

Nutritional, phytoconstituent and antioxidant potential of mucilage extract of Okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*)

¹Adetuyi, F. O. and ²Dada, I. B. O.

¹Biochemistry Unit, Chemical Sciences Department, Ondo State University of Science and Technology, PMB 353, Okitipupa, Ondo State, Nigeria

²Biochemistry Unit, Science Laboratory Technology Department, Rufus Giwa Polytechnic PMB 1019, Owo, Ondo State, Nigeria

Article history

Received: 13 February 2013

Received in revised form:

14 April 2014

Accepted: 15 April 2014

Keywords

Okra

Water leaf

Jews mallow

Nutrient

Zn bioavailability

Vitamin C

Total phenol

Antioxidant

Abstract

This study assessed the nutrient, phytoconstituent, minerals, Zn bioavailability and antioxidant properties of mucilage extracted from Okra, Water leaf and Jews mallow. The protein in the mucilage of these vegetables was significantly higher ($P \leq 0.05$) in water leaf (54.30%) than Jews mallow (44.80%) and okra (20.30%). Jews mallow had the highest fiber content (8.25%) and okra the lowest (2.00%). The calculated $[Ca]/[phytate]/[Zn]$ molar ratios for the mucilage of water leaf (0.59) and Jews mallow (1.51) were clearly above the critical level of 0.5 mol / kg while that of the mucilage of okra (0.14) was below 0.5 mol / kg. The vitamin C content ranged from 5.00 mg AAE/g in okra to 10.25 mg AAE/g in water leaf. The result revealed that okra mucilage had a significantly higher ($P \leq 0.05$) total phenol and reducing power than that of the mucilage of water leaf and Jews mallow. At the concentration of 100 and 300mg/ml, okra mucilage had the highest DPPH radical scavenging ability of 23.04% and 40.40% and the lowest OH radical scavenging ability of 13.16% and 59.03%. The mucilage of these vegetables could prevent protein malnutrition and serve as a natural antioxidant.

© All Rights Reserved

Introduction

Vegetables serve as indispensable constituents of the human diet supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy. Several vegetable species abound in Nigeria and most West African countries where they are used partly as condiments or spices in human diets or as supplementary feeds to livestock such as rabbits, poultry, swine and cattle. These vegetables are harvested at all stages of growth and fed either as processed, semi-processed or fresh to man while they are usually offered fresh to livestock. Leafy vegetables are known to add taste and flavour, as well as substantial amount of proteins, fibre, minerals and vitamins to the diet (Aja *et al.*, 2010).

Free radicals find their way into the human body via metabolic pathways within the body tissues and also from external sources such as food, drugs and pollution from the environment. There is strong evidence that suggests that free radicals, such as superoxide radical (O_2^-) and hydroxyl radical ($\cdot OH$) and non-free radical species, such as hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2) within the human body facilitate cellular injury, aging, development of neuro de-generative and cardiovascular diseases (Morrison and Twumasi, 2010). Dietary antioxidants

protect the human body against free radicals such as reactive oxygen species and prevent rancidity in foods (Obboh *et al.*, 2010). Free radicals are known to be a main contributor to major chronic diseases and degenerative diseases of ageing. Over the years, plant foods have been found to be rich in antioxidant phytochemicals such as phenolic compounds, ascorbic acid, carotenoids, anthocyanins, phytosterols and policosanols, known to significantly affect human health by combating/preventing the negative effect of free radicals. Increased consumption of fruits, cereal grains and vegetables is related to reduce risk of chronic diseases. Part of the antioxidant activities of plant foods are related to phenolic compounds (Obboh *et al.*, 2010).

In most part of Africa, when water leaf (*Talinum triangulare*) is to be cooked the leaves are squeezed with or without salt to remove the mucilage from the leaf before cooking, the resultant extracted mucilage are thrown away or consumed as food supplement. It has also been observed that the mucilaginous properties of okra and Jews mallow play an important role in their consumption. Also the mucilage of these vegetables are extracted and consumed naturally as food and herb in traditional folklore in West Africa. Although, a lot had been reported on the nutrient composition of okra fruit, water leaf and Jews

*Corresponding author.

Email: foluadetuyi@yahoo.co.uk

mallow leaves, still there is a dearth of information on the antioxidant properties of mucilage extracted from okra fruit, water leaf and Jews mallows. Hence, this study sought to evaluate the nutrient, phytoconstituent, minerals, Zn bioavailability and antioxidant potential of mucilage extracted from okra *Abelmoschus esculentus*, water leaf *Talinum triangulare* and Jews mallow *Corchorus olitorius*.

Materials and Methods

Materials

Okra fruit (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*), vegetables were freshly harvested and were identified and authenticated at the Agricultural Technology Department, Rufus Giwa Polytechnic, Owo. Ascorbic acid was from Merck (Darmstadt, Germany), gallic acid and quercetin was from Aldrich (Steinheim, Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 1,10-orthophenanthroline, trichloroacetic acid (TCA) were obtained from Sigma Chemical Inc. (St Louis, MO, USA). All other chemicals and solvent used are of analytical grade, glass-distilled water was used.

Preparation of okra, water leaf and Jews mallow mucilage

The mucilage was extracted according to the method of Woolfe *et al.* (1977) with slight modification. The leaves of water leaf and Jews mallow vegetables were removed from the stalk, cleaned, shredded and homogenized using rotor stator homogenizer with five times its weight of water, it was then filtered using membrane filter and centrifuged at 2,500 rpm for 30 minutes; the greenish, viscous solution is obtained. The solution was heated at 70°C for 5 minutes to inactivate enzymes and re-filtered. The mucilage was precipitated with three times volumes of ethanol and washed with more ethanol followed by acetone. The greenish coloured solid was dried in an oven at the temperature of 45°C for 12 hours; it was scraped and gave a yield of 21 g mucilage/Kg water leaf and 20 g mucilage/Kg Jews mallow. The seeds of okra do not contain any mucilage and were removed before the extraction. The okra fruit was sliced, before following the above extraction procedure; okra mucilage gave cream coloured solid and yielded 14 g mucilage/kg okra.

Nutrient analysis

Nutrient composition (fat, crude fibre, and ash) was determined by the standard method of the Association of Official Analytical Chemist AOAC (1990). The protein content was determined using the

micro-Kjedahl method ($N \times 6.25$) and carbohydrate determination was by difference. Food energy was calculated by the method of Jideani and Bello (2009) using the factor of $[(4 \times \text{Protein}) + (4 \times \text{Carbohydrate}) + (9 \times \text{Fat})]$.

Phytoconstituent analysis

The phytate content was determined by the method of Maga (1982), which depend on the ability of standard ferric chloride to precipitate phytate in diluted HCl extract of the mucilage. The tannin composition was determined by the colorimetric method of Van-Burden and Robinson (1981). Five hundred milligrams (500 mg) of the sample was weighed into a 50 ml plastic bottle. Fifty ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then, 5 ml of the filtrate was taken into a test tube and mixed with 2 ml of 0.1 M FeCl_3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm using JENWAY UV-Visible spectrophotometer (JENWAY 6305 Barloworld Scientific Ltd. Dunmow, Essex, UK) within 10 minutes. This was compared with the absorbance of standard solutions of tannic acid.

The Saponin was determined using the spectrophotometric method of Brunner (1984), in which the mixture of the mucilage and Isobutyl alcohol (2 g in 250 ml isobutyl alcohol) was filtered into 20 ml 40% saturated solution of magnesium carbonate. The mixture is filtered to get a colourless solution, 2 ml of 5% Iron (III) chloride is added to 1 ml of the colourless solution and made up to 50 ml mark with distilled water and the absorbance was measured after 30 minutes at 380 nm in a spectrophotometer (JENWAY 6305).

Mineral determination and Zn bioavailability calculation

The mineral contents were determined using an Atomic Absorption Spectrophotometer bulk scientific AAS (model 210/211 VGB). The method of Ferguson *et al.* (1988) was used for the calculation of phytate : zinc, calcium : phytate and $[\text{Ca}] [\text{phytate}] / [\text{Zn}]$ molar ratios and used for the Zn bioavailability prediction $[\text{Phytate} = 660, \text{Zn} = 65.40, \text{Ca} = 40]$.

Aqueous extract preparation

The aqueous extracts of okra, water leaf and Jews mallow mucilage were prepared using a modified procedure described by Oboh *et al.* (2010). About 10 g each of the powdered mucilage was homogenised in 100 ml distilled water in a Warring blender for 5 min. Thereafter, the mixture was centrifuged at

2000 g for 10 min. The supernatant was used for the determination of total phenolic content, vitamin C and antioxidant activity (reducing power and DPPH free radical scavenging ability).

Vitamin C content determination

The vitamin C content of the aqueous extract was determined using the method of Benderitter et al. (1998). 75 µl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg CuSO₄ · 5H₂O in 100 ml of 5 ml H₂SO₄) were added to 500 µl extracts mixture (300 µl of an appropriate dilution of the extract with 100 µl 13.3% trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 hours at 37°C, then 0.5 ml of 65% H₂SO₄ (v/v) was added to the medium and the absorbance was measured at 520 nm in a spectrophotometer (JENWAY 6305). The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard.

Phenolic content determination

The total phenol content was determined according to the method of Singleton *et al.* (1999). Appropriate dilutions of the extracts were mixed with 2.5 ml of 10% Folin–Ciocalteu's reagent (v/v) and neutralised 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 a spectrophotometer (JENWAY 6305). The total phenol content was subsequently calculated using gallic acid as standard.

Ferric reducing antioxidant power (FRAP)

The reducing property of the extracts was determined by assessing the ability of the extract to reduce FeCl₃ solution as described by Oyaizu (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min; thereafter 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 2 000 g 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 in a spectrophotometer (JENWAY 6305) and ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

DPPH free radical scavenging ability

The Free radical scavenging ability using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) as described by Singh *et al.* (2002). Different concentrations of the aqueous extract were taken in different test tubes

and the volume was made to 1 ml with distilled water. 4 ml of 0.1 mM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to stand for 20 min at room temperature. A control was prepared as above without the sample and distilled water was used for base line correction. Changes in absorbance of samples were measured at 517 nm in a spectrophotometer (JENWAY 6305). Free radical scavenging activity was expressed as percentage inhibition and was calculated using the following formula:

$$\text{Free radical scavenging activity (\%)} = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100$$

Degradation of deoxyribose (Fenton's reaction)

The ability of the extracts to prevent Fe²⁺/H₂O₂-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Freshly prepared aqueous extract (0–150 µl) was added to a reaction mixture containing 120 µl, 20 mM deoxyribose, 400 µl, 0.1 M phosphate buffer, 40 µl, 20 mM hydrogen peroxide and 40 µl, 500 M FeSO₄, and the volume made up to 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by the addition of 0.5 ml of 2.8% TCA; this was followed by the addition of 0.4 ml of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in spectrophotometer (JENWAY 6305).

Statistical analysis

The results of the three replicate readings were pooled and expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used to establish significant differences among samples. Mean separation were done where there was significant differences using Duncan multiple range test procedure. Significance was accepted at (P ≤ 0.05) (SAS, 2002).

Result and Discussion

The nutritional composition of the mucilage extracted from okra, water leaf and Jews mallow are presented in Table 1. The result revealed that the mucilage of these vegetables had high protein contents with water leaf having the highest protein content (54.30%) while okra had the lowest protein content (20.30%). The protein content of the mucilage was higher when compared with the protein of content of the whole vegetable. Adetuyi *et al.* (2011) reported 13.61 – 16.27% dry weight (DW) for 6 different

Table 1. Nutritional composition of the mucilage of okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*) (%)

| Mucilage content | Moisture | Protein | Fibre | Fat | Ash | Carbohydrate | Energy (Kcal) |
|------------------|------------|------------|-----------|------------|-----------|--------------|---------------|
| Okra | 14.50a±0.8 | 20.30c±2.3 | 2.00c±0.3 | 31.00a±1.6 | 5.00b±0.2 | 41.70a±1.9 | 527 |
| W. Leaf | 8.60c±0.3 | 54.30a±2.7 | 3.50b±0.2 | 29.00b±1.1 | 7.80a±0.1 | 5.40c±0.4 | 500 |
| J. mallow | 10.00b±0.5 | 44.80b±2.3 | 8.25a±1.0 | 33.80a±1.4 | 5.00b±0.5 | 8.15b±1.0 | 516 |

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$). W. Leaf = water Leaf, J. Mallow = Jews mallow

Table 2. Mineral composition of the mucilage of okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*) (ppm)

| Mucilage | Calcium | Magnesium | Iron | Zinc |
|-------------|-------------|------------|------------|-----------|
| Okra | 72.80b±1.0 | 66.55a±1.2 | 8.01c±0.9 | 1.59a±0.6 |
| Water leaf | 78.40b±1.6 | 27.45c±1.1 | 11.90b±0.6 | 0.76b±0.1 |
| Jews mallow | 216.00a±2.3 | 48.70b±1.2 | 13.30a±0.4 | 1.95a±0.3 |

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$).

Table 3. Phytoconstituent of the mucilage of okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*)

| Mucilage | Phytate g/100g | Saponin g/100g | Tannin mg/g |
|-------------|----------------|----------------|-------------|
| Okra | 1.26c±0.1 | 34.50a±1.3 | 15.50a±1.1 |
| Water leaf | 2.37b±0.5 | 9.25b±0.9 | 0.50c±0.0 |
| Jews mallow | 5.56a±0.3 | 7.50c±0.4 | 5.25b±0.5 |

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$).

varieties of okra fruits. Omale and Ugwu (2011) reported 1.58% wet weight (WW) for water leaf vegetable and 0.80% WW for Jews mallow vegetable while Adetuyi *et al.* (2010) reported 14.19% DW for Jews mallow vegetable. The protein content of the mucilage in water leaf 54.30% was higher than the protein content of Nigerian wild seeds 6.5 – 24.4% DW (Oboh and Ekperigin, 2004). The protein content of water leaf mucilage was also significantly higher ($P \leq 0.05$) than that of okra and Jews mallow mucilage. Cells are ruptured during homogenisation and cellular proteins are released into the mucilage, this could account for the high value of protein in the mucilage. The high value of protein in the mucilage could also be attributed to leaching of protein from the leaf into the mucilage. The fibre content of the mucilages was very low (okra, 2.00%, water leaf, 3.50% and Jews mallow, 8.25%) in comparison with the fibre content of the whole vegetable. The fibre content of a whole water leaf vegetable was found to be 12% DW and 8.5% WW (Aja *et al.*, 2010), the fibre content of 6 different varieties of okra fruit ranged from 10.25% - 11.63 % DW (Adetuyi *et al.*, 2011) while the fibre content of Jews mallow was 45.61% DW (Ezz El-Arab, 2009). Though, the fibre contents of the mucilages were very low, the fibre content of the mucilage of Jews mallow (8.25%) was significantly higher ($P \leq 0.05$) than the fibre present in the mucilage of okra and waterleaf. The main reason for the low fibre content in the mucilage could be attributed to the fact that the fibre constitutes the residue of the leaves of water leaf, Jews mallow and fruits of okra after homogenisation and sieving to

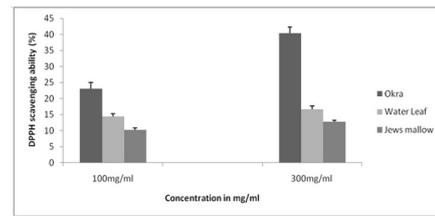


Figure 1. DPPH scavenging ability of the mucilage of okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*)

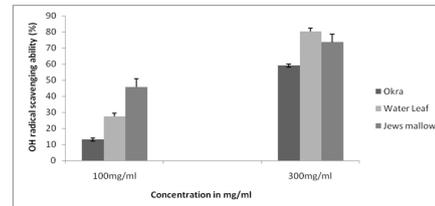


Figure 2. OH scavenging ability of the mucilage of okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*)

extract the mucilage. The ash content of the mucilage of water leaf (7.80%) and Jews mallow (5.0%) were very high when compared with the whole vegetable. Obahiagbon and Erhabor (2010) have reported 1.00% DW and 1.50% DW ash contents for water leaf and Jews mallow vegetables respectively, Saidu and Jideobi (2009) also reported 1.60% DW for Jews mallow vegetable, the ash content of the mucilage of okra (5.00%) was low in comparison with the ash content of 6 different varieties of okra fruit reported by Adetuyi *et al.* (2011) which ranged between 7.19% - 9.63% DW. The ash content of the mucilage of water leaf was significantly higher ($P \leq 0.05$) than the ash content of the mucilage of okra and Jews mallow. The high ash content of the mucilage of water leaf and Jews mallow could be attributed to leaching of nutrients into the mucilage during extraction but the low content of ash in the mucilage of okra could be due to high presence of minerals in the okra seed which has been removed before the mucilage extraction. Also Jideani and Bello (2009) described okra as amphipathic.

The result of the mineral composition of the mucilage of okra, water leaf and Jews mallow is shown in table 2. The result shows that mucilage of Jews mallow had the highest content of Ca (216.00 ppm), Fe (13.30 ppm) and Zn (1.95 ppm) and they were significantly different ($P \leq 0.05$) from that of okra and water leaf, while the mucilage of okra had the highest Mg (66.55 ppm). The mineral content in the mucilage of okra was higher than the mineral content of the whole okra fruit as reported by Adetuyi *et al.* (2011). It is to be noted that the Zn content of the mucilage of water leaf 0.76 ppm and Jews mallow 1.95 ppm were very low when compared to the Zn content of the whole vegetable 4.52 ppm water leaf

Table 4. Zn bioavailability estimation of the mucilage of okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*)

| Mucilage | Phy: Zn | Ca: Phy | [Ca][ph]/[Zn] ² mol/kg |
|-------------|------------|-----------|-----------------------------------|
| Okra | 7.92c±0.3 | 9.58a±0.7 | 0.14c±0.0 |
| Water leaf | 30.00a±1.1 | 5.44c±0.3 | 0.59b±0.0 |
| Jews mallow | 28.00b±1.0 | 6.43b±0.4 | 1.51a±0.1 |

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$).

Table 5. Vitamin C content, total phenol content and FRAP property of the mucilage of okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*)

| Mucilage | Vitamin C mgAAE/g | Total Phenol mgGAE/g | FRAP mgAAE/g |
|-------------|----------------------|-------------------------|-----------------|
| Okra | 5.00c±0.1 | 4.81a±0.2 | 0.95a±0.1 |
| Water leaf | 10.25a±0.2 | 2.98c±0.1 | 0.43b±0.1 |
| Jews mallow | 6.83b±0.1 | 3.50b±0.1 | 0.09c±0.0 |

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$).

and 2.56 ppm Jews mallow (Omole and Ugwu, 2011). The high content of minerals in the mucilage could be attributed to leaching of nutrients and minerals into the mucilage during extraction but that of the Zn in the water leaf and Jews mallow could have been that the Zn was trapped in the fibre of the leaves which constituted the residue.

The phytoconstituents of the mucilage of okra, water leaf and Jews mallow as presented in table 3. Phytic acid has been reported to have significant inhibitory effects against a variety of primary tumours, including colon and pancreatic cancer (Obloh *et al.*, 2010). Phytate forms an iron chelate that suppresses lipid peroxidation by blocking iron driven hydroxyl radical generation (Obloh, 2006). The phytate content of the mucilage of okra, water leaf and Jews mallow revealed that the phytate content ranged between 1.26 g/100 g and 5.56 g/100 g with Jews mallow having the highest value of 5.56 g/100 g which was significantly different ($P \leq 0.05$). The values reported in this study were generally higher than the phytate content of many tropical plant foods such as fruits, legumes and leafy vegetables (Obloh, 2006). Saponins are steroid or triterpenoid glycosides considered by animal nutritionists to be deleterious compounds but the biological effects of saponins include hypolipidemic, hypoglycaemic, anticarcinogenic and antioxidant properties (Elekofehinti *et al.*, 2012). The hypolipidemic activity of dietary saponins may be due to the formation of some complexes with dietary cholesterol or their bile salt precursors, which are then made unavailable for absorption. The saponin content of the mucilage showed that the mucilage of okra had the highest value of 34.50 g/100 g which was significantly different ($P \leq 0.05$) from the mucilage content of water leaf 9.25 g/100 g and Jews mallow 7.50 g/100 g. The values of saponin in these mucilage were lower than the saponin content of *Jatropha*

curcas (Oskoueian *et al.*, 2011) but higher than the saponin content of six varieties of okra (Adetuyi *et al.*, 2011). Tannin affects nutritive value of food products by forming a complex with protein (both substrate and enzyme), thereby inhibiting digestion and absorption (Obloh and Akindahunsi, 2003). Okra mucilage has the tannin content of 15.50 g/100 g; this value was significantly higher ($P \leq 0.05$) than the tannin content of water leaf 0.50 g/100 g and Jews mallow 5.25 g/100 g. It is expected that this high phytate, saponin and tannin content of the mucilage will greatly contribute to the antioxidant activity of the mucilage.

The effect of the phytate content on Zn bioavailability in the mucilage extracted from okra, water leaf and Jews mallow was estimated and is presented in table 4. The calculated [Phytate]/[Zn] molar ratios for the mucilage of water leaf (30) and Jews mallow (28) were far above 15.0, while that of okra (7.92) was below 15.0 considered to be the critical value for reduced zinc bioavailability (Ferguson *et al.*, 1988). The high value of [Phytate]/[Zn] molar ratios for the mucilage of water leaf and Jews mallow clearly indicated that the phytate present in them could reduce the Zn bioavailability to a critical level but the low value of [Phytate]/[Zn] molar ratios for the mucilage of okra indicates that the phytate level in the mucilage of okra will not reduce the bioavailability of zinc to a critical level.

The calculated [Ca]/[Phytate] molar ratios for the mucilage revealed that the values obtained for okra (9.58) and Jews mallow (6.43) mucilage were above 6.0 while the value for the mucilage of water leaf (5.44) was just below 6.0. It has been suggested that the solubility of phytate and proportion of zinc bound to the complex depend on the dietary calcium levels (Obloh and Ekperigin, 2004). Phytate precipitation is not complete until the dietary [Ca]/[Phytate] molar ratios attain a value of approximately 6.0. At higher ratio, phytate precipitation is complete, causing the solution to be free of dietary zinc. However, the calculated [Ca][phytate]/[Zn] molar ratio is a better index for predicting Zn bioavailability due to a kinetic synergism that exists between [Ca] and [Zn] ions resulting in Ca:Zn:phytate complex, which is less soluble than the phytate complex formed by either ions alone (Obloh *et al.*, 2010). The result of the present study clearly revealed that the calculated [Ca][phytate]/[Zn] molar ratio for the mucilage of water leaf (0.59) and Jews mallow (1.51) were above the critical level of 0.5 mol / kg, which is considered as the critical level for reduced Zn bioavailability (Obloh *et al.*, 2010). The calculated [Ca][phytate]/[Zn] molar ratio for the mucilage of okra (0.14) was

below 0.5 mol / kg, thus predicting bioavailability of dietary Zn in the mucilage of okra. This reduced Zn bioavailability in the mucilage of water leaf and Jews mallow could be attributed to low Zn content, or that the Ca content in them is not high enough to create a sparing effect for Zn from the phytate.

The vitamin C, total phenol and the Ferrous Reducing Antioxidant Power FRAP of the mucilage of okra, water leaf and Jews mallow is shown in table 5. The vitamin C content in mg Ascorbic Acid Equivalent/g ranged from 5.00 mg AAE/g (okra) to 10.25 mg AAE/g (water leaf). The vitamin C content in these mucilage were very high when compared to the vitamin C content reported for some commonly consumed and underutilized tropical legumes (0.5 – 0.9 mg/100 g) (Oboh, 2006). The vitamin C content of these mucilage were higher than the vitamin C content of commonly consumed green leafy vegetables (43.5 – 148.0 mg/100 g) and fruits (20 – 90 mg/100 g), also Indian green leafy vegetables (15.18 – 101.36 mg/100 g) (Oboh, 2006; Gupta and Prakash, 2009). The high vitamin C content in the mucilage could be attributed to the solubility of vitamin C in water. The total phenol content (mg Gallic Acid Equivalent/g) of the mucilage of okra was found to be 4.81 mgGAE/g, water leaf, 2.98 mgGAE/g and Jews mallow, 3.50 mgGAE/mg. These values were high; when that of okra was compared to the phenol content of the whole okra pod (Adetuyi *et al.*, 2008) and that of water leaf and Jews mallow were compared to their respective leafy vegetables (Morrison and Twumasi, 2010 and Adetuyi *et al.*, 2010). This could be due to the existence of most of the phenolics of vegetables in the polar forms since the mucilage extracted from these vegetables were in the polar form. The total phenol content of the mucilage of okra (4.81 mgGAE/g) was significantly higher ($P \leq 0.05$) than the total phenol content of the mucilage of water leaf and Jews mallow respectively (2.98 mgGAE/g and 3.50 mgGAE/mg). The total phenol of the mucilage of these vegetables was higher than the values reported for some tropical green leafy vegetables (Oboh, 2005) and some Chinese herbal medicine used to treat diabetes *Codonopsis pilosula*, *Alisma orientalis*, *Euryale ferox*, *Coix lacrymajobi*, *Pinella ternate*, *Radix trichosanthis*, *Atractylodes macrocephala* and *Poria cocoa* (Chen *et al.*, 2011). Reducing power is a novel antioxidation defense mechanism and the mechanisms that affect this property are electron transfer and hydrogen atom transfer. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Dastmalchi *et al.*, 2007; Lee *et al.*, 2007). The antioxidant effect exponentially

increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power (Oyaizu, 1986). The reducing power of the mucilage of okra, water leaf and Jews mallow were assessed based on their ability to reduce Fe^{3+} to Fe^{2+} . The result is shown as FRAP in mg Ascorbic Acid Equivalent/g. The reducing power was found to be 0.95 mgAEE/g in okra mucilage, 0.43 mgAEE/g in water leaf mucilage and 0.09 mgAEE/g in Jews mallow mucilage. The result revealed that okra mucilage had the highest reducing power which was significantly higher ($P \leq 0.05$) than the mucilage of water leaf and Jews mallow. The observed increase in the reducing power of okra mucilage over water leaf and Jews mallow could be due to the synergism between the extractable phytoconstituents and the phenol contents which was of course significantly higher ($P \leq 0.05$) in okra mucilage. The correlation analysis between the phytochemical composition of the extracts in Chinese herbal medicines used to treat diabetes and their antiglycation and antioxidant activity indicated that the total saponin and polyphenol contents may be responsible for the antiglycation and antioxidant activities (Chen *et al.*, 2011). Ferric-to-ferrous iron reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+} / Fe^{2+} ; the values in the FRAP assay will express the corresponding concentration of electron-donating antioxidants (Halvorsen *et al.*, 2002).

In the presence of hydrogen donors, DPPH is reduced and a free radical is formed from the scavenger. The reaction of DPPH is monitored by the decrease of the absorbance of its radical at 517 nm, but upon reduction by an antioxidant, the absorption disappears (Gupta and Prakash, 2009). DPPH radical scavenging ability of the mucilage of okra, water leaf and Jews mallow at 100 mg/ml and 300 mg/ml concentrations were measured and the results are presented in Figure 1. A dose-response relationship was found in the DPPH radical scavenging ability of the mucilage; the ability increased with an increase in the concentration of the mucilage of the vegetables. At the concentration of 100 and 300 mg/ml, okra mucilage had the scavenging ability of 23.04% and 40.40%, water leaf, 14.39% and 16.71% while Jews mallow, 10.29% and 12.76 %. It has been observed that concentrations of extracts have effect on the DPPH scavenging ability of extracts (Gupta and Prakash, 2009; Ademiluyi and Oboh, 2011). It showed from the result that okra mucilage had the highest DPPH scavenging ability of 40.40% which was significantly different ($P \leq 0.05$) when compared

with the mucilage of the other vegetable and Jews mallow with the lowest ability 12.76%. This could be because okra mucilage had the highest phenol and phytoconstituents than the mucilage of the other observed vegetables. It had been reported that correlation was established between total phenolic content, phytoconstituents and observed antioxidant activities (Ademiluyi and Oboh 2011, Chen *et al.*, 2011).

Fe²⁺ can also catalyze one-electron transfer reactions that generate reactive oxygen species (ROS) such as the very reactive OH•, which is formed from H₂O₂ through the Fenton reaction and OH• has been recognized to date as the most ROS. The overproduction of ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation (Ademiluyi and Oboh, 2011). Figure 2 showed the OH radical scavenging ability of the mucilage of okra, water leaf and Jews mallow. Water leaf mucilage had the OH radical scavenging ability of 80.20%, followed by Jews mallow with the ability of 73.60% and okra mucilage 59.03%. Considering the concentrations of the extracts, the OH radical scavenging ability of the mucilage of these vegetables were lower than the values reported for fermented and unfermented Bambara groundnut seed extracts (Ademiluyi and Oboh, 2011). The extracts of these mucilage scavenge OH• radical produced in Fe²⁺/H₂O₂⁻ induced decomposition of deoxyribose in Fenton reaction with increasing concentrations from 100 mg/ml to 300 mg/ml. It is expected that okra mucilage will have the highest OH scavenging ability considering its total phenol and phytoconstituent composition but it is to be noted that at 300 mg/ml water leaf mucilage recorded the highest OH scavenging ability of 80.20% which was significantly higher ($P \leq 0.05$) than the others. The reason for this could not be ascertained though the vitamin C content of water leaf mucilage was significantly higher than the other mucilage. Aqueous extract was used for this analysis and the total antioxidant activity of the aqueous extract cannot be predicted based on its total phenolic content alone, a synergism of soluble polyphenolic compounds, with one another, and/or other components present in the extracts, may contribute to the overall observed antioxidant activity (Moktan *et al.*, 2008).

Conclusion

This study showed that the mucilage of these vegetables is rich in protein and very low in fibre. The okra mucilage exhibited low [Ca]/[phytate]/[Zn] molar ratios than the mucilage of water leaf or Jews mallow.

The mucilage of these vegetables also exhibited a high content of vitamin C and total phenol which resulted in high antioxidant properties. It could be concluded from this study that the mucilage of these vegetables (okra, water leaf and Jews mallow) could prevent protein malnutrition in areas where animal protein are deficient and also could be a potential source of natural antioxidant that could have great importance as therapeutic agents in the management, prevention and/or slowing the progress of aging and age associated oxidative stress related degenerative diseases associated with free radical damage.

Acknowledgements

The authors wishes to specially thank Dr. Ganiyu Oboh of the Federal University of Technology, Akure, he gave us free access to the facilities of his personal laboratory, materials and chemicals during the antioxidant determination. The assistance rendered by Atunwa Israel, Ibiojo Funmi and Yakubu Rashidat in running around for the success of this work cannot be overlooked.

Reference

- Ademiluyi, A.O. and Oboh, G. 2011. Antioxidant properties of condiment produced from fermented Bambara groundnut (*Vigna subterranean* L. Verdc). *Journal of Food Biochemistry* 35: 1145–1160.
- Adetuyi, F.O., Osagie, A.U. and Adekunle, A.T. 2008. Antioxidant degradation in six indigenous Okra *Abelmoschus esculentus* (L) Moench varieties during storage in Nigeria. *Journal of Food Technology* 6 (5): 227-230.
- Adetuyi, F.O., Osagie, A.U. and Adekunle, A.T. 2011. Nutrient, antinutrient, mineral and zinc bioavailability of okra *Abelmoschus esculentus* (L) Moench Variety. *American Journal of Food and Nutrition* 1 (2): 49 – 54.
- Adetuyi, F.O., Agbaje, R.B. and Dada, I.B.O. 2010. Effect of storage method on nutrients composition, antioxidant content and consumer acceptability of *Corchorus olitorius*. *International Journal of Tropical Agriculture and Food System* 4 (2): 91 – 97.
- Aja, P.M., Okaka, A.N.C., Ibiyam, U.A., Uraku, A.J. and Onu, P.N. 2010. Proximate analysis of *Talinum triangulare* (Water Leaf) leaves and its softening principle. *Pakistan Journal of Nutrition* 9 (6): 524-526.
- A.O.A.C. 1990. *Methods of Analysis of the Association of Official Analytical Chemists*. 15th ed. Washington DC, USA.
- Benderitter, M., Maupoli, V., Vergely, C., Dalloz, F., Briot, F. and Rochette, L. 1998. Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid

- in plasma: Effects of iron chelators. *Fundamentals of Clinical Pharmacology* 12: 510-516.
- Brunner, J. H. 1984. Direct spectrophotometric determination of saponin. *Analytical Chemistry* 34: 1314 – 1326.
- Chen, Y., Roan, H., Lii, C., Yuan-Ching Huang, Y. and Wang, T. 2011. Relationship between antioxidant and antiglycation ability of saponins, polyphenols, and polysaccharides in Chinese herbal medicines used to treat diabetes. *Journal of Medicinal Plants Research* 5(11): 2322 – 2331.
- Dastmalchi, K., Dorman, H. J. D., Kosar, M. and Hiltunen, R. 2007. Chemical composition and *in-vitro* antioxidant evaluation of a water soluble Moldavian balm. *Leb. Wiss Technol.* 40: 239 – 248.
- Elekofehinti, O. O., Adanlawo, I.G., Komolafe, K. and Ejelonu, O.C. 2012. Saponins from *Solanum anguivi* fruits exhibit antioxidant potential in Wistar rats. *Annals of Biology Research* 3 (7): 3212-3217.
- Elmastas, M., Isildak, O., Turkekul, I. and Temur, N. 2007. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis* 20 (3-4): 337-345.
- Ezz El-Arab, A.M. 2009. A diet rich in leafy vegetable fiber improves cholesterol metabolism in high-cholesterol fed rats. *Pakistan Journal of Biological Science* 12 (19): 1299 – 1306.
- Fairweather-Tait, S., Lynch, S., Hotz, C., Hurrell, R., Abrahamse, L., Beebe, S., Bering, S., Bukhave, K., Glahn, R. P., Hambridge, M., Hunt, J., Lonnerdal, B., Miller, D., Mohktar, N., Nestel, P., Reddy, M., Sandberg, A. S., Sharp, P., Teucher, B. and Trinidad, T. 2005. The usefulness of *in vitro* models to predict the bioavailability of iron and zinc: a consensus statement from the harvest plus expert consultation. *International Journal of Vitamin Nutrition and Research* 75: 371-374.
- Ferguson, E. L., Gibson, R.S., Lilian, U. T., Ounpuu, S. and Berry, M. 1988. Phytate, zinc and calcium contents of 30 East African foods and their calculated phytate: Zn, Ca: phytate and [Ca] [phytate]/[Zn] molar ratios. *Journal of Food Composition and Analysis* 1: 316-325.
- Gibson, R.S. 2012. A Historical Review of Progress in the Assessment of Dietary Zinc Intake as an Indicator of Population Zinc Status1-3. *American Society of Nutrition. Advances in Nutrition.* 3: 772-782, doi:10.3945/an.112.002287.
- Gupta, S. and Prakash, J. 2009. Studies on Indian Green Leafy Vegetables for Their Antioxidant Activity. *Plant Food for Human Nutrition* 64: 39-45.
- Halvorsen, B. L., Holte, K. and Myhrstad, T. 2002. A systematic screening of total antioxidants in dietary plants. *The Journal of Nutrition* 132: 461-471.
- Halliwell, B. and Gutteridge, J. M. C. 1981. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. *FEBS Letter.* 128: 347-352.
- Hassimotto, N. M. A., Ine's Genovese, M. and Lajolo, F.M. 2009. Antioxidant capacity of Brazilian fruit, vegetables and commercially-frozen fruit pulps. *Journal of Food Composition and Analysis* 22: 394-396.
- Jideani, V.A. and Bello, B.M. 2009. Functional properties of okra protein products containing different levels of mucilage. *Journal of Food Agriculture and Environment* 7 (2): 252 – 255.
- Lee, Y.R., Woo, K.S., Kim, K.J., Son, J. and Jeong, H. 2007. Antioxidant Activities of Ethanol Extracts from Germinated Specialty Rough Rice. *Food Science Biotechnology* 16(5): 765 – 770.
- Maga, J.A. 1982. Phytate: its chemistry, occurrence, food interaction, nutritional significance and methods of analysis. *Journal of Agricultural and Food Chemistry* 3:1-9.
- Moktan, B., Saha, J. and Sarkar, P.K. 2008. Antioxidant activities of soybean as affected by *Bacillus*-fermentation to kinema. *Food Research International* 41: 586-593.
- Morrison, J.F. and Twumasi, S.K. 2010. Comparative studies on the *in vitro* antioxidant properties of methanolic and hydro-ethanolic leafy extracts from eight edible leafy vegetables of Ghana. *African Journal of Biotechnology* 9(32): 5177-5184.
- Obahiagbon, F.I. and Erhabor, J.O. 2010. The health implications of the dietary nutrients detected in the vegetable leaves intercropped with *Raphia hookeri* palms. *African Journal Food Science* 4(7): 440 – 443.
- Oboh, G. 2006. Antioxidant properties of some commonly consumed and underutilized tropical legumes. *European Food Research Technology* 224: 61-65. DOI 10.1007/s00217-006-0289-x.
- Oboh, G. and Akindahunsi, A.A. 2003. Biochemical changes in cassava products (flour & gari) subjected to *saccharomyces cerevisiae* solid media fermentation. *Food Chemistry* 82: 599 - 602
- Oboh, G. 2005. Effect of blanching on the antioxidant property of some tropical green leafy vegetables. *Lebensm. Wiss. u. Technol.*, 38: 513-517.
- Oboh, G and Ekperijin, M.M. 2004. Nutritional evaluation of some Nigerian wild seeds. *Nahrung/food* 2: 85 - 87
- Oboh, G., Ademiluyi, A.O. and Akindahunsi, A.A. 2010. The effect of roasting on the nutritional and antioxidant properties of yellow and white maize varieties. *International Journal of Food Science and Technology* 45: 1236-1242.
- Oboh, G., Raddatz, H. and Henle, T. 2008. Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. *Journal of Science Food and Agriculture* 88: 2486 – 2492. DOI,10.1002/jsfa.3367.
- Omale, J. and Ugwu, C.E. 2011. Comparative studies on the protein and mineral composition of some selected Nigerian vegetables. *African Journal of Food Science* 5(1): 22 – 25.
- Oskoueian, E., Abdullah, N., Saad, W., Omar, A., Ahmad, S., Kuan, W., Zolkifli, N., Hendra, R. and Ho, Y.W. 2011. Antioxidant, anti-inflammatory and anticancer

- activities of methanolic extracts from *Jatropha curcas* Linn. *Journal of Medicinal Plants Research* 5(1): 49-57.
- Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition* 44: 307–315.
- Saidu, A.N. and Jideobi, N.G. 2009. The Proximate and Elemental Analysis of some Leafy Vegetables Grown in Minna and Environs. *Journal of Applied Science and Environmental Management* 13(4): 21 – 22.
- Salunke, R., Rawat, N., Tiwari, V.K., Neelam, K., Randhawa, G.S., Dhaliwal, H.S. and Roy, P. 2012. Determination of bioavailable-zinc from biofortified wheat using a coupled *in vitro* digestion/Caco-2 reporter-gene based assay. *Journal of Food Composition and Analysis* 25: 149–159.
- SAS. 2002. Statistical Analysis System Proprietary software. Release 8.3. SAS Institute Inc., Carry, NC
- Siger, A., Czubinski, J., Kachlicki, P., Dwiecki, K., Lampart-Szczapa, E. and Nogala-Kaluvka, M. 2012. Antioxidant activity and phenolic content in three lupin species. *Journal of Food Composition and Analysis* 2: 190–197.
- Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Cioalteau Reagent. *Methods in Enzymology* 29: 152–178.
- Singh, R. P., Murthy, C.K.N. and Jayaprakasha, G.K. 2002. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* methods. *Journal of Agricultural and Food Chemistry* 50: 81– 86.
- Van-Burden, T.P. and Robinson, E.C. 1981. Formation of complexes between protein and tannin acid. *Journal of Agricultural and Food Chemistry* 1: 77 – 82.
- Wong, J.Y. and Chye, F.Y. 2009. Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis* 22: 269–277.
- Woolfe, M.L., Chaplin, M.F. and Otchere, G. 1977. Studies on the mucilages extracted from okra fruits (*Hibiscus esculentus* L.) and baobab leaves (*Adansonia digitata* L.). *Journal of science Food and agriculture* 28 (6): 519 – 529.