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Comparative study of the aqueous and ethanolic extract of *Momordica foetida* on the phenolic content and antioxidant properties

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Abstract

The present study was designed to evaluate and compare the phenolic (total phenols and flavonoid) contents and antioxidant activity of aqueous extract of *Momordica foetida* (AEMF) and ethanolic extract of *Momordica foetida* (EEMF) extract of *Momordica foetida* leaves; a popular medicinal plant used for traditional medicine preparations. The antioxidant activity of both extracts was assessed using nitric oxide (NO) radical scavenging and ferric reducing antioxidant power (FRAP) assay. The results revealed that EEMF had significantly ($p < 0.05$) higher total phenol and total flavonoid contents than AEMF. In the same vein, EEMF also exhibited significantly ($p < 0.05$) higher NO radical scavenging ability and ferric reducing antioxidant property than AEMF. The antioxidant properties of the vegetable revealed could be attributed to their phenolic contents. Moreover, EEMF showed better antioxidant activities when compared to AEFT.

Introduction

Productions of free radicals are essential to any biochemical process and represent an essential part of aerobic life and metabolism. The continuous generation of these radicals is produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions. A dynamic balance exists between the amount of free radicals generated in the body and antioxidants to scavenge them and protect the body against their perilous effects (Roberts *et al*., 2009; Ramjith *et al*., 2013). An imbalance between the generation of free radicals and the scavenging abilities of the antioxidants results in oxidative stress. Furthermore, oxidative stress has been implicated in the pathology of many diseases for example arteriosclerosis, cancer, inflammation, neurodegenerative diseases etc (Adesanoye *et al.*, 2012; Goveas and Abraham, 2013).

Several studies have been undertaken on how to delay or prevent the onset of these diseases. The most likely and practical way to fight against degenerative diseases is by boosting body's antioxidant status, which could be achieved by higher consumption of vegetables and fruits which are rich in natural antioxidants, especially phenolics and flavonoids hence the growing interest in research on plants and new plants with antioxidants ability and fit for human consumption (Oboh *et al.*, 2008; Salas *et al*., 2010). Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption (Jain *et al*., 2009)

Momordica foetida is a medicinal plant that belongs to the family of *Cucurbitaceae*. It is widely distributed in tropical Africa, South-Africa and also found in the Flora of west tropical Africa (Burkill, 1985; Baillie, 2007). The plant has both male and female flowers (Jeffrey, 1990). It has been shown to possess insecticidal properties (Baerts and Lehmann, 2002). The plant is eaten as vegetables in Gabon, Sudan, Uganda, Tanzania and Malawi (Olaniyi, 1975). The leaves have a bitter taste.The plant contains a number of bioactive compounds, including sitosterylglucoside, 5, 25-stigmastadien-3β-ylglucoside, and 1β-hydroxyfriedel-6-en-3-one, and several cucurbitane-type triterpenoid derivatives. (Mulholland, 1997).The plants are grazed by cattle and are said to be especially suitable for fattening rabbits. Recent study on the antioxidant potentials of *M. foetida* reveals its scavenging role against lipid peroxidation in rat homogenate (Oloyede and Aluko, 2012). Moreover, there is dearth of information on the antioxidant properties of the polar and non-polar extracts of the plant. Therefore, this study was designed to evaluate and compare the phenolic (total phenols and flavonoid) contents and antioxidant activity of aqueous (AEMF) and ethanolic (EEMF)

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extract of *Momordica foetida* leaves.

Materials and Methods

Sample collection

Fresh samples of *Momordica foetida* leaves were purchased from the market in Otun-Ekiti, Ekiti State Nigeria. The authentication of the leaves was carried out by a Plant Botanist at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

Chemicals and reagents

The following chemicals and reagents: quercetin, gallic acid, Folin–Ciocalteau's reagent, potassium ferricyanide, ferric chloride, potassium acetate, trichloroacetic acid (TCA), methanol, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sulphanilamide, and sodium carbonate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), Chemie GmbH (Steinheim, Germany) and BDH Chemicals Ltd., (Poole, England).

Aqueous extract preparation

The aqueous extract of the leaves were prepared by soaking the *Momordica foetida* leaves in water for about 24 hours; the mixture were filtered and the filtrate were evaporated under pressure at 40°C. The extracts were stored in the refrigerator for subsequent analysis.

Ethanolic extract preparation

The ethanolic extract of the leaves were prepared by soaking the *Momordica foetida* leaves in alcohol for about 24 hours; the mixture were filtered, and the filtrate were evaporated under pressure at 40°C. The extracts were stored in the refrigerator for subsequent analysis

Determination of total phenol content

The total phenol content was determined on the extracts using the method reported by (Singleton *et al.*, 1999). Appropriate dilutions of the extracts were oxidized with 2.5 mL of 10% Folin–Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated using Gallic acid as standard.

Determination of total flavonoid content

The total flavonoid content of both extracts was determined using a slightly modified method reported by (Meda *et al*., 2005). Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 µL of 10% $AlCl_3$, 50 µL of 1 mol L^{-1} potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard.

Nitric oxide (NO) radical scavenging assay

The scavenging effect of the phenolic extracts on nitric oxide ($NO^{\cdot}$) radical was measured according to the method of (Marcocci *et al*., 1994). 10 - 400 µl of the phenolic extracts was added in test tubes to 1 ml of sodium nitroprusside solution (25 mM) and tubes incubated at 37°C for 2 hours. An aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediaminedihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank with catechin (50 µg) used as standard. Results were expressed as percentage radical scavenging activity (RSA).

Determination of reducing property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5 mL aliquot was mixed with 2.5 mL of 200 mmol L^{-1} sodium phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferric cyanide. The mixture was incubated at 50°C for 20 min and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

Statistical analysis

The result of the triplicate experiments were pooled and expressed as mean ± standard deviation (SD). Student t-test was carried out to ascertain the level of significance. Significance was accepted at $p \leq 0.05$ (Zar, 1984).

Results

The results of the phenolic content of aqueous extract of *Momordica foetida* (AEMF) and ethanolic extract of *Momordica foetida* (EEMF) are shown in Table 1 and reported as gallic acid equivalent (GAE) for total phenol content and quercetin equivalent

Table 1. Phenolic contents of aqueous (AEMF) and ethanolic (EEMF) extract of *Momordica foetida*

	Total phenol (mgGAE/g)	Total flavonoids (mgQE/g)
AEMF aqueous	3.93 ± 0.17[a]	2.88 ± 0.04[c]
EEMF ethanolic	5.55 ± 0.46[b]	3.94 ± 0.07[d]

Values represent mean± standard deviation of triplicate readings, n = 3. Values with different superscript letters along the same column are significantly ($p < 0.05$) different

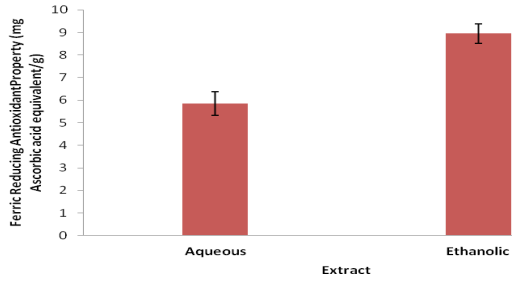

Figure 1. Ferric reducing antioxidant property of ethanolic and aqueous extract of *Momordica foetida*

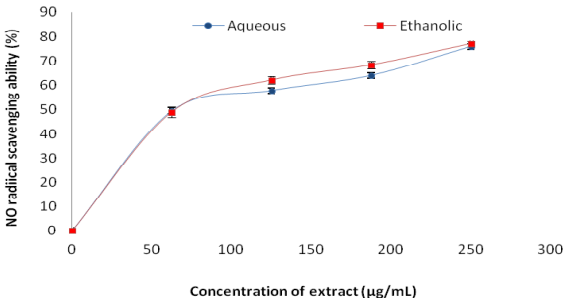

Figure 2. Nitric oxide radical scavenging ability of ethanolic and aqueous extract of *Momordica foetida*

antioxidant capacity (QEAC) for total flavonoid content. The result revealed that there is significant ($P < 0.05$) difference in total phenol contents of AEMF (3.93 ± 0.17 mg/g) and EEMF (5.55 ± 0.46 mg/g). Likewise, the total flavonoid contents of EEMF extract (3.94 ± 0.07 mg/g) was significantly higher ($P < 0.05$) than AEMF (2.88 ± 0.04 mg/g).The *in vitro* antioxidant indices (ferric reducing antioxidant property and nitric oxide scavenging property) of both extracts were presented in Figure 1 and 2 respectively. The trend of the result of the total phenol content of both extracts agrees with that of the total flavonoid content in that EEMF showed higher phenolic content than AEMF. Furthermore, the ferric reducing antioxidant property of the extracts was assessed for their ability to reduce Fe (III) to Fe (II) and expressed as ascorbic acid equivalent (AAE). as shown in Figure 1. The result revealed that both extracts possess good reducing potentials. However, Moreover, EEMF (8.95 mgAAE/g) had significant difference ($p < 0.05$) higher reducing power than AEMF (5.85 mgAAE/g). Also, the NO radical scavenging ability of both extracts is presented in Figure 2. Judging by the IC_{50} (extract concentration that caused 50% inhibition), the result revealed that there was no significant difference between EEMF (IC_{50} = 64.67 µg/ml) and AEMF (IC_{50} = 65.33 µg/ml). It is noteworthy that the phenolic contents of both extracts are consistent with their antioxidant properties in that EEMF exhibited higher phenolic contents and antioxidant properties than AEMF.

Discussion

Strong research evidences has shown that most of the biological actions of plant foods are linked to their phenolic contents (Scalbert *et al.*, 2005; Oboh and Rocha, 2007). Phenolic compounds exert their antioxidant properties by scavenging free radicals, chelating metals, reducing action and inhibition of lipid peroxidation (Alia *et al.*, 2003).

Total phenolic compounds are reported as milligram gallic acid equivalents/g equivalents (mgGAE/g) as shown in Table 1. The total phenolic contents of AEMF and EEMF were 3.93 ± 0.01 and 5.55 ± 0.46 mgGAE/g of sample, respectively. The total flavonoid contents of AEMF and EEMF were 2.88 ± 0.04 and 3.94 ± 0.07 mg quercetin equivalent/g of sample. EEMF had higher total phenol and flavonoid contents than AEMF ($P < 0.05$). It has been reported that green leafy vegetables, soft fruits and medicinal plants exhibited higher levels of flavonoid (Sultan and Anwar, 2009). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Van Acker *et al*., 1986).

The ability of the extracts to reduce ferric ion to ferrous ion was determined and the result was presented as ascorbic acid equivalent. The result revealed that EEMF was found to be significantly higher ($p < 0.05$) as compared to AEMF (Figure 1) Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, it can be reported that AEMF and EEMF may act as free radical scavenger, capable of transforming reactive free radical species into stable non radical products.

Figure 2 shows the Nitric oxide radical scavenging ability of ethanolic and aqueous extract of *Momordica Foetida.* The extract effectively reduced the generation of nitric oxide from sodium nitroprusside (Figure 2). Inhibition of nitric oxide radical is a measure of antioxidant activity of plant extracts. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent (Marcocci *et al.*, 1994). Both AEMF and EEMF decreased the amount of

nitrite generated from the decomposition of sodium nitroprusside which indicated the presence of antioxidant principles in the extract. The percentage scavenging activity increased with increasing concentration of the extract. Moreover, there was no significant difference ($p \geq 0.05$) in the Nitric oxide radical scavenging ability of AEMF and EEMF.

Conclusion

In this study, we have demonstrated that both ethanolic and aqueous extract of *Momordica foetida* exhibited antioxidants properties. The antioxidant properties of the vegetable revealed could attributed to their phenolic contents. Moreover, Ethanolic extract of *Momordica foetida* showed better antioxidant activities when compared to aqueous extract of *Momordica foetida.*

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