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Chemical Composition and Phytochemical Screening of *Ficus exasperata* (Vahl) Leaf

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ABSTRACT

Ficus exasperata Vahl leaf is a potent ethno-medicine in Ubiaja, Edo State, Nigeria, where it is used in treating diabetic persons. This study evaluates its chemical composition and screens its aqueous extract for phytochemicals using standard methods. Proximate analysis revealed a high level of ash (13.76) in g% dry weight suggestive of a reasonably good source of minerals. Others were crude fibre (12.50), crude protein (11.38), crude fat (2.10), while moisture content was 57.20 g% wet weight. Preliminary phytochemical screening showed the presence of tannins, flavonoids, alkaloids, anthraquinones, steroids and cardiac glycosides, though the frothing test failed to reveal the presence of saponins. The results support the folkloric use of *Ficus exasperata* leaf as a therapeutic agent and potential source of novel drugs.

KEY WORDS: *Ficus exasperata*, proximate analysis, phytochemicals.

INTRODUCTION

The use of plants for medicinal purposes, usually in the form of traditional medicine has been recognized by the World Health Organization (WHO, 1999). The healing power of herbs has been exploited since antiquity by various cultures and the study of medicinal plants have yielded well known chemical substances such as quinine, aspirin, digoxin, cocaine and morphine among others, which have physiological and biochemical actions in man and animals (Farnsworth and Bingel, 1997).

Ficus exasperata Vahl (Family: Moraceae) is widespread in tropical Africa and Arabia, and usually inhabits dryer types of forests. It is a small tree growing as high as 21 meters, but usually smaller and deciduous. The leaves are variable, mostly entire but sometimes toothed, and are often deeply three-lobed in young trees (Keay, 1989).

Decoctions of the root bark are used to treat asthma orally in Tanzania (Chhabra *et al.*, 1984), while the fresh leaf is used to treat inflammations of tonsils and throat. Aqueous extracts of the leaves are used for ophthalmic conditions and as an anthelmintic. In Sierra Leone, powdered leaves are used for vaginal rash (Macfoy and Sama, 1983), and an infusion of the crushed leaves is taken as an abortifacient. Also in Sierra Leone, the dried leaf is used as a haemostatic (Kone-Bamba *et al.*, 1987) and also said to have anticoagulant activity. In Ubiaja, a settlement in Esan South East Local Government Area of Edo State in Nigeria, the aqueous leaf extract from *F.exasperata*, locally known in this area as "Amemele" had found use in the oral treatment of diabetes mellitus (Nimenibo-Uadia and Osagie, 2001). Phytochemical screening has revealed the presence of essential oils, flavonoids, quinones, sterols and tannins in the root bark (Chhabra *et al.*, 1984). Literature on the phytochemical and proximate components of the leaves appears to be non-existent.

MATERIALS AND METHODS

Chemicals

All chemicals/reagents used in this study were of analytical grade.

Plant Material

Fresh leaves were collected from *Ficus exasperata* in and around University of Benin, Benin City, grounds and taxonomically identified and authenticated at the herbarium of the Department of Plant and Biotechnology of the same university.

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The leaves were washed with distilled water, air-dried indoors (25±2 ^{0}C), for a week and then oven-dried at 40 ^{0}C to a constant weight (6 h) and milled to powder (Corona, Landers Y CIA, SA) to pass through a 0.8mm sieve and stored in air-tight stoppered glass bottles until needed.

Chemical Composition

The proximate analysis (moisture, protein, lipid, ash and carbohydrate) of *F.exasperata* leaf was determined based on the methods of the Association of Official Analytical Chemists (AOAC, 2000). All experiments were done in triplicate.

Moisture content of the sample was determined using the thermal drying method (evaporation of moisture). 2.0 g of the fresh leaf (cut in bits) was weighed and placed in a washed oven-dried and weighed crucible. This was placed in an oven (Gallenkamp, London, UK) and dried at 40 ^{0}C for 5 hours, after which it was allowed to cool in a desiccator and then re-weighed at room temperature. This procedure was repeated until a constant weight was obtained. The percentage moisture content (fresh weight) was expressed as the loss in weight on drying as a fraction of the initial weight of sample, multiplied by 100.

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (g)}}{\text{Initial weight of sample (g)}} \text{ x } 100$$

Total ash content was determined using the ignition method (furnace incineration). The crucible used was washed, pre-ignited in a muffle furnace (Gallenkamp, London, UK) at 600 ^{0}C for 2 hours, cooled and weighed with the lids on. 2.0 g of the milled leaf sample was weighed into a crucible with the lid on and placed in the cold furnace with temperature rising to 600 ^{0}C. The ashing was carried out for 6 h, and the crucible and contents cooled in a desiccator and re-weighed at room temperature. The percentage ash content was calculated as

$$\text{Ash (\% dry weight)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \text{ x } 100$$

The soxhlet extract procedure as described by Shirlaw (1976) was adopted for the estimation of **crude fat/lipid content** of the leaf sample. The procedure is based on the extraction of fats by non-polar solvents like petroleum ether or n-hexane. The weight of the fat present is obtained after evaporating the solvent from the extract. 2.0 g of the milled sample was weighed and secured in an extraction thimble which was then plugged with cotton wool, to avoid loss of sample. The thimble was placed in the extractor that was connected to a condenser and an already weighed, clean and dry round-bottomed soxhlet flask was attached to the bottom of the extractor. 200 ml of n-hexane was poured into this dry soxhlet flask and the heating mantle switched on so that the hexane just boils. Anti-bumping granules were introduced into the soxhlet flask and heating continued for at least 6 hours (naked flames were not allowed near this set-up). The content of the flask was taken to dryness using a vacuum rotary evaporator.

The flask was removed and then dried to a constant weight in a water bath at 60 ^{0}C. The flask was cooled, re-weighed and the amount of fat extracted calculated from the difference between the weight of the flask before and after extraction.

$$\text{Ether Extract (\% dry weight)} = \frac{\text{Weight of lipid (g)}}{\text{Weight of sample (g)}} \text{ x } 100$$

Crude protein was estimated by the semi-micro Kjeldahl method of Markham (1942) as reported by Pearson (1973). It involves digestion, distillation and titration. 2.0 g of the milled sample was weighed and placed in a digestion flask with 3.0 g catalyst mixture (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide), 30 ml concentrated H_2SO_4 and anti-bumping granules added. The flask was placed in an inclined position and heated gently (heating mantle) until the initial frothing had ceased. The flask was shaken from time to time and digestion continued for about one hour after the liquid had become clear. It was then cooled and the digest filtered and quantitatively transferred into 100 ml volumetric flask and made up to the mark with distilled water. For distillation, 10 ml of the diluted digest was transferred into a round-bottomed flask and made alkaline with 25 ml of 40 % NaOH solution. The flask was placed on a heating mantle and connected, using a Liebig condenser, to a beaker (the receiving flask) containing 10 ml of 2% boric acid to which 2 – 3 drops methyl orange indicator had been added. Care was taken to avoid leakage of the gas by connecting up the apparatus with the

delivery tube dipping below the boric acid solution and closing all joints tightly. Distillation of the ammonia formed was commenced by heating the flask. The distillation continued until the boric acid mixture (containing the ammonium borate complex formed) completely changed from purple to a greenish-yellow colour. The distillate was then titrated against a standardized HCl solution (0.1M HCl) to a colourless endpoint and the titre noted. The percentage crude protein was calculated by first calculating the total organic nitrogen (N) and multiplying by 6.25.

$$1000\text{ml of 1M HCl} \equiv 14\text{g of NItrogen (N)}$$

$$\text{OR } 1000\text{ml of 0.1M HCl} \equiv 1.4\text{g N}$$

$$\therefore 1\text{ml of 0.1M HCl} = \frac{1.4\text{g}}{1000} \text{ of N} = 0.0014\text{g N}$$

$$\text{i.e. } 1\text{ml of 0.1M HCl} \equiv 1.4\text{mg N}$$

$$\%\text{ Nitrogen} = \frac{\text{Titre value x 1.4 x Dilution factor}}{\text{Weight of sample (mg)}} \text{ x } 100$$

$$\therefore \%\text{ Crude protein} = \%\text{N x } 6.25$$

Note: 1.40 is nitrogen value equivalent to 0.1M HCl used in titration while 6.25 is the protein conversion factor.

Crude fibre is the plant polysaccharide and lignin, which are resistant to hydrolysis by digestion with acid and alkali. 2.0 g of a dried fat-free residue was transferred to an Erlenmeyer flask. 200ml of 1.25% H_2SO_4 solution was added and the flask connected with a reflux condenser. The content of the flask was boiled gently for 30 min and rotated at 5 min intervals for thorough mixing. A blast of air was occasionally directed into the flask to reduce frothing. After digestion, the content of the Erlenmeyer flask was filtered through cheese cloth supported in a funnel and washed free from acid with boiling distilled water. Washed residue on the cheese cloth was gently transferred back to the Erlenmeyer flask. 200 ml of 1.25% NaOH solution was added and the flask was connected to the reflux condenser. The sample was boiled for 30 min, rotating the flask at 5 min intervals. The content of the flask was again filtered through the same linen filtering cloth and washed thoroughly with boiling distilled water. The residue was then transferred to a clean and dry crucible. The crucible and contents were dried at 100 ^{0}C to a constant weight and cooled before weighing. The crucible with its content was then ignited in the furnace at 600 ^{0}C for 20 min. The crucible containing the ash was cooled in a desiccator and re-weighed. The loss in weight during incineration is equivalent to the weight of crude fibre in the sample.

$$\text{Crude Fibre (\%)} = \frac{\text{Weight of fibre (g)}}{\text{Weight of sample (g)}} \text{ x } 100$$

Carbohydrate content of the sample was determined by "difference". This was done by summing up percentages of all proximate components and subtracting from 100.

$$\text{Total Carbohydrate (\%)} = 100 - (\%\text{ moisture} + \%\text{ ash} + \%\text{ fat} + \%\text{ protein})$$

The **Calorific value** was calculated as kcal/100g sample, using the Atwater factors (4, 9, 4 for protein, fat and carbohydrate respectively) based on the following formula (FAO/WHO/UNU, 1991):

$$\text{Calorific value} = (\%\text{ carbohydrate x } 4) + (\%\text{ crude fat x } 9) + (\%\text{ crude protein x } 4)$$

Phytochemical Composition

Preliminary evidence for the presence of secondary products in *F.exasperata* (Vahl) leaf sample was obtained using standard phytochemical procedures (Harborne, 1973; Odebiyi and Sofowora, 1978; Sofowora, 1993).

Statistical Analysis

Means and standard error of means were calculated for three independent determinations of each proximate component except for total carbohydrate which was by "difference".

RESULTS AND DISCUSSION

A good number of plants are being used locally in the management of diabetes mellitus, partly to cut costs of orthodox medicines or avoid their attendant side effects. *Ficus exasperata* is one of such plants and

our earlier studies with diabetic animals confirmed the leaves have antihyperglycaemic activity (Nimenibo-Uadia and Osagie, 2001). The efficacy of these medicinal plants may be due to the presence of bioactive components in the plant. Thus, this study set out to determine the proximate composition and screen for presence of phytochemicals in the leaf of *F.exasperata* as an initial step in unraveling the biochemical principles responsible for its antidiabetic activities.

Figure 1 shows the proximate chemical composition of the *F.exasperata* leaf. Other results not on the table are carbohydrate content (15.56 g %), which was calculated by "difference" and calorific value (126.66 kcal/100g). It should be clear that carbohydrate estimated this way includes fibre, as well as some components that are not strictly speaking carbohydrate e.g. organic acids (Merrill and Watt, 1973). Leafy vegetables are not good sources of dietary energy as this is a reflection of low dry matter (DM)) content of many leaves (Oguntona, 1998). The percentage moisture content of the leaf was determined on a fresh weight basis. The moisture content is a measure of susceptibility to microbial spoilage. *F.exasperata* had a moderate moisture content of 57.20±0.0 % which means shelf – life may not be long. Most fresh leafy vegetables have moisture content in the range of 70 to 90% (Oguntona, 1998).

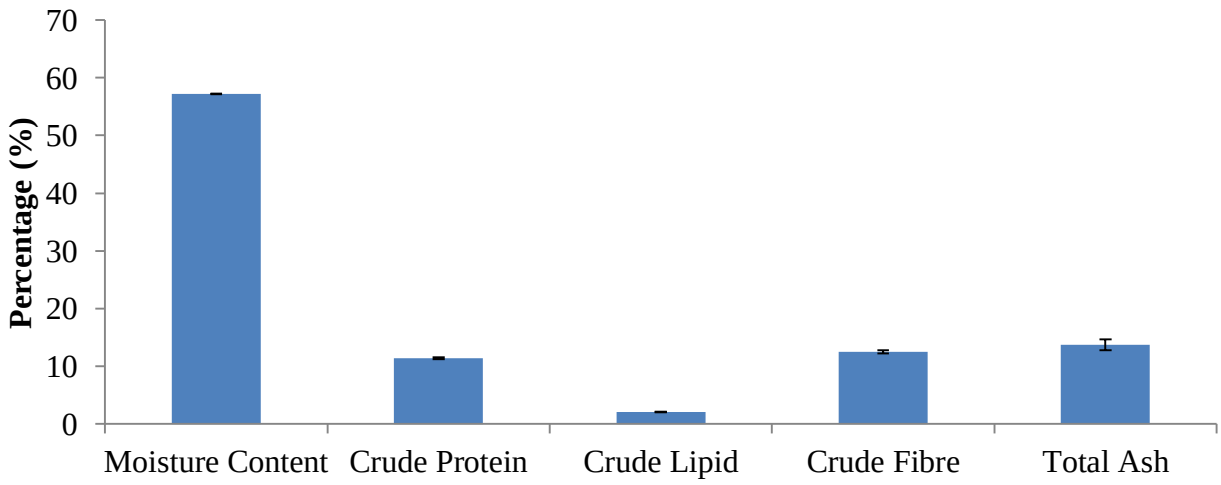

Figure 1: Proximate composition (%) of *Ficus exasperata* leaf

The observed protein content was 11.38±0.16%. This compares favourably with 13.42% for *Azadirachta indica* reported by Atangwho *et al*. (2009), but higher than the 5.58% for *Euphorbia heterophylla* leaf reported by James and Friday (2010). Most leafy green vegetables are not good sources of protein. Crude protein in the dried leaf samples can range from 15.0 to 30% (Aletor and Adeogun, 1995).

The lowest recorded nutrient value was crude lipid (Table 1). A value of 2.10±0.06% is low and compares favourably with that of *Vernonia amygdalina* leaf (3.53%) reported by Atangwho *et al*. (2009), and 1.25% for *E.heterophylla* leaf reported by James and Friday (2010). Leafy vegetables are poor sources of lipids (Oguntona, 1998; Ejoh *et a*l., 2007).

Crude fibre of *F.exasperata* leaf recorded in this study was 12.50±0.29% which is much higher than the 3.0% for *E.heterophylla* leaf reported by James and Friday (2010), but comparable to 13.69% for *Gongronema latifolium* reported by Atangwho *et al*. (2009). Leafy vegetables are important sources of fibre intake in Nigeria (Oguntona, 1998). Crude fibre plays an important role in human nutrition. Increased fibre consumption reduces the incidence of certain diseases such as colon cancer, coronary heart disease, diabetes mellitus, obesity, hypertension and various digestive disorders including constipation (Chaney, 2006; SACN, 2008).

The current study revealed a high ash content for *F.exasperata* leaf (13.76±0.95%). It is higher than the 11.93% for *A.indica*, 10.01% for *V.amygdalina*

leaves reported by Atangwho *et al*. (2009) and those of commonly consumed leafy vegetables such as *Occimum graticimum* (8%) and *Hibiscus esculentus* (8%) but much lower than the 20% of *Talinum triangulare* (Akindahunsi and Salawu, 2005).

Table 1: Proximate composition of *Ficus exasperata* leaf

Parameter	Value (g% composition)
Moisture content	57.20±0.03
Crude protein	11.38±0.16
Crude lipid	2.10±0.06
Crude fibre	12.50±0.29
Total ash	13.76±0.95

Values are Means ± SEM of triplicate determinations

The ash content is a reflection of the concentration of mineral elements present in the plant part. Thus, a large concentration of minerals may be present in *F. exasperata* leaf. This needs to be ascertained.

Phytochemical analysis showed that *F.exasperata* leaf has tannins, flavonoids, alkaloids, anthraquinones, cardiac glycosides (Table 2), but saponins were not detected.

The presence of phytochemicals in the aqueous extract of *F.exasperata* is suggestive of possible physiological and biochemical activities. The aqueous extract was reported to lower blood cholesterol, triacylglycerols and ketones (Nimenibo-Uadia, 2003). Saponins were found useful in the treatment of hypercholesterolaemia (Oakenful and Sidhu, 1990), an attendant derangement of diabetes mellitus. However, in the present study, saponins were not detected in the aqueous leaf extract of *F.exasperata* which clearly rules out saponins as the effective agent in *F.exasperata* that ameliorates the diabetic syndrome.

The presence of alkaloids (Table 2) in the leaf of *F.exasperata* may also play a role in its antidiabetic property. Hypoglycaemic alkaloids have been found in a number of plants including *Tecoma stans*, *Trigonella foenum-graecum* and *Catheranthus roseus* (Oliver-Bever, 1980).

The leaf of *F.exasperata* also contains flavonoids (Table 2) which are well-known anti-oxidants which could ameliorate diseased states by scavenging free radicals (Tiwari and Rao, 2002). *Ginkgo biloba* is reported to owe its anti-diabetic activity to flavone glycosides (Shane-McWhorter, 2001). Besides, flavonoids also have anti-inflammatory properties (Kenner and Requena, 1996), lending credence to the use of the fresh leaf of *F.exasperata* in the treatment of inflammation of tonsils and throat.

The present study also reports the presence of tannins (Table 2). Tannins have astringent properties and aid wound healing (Okuda *et al.*, 1982). In addition, tannins may lower the rate of starch digestion and hence blood glucose response by binding directly with the amylase enzyme thus inactivating it (Thompson, 1988). Tannins may thus play a role in the blood glucose lowering capacity of the aqueous leaf extract of *F.exasperata*. The use in Sierra Leone of the powdered leaves of *F.exasperata* for vaginal rash (Macfoy and Sama, 1983) may be due to the presence of tannins (Table 2). Several phenolic compounds, e.g. monomeric hydrolysable tannins, oligomeric ellagitannins and condensed tannins, having galloyl groups or hexahydroxydiphenoyl groups have potent inhibitory effect on herpes simplex virus types 1 and 2 infection (Fukuchi et al., 1989). The present study also revealed the presence of anthraquinones (Table 2). Anthraquinone glycosides are cathartic, acting on the large intestine (colon), which may be used as purgatives (Harborne, 1973). Cardiac glycosides also known as cardenolides were detected in the leaf of *F.exasperata* (Table 2). Most cardiac glycosides (a group of triterpenoids) are toxic but many have pharmacological activity.

Table 2: Phytochemical screening of the aqueous extract of *Ficus exasperata* leaf

Phytochemical	Test	Result
Tannins	Ferric chloride	+
Flavonoids	Sodium hydroxide	+
	Ferric chloride	+
	Lead acetate	+
Alkaloids	Mayer's	+
	Dragendorff's	+
Saponins	Frothing	-
Anthraquinones	Bontrager's	+
Cardiac glycosides	Salkowski's	+
	Keller-Killiani's	+

Key: + = Present - = Not Detected

In therapeutic doses they strengthen the beat of a weak heart muscle (Brewer and Scott, 1983). Thus, the levels of these bioactive molecules in *F.exasperata* leaf need to be ascertained.

CONCLUSION

Tannins, flavonoids, alkaloids, anthraquinones and cardiac glycosides were present in the plant. Their presence may have contributed to its antidiabetic property. The study has also provided some biochemical basis for its other ethnopharmacological uses, thus revealing further potentials of the leaf of *F.exasperata* as a novel source of useful drugs. Apart from the array of phytochemicals present, the high fibre content as revealed in this study may also play a role in its antidiabetic activities.

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