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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING OF AQUEOUS, ETHANOL AND METHANOL EXTRACTS OF *FLACOURTIA INDICA* LEAF AND RIPE FRUIT

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Abstract

Aim and Objective: Plants have been exploited over the years for their therapeutic benefits, because they contain a lot of bioactive compounds that have potentials and ability to treat or manage diseases. Thus, this study investigated the qualitative and quantitative phytochemicals in *Flacourtia indica* (*F. indica*) aqueous, ethanol and methanol leaf and ripe fruit extracts.
Methods: *F. indica* leaf and ripe fruit were harvested from a fruiting tree in Emene, Enugu state, Nigeria, and the analyses were done following standard methods.
Results: Qualitative screening revealed that all tested extracts contained saponin, tannin, flavonoids, alkaloids, glycosides and phenol. While proteins and steroids were not detectable in aqueous and ethanol leaf and ripe fruit extracts, they were present in moderately high (++) amount and in trace amount (+) in methanol leaf extract of *F. Indica*. The quantitative screening comparatively revealed that concentrations of glycosides (mg/ml), flavonoids (mg/ml), alkaloids (mg/ml) and tannins (mg/ml) were higher and phenol (mg/ml) lower in ethanol leaf extract, while in the aqueous leaf extract, phenol (mg/ml) was higher; in the ethanol fruit extract, flavonoids (mg/ml) and alkaloids (mg/ml) were higher, and in the aqueous fruit extract, phenol (mg/ml) and tannin (mg/ml) were higher.
Conclusion: In conclusion, quantitatively, ethanol was a better solvent of extraction than water and methanol and F. *indica* leaf and ripe fruit are rich sources of secondary bioactive phytomolecules, which could be consumed for their health and therapeutic benefits.
Keywords: Alkaloids, extract, flavonoids, glycosides, phytochemical, tannin.

INTRODUCTION

Herbal pharmacological and therapeutic applications are significantly tied to plants' endowed phyto-metabolites, and the management and treatment of diseases with herbs in conventional medicine dated to the ancient age. Plants are composed of several chemicals which they synthesis for self defense or for their bio-physiological benefits. In Greek, plant means phyto and thus phytochemicals are plant chemicals[1]. Some of these plant chemicals or biomolecules are needed for proper body's physiological performance and are thus tagged as indispensable part in dieting[2]. Some phytochemical are known to be anti-nutrient (anti-absorptive) and toxic agents such as phytate in legumes[3]. Solvents are used for the extraction, screening, identification, quantification and isolation of phytochemicals in plants for pharmacological applications. Plants' bioactive molecules are rich in quantifiable measures and compositions[4], and Nwali *et al.*,[1] reported that *Azadirachta indica* contained phenols, alkaloids, saponin, tannin, reducing sugars, flavonoids, anthraquinones, glycoside (cyanogenic and cardiac) and steroids. Similarly, the phytochemical contents of *Phaseolus vulgaris* (Kidney Beans) were reported by Idoko *et al.*,[3] to include alkaloids, cyanogenic glycoside, phenols, saponins, terteoids and tannin. Several discipline involving Life Sciences, pharma-ceutical Sciences and Botany have delved into the practice of screening plant for their antioxidants, antifungal, anti-pathogenic, medicinal and general pharmacological properties; for the health benefit of man and animals[5,6]. Researchers have continued to explore quite a lot of plants for their therapeutic and medicinal values[7]. Several plants have been reported for their pharmacological and medicinal actions; for example, aqueous root extract of *Cassia occidentalis* exerted hepatoprotective and renal-protective activity on acetaminophen induced hepatorenal toxicity in rats[8]; *Ziziphus mauritiana* and *Ziziphus spina* Christi fruit extracts exhibited hypoglycaemic and hypolipidaemic activities in alloxan induced diabetic rats[9]; aqueous

fresh leaf extract of *Chromolaena odorata* (linn) demonstrated hypoglycemic and lipid lowering effects in albino wistar rats fed different concentrations of cholesterol enriched diet[10]; fresh lime juice and honey exerted hypocholesterolaemic activity in albino wistar rats[11]; and methanol extract of *F. indica* exhibited analgesic, anti-inflammatory, and diuretic activities[12]. *F. indica* is a shrub-like tree plant with all its parts possessing several medicinal activities. Its root extract was reported to exhibit antibacterial and anti-pathogenic abilities[13]; fresh leaf aqueous extract of *F. indica* possessed hepatoprotective, anti-anemic and hypoglycemic abilities in CCl_4 induced hepatotoxicity in albino wistar rats[14]; ethanol extract of stem bark of *F. indica* exerted hepatocurative and gluco-stabilizing abilities in aluminium chloride induced toxicity in albino Wistar rats[15]. Others include protection of the liver against paracetamol liver toxicity[16]; anticancer and antioxidant properties[17]; diuretic, analgesic and anti-inflammatory activities[12]; and anti-diabetic ability in streptozotocin induced diabetic rats[18].

Therefore, this study investigated the qualitative and quantitative phytochemicals in *F. indica* aqueous, ethanol and methanol leaf and ripe fruit extracts.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh undamaged leaf and fruit samples were harvested from several parts of the innermost canopies of fruiting plants from Emene, Enugu, Enugu State Nigeria in June, 2021. The collected samples were authenticated by Mr. Okafor, C.U., a botanist in plant tissue culture and biotechnology department, Faculty of Biological Sciences, University of Nigeria, Nsukka and a voucher number of PSB/109-12 A was written. The collected samples were prepared according to methods of Idoko *et al*[3]., The leaf and fruit collected were shade-dried at 25-30^{0}C (room temperature). The dried fruit and leaf samples were macerated into powder employing electrical grinder, and from each powdered sample, 200 g was weighed and dissolved in aqueous, ethanol and methanol solvents respectively for 72 hours to allow adequate extraction. With the use of a mesh and Whatman's filter paper (No 1) in a separating funnel, the mixture was filtered. The water bath was adjusted to 45^{0}C and concentration of the filtrate to semi-solid residue was achieved. The concentrates were kept in airtight containers at room temperature for subsequent analysis.

Qualitative and quantitative phytochemical screening

The aqueous, ethanol and methanol leaf and fruit extracts of *F. indica* were screened qualitatively for their phytochemicals identification using the method of Harborne[19]; while quantitative phytochemical screening by the methods described in previous studies [19,20,21], with slight modification.

Quantitative determination of Alkaloids in extracts of *F. indica*

The method of Harborne[19] was used to determine alkaloids in *F. indica* samples. Into 250 ml beaker was 5g of powered sample loaded, and 200 ml of 10% acetic acid in ethanol was added. At room temperature, the mixture was allowed to stand for 4 hours while covered, and then filtered. Alkaloid was precipitated after a drop wise addition of concentrated aqueous ammonium solution into the collected filtrate, which was concentrated to solid by evaporation on a water bath. Putting the alkaloid precipitated in a filter paper, it was weighed as W1, and content washed by 1% ammonium solution, dried at 80ºC in the oven. After drying and cooling, weighing the filter paper and residue gave W2. The formula below was used to calculate alkaloids in *Flacourtia indica* samples and was expressed in percentage weight.

Quantitative determination of Flavonoid in extracts of *F. indica*

Flavonoid in samples was determined by the method of Obadoni and Ochuko[21]. Briefly, into a 250 ml conical flask was 10 g of samples placed and 100 ml of 80% aqueous methanol added, and on an electronic shaker, the mixture was properly mixed for three hours. Into a weighed beaker was the mixture filtered and on a water bath, it was evaporated to dryness and weighed again until a constant weight was obtained.

Quantitative determination of Tannin in extracts of *F. indica*

Tannin in *F. indica* samples was determined using Trease and Evans[20] method, with some modifications. From the prepared samples, was 0.5 g weighed into a conical flask where 50 ml of distilled water was added and content filtered after mixing for 1 hour. Into a 50 ml volumetric flask, 5 ml of the filtrate and 5 ml of 0.1% tannic acid were respectively poured. Five (5) ml of distilled water in a 50 ml volumetric flask was used as the blank, and at 20ºC, in a water bath, the three flasks were filled up with distilled water to the 50 ml mark and incubated for 1 hour 30 minutes. Using UV-Vis Spectrophotometer, DHG-9101, tannin concentration in samples was determined at 760 nm.

Quantitative determination of Phenol in extracts of *F. indica*

Quantification of phenol in extracts of *F. indica* was determined using Obadoni and Ochuko[21] method. Briefly, 1 g of sample was weighed into a 150 ml beaker, and then 20 ml of 80% ethanol was added, mixed and filtered with Whatman's filter paper (No 1) in a separating funnel. Into another 150 ml beaker was 5 ml of the filtrate loaded and 0.5 ml folinciocalteus reagent added and 2 ml of 20% sodium carbonate was added after half an hour and mixed. At 650 nm, absorbance was measured and phenol was calculated in mg/ml.

Quantitative determination of Glycosides in extracts of *F. indica*

The quantification of glycosides in *F. indica* extracts were achieved by the method of Obadoni and Ochuko[21]. Into a 250 ml conical flask was 5 g of the extracts loaded and 100 ml distilled water was added, stirred for three hours and filtered. Into a test tube was 2 ml of filtrate added followed by the addition of 2 ml of 10% DNS reagent. The test tube and its content inside a beaker of boiling water were left to boil for 20 minutes and then cooled in cold water. At 540 nm, the

absorbance was read using UV-Vis Spectrophotometer, DHG-9101 and glycoside in sample was calculated.

Statistical Analysis

Data were analyzed in triplicate and calculated averages presented in the required units. The IBM statistical package for social sciences (SPSS) for Windows version 17.0 was used to analyze collected data. Results are presented as mean±standard deviation at $p<0.05$.

RESULTS

Percentage Yield of Aqueous and Ethanol extracts of *F. indica*

Table 1 represents the percentage yield of the aqueous and ethanol extracts of *F. indica* leaf and fruit. The result shows that 401.98 g of dried *F. indica* after extraction with aqueous and ethanol yielded 21.05% and 19.19% respectively.

Table 1: Percentage yield of aqueous and ethanol extracts of *F. indica* leaf and fruit.

Extraction solvent	Ground leaf (g)	Ground fruit (g)	Yield after extraction (g)	% yield
Aqueous	100.09	100.60	42.25	21.05
Ethanol	101.06	100.23	38.62	19.19

Key: + = Present; ++ = Moderately present; +++ = Highly present; ND = Not detectable

Qualitative Phytochemical Analyses of Ethanol, Aqueous and Methanol leaf and fruit extracts of *F. indica*

Table 2 shows the qualitative phytochemical results of ethanol, aqueous and methanol leaf and fruit extracts of *F. indica*. Protein was not detectable in both ethanol and aqueous extracts of leaf and fruit, but was moderately high in methanol leaf extract. Saponin was present (+) in both aqueous and ethanol leaf extract, was not detected in either solvents of fruit extract but was moderately high (++) in methanol leaf extract. Tannin was very high (+++) and moderately high (++) in ethanol leaf and aqueous leaf extracts respectively; present (+) and moderately high (++) in ethanol fruit and aqueous fruit extracts respectively; and was very high (+++) in methanol leaf extracts. Flavonoids were present (+) in both ethanol leaf and fruit extracts but were moderately high (++) in methanol leaf extract; and not detectable in both aqueous leaf and fruit extracts. Alkaloids were very high (+++) in both ethanol leaf and fruit extracts; moderately high (++) in both aqueous leaf and fruit extracts; and moderately high (++) in methanol leaf extract. Terpeniods and steroids were not detected in ethanol and aqueous solvents of leaf and fruits extracts but steroids were present (+) in methanol extract. Glycosides were very high (+++) in both ethanol and aqueous leaf extracts; moderately high (++) in both ethanol and aqueous fruit extracts; and very high (+++) in methanol leaf extract. Phenol was moderately high (++) in ethanol leaf and fruit, and aqueous fruit extracts; and was very high (+++) in aqueous and methanol leaf extracts.

Quantitative Phytochemical Analyses of Ethanol and Aqueous leaf and fruit extracts of *F. indica*

The quantitative phytochemical analysis results are shown in Table 3. The results of the analysis show that *F. indica* ethanol leaf extract contains high concentrations of alkaloids, flavonoids, tannins and glycosides, with low concentration of phenols, than aqueous leaf extract. While ethanol fruit showed higher concentrations of alkaloids and flavonoids, with low concentrations in aqueous fruit extract, the concentrations of tannin, phenol and glycosides were higher in aqueous fruit extracts than ethanol fruit extract.

Table 2: Results of qualitative phytochemical analysis of ethanol, aqueous and methanol leaf and fruit extracts of *F. indica*.

S. N.	Constituent	Ethanol extract of leaf	Aqueous extract of leaf	Ethanol extract of fruit	Aqueous extract of fruit	Methanol extract of leaf
1	Protein	ND	ND	ND	ND	++
	Amino Acid	ND	ND	ND	ND	
	Secondary Metabolites					
2	Saponins	+	+	ND	ND	++
3	Tannin (Catecholic)	+++	++	+	++	+++
4	Flavonoids	+	ND	+	ND	++
5	Alkaloids	+++	++	+++	++	++
6	Steroids	ND	ND	ND	ND	+
7	Terpeniods	ND	ND	ND	ND	
8	Glycosides (Cyanogenic and Cardiac)	+++	+++	++	++	+++
9	Phenol	++	+++	++	++	+++

Table 3: Quantitative phytochemical analysis results.

Samples	Alkaloids mg/ml	Flavonoids mg/ml	Tannins mg/ml	Phenol mg/ml	Glycosides mg/ml
Ethanol leaf extract	0.58±0.10	0.61±0.10	0.53±0.10	0.32±0.10	1.08±0.30
Aqueous leaf extract	0.49±0.00	0.00±0.00	0.46±0.00	0.71±0.00	0.06±0.00
Ethanol fruit extract	0.23±0.01	0.45±0.01	0.34±0.01	0.31±0.01	0.06±0.01
Aqueous fruit extract	0.19±0.01	0.00±0.00	0.60±0.01	0.66±0.01	0.02±0.01

Results are Mean ± Standard deviation; the mean difference is significant at $p<0.05$.

DISCUSSION

The results of this study showed that proteins, saponins, catecholic tannin, flavonoids, steroids, glycoside (cynogenic and cardiac) and phenol are present in the leaf and fruit of *F. indica*. This is consistent with the findings of a previous study[22], who reported the presence of proteins, saponins, tannin, flavonoids, alkaloids, sterols, tepernoids, lignans, phenol and carbohydrates in fruits methanol extract of *F. indica*. Similarly, phytochemical contents in other plants have been reported such that in previous studies[1,23] reported that *Azadirachta Indica* contain alkaloids, saponin, tannin, flavonoids, glycoside (cyanogenic and cardiac), phenols, steroids, reducing sugars and anthraquinones; Alhassan *et al*.,[24] reported the presence of alkaloid, tannins, saponins, flavonoids, steroids and glycosides in *Balanites aegyptiaca* kernel. Physiologically, the advantage of alkaloids in pharmaco-therapeutics is associated to their non toxic properties and thus the rich content of alkaloids in all solvents of extraction in this study suggest that *F. indica* leaf and fruit could serve as sources of non toxic medicinal formulations[25]. The significance of tannins, saponins and flavonoids in diet, from fruits, vegetables and herbs are reported to be the major active ingredients in herbal recipes for the exertion of the several pharmacological activities such as anti-diabetic, anti-inflammatory, antioxidant, anti-obesity and various organ/tissue protective properties in plants[26,27]. Owing to the protein content in *F. Indica*, it consumption could provide a healthy source for possible reduction in total cholesterol, hypoglyceamic, decrease in body weight, reduction in blood pressure and enhancement of insulin sensitivity[3,14]. The antioxidant and medicinal properties of flavonoids, particularly, quercetin was reported to include; anticancer, inhibitory and free radical scavenging, improvement on cognitive ability and histamine production inhibition; and that they improve the characteristics flavour, taste and colour of diet[28-30].

CONCLUSION

In conclusion, *F. indica* leaf and fruit essentially contain plant secondary metabolites and protein which could be responsible for their reported pharmacological properties by researchers.

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AUTHOR'S CONTRIBUTION

Idoko A: writing original draft, study conception and design. **Emmanuel UEG:** conceptualization, literature survey, methodology. **Catherine OI:** formal analysis, research design. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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