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Original Research Article

Influence of different solvent polarities on the phenolics, flavonoids and antioxidant properties of the fruit of *Xylopia aethiopica* (Dunal) A. Rich

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ABSTRACT

The aim of the present study was to evaluate the influence of different solvents with different polarities on the antioxidant properties of the fruit of *Xylopia aethiopica* (Dunal) A. Rich. The phenolics and flavonoids contents of the studied extracts were quantified through colorimetric tests, while the *in vitro* antioxidant capacity of the extracts was evaluated through the assessments of the Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging potential. The quantitative evaluations of the phytochemical compositions indicated that there were significant differences ($p<0.05$) in the amounts of phenolics and flavonoids of the extracts. The antioxidant activities measured by FRAP, TEAC and DPPH• scavenging methods revealed significant ($p<0.05$) differences amongst the extracts. Accordingly, methanolic extract was found to have the highest TEAC and FRAP contents with the values of 244.12 ± 21.76 mg/100g and 304.78 ± 5.71 mg/100g, respectively and the lowest IC_{50} value in the results obtained from DPPH• scavenging assay (64.33 ± 2.63 µg/mL). The results showed that methanol could be an appropriate extraction solvent for phytomedicines from *X. aethiopica* (Dunal) A. Rich that could be helpful in the treatment and management of free-radical-associated oxidative damage.

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1. Introduction

Plants-based products have been employed for centuries in the treatment and management of various diseases. The health promoting potentials of plant-based natural products have been related to a variety of chemical constituents and structures of secondary metabolites in the genus in which the botanicals belong (Camilo et al., 2017; Mohammadhosseini, 2017a, Mohammadhosseini et al., 2017). *Xylopia aethiopica* (Dunal) A. Rich, an angiosperm belonging to the genus *Xylopia* (Annonaceae), is a tropical evergreen tree bearing aromatic seeds. It is locally known as Kimbaa (Hausa), Uda (Igbo), Erínje or Èèrù (Yoruba) and commonly called Negro pepper or Ethiopia pepper (Fig. 1). It is natural to the coastal rain forest and moist border forest in the Savannah zones of Africa, but mainly located in Central, Western and Southern Africa (Tairu et al., 1999). The fruit has been used throughout the history in local preparations of "pepper soup" and "kunnu" in Nigeria. Various parts of the vegetal have been used in traditional medicine for the treatment of several diseases. The leaves and the bark of the stem are regularly used as wound healing agents (Busia, 2007). Besides, the stem bark is usually employed in the treatment of skin infections and postpartum breast infections (Kadiri et al., 2015). The decoction of the dried fruit is used as tonic in the treatment and management of bronchitis, asthma, infertility, wounds, arthritis and rheumatism, post-natal pains and dysenteric conditions (Burkill, 1985; Fall et al., 2003; Ogunkunle and Ladejobi, 2006; Ezekwesili et al., 2010). Metabolomic profiling of the ethanolic extract of the fruit revealed the presence of xylopic acid, caffeic acid, chlorogenic acid, ellagic

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Fig. 1. The dried fruit of *X. aethiopica* (Dunal) A. Rich.

acid, apigenin, rutin, kaempferol and quercetin (Oso et al., 2018). The acetone extract of the fruit has been suggested to possess antioxidant potential which could be used to ameliorate disorders associated with oxidative stress (Mohammed and Shahidul Islam, 2017). Moreover, its influence on storage and retention of antioxidant compounds in fruits has been reported and consequently, it has been proposed to have prospective influence on general acceptability and marketability of perishable fruits such as tomato (Babarinde and Adegoke, 2015). The medicinal effects of the plant with respect to its ethnobotanical claims could be attributed to the presence of various bioactive compounds in the botanical (Aggarwal et al., 2006; Oso et al., 2017). However, several factors that could influence the potencies and the biological functions of these bioactive compounds had been identified through various systematic studies; these include processing, handling and extraction techniques (Nwozo et al., 2015; Mohammadhosseini, 2017b). Additionally, extraction yield and biological activities of phytochemicals have been reported to be influenced generally by the polarity and chemical characteristics of the solvent used for the extraction (Do et al., 2014; Złotek et al., 2016). Therefore, the aim of this study was to evaluate the influence of solvents of different polarities on the antioxidant properties of *X. aethiopica* (Dunal) A. Rich.

2. Experimental

2.1. Chemicals and reagents

Trolox, quercetin, sodium nitrite, 2,2-diphenyl-1-picrylhydrazyl(DPPH),2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and Gallic acid were purchased from Merck company and were of highest purity. These reagents were used without further purification under the experimental conditions.

2.2. Extraction and preparation of sample

Plant material was collected from farms located in the north central zone of Nigeria. The biomass was air-dried under darkness at room temperature (30 ± 2 ºC). The sample was authenticated at the Department of Plant Biology, University of Ilorin with the voucher number UIH001/1089. Dried fruits were ground and stored in tight-sealing dark containers until needed. The powdered fruits (100 g) of *X. aethiopica* (Dunal) A. Rich were mixed with different solvents (1.0 L) of different polarities including distilled water, acetone, methanol and petroleum ether for seventy-two hours (72 hours) at ambient temperature (30 ºC) and solubilised with the aid of a shaker. The filtrate of each mixture was isolated by filtration to remove liquid phase containing the extract from the plant residue. The crude extracts were dried at 40 ºC. Each extract was diluted in its original solvent for the subsequent evaluations of phytochemical profile and antioxidant activity.

2.3. Phenolics contents

The assay principle is based on the reactions of phenolic groups present in the samples with Folin-Ciocalteu reagent (FCR) in an alkaline medium. This gives a blue colour with a maximal absorption at 760 nm. The assay was carried out as described by Singleton et al. (1999) with slight modifications. About 1.0 mL of the extract (105 µg/mL) was mixed with 0.5 mL of an aqueous solution of FCR (FCR: water, 1:10 v/v). The mixture was allowed to stand at room temperature (29 ºC) for 1 hour. After that, 1.5 mL of sodium carbonate (7.5%) was added and the reaction solution was made up to 5.0 mL with distilled water. The absorbance was read at 765 nm against a blank which contained all reagents without the samples. The results were calculated from the standard curve ($y=0.026x+0.6234$; $r^2=0.9829$) obtained using varying concentrations of Gallic acid (5-20 µg/mL) in methanol and expressed in mg/100g Gallic acid equivalents (GAE).

2.4. Flavonoids contents

The flavonoids were measured by aluminium chloride colorimetric assay based on the development of flavonoid-aluminium complex with a maximal absorption at 510 nm, and performed as described by Zhishen et al. (1999) with slight modifications. Exactly, 1.0 mL of the extract (105 µg/mL) was mixed with 1.0 mL of AlCl3 (5%). The mixture was allowed to stand at room temperature (29 ºC) for 5 minutes after which 2.0 mL of $NaNO_2$ (7%) was added. Afterwards, 1.0 mL of sodium hydroxide (1%) was added to the reaction mixture. The absorbance was determined at 510 nm against a blank which contained all reagents without the samples. The results were calculated from standard curve ($y=0.039x+0.4542$; $r^2=0.9852$) obtained using varying concentrations of quercetin (5-20 µg/mL) in methanol and expressed in mg/100g quercetin equivalents (QE).

2.5. Trolox Equivalent Antioxidant Capacity (TEAC)

The principle is based on the measurement of the antioxidant capacity of the extracts, as compared to the standard, Trolox, using the $ABTS^+$ decolourisation assay measured at 734 nm. The $ABTS^+$ decolourisation assay was performed as defined by Re et al. (1999). Exactly, 0.10 mL of each extract (150 µg/mL) was added to the wells of the micro-plate and the reaction was initiated by adding 0.1 mL of $ABTS^+$ solution. This solution was prepared by addition proper amounts of ABTS (7 mM) in ammonium persulphate (2.45 mM) overnight. The absorbance of the resulting solution was recorded at 734 nm. The antioxidant activity of each of the extract was calculated from the standard curve ($y=-0.0352x+0.6756$; $r^2=0.7438$) obtained from varying concentrations of Trolox (5-20 µg/mL) and expressed in mg/100g TEAC.

2.6. Ferric Reducing Antioxidant Power (FRAP)

The assay was carried out in accordance with the method described by Oyaizu (1986) with slight modifications. The principle is based on the reduction of potassium ferricyanide to potassium ferrocyanide which forms a Prussian blue complex with excess ferric chloride and measurement of the corresponding absorbance at 700 nm. Precisely, 2.5 mL of each solution of the extracts (150 µg/mL) was added to 2.5 mL of sodium phosphate buffer (0.2 M) and 2.5 mL of potassium ferricyanide (1%). The mixture was allowed to incubate at 50 ºC for 20 minutes. Afterwards, 2.5 mL of trichloroacetic acid solution (5% w/v) was added and the mixture was centrifuged at 650×g for 10 minutes. Exactly, 5.0 mL of the supernatant was carefully taken and mixed with equal volume of distilled water and 1.0 mL of ferric chloride solution (0.1%) was added to the reaction mixture. The absorbance of the resulting Prussian blue complex was read at 700 nm. The ferric reducing antioxidant property was calculated from the standard curve ($y=0.0044x+0.0046$; $r^2=0.9337$) obtained from varying concentrations of ascorbic acid (10-50 µg/mL). The reducing power was expressed as mg/100g of the extract.

2.7. DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) scavenging activity

The hydrogen atom donating capacities of the samples were measured from the decolourisation of the purple solution of DPPH•. The assay was performed as described Gyamfi et al. (1999). Exactly, 1.0 mL of DPPH (0.2 mM) in ethanol solution was added to 1.0 mL of sample at varying concentrations (10-50 µg/mL). The mixture was allowed to stand for 30 minutes in the dark at room temperature (29 ºC). The absorbance was read at 516 nm and percentage DPPH• scavenging was calculated as follows (Eqn. 1):

$$\text{Free radical percentage scavenging activity} = \frac{A_0 - A_1}{A_1} \times 100\% \quad \text{(Eqn. 1)}$$

A_0 was the absorbance of the control, while A_1 was the absorbance of sample. The inhibition concentration at 50% (IC_{50}) was calculated from graph of percentage scavenging activity against the concentrations and expressed in µg/mL.

2.8. Statistical analysis

Three determinations of each sample were used for statistical analysis and values were reported as mean ± standard deviation. Data were subjected to one way analysis of variance (ANOVA) and mean values were compared using Duncan's post-hoc multiple comparison tests. Differences at $p<0.05$ were considered to be significant.

3. Results and Discussion

3.1. Phytochemical contents and antioxidant potentials

The results of this study showed that the physical and chemical properties of the solvents had significant influence on the phenolics, flavonoids and antioxidant properties of the extracts (Fig. 2 to Fig. 6). The polarities of the extracting solvents significantly ($p<0.05$) influenced the measured phytochemicals and antioxidant activities. Among the different solvents used, methanol extracted most of the phenolic compounds (974.66 ± 7.61 mg/100g), whereas the petroleum ether extract had the lowest amount of phenolics (24.43 ± 2.11 mg/100g) (Fig. 2). Similar trends were observed in the amounts of flavonoids, FRAP and TEAC across the various solvents (Fig. 3 to Fig. 5). Flavonoids were not detected in the petroleum ether extract (Fig. 3). Furthermore, the assessment of the antioxidant activities of the extracts using TEAC, FRAP and DPPH• scavenging assays showed the impact factor of different extracting solvents on *X. aethiopica* (Dunal) A. Rich as shown by the significant

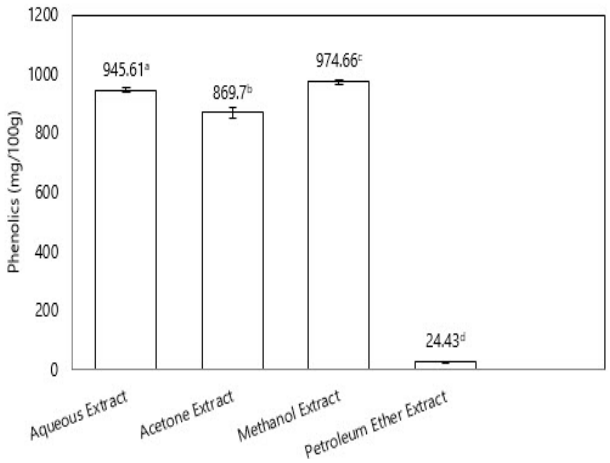

Fig. 2. Phenolic contents of *X. aethiopica* (Dunal) A. Rich extracts from different extraction solvents.

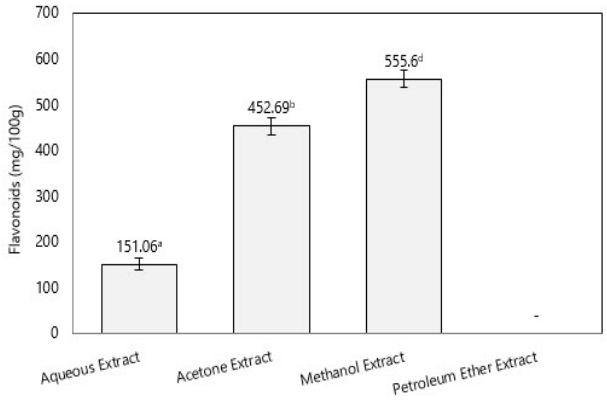

Fig. 3. Flavonoid contents of *X. aethiopica* (Dunal) A. Rich extracts from different extraction solvents.

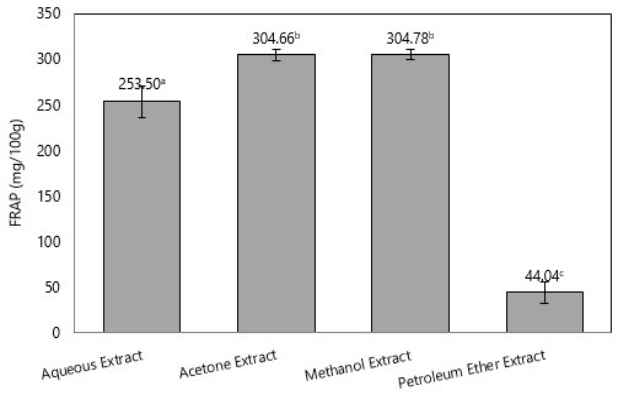

Fig. 5. Ferric reducing antioxidant power (FRAP) of the different extracts of *X. aethiopica* (Dunal) A. Rich fruit.

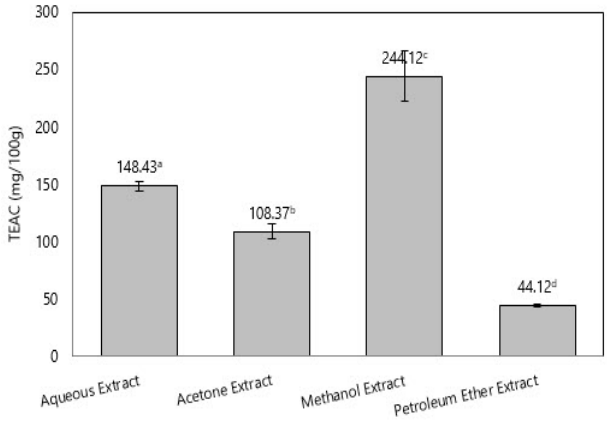

Fig. 4. Trolox equivalent antioxidant capacity (TEAC) of the different extracts of *X. aethiopica* (Dunal) A. Rich fruit.

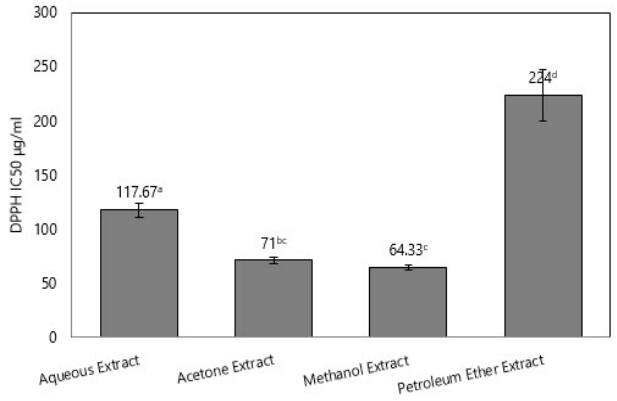

Fig. 6. IC_{50} values of the DPPH• (1,1-diphenyl-2-picryhydrazyl radical) scavenging activity of different extracts of *X. aethiopica* (Dunal) A. Rich fruit.

($p<0.05$) differences in the antioxidant capacities among the extracts. Methanolic extract exhibited the highest antioxidant activity marked with increased concentration of TEAC and FRAP followed by those of aqueous, acetone and then petroleum ether extracts the (Fig. 4 and Fig. 5). In addition, the methanolic extract had significantly ($p<0.05$) the lowest IC_{50} (64.33 ± 2.63 µg/mL) as regards the DPPH• scavenging potential (Fig. 6); this indicates that it has the utmost capacity for radical scavenging activity among the extracts as the IC_{50} is inversely related to the percentage scavenging activities of the extract.

Intake of plant-derived antioxidant certainly plays an important role in health across a population as it checkmates the onset of many degenerative processes and diminishes the severity of degenerative diseases in which inflammation plays a role (Ames et al., 1993). The fruit of *X. aethiopica* (Dunal) A. Rich had been shown to play protective roles against inflammatory disorders; this biological property has been suggested to be partly due to the antioxidant capacity of the extract of the fruit (Oso et al., 2017). Moreover, several studies have related the therapeutic effects of plant parts to the wide distributions of phenolics and flavonoids among other phytochemicals in the plant kingdom (Złotek et al., 2016; Mohammadhosseini et al., 2017). Phenolics and flavonoids are some of the naturally occurring antioxidants found in plants and their contents and subsequent health benefits are found to be influenced by several factors such as cultivars, harvesting time, plant maturity, extraction techniques and processing methods (Nwozo et al., 2015; Mohammadhosseini, 2017b). Further investigations on these phytochemicals and their isolations from plant materials are of prime importance because of their positive correlations to wellness in connection with maintenance of oxidant-antioxidant balance through scavenging of excessive oxidants in the cells. In the present study, the solubility and extraction of the antioxidant compounds were significantly influenced by the polarities and chemical characteristics of the solvents. Water and methanol are polar protic solvents with high dielectric constants and polarities, whereas acetone and petroleum ether are polar aprotic and non-polar solvents, respectively. The findings of this report revealed that the extraction of antioxidant compounds is closely related to the type of solvent used. Methanol was the most effective solvent in extractions of the phenolics and flavonoids as it presented the highest phenolics and flavonoids values followed by the aqueous extract. In a study conducted

by Sowndhararajan and Kang (2013), methanol was also more effective in extractions of phenolic compounds from *Bauhinia vahlii*. However, in Złotek et al. (2016), the best solvent for phenolic extraction from basil leaves (*Ocimum basilicum* L.) was acetone mixtures with acetic acid.

3.2. Solvent polarities and the efficacy of extracted compounds

The nature of the extraction solvent ultimately influences the efficacy of the extracted bioactive compounds from plant materials (Roby et al., 2013; Ye et al., 2015). Antioxidant phytochemicals have varying degrees of polarities from very polar to very non-polar. Their solubilities are probably influenced by the dielectric constant and chemical structure of respective solvents. This study indicated that methanol might be the preferred solvent for extracting bioactive compounds with antioxidant activities from the fruit of *X. aethiopica* (Dunal) A. Rich probably due to its ability to extract both polar and non-polar compounds and consequently the synergistic effect of polar and non-polar bioactive compounds in the sample followed by water. This was revealed by the values for TEAC and FRAP and DPPH• scavenging potential. Equally, Sowndhararajan and Kang (2013) reported the preference of methanol for the extraction of antioxidant compounds from *B. vahlii* leaves. In contrast, the antioxidant potential of acetone extract of *Limnophila aromatica* was higher than that of methanolic extract (Do et al., 2014). The contradictions in this report and the reports from other authors could be associated with the differences in the plant structures and conditions of extraction (Michiels et al., 2012; Do et al., 2014; Złotek et al., 2016).

In addition, it is noteworthy to indicate that the phenolic and flavonoid contents of *X. aethiopica* (Dunal) A. Rich were directly proportional to the antioxidant activities. This was in accordance with the reports of Liu et al. (2009) and Do et al. (2014) on the phytochemical components and antioxidant properties of various solvent extracts from *Litchi chinenesis* and freeze-dried *L. aromatica*, respectively. It could thus be assumed that the phenolic and flavonoid contents of *X. aethiopica* (Dunal) A. Rich may be accountable for its antioxidant activities as assessed through diverse *in vitro* models.

4. Concluding remarks

The results of the present study showed that the extraction of the antioxidant compounds in *X. aethiopica* (Dunal) A. Rich is influenced greatly by the properties of the solvents used. Generally, methanol was found to be more effective than water, acetone and petroleum ether in extracting bioactive compounds from *X. aethiopica* (Dunal) A. Rich; this might be attributed to the protic nature of the methanol favouring the extractions of polar and non-polar antioxidant compounds. Furthermore, the phenolic and flavonoid contents correlated significantly with the antioxidant activities of the extracts. The establishment of methanol as the appropriate extraction solvent in this study could facilitate the discovery of phytomedicines from *X. aethiopica* (Dunal) A. Rich that could alleviate free-radical-associated oxidative damage. However, further studies are recommended to define the overall pharmacokinetic and pharmacodynamic mechanisms of the phytochemical components of the methanolic extract using animal models.

Conflict of interest

The authors declare that there is no conflict of interest.

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