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BIOACTIVE PROPERTIES OF *MORINGA OLEIFERA* SEED OIL EXTRACTED USING COLD PRESS AND SOXHLET EXTRACTION METHOD

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ABSTRACT

This study investigates the bioactive properties of Moringa oleifera seeds oils extracted by cold press and Soxhlet method. The result showed that the values of mineral element per 100g for cold press and Soxhlet extraction ranged from 455.22 and 356.76 mg/100g calcium, 1325.4 and 1164.30 mg/100g potassium, 33.62 and 21.46 mg/100g iron, and 91.64 and 84.54 mg/100g magnesium. The study demonstrated that Moringa seeds oils has total phenolics and tocopherol content which ranges from 21.63 to 24.84 01mg/100g /100 g and 113.55 to 175.62 mg/100g respectively, with oils extracted using cold press extraction method having highest total tocopherol content. Moringa seeds have potent antioxidant activities, with oxygen radical absorbance capacity ORAC and DPPH ranging from 27.66 to 32.66 µM TE/g and 32.47 to 41.64µM TE/g respectively. This study has shown that cold press extracted Moringa seed oil has good bioactive components with a great potential for industrial, nutritional and health applications

Keywords: antioxidant, edible oil, nutrition, underutilized.

INTRODUCTION

Lipids are recognized as vital nutrients to our diet. They possess Minerals and trace elements which are essential to the good functioning of the body and they have to be supplied by the food. Many phytochemicals that are antioxidants, especially phenolic compounds, have been isolated from extracts of different parts of plants, such as seeds, fruits, leaves, stems, and roots (Malecka, 2002, Schmidt et al., 2003). Ammar et al., (2017) reported that high concentrations of phytochemicals, which may protect against free radical damage, accumulate in different plant parts. Plants containing valuable phytochemicals such as Tocopherols which are fat soluble antioxidants that function as scavengers of lipid peroxyl radicals (Knekt et al., 1994) may supplement the needs of the human body by acting as natural antioxidants. Antioxidants control and decrease the oxidative damage in foods by impeding or inhibiting oxidation caused by reactive oxygen species (ROS), thereby increasing the shelf-life and quality of these foods, Ammar et al., (2017). Moringa oleifera oil is clear, sweet and odourless oil that is rich in antioxidants and is similar to olive oil in terms of its nutritional profile. It does not turn rancid like other oils hence, has an indefinite shelf life (Drew, 2014). Bukar et al., (2010) reported that some phytochemicals particularly polypeptide found in Moringa seed extracts (oil) may act directly on microorganisms and result in growth inhibition through disrupting cell membrane synthesis or synthesis of essential enzymes.

Over the recent years, much attention has been focused on the edible oils which exhibit high oxidative stability. Despite the high content of phytochemicals, Moringa oil is underutilized in Nigeria and it's just gaining exposure. The study on bioactive compounds in Moringa oil is limited. This present study was carried out to quantify the total phenolic, tocopherol, mineral content and evaluate the antioxidant potential of Moringa oil.

MATERIALS AND METHODS

Materials

Moringa oleifera seeds that were used for this work was procured from *Moringa* research farm, University of Agriculture, Makurdi. Chemicals and all other reagents of analytical grade were provided by chemistry department Benue State University. Extraction was done by cold press and Soxhlet extraction using food grade hexane solvent (AOAC 2012). Pre-treatment of oil seed (*Moringa oleifera*) was done as described by (Evbuomwan, 2015). The cold press extraction of *Moringa* seed oil was done using the local extraction method as described by Kate *et al*., (2014) with slight modifications. The oil was stored in amber bottles or plastic at 4 °C until analyzed and Soxhlet method was done as described by (Evbuomwan 2015).

Parameters

Mineral determination

The quantitative estimation of micronutrients was done using UNICAM solar 969 atomic absorption spectrophotometers as described by Sharma 2009.

Determination of Tocopherols

The method described by Gliszczyńska-Świgło&Sikorska (2004) was use in quantifying the Tocopherols content.

Determination of oxygen radical absorbance capacity (ORAC)

ORAC values of the oils were determined according to Robles-Sánchez *et al.* (2009). The values were calculated using a regression equation between the trolox concentration and the net area under the fluorescein decay curve, and results were expressed as trolox equivalents (μmol TE)/g of fresh weight.

Determination of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity

Determination of DPPH radicals scavenging activity was estimated with the method used by Robles-Sánchez *et al.* (2009). Percentage inhibition of free radical DPPH was calculated based on control reading by following equation [1].

$$\text{DPPH Scavenged (\%)} = \frac{(\text{Acon} - \text{A test})}{Acon} x\ 100 \qquad [1]$$

A con - is the absorbance of the control reaction
A test - is the absorbance in the presence of the sample of the extracts.

RESULTS AND DISCUSSION

Potassium

The most abundant elements out of the four minerals determined in this study were potassium. Cold pressed moringa oil had a value of 1325.4 mg/100g and Soxhlet extracted oil had a value of 1164.30 mg/100g; this showed that the value of the oils extracted by cold press method was higher than that extracted by Soxhlet method as can be seen in Figure 1. The oil is a rich source of potassium when compared to the daily requirement of 2000-3500 mg/day according to FAO/WHO, (1987). The value of potassium in *Moringa* seed oil (CPMSO and SSMSO) in this study falls within the range of 1045.24 -1278.65 mg/100g as reported for *Moringa* seeds by Barakat and Ghazal (2016). Nzikou *et al.* (2009) reported that Potassium is an essential nutrient and play an important role in the synthesis of amino acids and proteins. Aslam *et al.* (2005) also reported that potassium is important for reducing blood pressure and increasing blood circulation, as well as preventive aid on general health of the heart.

Calcium (Ca)

The amount of Calcium (Ca) in the sample of cold pressed *Moringa* seed oil (CPMSO) and Soxhlet extracted *Moringa* seed oil (SEMSO) were found to be 455.22 and 358.76 mg/100g respectively (Figure 1). Least amount of Ca (358.76±0.01 mg/100g) was found in SEMSO. Ca according to Aslam *et al*. (2005) helps in transporting long chain fatty acid which helps in prevention of heart diseases, high blood pressure and other cardiovascular diseases. The values of Ca in cold pressed and Soxhlet extracted *Moringa* oils falls within the range (254.19- 478.63 mg/100g) as reported by Barakat and Ghazal (2008).

Magnesium (Mg)

The value of magnesium (Mg) in the cold pressed sample of *Moringa* oil was 91.64 mg/100g and that of Soxhlet extracted moringa oil was 84.54 mg/100g respectively. The lowest value was observed in SEMSO. The two oils were significantly ($p<0.05$) different from each other. Nzikou *et al.* (2009) reported that Calcium and Magnesium plays a significant role in photosynthesis, carbohydrate metabolism, nucleic acids and binding agents of cell walls.

Iron (Fe)

The amount of Fe in the samples of oil of CPMSO and SEMSO were found to be 21.46 and 33.62 mg/100 g respectively (Figure 1). Fe content of oils extracted from *Moringa* seeds by Soxhlet method was found to be significantly ($p<0.05$) less than that of cold pressed method. The values (21.46 and 33.62 mg/100 g) of Fe in *Moringa* seed oil (CPMSO and SEMSO) reported in this study were lower than the range of values (182.28 - 283.79 mg/100 g) reported by Barakat and Ghazal, (2016) for *Moringa* seed. Generally, the difference in mineral composition can be as a result of compositional differences which exist among the different varieties of *Moringa* seeds, (Zebib *et al.* 2015).

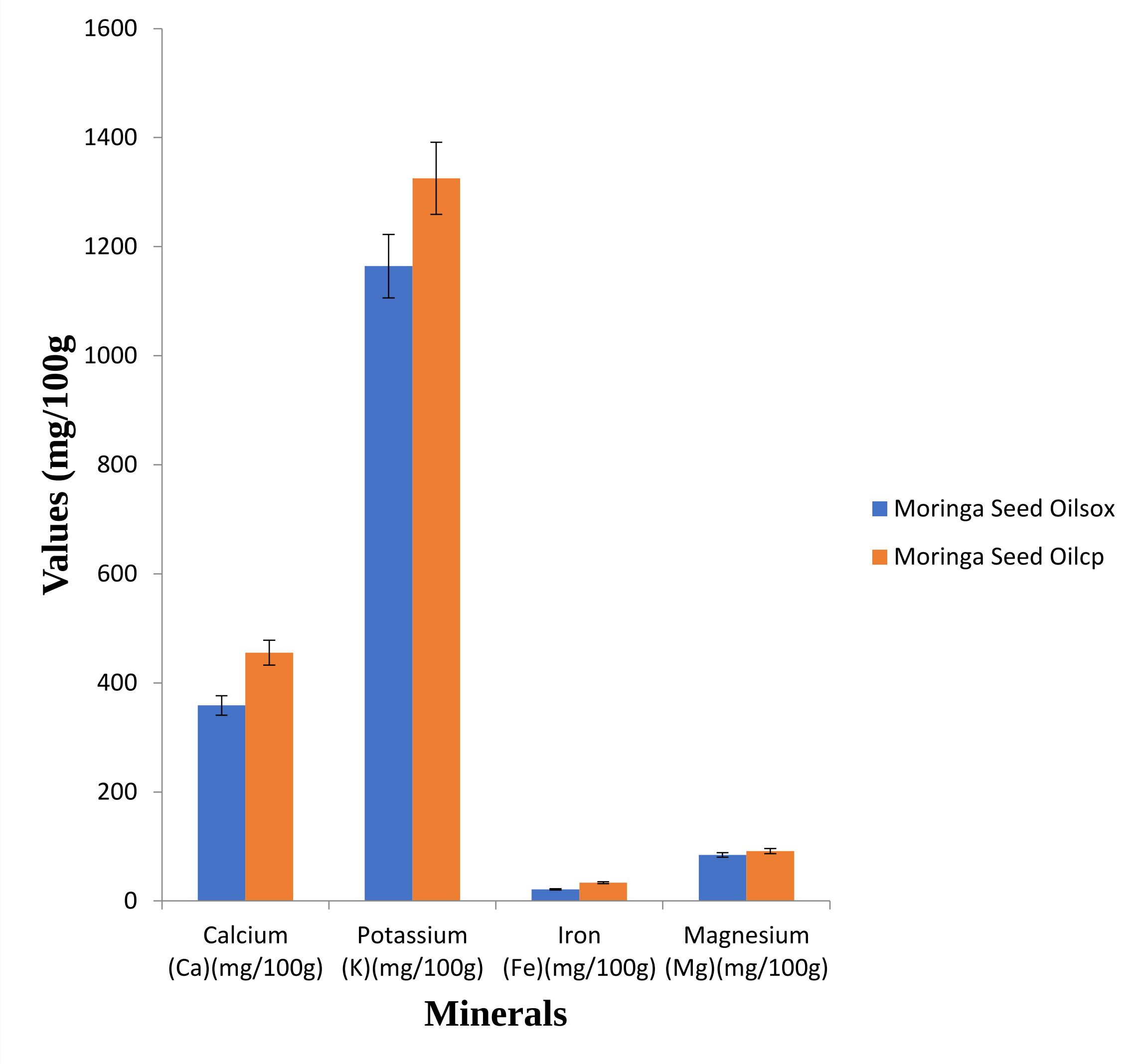

Figure 1. Mineral Composition of *Moringa* seed oils extracted using cold pressed and hexane solvent extraction methods

Key

Cp = cold press extraction method
Sox = Soxhlet extraction method

Total Phenolic Content (TPC) of Extracted Moringa Oils

The TPC value of oil (*Moringa* seed) derived from Soxhlet extraction were higher than that derived from cold press extraction method (Figure 3.2). SEMSO recorded the highest value of 24.84 mg/100 g. That for *Moringa* oils in this study 24.84 and 21.63 mg/100 g were higher than the value (160.00 µg/g which is the same as 16.00 mg/100 g of extract) reported by Basuny and Al-Marzour (2015). From this study it was observed that the TPC was affected by the method and solvent used for extraction. Phenolic compounds have been proved to be responsible for antioxidant and antimicrobial activities on many vegetable seed oils (there is a strong relationship between phenolic content and antioxidant activity in selected fruits and vegetables); it is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Beckles, 2012). From this result the converse is the case for

SEMSO with higher TPC, had lower antioxidant activity properties as represented by the TTC, DPPH and ORAC values. According, to Awatif *et al.*, (2013) temperature during extraction can affect the extractable compounds differently: In addition to thermal decomposition, phenols can react with other plant components, to impede their extraction. The presence of phenolic compounds in *Moringa* seed oil is an added value to its nutritional potential.

The Total Tocopherol Content (TTC)

The values were significantly ($p<0.05$) different, SEMSO had the lowest value of 113.55 mg/100 g, CPMSO value (175.62 mg/100 g) was higher than SEMSO. This was corroborated by Alessandro *et al.* (2016) who reported a TTC of 365.80 mg/kg for CPMSO and 248.00 mg/kg for SEMSO. Alessandro *et al.* (2016) reported a range of 24.70 – 36.58 mg/100 g for *Moringa oleifera* oil. Also, they were lower than that of the ranges (33.00 mg/100 g to 101.00 mg/100 g) reported by the Codex standard (2001).

Antioxidant Properties of the Oils

As a result of the complex components in Moringa seeds oil; two *in vitro* antioxidant activity assays (ORAC and DPPH) were used in the present study.

ORAC

The measured values of ORAC are given in Figure 2. ORAC values ranged from 27.66 (SEMSO) to 32.66 μmol TE g^{-1} (CPMSO) in *Moringa* seed oils, SEMSO showed the lowest total ORAC value (27.66 μmol TE g^{-1}). According to Berger *et al.*, (2011) the rapid ORAC assay gives results that often coincide with the total phenols present as determined by the Folin-Ciocalteu reagent. However, total ORAC values in this study for the oils showed a weak association with contents of total phenolics when considering the extraction methods. While there is a strong relationship between TTC of the *Moringa* oils with the ORAC values. Though specific reason is not clear, none the less, synergistic effects of various phytochemicals present in the oil may involve complex antioxidant behaviour of these constituents.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity.

The DPPH radical scavenging is the most widely used antioxidant activity assays to evaluate the activity of bioactive components of plant extracts. The percentage DPPH scavenging activity of the various oils (SEMSO and CPMSO) is shown in Figure 2. Soxhlet extracted oil SEMSO showed lower activities (32.47 trolox equ TE/g). The DPPH value of cold pressed extracted oil (CPMSO) was higher (41.64 trolox equ TE/g) than that of the hexane extracted oil (SEMSO). Nawirska-Olszanska *et al.*, (2013), attributed the variation in antioxidant activities among samples to the tocopherol contents, the presence of phenolic compounds and the form of antioxidant compounds (hydrophilic and lipophilic). Also, Choe and Min (2006) reported that the quality of natural extracts and their antioxidative performances depends not only on the quality of the original plant, the geographic origin, climatic condition, harvesting date and storage, but also environmental and technological factors affecting the activities of antioxidants from residual sources. They claimed that different compounds are responsible for this activity such as phenolic compounds, metals, chlorophyll, peptides/ amino acids and phospholipids.

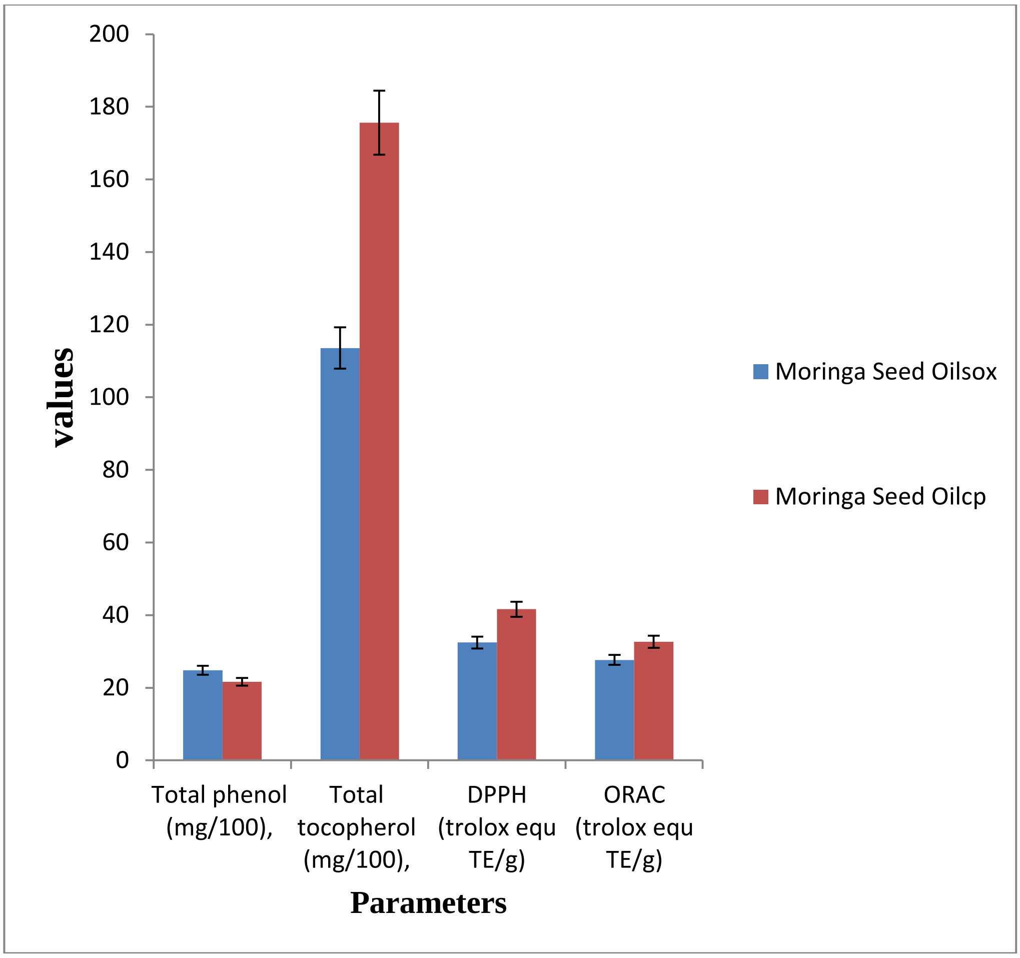

Figure 2. Mineral Composition of *Moringa* seed oils extracted using cold pressed and hexane solvent extraction methods
Keys
Cp = cold press extraction method
Sox = Soxhlet extraction method

CONCLUSION

This study has highlighted the unique bioactive components of *Moringa* seed oil from Benue State, Nigeria and the results indicated good bioactive components of the oils with oils extracted using cold pressed method having higher bioactive components when compared to the oil extracted using Soxhlet extraction method. A well detailed scientific knowledge regarding the bioactive composition of this seed oil is of utmost importance for the development and utilization of this oil for nutrient enrichment.

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