



Determination of Antioxidant Activities of *Moringa oleifera* Leaves from Selected Countries

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Authors' contributions

This work was carried out in collaboration among all authors. Author OMM and WLT designed the study, performed the statistical analysis and wrote the protocol. Authors KCO and OMM wrote the first draft of the manuscript. Authors OMM, WLT and OS managed the analyses of the study. Authors OMM, WLT and KCO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Moringa oleifera is a native plant from Asia, grown in the tropics. The leaves, bark, flowers, fruits, seeds, and roots are rich sources of phytochemicals and antioxidants; hence, they have been extensively utilized for medicinal purposes. This study aimed to determine the Antioxidant Activities of methanolic and ethanolic extracts of five varieties of Moringa leaves from Nigeria, Ghana, Haiti, India, and USA. The leaves were extracted using aqueous methanol and ethanol. 2, 2, -diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP), and Trolox equivalent antioxidant capacity (TEAC) were then determined. Results indicated there were significant differences were recorded in DPPH, FRAP, and TEAC respectively. A significant

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difference in DPPH radical scavenging activities in the leaves from Haiti (94.83 $\mu\text{mol TE g}^{-1}$) and Ghana (86.69 $\mu\text{mol TE g}^{-1}$), Haiti (94.83 $\mu\text{mol TE g}^{-1}$) and Nigeria (84.94 $\mu\text{mol TE g}^{-1}$) respectively. Highest activity was observed in the ethanolic extract from the USA (123.48 $\mu\text{mol TE g}^{-1}$), while the lowest was in the methanolic extract from Nigeria (84.94 $\mu\text{mol TE g}^{-1}$). A similar result was also recorded for TEAC with ethanolic extracts from Ghana (61.59 $\mu\text{mol TE g}^{-1}$) significantly different from Nigeria (63.36 $\mu\text{mol TE g}^{-1}$), India (63.34 $\mu\text{mol TE g}^{-1}$), Haiti (62.42 $\mu\text{mol TE g}^{-1}$) and USA (62.36 $\mu\text{mol TE g}^{-1}$). FRAP methanolic extracts from Nigeria (232.96mg GAE g⁻¹), USA (214.24mg GAE g⁻¹) and India (201.81mg GAE g⁻¹) were significantly different, while extracts from Haiti (222.16mg GAE g⁻¹) and Ghana (221.35mg GAE g⁻¹), USA (214.24mg GAE g⁻¹) and Ghana (221.35mg GAE g⁻¹) were in range.

Keywords: *Moringa oleifera* leaves; antioxidants activity; reducing power; radical scavenging activity.

1. INTRODUCTION

Moringa is a native plant from Asia. It is from the genus of *Moringaceae*. It has 13 species from tropical and sub-tropical climates. *Moringa oleifera* (popularly known as Moringa) is the most cultivated species and is often called the “miracle tree.” It is grown for its pods, seeds, edible leaves, bark, gum, roots, and flowers which can all be used for nutrition [1], medicine, water purification, and livestock feed among other uses [2,3]. Several studies have shown the beneficial effects of Moringa in humans. It is reported to aid in the treatment of more than 300 diseases and chronic conditions including diabetes, scurvy, diarrhea, kidney pain, asthma, tumors, and tuberculosis [4]. Moringa is known to have more than 90 nutrients and 46 types of antioxidants [5]. According to, Moringa contains a large number of bioactive compounds including vitamins, carotenoids, polyphenol, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponin [6]. Moringa leaves contain 7 times the amount of vitamin C typically found in oranges, 4 times the amount of vitamin A in carrots, 36 times the amount of magnesium in eggs, 25 times the amount of iron in spinach, 50 times the amount of vitamin B₃ in peanuts, and 50 times the vitamin B₂ in bananas [7,8]. It is eaten fresh or cooked and can be stored as dried powder for months without refrigeration and reportedly no loss of nutritional values [7].

Moringa, being a functional food, has a positive effect on health beyond basic nutrition [9]. Functional foods are said to promote optimal health and help reduce the risk of diseases [10,11]. Antioxidants present in Moringa leaves inhibit cell proliferation and protect the body from the effects of various free radicals, pollutants, and toxins [12]. Flavonoids are described as a large group of biologically active (water-soluble)

plant compounds. They include compounds such as anthocyanins and flavones. Moringa is reported to have an abundance of flavonoids which have been shown to protect against chronic diseases associated with oxidative stress, including cardiovascular disease and cancer [13]. This study will examine phytochemical content, antioxidant potential, and individual phenolic compounds in different varieties of *Moringa oleifera* leaves [14].

2. METHODOLOGY

2.1 Moringa Oleifera Leaves

Fresh leaves of five varieties of Moringa were retrieved from the Winfred Thomas Agricultural Research Station (WTARS) at Alabama A&M University. The fresh leaves were allowed to dry at room temperature, ground using a laboratory Micro-Mill (Bel-Art Products, Pequannock, NJ 07440 USA) and kept in sealed air-tight Ziploc bags until further analyses.

2.2 Chemicals

Gallic acid, Catechin, Folin & Ciocalteu's phenol reagent, Methanol, Trolox, ABTS salt, Aluminium Chloride, Sodium Hydroxide, Sodium Nitrite, Sodium Carbonate, Acetic acid, Ethanol, Potassium Persulfate, Hydrochloric acid, TPTZ (tripyrindyl-S-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl), Iron Chloride were purchased.

2.3 Sample Extraction

For the preparation of extracts, Moringa leaves were dissolved in methanol and ethanol. The mixture was stirred using a magnetic stir bar and VMR Standard Multi-Position Stirrer for 3 hours at room temperature. Each sample was filtered using Whatman filter paper No. 4 and the filtrate was evaporated to dryness under reduced

pressure using Buchi Rotavapor at 50°C [15,16]. The samples were dissolved with deionized water and kept in the -80°C freezer overnight. The frozen samples were kept in the freeze dryer for 48 hours. The freeze-dried samples were kept at room temperature for further analysis [17,18]

2.4 Determination of Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity: The radical scavenging activity of the extracts and fractions against DPPH free radical was measured using the [19] method with slight modification. 20 µl of moringa oleifera leaves extract or Trolox standard solution with different concentrations (10, 20, 40, 80, 160, 240 µg/mol) was added in a well of a 96-well plate. 230 µl of DPPH solution was added to the 96-well plate. The mixture was mixed gently by shaking and absorbance was read at 517 nm (0 min). The mixture was allowed to sit in the dark at room temperature for 90 min and the absorbance of the mixture was measured again at 517 nm. Result was calculated from the standard curve of Trolox and expressed as micromoles of Trolox Equivalent (TE) per gram of sample (µmol TE/g).

Trolox Equivalent Antioxidant Capacity (TEAC): Antioxidant activity was measured using the method described by [11] with slight modification. ABTS radical cation was prepared by adding solid manganese dioxide to the stock solution of ABTS. Trolox was used standard and a calibration curve was obtained for Trolox at different concentration (0, 50, 100, 150, 200, 250, 300 and 350 µM). Samples was diluted appropriately according to antioxidant activity in Sodium and Potassium Buffer with pH, 7. Briefly, 10 µl of appropriately diluted samples was added in a well of a 96-well plate, 190 µl of ABTS solution was added to the 96-well plate. The mixture was incubated for 30 min at room temperature and the absorbance was read at 734 nm. Result was calculated from the standard curve of Trolox and expressed as micromoles of Trolox Equivalent (TE) per gram of sample (µmol TE/g).

Ferric Reducing Antioxidant Power (FRAP): The ferric reduction ability of plasma was measured using the [20] method with slight modification. Frap reagent was prepared by mixing 10 volumes of 250mM acetate buffer (pH 3.6), with 1 volume of 10 mM TPTZ in 40 Mm Hydrochloric acid and 1 volume of 20 mM of Iron (III) Chloride Hexahydrate. Ascorbic acid was

used as standard at different concentrations (10, 20, 40, 80, 100 µg/ml). 10 µl of properly diluted sample was added in a well of a 96-well plate, 30 µl of deionized water was added to the 96-well plate and 260 µl of FRAP reagent was added to the 96-well plate. The mixture was incubated at 37°C throughout the reaction. The mixture was incubated for 8 min at 37°C and the absorbance was read at 593 nm. The antioxidant capacity values were expressed in mg AAE (Ascorbic Acid Equivalent)/ 100 g.

2.5 Statistical Analysis

The data was subjected to one-way analysis of variance (ANOVA) and the significance of difference of the difference between means was determined by Duncan's multiple-range test ($p < 0.05$) using by SAS. The average content of total phenolic, total flavonoid, DPPH (1/IC50) of the extracts were statistically investigated using one-way analysis of variance (ANOVA) with least significant difference (LSD) by SAS for concentration are expressed ($p < 0.05$) differences. Values expressed are means of three replicate determinations \pm standard deviation.

3. RESULTS AND DISCUSSION

3.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

Figs. 1, 2, and 3 show the antioxidant activities recorded differences in the DPPH radical scavenging in leaves from Nigeria (84.94 µmol TE g⁻¹), Ghana (86.69 µmol TE g⁻¹), India (89.65 µmol TE g⁻¹), USA (90.71 µmol TE g⁻¹) and Haiti (94.83 µmol TE g⁻¹) with a significant difference in the scavenging activities in the leaves from Haiti (94.83 µmol TE g⁻¹) and Ghana (86.69 µmol TE g⁻¹), Haiti (94.83 µmol TE g⁻¹) and Nigeria (84.94 µmol TE g⁻¹) respectively. The ethanolic extract leaves range from Nigeria (104.10 µmol TE g⁻¹), Ghana (109.35 µmol TE g⁻¹), India (112.24 µmol TE g⁻¹), Haiti (117.52 µmol TE g⁻¹) and USA (123.48 µmol TE g⁻¹). The ethanolic extracts showed higher DPPH radical scavenging activity compared to methanolic extracts across all regions. This suggests that ethanol is a more efficient solvent for extracting antioxidant compounds from Moringa oleifera leaves. [21] found similar results, indicating that ethanol generally enhances the extraction of antioxidant compounds better than methanol due to its higher polarity and ability to solubilize a wider range of phytochemicals

The highest DPPH radical scavenging activity was observed in the ethanolic extract from the USA ($123.48 \mu\text{mol TE g}^{-1}$), while the lowest was in the methanolic extract from Nigeria ($84.94 \mu\text{mol TE g}^{-1}$). Environmental factors such as soil quality, climate, and agricultural practices can influence the antioxidant content in plants. [22] highlighted that these factors significantly affect the antioxidant capacity of plant leaves [23].

The high DPPH radical scavenging activity in ethanolic extracts, especially from the USA and Haiti, indicates strong antioxidant properties. This suggests that these extracts have a higher potential for reducing oxidative stress and preventing related diseases [24].

emphasized the importance of antioxidants in neutralizing free radicals and protecting against chronic diseases such as cancer and cardiovascular diseases. [25] reported that ethanol is more effective than methanol in extracting antioxidant compounds from *Moringa oleifera* leaves, which is consistent with the current study's findings.

[26] found that environmental conditions such as temperature, humidity, and soil composition significantly affect the antioxidant capacity of plant extracts. The variations in DPPH radical scavenging activity among different countries observed in this study can be explained by these environmental differences.

Higher antioxidant activity indicates better potential health benefits, such as anti-inflammatory and anti-cancer properties. [27] discussed the health-promoting properties of antioxidants, reinforcing the importance of optimizing extraction methods to maximize antioxidant activity.

3.2 Trolox Equivalent Antioxidant Capacity (TEAC)

Figs. 4, 5 and 6 show the trolox equivalent antioxidant capacity of methanolic extract of leaves with India ($61.27 \mu\text{mol TE g}^{-1}$), Nigeria ($60.87 \mu\text{mol TE g}^{-1}$), Ghana ($60.67 \mu\text{mol TE g}^{-1}$), USA ($60.40 \mu\text{mol TE g}^{-1}$) and Haiti ($60.35 \mu\text{mol TE g}^{-1}$). While there were no significant difference in the selected countries, ethanolic extracts from Ghana ($61.59 \mu\text{mol TE g}^{-1}$) was significantly different from Nigeria ($63.36 \mu\text{mol TE g}^{-1}$), India ($63.34 \mu\text{mol TE g}^{-1}$), Haiti ($62.42 \mu\text{mol TE g}^{-1}$) and USA ($62.36 \mu\text{mol TE g}^{-1}$), while extracts from Nigeria ($63.36 \mu\text{mol TE g}^{-1}$) and India ($63.34 \mu\text{mol TE g}^{-1}$), extracts from Haiti

($62.42 \mu\text{mol TE g}^{-1}$) and USA ($62.36 \mu\text{mol TE g}^{-1}$).

The ethanolic extracts generally displayed higher TEAC values compared to methanolic extracts. This suggests that ethanol is more effective than methanol in extracting antioxidant compounds from *Moringa oleifera* leaves. [21] confirmed that ethanol, being a more polar solvent, typically extracts a broader range of antioxidant compounds, which could explain the higher TEAC values. The highest TEAC in methanolic extracts was recorded for leaves from India ($61.27 \mu\text{mol TE g}^{-1}$), while the lowest was from Haiti ($60.35 \mu\text{mol TE g}^{-1}$). For ethanolic extracts, the highest TEAC was from Nigeria ($63.36 \mu\text{mol TE g}^{-1}$), and the lowest from Ghana ($61.59 \mu\text{mol TE g}^{-1}$). Environmental factors such as soil composition, climate, and cultivation practices can significantly influence the antioxidant content in plant leaves. [22] highlighted that these factors affect the antioxidant capacity of plant materials.

Antioxidants are critical in mitigating oxidative stress, which is implicated in various chronic diseases. The higher TEAC values in ethanolic extracts, particularly from Nigeria and India, suggest these extracts have stronger antioxidant properties. [9] discussed the importance of antioxidants in protecting against oxidative damage and reducing the risk of diseases such as cancer and cardiovascular diseases.

[28] found that ethanol is generally more effective than methanol in extracting antioxidant compounds from *Moringa oleifera* leaves, supporting the current findings of higher TEAC in ethanolic extracts. [29] demonstrated that environmental conditions significantly impact the antioxidant capacity of plant extracts. The observed variations in TEAC among different countries in this study align with this understanding.

Higher TEAC values indicate a greater ability to neutralize free radicals, contributing to better health outcomes. [27] emphasized the health benefits of antioxidants, including anti-inflammatory and anti-cancer effects.

3.3 Ferric Reducing Antioxidant Power (FRAP)

Figs. 7, 8, and 9 indicate the methanolic extracts from Nigeria ($232.96 \text{mg GAE g}^{-1}$), USA ($214.24 \text{mg GAE g}^{-1}$), and India ($201.81 \text{mg GAE g}^{-1}$) were significantly different in their FRAP

content, while extracts from Haiti (222.16mg GAE g⁻¹). The significant differences in FRAP content among the methanolic extracts from different countries highlight the variability in antioxidant capacity. This variability can be influenced by several factors including genetic differences, environmental conditions, soil composition, and post-harvest processing methods. [20] developed the FRAP assay, which is widely used to assess the antioxidant power of plant extracts. This assay measures the ability of

antioxidants to reduce ferric ion (Fe³⁺) to ferrous ion (Fe²⁺), providing an indication of the reducing power of the extract. The Nigerian variety exhibited the highest FRAP content (232.96 mg GAE g⁻¹), suggesting a superior antioxidant capacity compared to the other countries [23]. This high antioxidant potential could be due to the presence of specific phenolic compounds in higher concentrations, which are known for their reducing power [30].

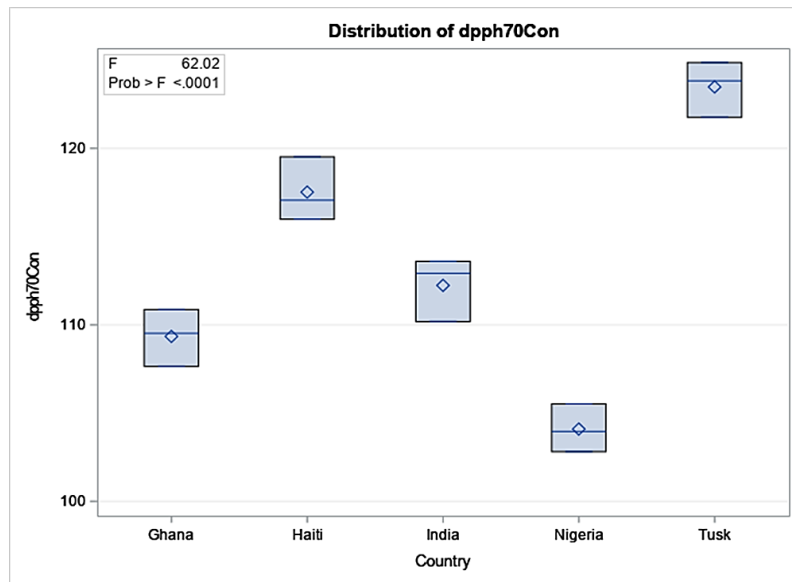


Fig. 1. DPPH radical scavenging activities in five varieties of moringa oleifera leaves dissolved in 70% ethanol

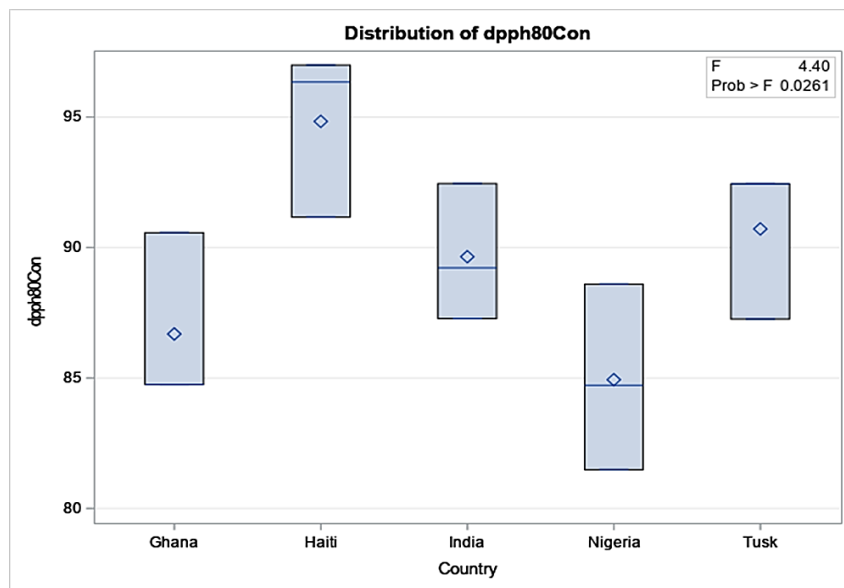


Fig. 2. DPPH radical scavenging activities in five varieties of moringa oleifera leaves dissolved in 80% methanol

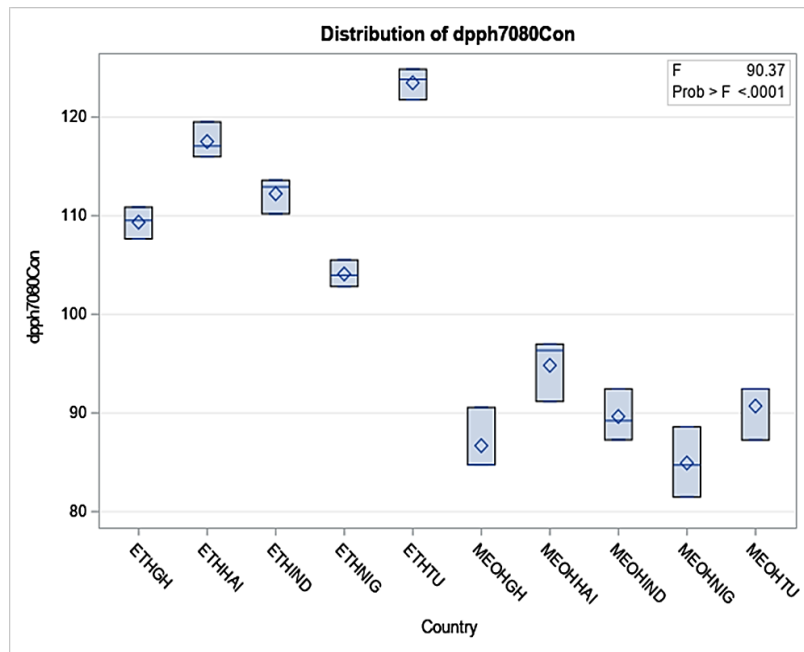


Fig. 3. DPPH radical scavenging activities in five varieties of moringa oleifera leaves dissolved in 70% ethanol and 80% methanol

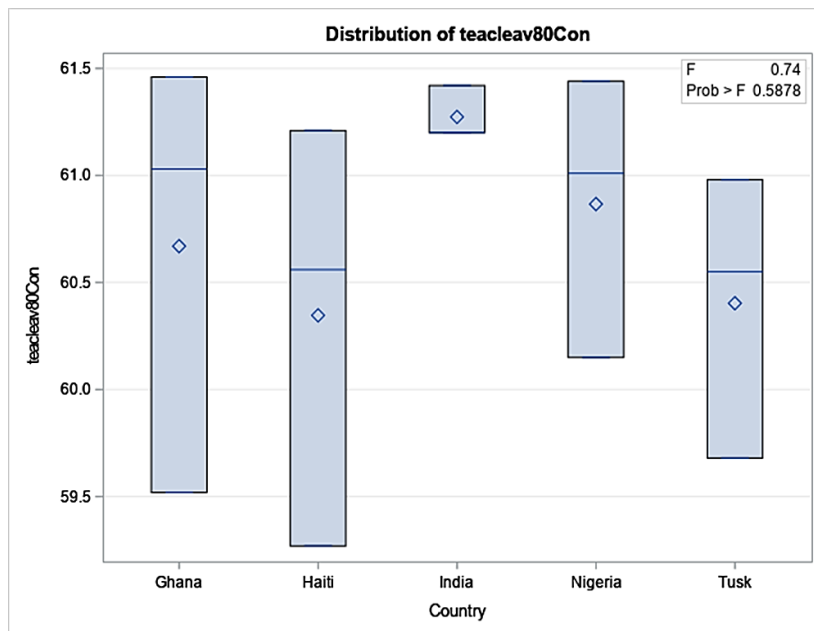


Fig. 4. Trolox equivalent antioxidant capacity in five varieties of moringa oleifera leaves dissolved in 80% methanol

Salawu [31] and [20] reported that Nigerian medicinal plants, including *Moringa oleifera*, have high antioxidant activities due to their rich phenolic content. The extracts from the USA (214.24 mg GAE g⁻¹) and India (201.81 mg GAE g⁻¹) were significantly lower in FRAP content compared to Nigeria [30]. This difference might

be attributed to variations in climatic conditions and soil nutrients that affect the synthesis of antioxidant compounds. [21] discussed how environmental factors such as sunlight exposure and soil composition influence the antioxidant levels in plants. The extracts from Haiti (222.16 mg GAE g⁻¹) and Ghana (221.35 mg GAE g⁻¹)

showed intermediate FRAP values. These results indicate that these varieties also possess substantial antioxidant capacity, which could be due to favorable growing conditions and efficient extraction methods. The high DPPH radical scavenging activity in ethanolic extracts, especially from the USA and Haiti, indicates strong antioxidant properties. This suggests that

these extracts have a higher potential for reducing oxidative stress and preventing related diseases.

Similar trends in antioxidant capacities among *Moringa oleifera* leaves from different regions, highlighting the influence of geographical factors.

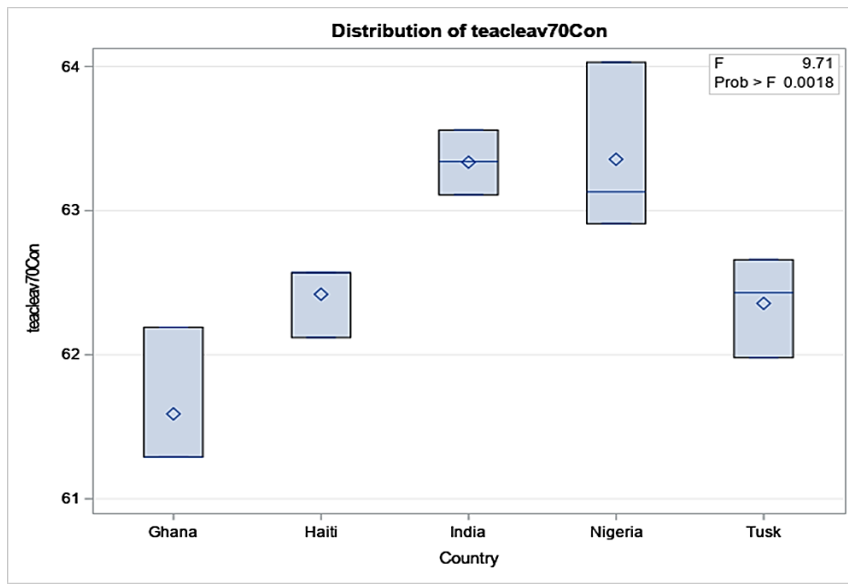


Fig. 5. Trolox equivalent antioxidant capacity in five varieties of moringa oleifera leaves dissolved in 70% ethanol

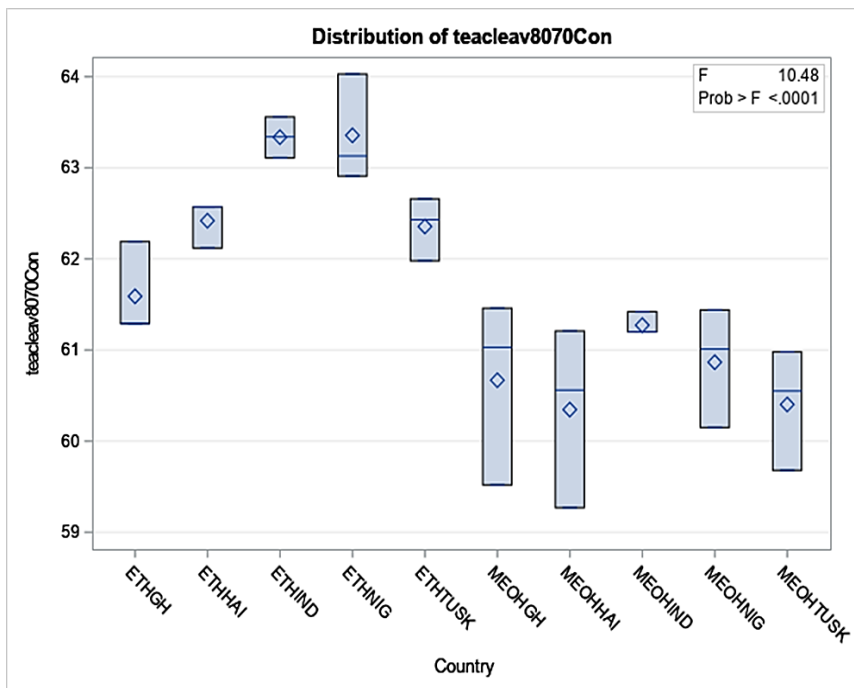


Fig. 6. Trolox equivalent Antioxidant capacity in five varieties of moringa oleifera leaves dissolved in 80% methanol and 70% ethanol

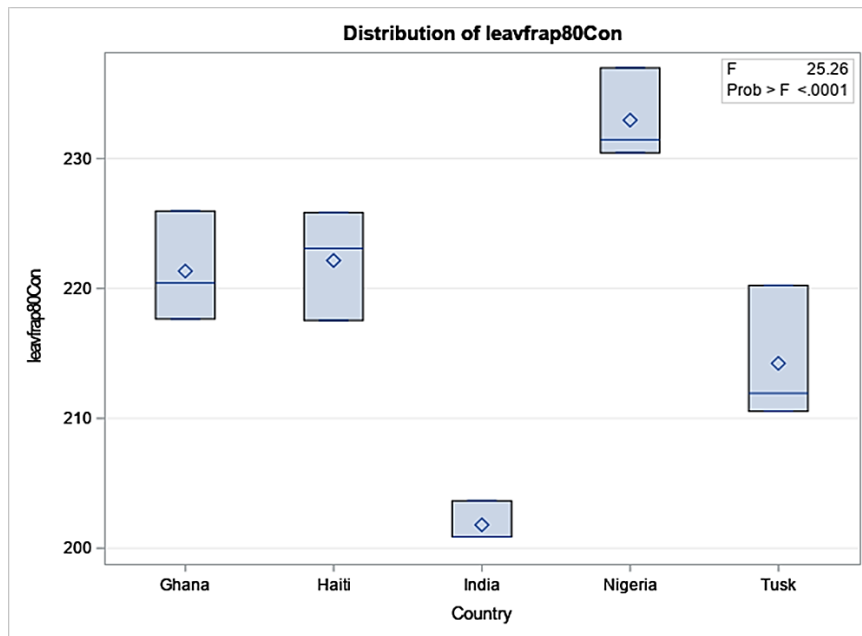


Fig. 7. Ferric reducing antioxidant power in five varieties of moringa oleifera leaves dissolved in 80% methanol

The extract from India exhibited the lowest FRAP content among the compared countries. This could be due to diverse factors such as soil type, water availability, and traditional farming techniques. Despite this, the antioxidant activity remains significant, emphasizing the health-promoting properties of Moringa leaves in Indian traditional medicine and diet. [32]. The

extract from Haiti showed a higher FRAP content than India but lower than Nigeria. This intermediate value suggests that the Moringa leaves from Haiti possess robust antioxidant properties, likely influenced by the local growing conditions which include tropical climate and potentially organic farming practices [30].

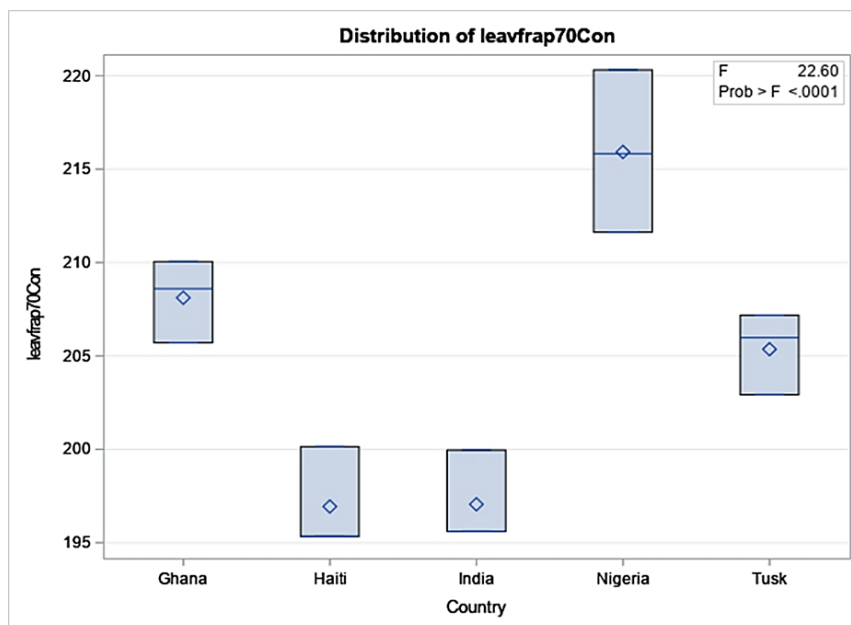


Fig. 8. Ferric reducing antioxidant power in five varieties of moringa oleifera leaves dissolved in 70% ethanol

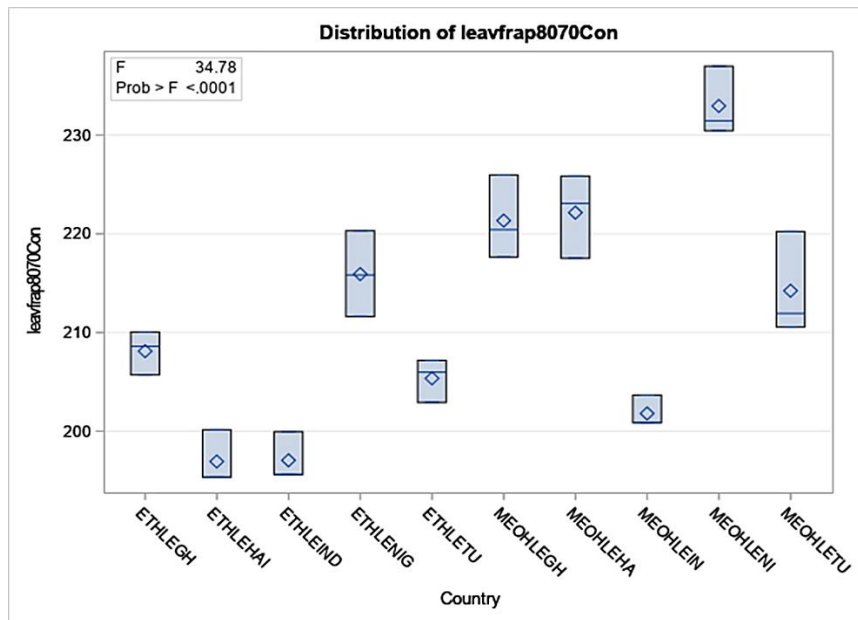


Fig. 9. Ferric reducing antioxidant power in five varieties of moringa oleifera leaves dissolved in 80% methanol and 70% ethanol

Methanolic extraction is known to be effective in isolating phenolic compounds due to its polarity [33]. The choice of methanol as the extraction solvent might have contributed to the high FRAP values observed [34]. emphasized that the efficiency of antioxidant extraction is highly dependent on the solvent used, with methanol being particularly effective for extracting phenolic compounds. [35] reported significant variations in the antioxidant capacities of *Moringa oleifera* leaves from different regions, attributing these differences to genetic and environmental factors. The study observed similar trends, with certain regions producing leaves with higher antioxidant capacities.

[30] noted that *Moringa oleifera* leaves contain high levels of phenolic compounds, which contribute to their antioxidant capacity. The variability in FRAP values among different countries can be linked to the varying levels of these compounds. [36] discussed how harvesting time and post-harvest handling practices influence the antioxidant properties of *Moringa oleifera* leaves. Leaves harvested during peak growth periods typically exhibit higher antioxidant activities. The high FRAP values in the Nigerian and Haitian varieties suggest their potential use in nutraceuticals and functional foods aimed at combating oxidative stress [37]. Antioxidants from these sources could play a significant role in preventing chronic diseases such as cardiovascular diseases and cancers [38,39]

highlighted the potential of *Moringa oleifera* leaves as a natural source of antioxidants for health promotion and disease prevention [40,41,42].

4. CONCLUSION AND FUTURE RESEARCH

Moringa oleifera Lam. is a fast growing tree with interesting benefits for human health. It is traditionally cultivating in its origin region, India, as well as Asian countries. *Moringa* was also introduced to other tropical regions as an interesting agricultural crop. Our investigation demonstrates that ethanolic extracts of *Moringa oleifera* leaves exhibit higher DPPH radical scavenging activity compared to methanolic extracts, the ethanolic extracts of *Moringa oleifera* leaves exhibited higher Trolox equivalent antioxidant capacity compared to methanolic extracts, with significant variations were observed among different geographical regions. The study also reveals significant variability in the FRAP content of *Moringa oleifera* leaves from different countries, with Nigeria exhibiting the highest antioxidant capacity. This variability is influenced by genetic, environmental, and post-harvest factors. Understanding these differences is crucial for optimizing the use of *Moringa oleifera* leaves in nutraceutical and functional food applications, enhancing their potential as a natural source of antioxidants. These findings are consistent with previous research and highlight

the importance of both solvent type and environmental factors in determining antioxidant activity. The results underscore the significant health benefits associated with the antioxidant compounds in *Moringa oleifera* leaves and emphasize the need for optimized extraction methods to enhance their nutritional and therapeutic value. This study also elucidates the significant variability in phenolic compounds among different varieties of *Moringa oleifera* leaves, highlighting their diverse antioxidant and health-promoting properties. Geographical factors and genetic diversity contribute to these variations, influencing the composition and potential benefits of *Moringa oleifera* as a functional food and medicinal plant. Further studies should optimize solvent extraction methods to maximize the yield of antioxidant compounds from *Moringa oleifera* leaves. Further research should focus on identifying the specific phenolic compounds contributing to the high FRAP values in Nigerian *Moringa oleifera* leaves. Research should also investigate the bioavailability and metabolism of antioxidant compounds in different solvent extracts to better understand their health impacts. Also, exploring the influence of specific environmental factors on antioxidant content can help in understanding and enhancing the nutritional and therapeutic value of *Moringa oleifera* leaves. Furthermore, research should also investigate the bioavailability and metabolism of antioxidant compounds in different solvent extracts to better understand their health impacts.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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