6.00mg/100g observed by Kubmarawa *et al*., (2007). Zinc is essential constituents of enzymes involve in carbohydrate and protein metabolism and nucleic acid synthesis. Its deficiency results in impaired growth and development, skin lesion and loss of appetite. Zn in excess can be toxic and it causes health problems such as stomach cramps, skin irritations, vomiting and pancrease damage.

CONCLUSION

From a phytochemical point of view, the analysis of the ethanol extract and aqueous extracts did not show significant qualitative differences. Only the HPLC fingerprinting revealed subtler quantitative differences in terms of phenolic metabolites.

There was also no difference in the amount of elements detected in each of the extracts. This is evident in the results in the tables above. It can be concluded that *Ficus polita* and *Ficus platyphylla* can be used in traditional medicine because the presence of these essential elements plays a very important role in the maintenance of human health.

Recommendations

The result of this study is an important result as human health is directly affected by ingestion of plant parts. Bio-monitoring of essential and trace elements in the leaves and stem bark of plants need to be investigated because these are the main source of food and medicine for humans in most of the African countries.

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