DETERMINATION OF ANTIOXIDANT ACTIVITIES OF *MORINGA OLEIFERA LEAVES* FROM SELECTED COUNTRIES

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 difference in DPPH radical scavenging activities in the leaves from Haiti (94.83µmol TE g-1) and Ghana (86.69µmol TE g-1), Haiti (94.83µmol TE g-1) and Nigeria (84.94µmol TE g-1) respectively. Highest activity was observed in the ethanolic extract from the USA (123.48 µmol TE g^{-1}), while the lowest was in the methanolic extract from Nigeria (84.94 µmol TE g^{-1}). A similar result was also recorded for TEAC with ethanolic extracts from Ghana (61.59µmol TE g-1) significantly different from Nigeria (63.36µmol TE g-1), India (63.34µmol TE g-1), Haiti (62.42µmol TE g-1) and USA (62.36µmol 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

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, medicine, water purification, and livestock feed among other uses (Anwar et al., 2007). 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 (Lockett & Clavert, 2000). Moringa is known to have more than 90 nutrients and 46 types of antioxidants. According to Sanchez et al. (2010), Moringa contains a large number of bioactive compounds including vitamins, carotenoids, polyphenol, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins. 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 B_3 in peanuts, and 50 times the vitamin B_2 in bananas (Abdulsalam et al., 2018). It is eaten fresh or cooked and can be stored as dried powder for months without refrigeration and reportedly no loss of nutritional values (Abdulsalam et al., 2018).

Moringa, being a functional food, has a positive effect on health beyond basic nutrition. Functional foods are said to promote optimal health and help reduce the risk of diseases (Okwori et al., 2009). Antioxidants present in Moringa leaves inhibit cell proliferation and protect the body from the effects of various free radicals, pollutants, and toxins. 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 (Pandey et al., 2009). This study will examine phytochemical

content, antioxidant potential, and individual phenolic compounds in different varieties of *Moringa oleifera* leaves.

METHODOLOGY

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.

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 (tripyridyl-S-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl), Iron Chloride were purchased.

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. 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.

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 Brand-Williams method (1995) 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 Seeram et al. (2006) 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 Benzie and Strain method (1999) with slight modification. Frap reagent was prepared by mixing 10 volumes of 250mM acetate

buffer (pH 3.6), with I volume of 10 mM TPTZ in 40 Mm Hydrochloric acid and 1volume 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.

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 ± standard deviation.

RESULTS AND DISCUSSION

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

Figures 1, 2, and 3 show the antioxidant activities recorded differences in the DPPH radical scavenging in leaves from Nigeria (84.94µmol TE g^{-1}), Ghana (86.69µmol TE g^{-1}), India (89.65µmol TE g-1), USA (90.71µmol TE g-1) and Haiti (94.83µmol TE g^{-1}) with a significant difference in the scavenging activities in the leaves from Haiti (94.83µmol TE g-1) and Ghana

(86.69µmol TE g-1), Haiti (94.83µmol TE g-1) and Nigeria (84.94µmol TE g-1) respectively. The ethanolic extract leaves range from Nigeria (104.10µmol TE g-1), Ghana (109.35µmol TE g-1), India (112.24µmol TE g-1), Haiti (117.52µmol TE g-1) and USA (123.48µmol 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. Do et al. (2014) 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 µmol TE g^{-1}), while the lowest was in the methanolic extract from Nigeria (84.94 µmol TE g^{-1}). Environmental factors such as soil quality, climate, and agricultural practices can influence the antioxidant content in plants. Sreelatha and Padma (2009) highlighted that these factors significantly affect the antioxidant capacity of plant leaves.

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.

Scalbert, Johnson, and Saltmarsh (2005) emphasized the importance of antioxidants in neutralizing free radicals and protecting against chronic diseases such as cancer and cardiovascular diseases. Makkar and Becker (1996) 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.

Alothman, Bhat, and Karim (2009) 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. Middleton, Kandaswami, and Theoharides (2000) discussed the health-promoting properties of antioxidants, reinforcing the importance of optimizing extraction methods to maximize antioxidant activity.

Trolox Equivalent Antioxidant Capacity (TEAC)

Figures 4, 5 and 6 show the trolox equivalent antioxidant capacity of methanolic extract of leaves with India (61.27µmol TE g-1), Nigeria (60.87µmol TE g-1), Ghana (60.67µmol TE g-1), USA (60.40µmol TE g-1) and Haiti (60.35µmol TE g-1). While there were no significant difference in the selected countries, ethanolic extracts from Ghana (61.59µmol TE g-1) was significantly different from Nigeria (63.36µmol TE g-1), India (63.34µmol TE g-1), Haiti (62.42µmol TE g-1) and USA (62.36µmol TE g-1), while extracts from Nigeria (63.36µmol TE g-1) and India (63.34µmol TE g-1), extracts from Haiti (62.42µmol TE g-1) and USA (62.36µmol 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. Do et al. (2014) 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 µmol TE g^{-1}), while the lowest was from Haiti (60.35 µmol TE g^{-1}). For ethanolic extracts, the highest TEAC was from Nigeria (63.36 µmol TE g^{-1}), and the lowest from Ghana (61.59 µmol TE g^{-1}).

Environmental factors such as soil composition, climate, and cultivation practices can significantly influence the antioxidant content in plant leaves. Sreelatha and Padma (2009) 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. Scalbert, Johnson, and Saltmarsh (2005) discussed the importance of antioxidants in protecting against oxidative damage and reducing the risk of diseases such as cancer and cardiovascular diseases.

Makkar and Becker (1996) 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. Alothman, Bhat, and Karim (2009) 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. Middleton, Kandaswami, and Theoharides (2000) emphasized the health benefits of antioxidants, including anti-inflammatory and anti-cancer effects.

Ferric Reducing Antioxidant Power (FRAP)

Figures 7, 8, and 9 indicate the 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 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. Benzie and Strain (1996) 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 (Fe^{3+}) to ferrous ion (Fe^{2+}), 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. This high antioxidant potential could be due to the presence of specific phenolic compounds in higher concentrations, which are known for their reducing power Shiv Kumar et al., (2022).

Odukoya et al. (2007), and Shiv Kumar et al., (2022) 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 Shiv Kumar et al., (2022). This difference might be attributed to variations in climatic conditions and soil nutrients that affect the synthesis of antioxidant compounds.Dillard and German (2000) 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. Sánchez-Machado et al. (2009) found similar trends in antioxidant capacities among Moringa oleifera leaves from different regions, highlighting the influence of geographical factors.

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. Olaoye, A.B.et al., (2021). 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. Shiv Kumar et al., (2022.

Methanolic extraction is known to be effective in isolating phenolic compounds due to its polarity. The choice of methanol as the extraction solvent might have contributed to the high FRAP values observed.Dorman et al. (2003) emphasized that the efficiency of antioxidant extraction is highly dependent on the solvent used, with methanol being particularly effective for extracting phenolic compounds. Vongsak et al. (2013) 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.

Kasolo et al. (2010) 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. Moyo et al. (2011) 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. Antioxidants from these sources could play a significant role in preventing chronic diseases such as cardiovascular diseases and cancers. Ghebremariam et al. (2014) highlighted the potential of Moringa oleifera leaves as a natural source of antioxidants for health promotion and disease prevention.

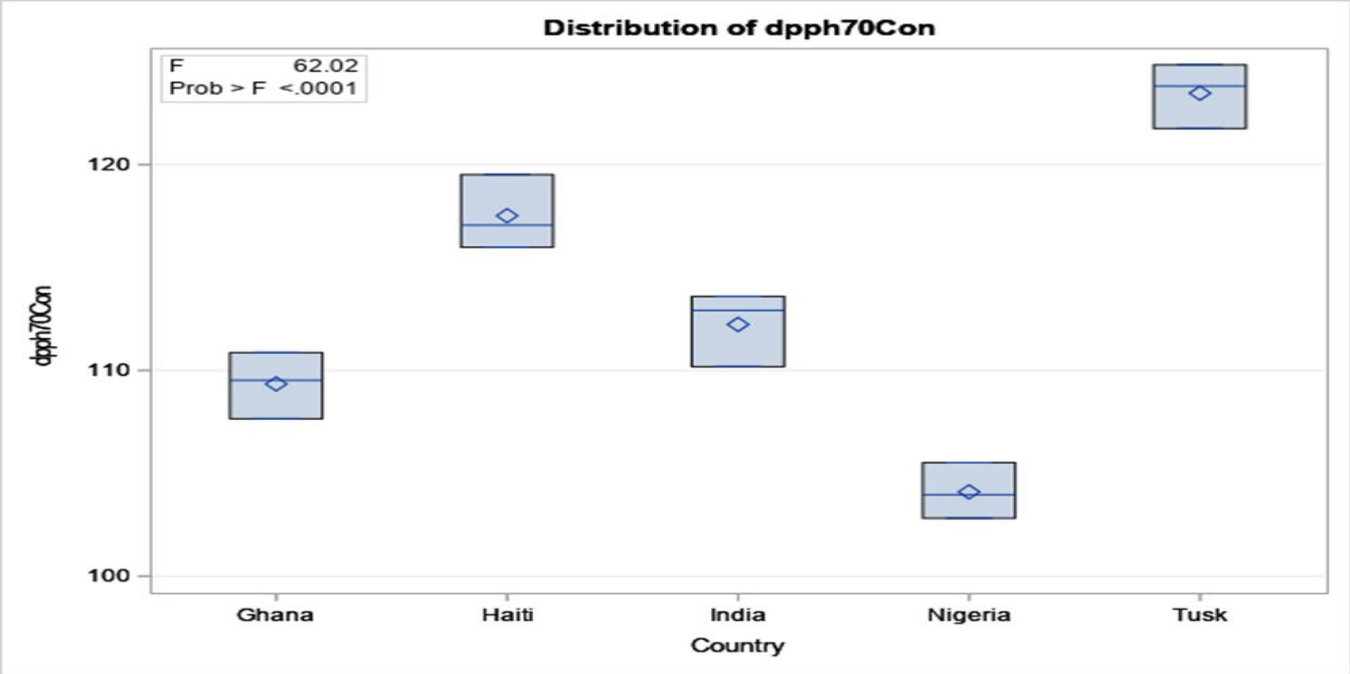

Figure 1: DPPH RADICAL SCAVENGING ACTIVITIES IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 70% ETHANOL

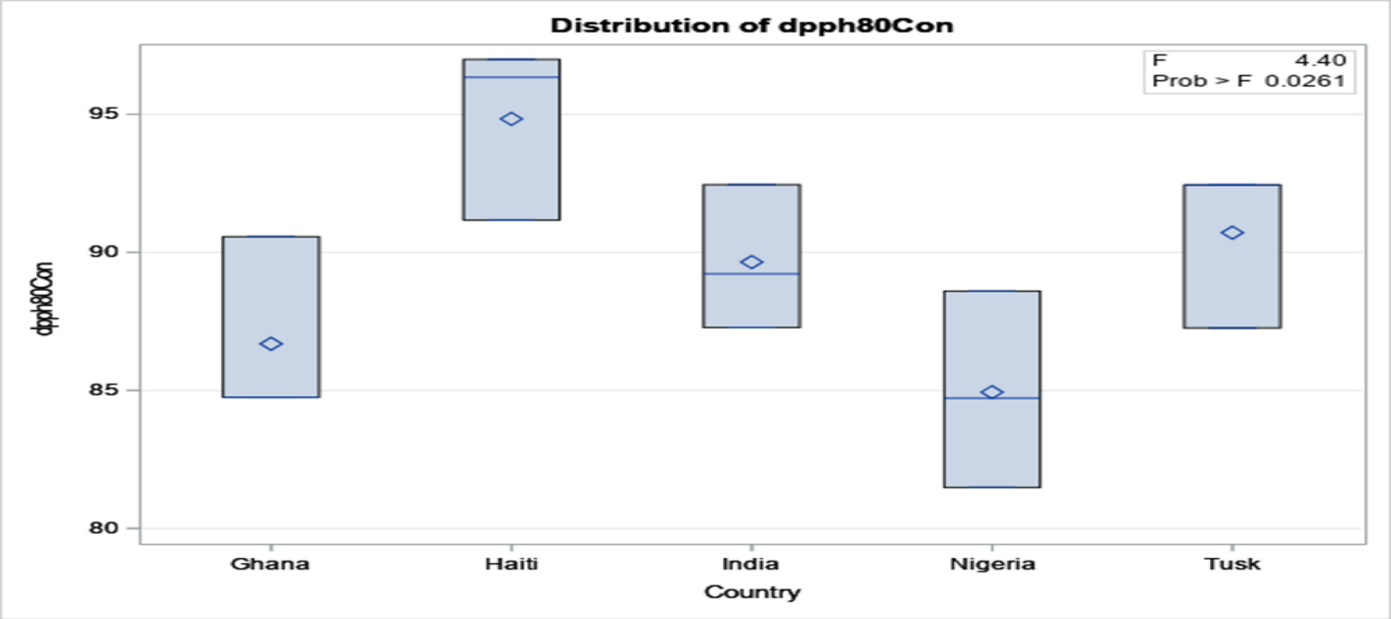

Figure 2: DPPH RADICAL SCAVENGING ACTIVITIES IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 80% METHANOL

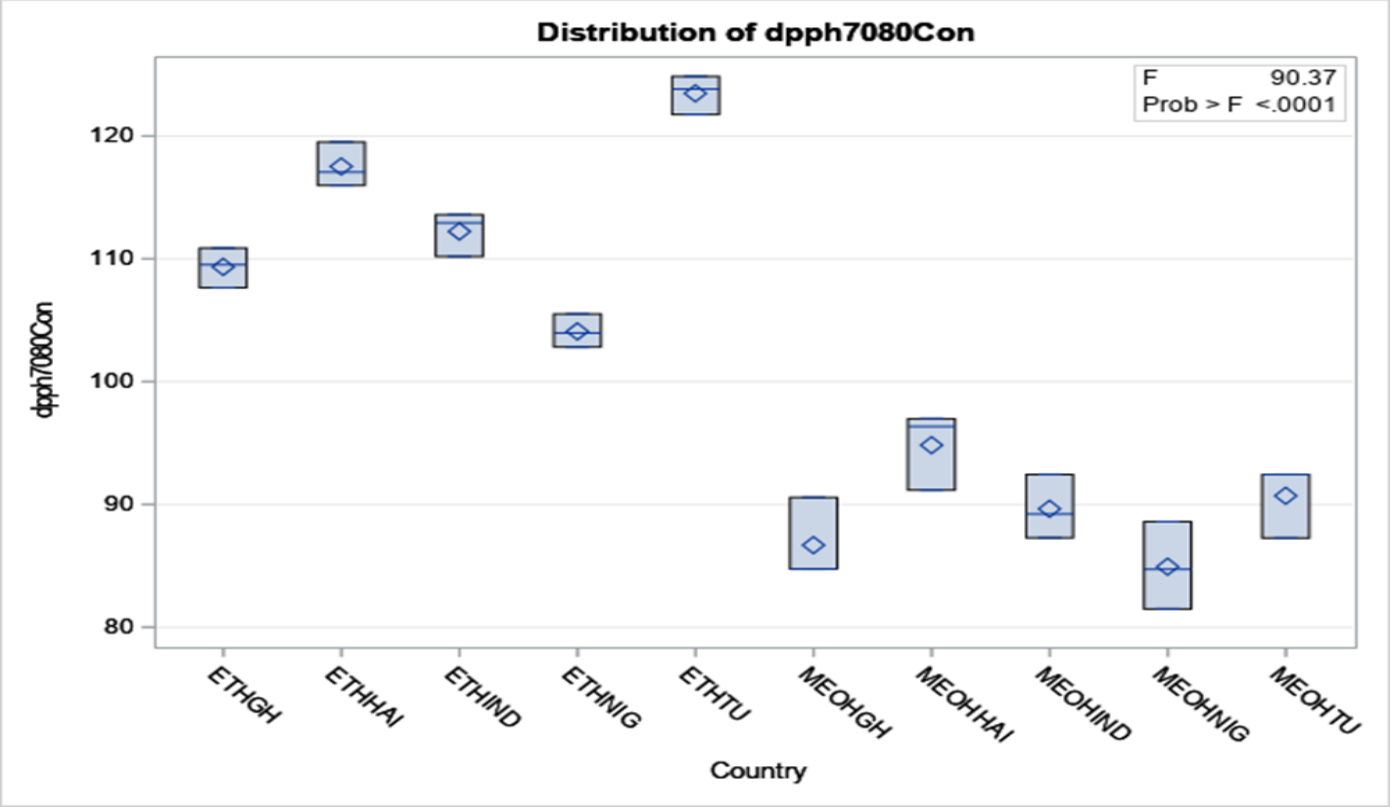

Figure 3: DPPH RADICAL SCAVENGING ACTIVITIES IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 70% ETHANOL AND 80% METHANOL

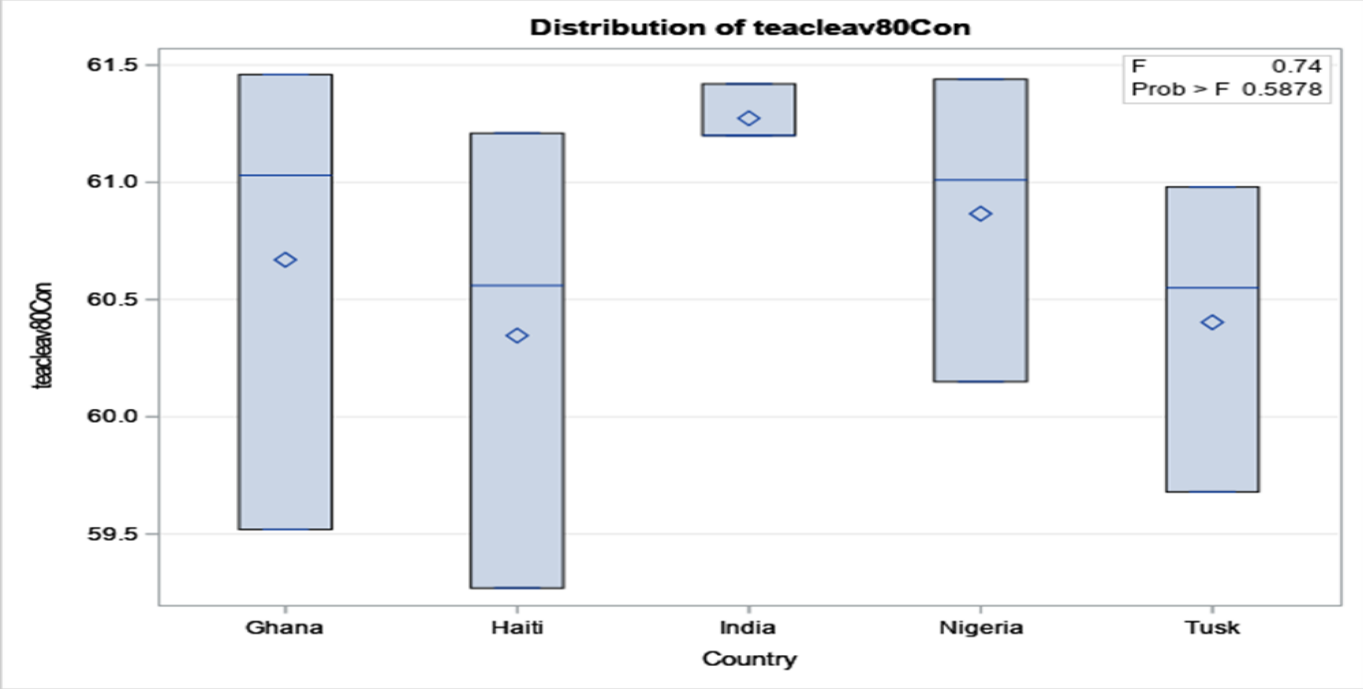

Figure 4: TROLOX EQUIVALENT ANTIOXIDANT CAPACITY IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 80% METHANOL

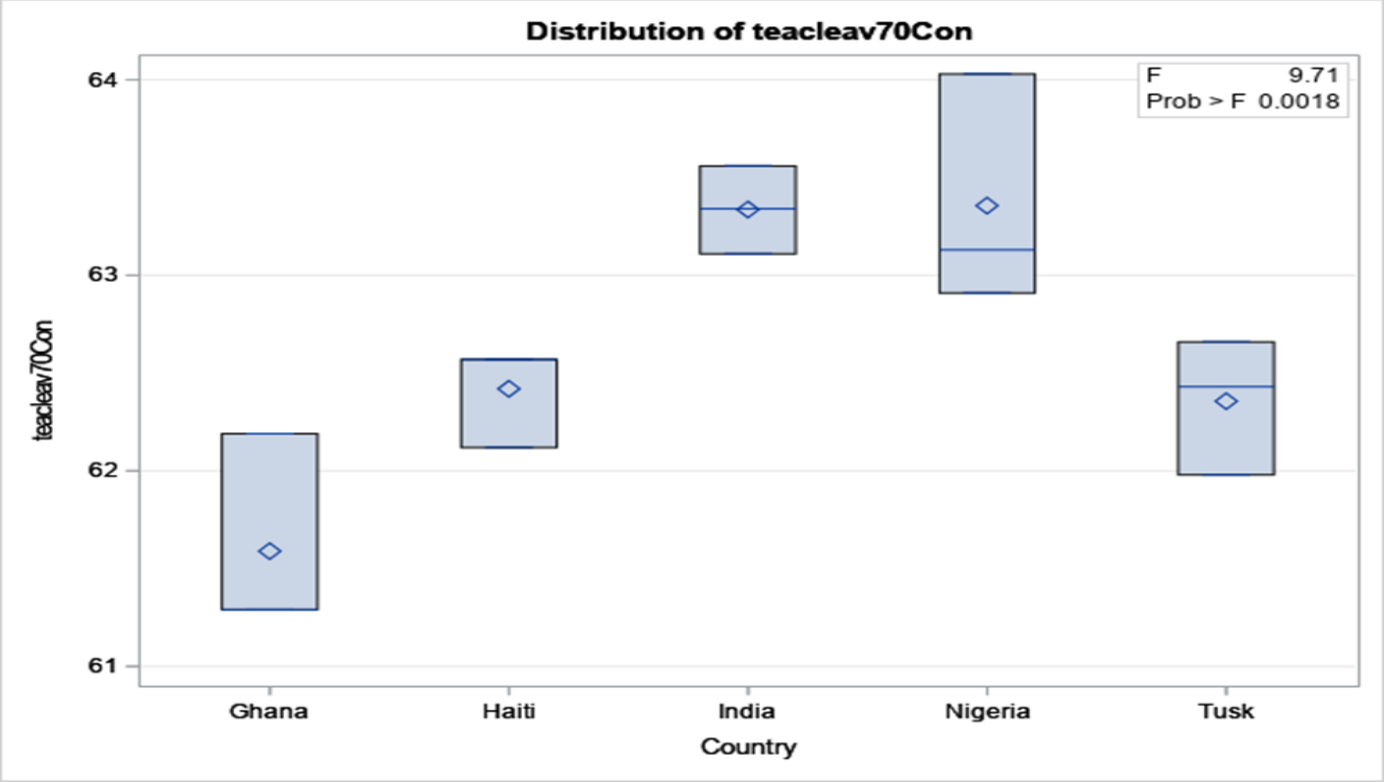

Figure 5: TROLOX EQUIVALENT ANTIOXIDANT CAPACITY IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 70% ETHANOL

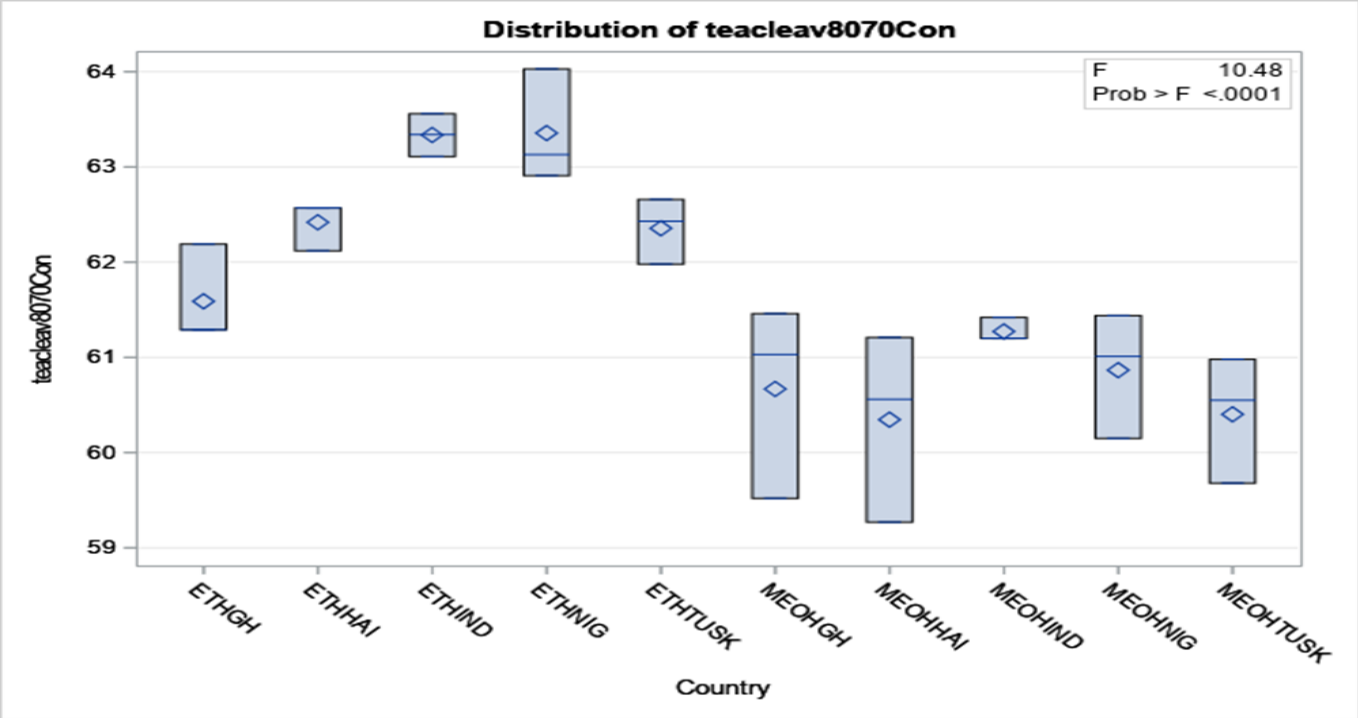

Figure 6: TROLOX EQUIVALENT ANTIOXIDANT CAPACITY IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 80% METHANOL AND 70% ETHANOL

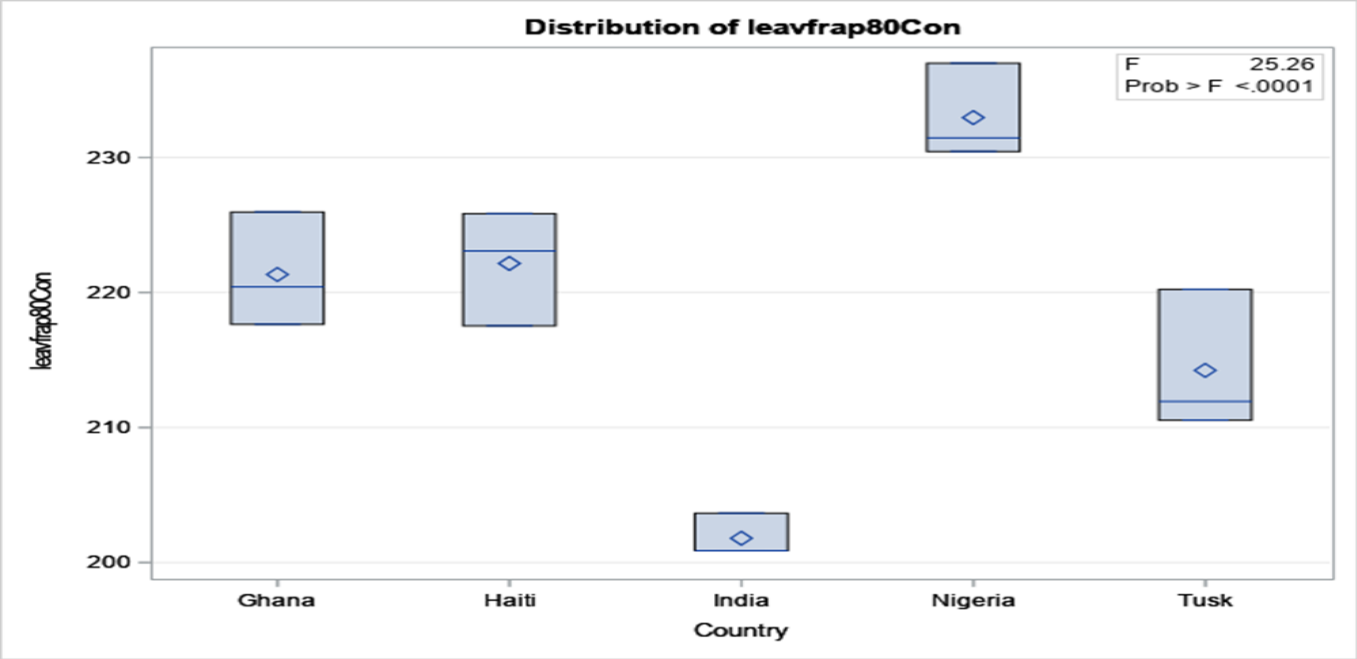

Figure 7: FERRIC REDUCING ANTIOXIDANT POWER IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 80% METHANOL

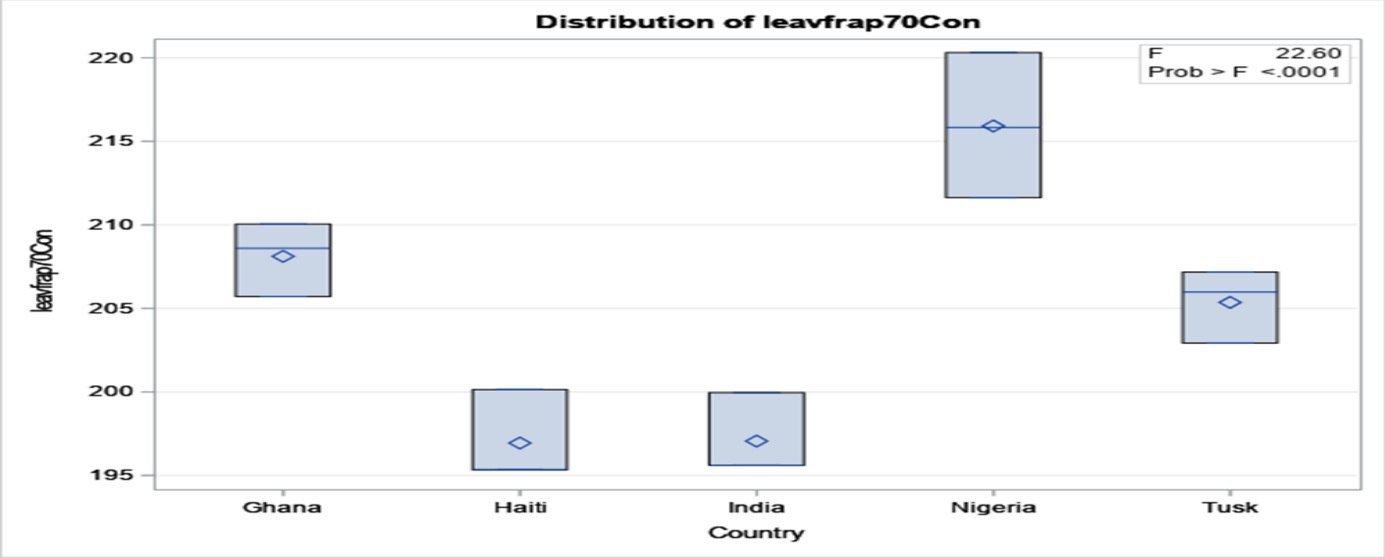

Figure 8: FERRIC REDUCING ANTIOXIDANT POWER IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 70% ETHANOL

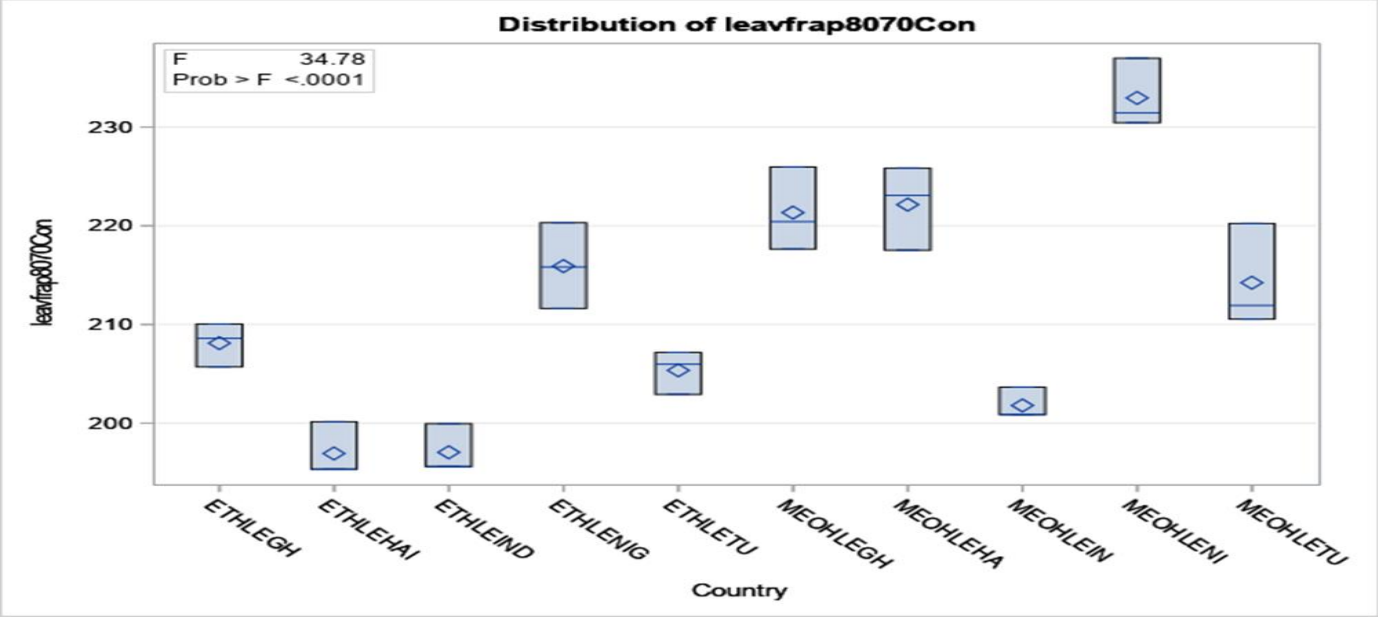

Figure 9: FERRIC REDUCING ANTIOXIDANT POWER IN FIVE VARIETIES OF MORINGA OLEIFERA LEAVES DISSOLVED IN 80% METHANOL AND 70% ETHANOL

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

Details of the AI usage are given below:

1. THE VERSION OF ChatGPT-4 IS RELEASE BY OpenAI..
2. USED FOR SOME PART OF THE DISCUSSION
3. USED FOR THE COMPARISON OF RESULTS WITH PREVIOUS STUDIES

REFERENCES

Adedapo, A.A., Mogbojuri, O. M. & Emikpe, B. O. (2009). Safety Evaluations of the Aqueous Extract of the leaves of *Moringa Oleifera* in Rats. *Journal of Medicinal Plant Research, 3*(8), 586-591.

Ademiluyi A. O., Oyeleye, S. I., & Oboh, G. (2016). Biological activities, antioxidant properties and phytoconstituents of essential oil from sweet basil (Ocimum basilicum L.) leaves. *Comparative Clinical Pathology, 25*, 169-176.

Aekthammarat.D., Pannangpetch, P., & Tangsucharit, P. (2019). *Moringa Oleifera* leaf extract lowers high blood pressure by alleviating vascular dysfunction and decreasing oxidative stress in L-NAME hypertensive rats. *Phytomedicine, 54*(15), 9-16. https://doi.org/10.1016/j.phymed.2018.10.023

Alothman, M., Bhat, R., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry, 115(3), 785-788.

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). *Moringa Oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Research, 21*(1), 17-25. doi: 10.1002/ptr.2023

AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemist 15th Ed., Arlington Virginia, U.S.A.

AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry, 99(1), 191-203.

Benzie, I. F. F. & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. doi: 10.1006/abio.1996.0292.

Brand-Williams, W., Cuvelier, M. E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Brahmachari, G. (Ed.). (2012). Bioactive Natural Products: Opportunities and Challenges in Medicinal Chemistry. Elsevier.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of Food and Drug Analysis, 22(3), 296-302.

Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. Journal of the Science of Food and Agriculture, 80(12), 1744-1756.

Dorman, H. J., Peltoketo, A., Hiltunen, R., & Tikkanen, M. J. (2003). Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chemistry, 83(2), 255-262

Fahey, Jed W. (2005). *Moringa Oleifera*: A Review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for Life Journal, 1(*5). https://www.tfljournal.org/article.php/20051201124931586

Fugile, L. J. (1999). *The miracle tree:* Moringa oleifera*: Natural nutrition for the tropics.* Church World Service.

Ghebremariam, Y. T., Boadi, W., & Adunyah, S. E. (2014). Moringa oleifera plant: Its potentials to improve health and manage diseases. Future Science OA, 1(2), FSO44.

Goveta A., Ottavian J. I., Keen C. L. & Fraga C. G. (2003). Inhibition of ACE activity by flavan-3-ols and procyanidins. *FEBS Letters, 555*(3):597-600. doi: 10.1016/s0014-5793(03)01355-3.

Jang, J. H., Jeong, S. C., Kim, J. H., Lee, Y. H., Ju, Y. C., & Lee J. S. (2011). Characterization of a new antihypertensive angiotensin I-converting enzyme inhibitory peptide from *Pleurotus cornucopiae*. *Food Chemistry, 127*(2), 412-418. https://doi.org/10.1016/j.foodchem.2011.01.010

Kashyap, P.; Kumar, S.; Riar, C.S.; Jindal, N.; Baniwal, P.; Guiné, R.P.F.; Correia, P.M.R.; Mehra, R.; Kumar, H. Recent Advances in Drumstick (Moringa oleifera) Leaves Bioactive Compounds: Composition, Health Benefits, Bioaccessibility, and Dietary Applications. Antioxidants 2022, 11, 402. https://doi.org/ 10.3390/antiox11020402

Kasolo, J. N., Bimenya, G. S., Ojok, L., Ochieng, J., & Ogwal-Okeng, J. W. (2010). Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities. Journal of Medicinal Plants Research, 4(9), 753-757

Kwon, Y. I., Jang, H. D., & Shetty, K. (2006). Evaluation of *Rhodiola crenulate* and *Rhodiola rosea* for managementof type II diabetes and hypertension. *Asia Pacific Journal of Clinical Nutrition, 15*(3):425-32.

Lockett, C. T. & Calvert, C.C. (2000). Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria. *International Journal of Food Sciences and Nutrition, 51(*3), 195-208. doi: 10.1080/09637480050029700.

Makkar, H. P. S., & Becker, K. (1996). Nutritional value and antinutritional components of whole and ethanol extracted Moringa oleifera leaves. Animal Feed Science and Technology, 63(1-4), 211-228.

Mehta, J., Shukla, A., Bukhariya, V., & Charde, R. (2011). The magic remedy of *Moringa oleifera*: An overview. *International Journal of Biomedical and Advance Research, 2(*6), doi:10.7439/ijbar.v2i6.35

Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews, 52(4), 673-751.

Moyo, B., Masika, P. J., Hugo, A., & Muchenje, V. (2011). Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves. African Journal of Biotechnology, 10(60), 12925-12933.

Nabavi, S. F., Same, M. R., Nabavi, S. M., & Ebrahimzadeh, M. A. (2012). The antioxidant activity of various leaves extracts from temperate trees in northern Iran. Pharmaceutical Biology, 50(9), 1139-1145

Ndhlala, A. R., Moyo, M., & Van Staden, J. (2010). Natural antioxidants: fascinating or mythical biomolecules? Molecules, 15(10), 6905-6930.

Oboh, G., Agunloye, O. M., Adefegha, S. A., Akinyemi, A. J. & Ademiluyi, A. O. (2015) Caffeic and Chlorogenic acids inhibit key enzymes linked to type 2 diabetes (in vitro): A comparative study. *Journal of Basic and Clinical Physiology and Pharmacology, 26*(2), 165-70. doi:10.1515/jbcpp-2013-0141

Odukoya, O. A., Inya-Agha, S. I., Segun, F. I., & Sofidiya, M. O. (2007). Antioxidant activity of selected Nigerian green leafy vegetables. American Journal of Food Technology, 2(3), 169-175.

Ogawa, K., Kawasaki, A., Omura, M., Yoshida, T., Ikoma, Y., & Yano, M. (2001). 3' ,5'-Di-C-β-glucopyranosylphloretin, a flavonoid characteristic of the genus *Fortunella*. *Phytochemistry, 57*(5), 737–742. https://doi.org/10.1016/S0031-9422(01)00132-7

Okwari, O., Dasofunjo, K., Asuk, A., Alagwu, E., & Mokwe, C. (2013). Anti-hypercholesterolemic and hepatoprotective effect of aqueous leaf extract of *Moringa oleifera* in rats fed with thermoxidized palm oil diet. *IOSR Journal of Pharmacy and Biological Sciences 8*(2):57-62 doi:10.9790/3008-0825762

Okwori, E., Onu, R. O., & Onagwa, G. I. (2009). Benefit of garlic as a functional food in health and diseases. *West African Journal of Physical and Health Education, 13*, 220-227.

Olaoye, A.B., Ologunde, C.A., Molehin, O.R. *et al.* Comparative Antioxidant Analysis of *Moringa oleifera* Leaf Extracts from South Western States in Nigeria. *Futur J Pharm Sci* **7**, 68 (2021). https://doi.org/10.1186/s43094-021-00204-8

Omodanisi, E. I., Aboua, Y. G. & Oguntibeju, O. O. (2017). Assessment of the anti-hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of *Moringa Oleifera* in diabetes-induced nephrotoxic male wister rats. *Molecules, 22*(4), 439. doi: 10.3390/molecules22040439.

Oyedepo, T. A., Babarinde, S. O., & Ajayeoba, T. A. (2013). Evaluation of the antihyperlipidemic effect of aqueous leaves extract of *Moringa Oleifera* in alloxan induced diabetic rats. *International Journal of Biochemistry Research & Review 3(*3), 162-170. doi:10.9734/IJBCRR/2013/3639

Pandey, K. B. & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and diseases. *Oxidative Medicine and Cellular Longevity, 2*(5): 270–278. doi: 10.4161/oxim.2.5.9498

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum-grain. *Journal of Agricultural and Food Chemistry, 26(*5), 1214–1218. https://doi.org/10.1021/jf60219a031

Prior, R. L., Hoang, H., Gu, L. W., Wu, X. L., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D. J., Ou, B. X., & Jacob, R. (2003). Assay for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL) of plasma and other

biological and food samples. *Journal of Agricultural and Food Chemistry, 51*(11), 3273–3279.

Ratti, C. (2001). Hot air and freeze-drying of high-value foods: a review. Journal of Food Engineering, 49(4), 311-319.

Salawu, O. A., Chindo, B. A., Tijani, A. Y., Obidike, I. C., Salawu, T. A., & Akingbasote, A. J. (2009). Acute and sub-acute toxicological evaluation of the methanolic stem bark extract of *Crossopteryx febrifuga* in rats. A*frican Journal of Pharmacy and Pharmacology 3*(12), 621–626.

Sanchez-Machado, D. I, Nunez-Gastelum, J. A, Reyes-Moreno, C., Ramírez-Wong, B. & López-Cervantes, J. (2010). Nutritional Quality of Edible Parts of *Moringa Oleifera*. *Food Analytical Methods, 3*, 175-180.

Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. American Journal of Clinical Nutrition, 81(1), 215S-217S.

Seeram, N. P., Henning, S. M., Lee, R., Niu, Y., Scheuller, H. S., & Heber, D. (2006). Catechin and caffeine contents of green tea dietary supplements and correlation with antioxidant activity. *Journal of Agricultural and Food Chemistry, 54*(5), 1599–1603.

Sengev A. I, Abu1, J. O., & Gernah, D. I. (2013). Effect of *Moringa oleifera* leaf powder supplementation on some quality characteristics of wheat bread. *Journal of Food and Nutrition Sciences. 443036*(4), 270-275. DOI:10.4236/fns.2013.43036

Shin, Y., Ryu, J. A., Liu, R. H., Nock, J. F., Polar-Cabrera, K., & Watkins, C. B. (2008). Fruit quality, antioxidant contents and activity, and antiproliferative activity of strawberry fruit stored in elevated CO2 atm. *Journal of Food Science, 73*(6), S339–S344. doi: 10.1111/j.1750-3841.2008.00857.x.

Shodehinde, S. A., Oyeleye, S. I., Olasehinde, T. A., Adebayo, A. A., Oboh, G. & Boligon, A. A. (2017). *Lasianthera Africana* leaves inhibits α-amylase α-glucosidase, angiotensin-1 converting enzyme activities and Fe2+-induced oxidative damage in pancreas and kidney homogenates. *Oriental Pharmacy and Experimental Medicine, 17*, 41-49.

Singleton, V. L. & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymology, 299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sreelatha, S., & Padma, P. R. (2009). Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant Foods for Human Nutrition, 64(4), 303-311.

Takasaki, Y. (2005). Serum lipid levels and factors affecting atherogenic index in Japanese children. *Journal of Physiological Anthropology and Applied Human Science, 24*(4), 511-515. doi: 10.2114/jpa.24.511

Vongsak, B., Sithisarn, P., & Gritsanapan, W. (2013). Simultaneous HPLC quantitative analysis of active compounds in leaves of Moringa oleifera Lam. Journal of Chromatographic Science, 51(6), 593-598.

Waterman, C., Cheng, D. M., Rojas-Silva, P., Poulev, A., Dreifus, J., Lila, M. A., & Raskin, I. (2014). Stable, bioavailable isolated phenolic compounds from Moringa oleifera with increased antioxidant and anti-inflammatory activity. PLoS One, 9(2), e89102.

Waridel, P., Wolfender, J. L., Ndjoko, K., Hobby, K. R., Major, H. J., & Hostettmann, K. (2001). Evaluation of quadrupole time-of-flight tandem mass spectrometry and ion-trap multiple-stage mass spectrometry for the differentiation of C-glycosidic flavonoid isomers. *Journal of Chromatography A, 926*(1), 29–41.

Zhu, F., Cai, Y. Z., Sun, M., Ke, J., Lu, D., & Corke, H. (2009). Comparison of major phenolic constituents and in vitro antioxidant activity of diverse *Kudingcha* genotypes from *Ilex kudingcha*, *Ilex cornuta*, and *Ligustrum robustum*. *Journal of Agricultural and Food Chemistry, 57(*14), 6082–6089. doi: 10.1021/jf901020h