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RESEARCH ARTICLE

Assessment of Antioxidant Potential, Phenolic and Flavonoid Contents of Different Solvent Extracts from Dried Leaves of *Ficus Exasperata* Vahl

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Abstract The therapeutic properties of plant-based natural products have been shown to be determined by the nature of the intrinsic secondary metabolites. Equally, the quantities and qualities of the secondary metabolites depend on the nature of the selected extraction solvent. The aim of the present study was to evaluate the antioxidant potential and to determine the total phenolic and total flavonoid contents of various solvent extracts from dried leaves of *Ficus exasperata* Vahl. The in vitro estimations of their antioxidant properties were carried out through the ascorbic acid equivalent antioxidant capacity (AEAC) assay which measures the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical discoloration abilities and the ferric ion reducing potential (FRAP) assay. The amounts of phenolics and flavonoids in the extracts were determined through spectrophotometric analyses. The data obtained were analysed using a one-way analysis of variance.

The results showed variances in their antioxidative activities with respect to the antioxidant assessments. The extract obtained from n-butanol had the highest AEAC value, followed by the ethanol extract while FRAP values were not significantly ($p < 0.05$) different in the ethanol and aqueous extracts. The least amount of FRAP was estimated in the n-butanol extract. The quantities of the phenolics obtained from aqueous extract were higher ($p < 0.05$) than those in other extracts. Likewise, the extract obtained from n-butanol was shown to have the highest amounts of flavonoids compared to other selected extractives. The study showed that the physical and chemical natures of these solvents could play a critical role in extractions of the antioxidative compounds of the dried leaves of *F. exasperata* which could invariably influence the biological applications of the respective extracts.

Keywords *Ficus exasperata* Vahl · Phytochemicals · Polarity · Antioxidant activity

Significance statement The study assessed the influence of solvents with varying polarities on the anti-oxidative potential of dried leaves of *Ficus exasperata* Vahl and the results revealed that the properties of extracting solvents could influence the antioxidant properties of the leaves.

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Introduction

Ficus exasperata Vahl. (Moraceae) is a deciduous shrub belonging to the genus *Ficus*. *F. exasperata* is native to tropical Africa. The leaf is commonly called sandpaper leaf due to its appearance and coarse nature. Different parts of the botanical are used in folklore medicine for the treatments of various ailments such as skin infections, inflammation, anxiety and malaria [1, 2]. In South-Western region of Nigeria, infusion of *F. exasperata* leaf has been used in the traditional management of cardiovascular dysfunctions and hypertension [3]. Analyses of the components of the *F. exasperata* leaf revealed that it contains phytochemicals such as quercitrin, chlorogenic acid, caffeic acid and cinnamoyl derivatives as the major phenolics which have been described to have antiaging and antioxidative properties [3]. Furthermore, the aqueous extract possesses hypoglycaemic, hypotensive, and sedative-hypnotic properties with no reports of cytotoxicity [4–6]. Most of the secondary metabolites, though commonly

present in low concentrations, contribute to the acclaimed medicinal usefulness of many plants including *F. exasperata* [3]. The yields and qualities of these compounds are dependent on the protocol of extraction with reference to the nature of the solvents used and methods of extraction [7]. These could also be affected by the physical properties, chemical natures and the stability of the varied secondary metabolites in the plant matrix. Thus, diverse extracting solvent and procedures are used to optimise extraction purposes. Earlier studies had reported pharmacological use of various extracts of *F. exasperata* [3–6]. However, these reports were obtained from independent studies that could not account for comparative evaluation of the pharmacological potentials of the extracts of *F. exasperata* prepared with different solvents. This study was therefore aimed at evaluating and comparing the impacts of n-butanol, ethanol, and water on the extraction effectiveness of antioxidative compounds of the leaves of *F. axasperata.*

Material and Methods

Chemicals

Ethanol and n-butanol were products of Guangzhou JHD Chemical Reagent Co., Ltd, China and MRS Scientific, Essex, UK, respectively. 1,1-diphenyl-2-picrylhydrazyl was obtained from E-Labscience, China while Folin Ciocalteu's phenol reagent was a product from Loba Chemie, India. All other chemicals used for the study were of analytical grade.

Preparation of Plant Samples

The leaves of *F. exasperara* were collected from the surroundings of McPherson University in Seriki Sotayo, Ogun State, Nigeria and authenticated at the Department of Biological Sciences, McPherson University. The samples were air-dried at room temperature of 28 ± 1 ℃ and pulverized. The extracts were obtained by soaking 6.0 g of the pulverized sample separately in 60 mL of distilled water, 99.7% ethanol and 99.5% butanol for 24 h. The samples were filtered and the filtrates were used for the assessment of the biological properties.

Assessment of in vitro Antioxidant Properties

Ascorbic Acid Equivalent Antioxidant capacity (AEAC)

The ascorbic acid equivalent antioxidant capacities of the samples were assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) discoloration assay as described by Oso and Ogidi [8]. Precisely 0.5 mL of each extract was transferred into a tube containing 0.5 mL phosphate buffer (0.05 M, pH 7.0) after which 2.0 mL DPPH (0.1 mM, freshly prepared in methanol) was added. The absorbance was measured at 517 nm after 20 min of incubation in the dark at the room temperature (RT) of 29 ℃. The radical scavenging capacity of each extract was calculated from the equation ($y = -0.004x + 0.209$) obtained from a curve prepared using ascorbic acid. The results were presented in µg/100 g of dry weight.

Hydrogen Peroxide Scavenging Capacity

Hydrogen peroxide scavenging capacity of each of the extracts was evaluated as percentage induced-decomposition of hydrogen peroxide by the extract into water and oxygen gas following the method described by Sinha [9] with slight modification. The extract (0.5 mL) was added to a test_tube containing 0.3 mL of 5 mM H_2O_2 and 1.0 mL of distilled water. The mixture was allowed to stand at room temperature for 3 min. 0.2 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid (1:3 v/v)) was added and the reaction solution was made up to 5.0 mL with distilled water. The tube was boiled in water bath at 100 ℃ for 10 min and cooled. The absorbance of the undecomposed hydrogen peroxide was read at 570 nm against a blank which contained all reagents without the samples. The percentage of hydrogen peroxide scavenged was calculated as the percentage difference in the absorbance of the blank and the test sample.

Ferric Reduction Antioxidant Potential

Ferric ion reducing potential of each extract was carried out according to Oyaizu [10]. The filtrate (2.5 mL) was added into a tube containing 2.5 mL phosphate buffer (pH 6.6, 0.1 M) and 2.5 mL of potassium ferricyanide (0.5% (w/v)). The tube was allowed to stand for 25 min at room temperature (RT) of 29 ℃. 1.0 mL of 4% trichloroacetic acid was added to the tube and centrifuged at $600 \times g$ for 5 min. Precisely 2.0 mL of the supernatant was transferred into a separate tube containing 0.1 mL ferric chloride (0.2% (w/v)). The absorbance was measured at 760 nm. The results were calculated and presented in µg/100 g of dried weight of the leaves.

Determination of Phytochemical Compositions

Total Phenolic Content

The total phenolic content was determined through the method presented by Singleton et al. [11]. The test extract (0.1 mL) was added to a tube containing 500 µL of the Folin Ciocalteu reagent (prepared in ratio of 1 to 10 with distilled water). Afterward, 5.0 mL of 10% (w/v) sodium

carbonate was added after it had been allowed to stand at room temperature (29 ℃) for 20 min. The absorbance was taken at 765 nm after 20 min and the results were expressed in µg/100 g of dried weight of the leaves.

Total Flavonoid Content

Total flavonoids content was assessed as described by Zhishen et al. [12]. The test extract (0.1 mL) was added into a test tube containing 1.5 mL 2% sodium nitrite and allowed to stand at room temperature of 29 ℃ for 5 min after which 1.5 mL of aluminium chloride (7.5%) was added into the tube. After 20 min, 2.0 mL of 0.5 M sodium hydroxide was added to the tube. The absorbance was measured at 510 nm. The results were presented in µg/100 g of the dried weight of the leaves.

Statistical Analysis

The data obtained were analysed using a one-way analysis of variance. The data were expressed in mean ± standard deviation of three determinations and differences between the means were examined by Duncan's New Multiple post-hoc tests using the IBM SPSS Statistics 20 software.

Results and Discussion

Health-promoting properties of plant materials with reference to their extensive use in folklore medicine have been attributed to their high contents of bioactive compounds which are influenced by the physical and chemical natures of their extracting solvents among other factors [7, 13–15]. Three solvents of varying polarities were chosen in this study to investigate the potential of solubilization of antioxidative compounds present in the dried leaves of *F. exasperata*. The extract of n-butanol had the highest AEAC value, followed by the ethanol extract. The degradation of hydrogen peroxide by the extracts showed that the butanol and ethanol extract had competitive degradation potentials followed by the aqueous extract. The ethanol and aqueous extract displayed the highest ferric reducing potential with the values of 27.60 ± 3.31 and 24.71 ± 3.32 (µgAAE/100 g), respectively (Table 1).

Polar solvents could enhance adequate extraction of compounds with antioxidant potentials from medicinal plants [16]. The influence of the extracting solvents on the antioxidative capacities of the dried leaves of *F. exasperata* was revealed by the capacity of each of the extracts to reduce ferric ion. The observations are consistent with previous reports that the chemical and physical properties of the extracting solvents could be responsible for the reported variances in the antioxidative properties of *F. exasperata* [7, 17].

The results of phenolic and flavonoid contents of the extracts showed the amounts of the phenolic contents in the n-butanol, ethanol, and aqueous extracts varied significantly with the values of 16.26 ± 0.44, 14.52 ± 1.88 and 19.78 ± 1.37 (µgGAE/100 g), respectively (Table 2). It could be observed that the extract of n-butanol had higher flavonoid content than that of the other extractives. This could unvaryingly affect the biological efficacy of the extracts.

N-butanol had the strongest capacity for the extraction of antioxidant compounds followed by ethanol comparable to water. The AEAC decreased probably because of the prevalence of the non-polar part of n-butanol over the polar hydroxyl group. The observed variances might also be due to differences in the structures and properties of the compounds. The AEAC corresponded to the apparent amounts of flavonoids in the extracts. This substantiates the earlier report of Varghese et al. [18] though, on different specie of plant material. The polar character of the ethanol and its respective affinity to water-soluble compounds could be

Table 2 Amount of Phenolic and Flavonoid Contents of the extracts of dried leaves of *F. exasperata*

	Phenolics (µgGAE/100 g)	Flavonoid (µgQE/100 g)
Butanol Extract	16.26 ± 0.44^{a}	50.54 ± 5.71^{a}
Ethanol Extract	14.52 ± 1.88^{b}	10.03 ± 2.34^{b}
Water Extract	19.78 ± 1.37^{c}	10.10 ± 1.38^{b}

GAE Gallic acid equivalent, *QE* Quercetin equivalent

Data expressed as mean ± standard deviation of triplicate experiments. Values with different alphabets within a column are significantly different at $p < 0.05$

Table 1 Antioxidant potential of the extracts of dried leaves of *F. exasperata*

	AEAC (µg/100 g)	H_2O_2 Scavenging (%)	FRAP (µgAAE/100 g)
Butanol Extract	63.22 ± 10.82^{a}	22.01 ± 1.71^{a}	17.75 ± 2.73^{a}
Ethanol Extract	45.62 ± 3.84^{b}	19.39 ± 4.78^{ab}	27.60 ± 3.31^{b}
Water Extract	34.07 ± 3.02^{c}	10.92 ± 6.38^{b}	24.71 ± 3.32^{b}

AEAC Ascorbic acid equivalent antioxidant capacity, *FRAP* Ferric reducing potential

Data expressed as mean ± standard deviation of triplicate experiments. Values with different alphabets within a column are significantly different at $p < 0.05$

an essential factor for the observed results. In contrast, the solubility of polar compounds that could be accountable for the reduction of ferric ion in n-butanol could be limited. On the other hand, the aqueous extract presented the highest amount of phenolics followed by the butanol extract.

The properties of these solvents with reference to polarity, viscosity, and dielectric constant could play an essential role in the extraction of phenolic and flavonoid contents in the dried leaves of *F. exasperata*. The influence of the extractants on the extraction of the antioxidant compounds might conceivably be due to the differences in the dielectric constants and polarities of the solvents. Antioxidant compounds have varying degrees of polarities [17]. Similarly, the solubility and recovery of these bioactive compositions could probably be influenced by other physicochemical parameters of the solvents such as viscosity, dielectric constant and polarity of respective solvents. Dielectric constant measurement is often used for assessment of the polarity of solvents as it roughly categorizes a solvent into either polar or non-polar [19]. According to Gregory and Clarke [20] and Mohsen-Nia et al. [21], water had the highest dielectric constant value followed by ethanol. This corresponds to the order of the polarity of these solvents. Moreover, the kinematic viscosity of the solvents increases as the polarity decreases [22]. The potentials of antioxidant compounds in the solvents could represent the interaction of the solutes based on differences in viscosity and dielectric properties. Thus, these physicochemical properties could inclusively influence the biological applications of the solvents and the subsequent antioxidant activity of the extracts.

The reported antioxidant potentials of the extracts could possibly be related to the nature and quantities of the antioxidant compounds in the respective extracts. Thus, butanol could be a preferred solvent for extracting flavonoids and other antioxidant compounds with hydrogen atom donating potential from the dried leaves of *F. exasperata* whereas ethanol might be preferred, as a solvent, for the antioxidant properties that relate to the reduction of ferric ion. The potencies of the n-butanol extract could be related to the ability of the extract to abstract compounds that could partition solvents with relative intermediate polarities. Moreover, the consequential influence of the n-butanol extract on the discoloration of DPPH radicals could be associated with the synergistic effect of polar and non-polar bioactive compounds in the sample.

Conclusion

This work demonstrated that extracts of the leaves of *F. exasperata* exhibited considerable antioxidants properties. The study showed that the chemical properties of selected solvents could play a critical role in the extraction of antioxidant compounds from the dried leaves of *F. exasperata* which could invariably influence the biological applications of the plant material.

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Author Contributions BJO conceived, designed and performed the experiments with the assistance of IFO. BJO wrote the manuscript draft. Revision of the manuscript was done by all the authors. All authors read and approved the final manuscript.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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