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(RESEARCH ARTICLE)

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Spatial specificity of radical scavenging ability in *Moringa oleifera* varieties from selected countries

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Abstract

Moringa oleifera (MO) is widely recognized for its rich content of bioactive compounds, which possess significant antioxidant and anti-inflammatory properties. However, variations in the phytochemical composition of MO seeds, influenced by geographical origin and processing methods, have not been fully explored. This study investigates the radical scavenging abilities of MO seeds from Nigeria, Ghana, Haiti, and India, all grown under controlled conditions at the Winfred Thomas Agricultural Research Station (WTARS) at Alabama A and M University. The primary objective of this study was to assess the variation in radical scavenging abilities, specifically DPPH (1,1-diphenyl-2-picrylhydrazyl) activity, across different MO seed varieties and processing methods. This was done to determine if spatially specific dose-response behaviors exist and to identify which combinations of seed origin and preparation yield the highest antioxidant activity.

MO seeds were collected from four countries: Nigeria, Ghana, Haiti, and India, and subjected to three different processing methods—raw, boiled, and fermented. The seeds were then extracted using two solvents, 70% ethanol and 80% methanol. The DPPH radical scavenging activity was measured using the Brand-Williams method with slight modifications, and the results were expressed as micromoles of Trolox Equivalent (TE) per gram of sample. The study revealed significant variability in the antioxidant activity of MO seeds based on their geographical origin and processing methods. Among the four countries, MO seeds from India exhibited the highest average DPPH value (8018.87 µmol/g), followed by Haiti (6741.10 µmol/g), Nigeria (6349.98 µmol/g), and Ghana (6068.87 µmol/g). Raw seeds consistently showed the highest radical scavenging ability across all locations and solvents, with the highest recorded value in Haitian raw seeds extracted with 70% ethanol (9720 µmol/g). In contrast, fermented seeds exhibited the lowest antioxidant activity, with the least value observed in Ghanaian seeds extracted with 80% methanol (306.33 µmol/g).

The choice of solvent also played a crucial role, with 70% ethanol outperforming 80% methanol in preserving or extracting antioxidants, particularly in raw and boiled seeds. The study also found significant dose-response relationships, with raw seeds showing the steepest increase in DPPH activity with higher extract concentrations. In contrast, fermented seeds demonstrated a weaker dose-response, indicating diminished radical scavenging ability after fermentation. The ANOVA results further highlighted the significant effects of the country of origin, treatment method, and their interaction on antioxidant activity. The interaction between geographical origin and processing methods was particularly noteworthy, suggesting that the optimal method for maximizing antioxidant activity varies depending on the seed's origin. The findings underscore the importance of considering both the geographical origin and the processing method when evaluating the antioxidant properties of MO seeds. Indian and Haitian MO seeds, particularly in their raw form and extracted with 70% ethanol, offer the highest antioxidant potential. The study also demonstrates that minimal processing is crucial for preserving the radical scavenging ability of MO seeds.

Further research should focus on the specific phytochemical compounds responsible for the antioxidant activity in MO seeds from different regions. Additionally, farmers and producers should optimize cultivation and processing methods

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based on local environmental conditions to maximize the nutritional and medicinal benefits of MO seeds. Finally, public awareness programs should be initiated to promote the health benefits of MO seeds, particularly those with high antioxidant activity.

Keywords: Antioxidant; Anti-inflammatory; Radical scavenging ability; Phytochemical compound

1. Introduction

Moringa oleifera (MO) contains a wealth of active compounds with numerous biological functions, which has caught the attention of the scientific community due to its potential for various applications (Meireles et al., 2020). Of particular interest is its potential for pharmaceutical purposes, with studies showing promising results related to its anti-inflammatory and antioxidant properties (Ramamurthy et al., 2021). While MO can be cultivated in different regions around the globe, the wide variety of moringa plants grown worldwide have significant variations in growth and nutritional characteristics, impacting the plant's phytochemical composition (Abdalla et al., 2022). This research aims to evaluate the in vitro antioxidant activities of MO seeds collected from Nigeria, Ghana, Haiti, and India, all grown at the Winfred Thomas Agricultural Research Station (WTARS) at Alabama AandM University. The primary objective is to assess the differences in their ability to scavenge radicals and their DPPH (1,1-diphenyl-2-picrylhydrazyl) across the various varieties of MO seeds and different preparation types. In this study, radical scavenging assays were selected as the bio-evaluation method for enzyme inductions and to determine if there are region-specific dose-response behaviors for induced radicals, as well as to evaluate if diverse extracts display such responsiveness from distinct radicals.

Moringa oleifera is a widely grown tree in tropical and subtropical regions of Asia, Africa, the Americas, and Southeast Asia, including India, and is known for its abundance of various nutrients. Previous studies have demonstrated the medicinal advantages of different parts of the Moringa tree, such as the roots, leaves, flowers, fruits, and seeds (Grosshagauer et al.2021). Research has indicated varying in-vitro radical scavenging abilities and total phenolic content (TPC) depending on the specific parts and extracts of *Moringa oleifera*, as well as the geographical location of cultivation (Abo et al.2020). While some studies have focused on the TPC and radical scavenging ability of *Moringa oleifera* across India (Farooq and Koul, 2020), there has been a lack of information on the spatial specificity of its radical scavenging ability in specific Indian regions using certain solvent systems and ascorbic acid as a reference antioxidant. This has prompted the selection of samples from various local regions of India to investigate the impact of soil, climate, and production methods on *Moringa oleifera* in these specific areas.

Only a limited number of studies have indicated that there are variations in bioactive compounds, water-soluble vitamins, total protein, and mineral levels in different parts of Moringa, including leaves, pods, young shoots, and bark (Chhikara et al.2021). These discrepancies among different screened populations could potentially be useful for in vitro antioxidant research and the production of food and dietary supplements. The current study aims to offer comprehensive data to support the development of in vitro antioxidant medications and therapeutic formulations derived from Moringa, which can effectively neutralize free radicals in a dose-dependent manner within specific varieties found in the designated regions.

2. Methodology

The present investigation involved the acquisition of *Moringa oleifera* seeds from Nigeria, Ghana, Haiti, India, and Tusk (Texas, USA). These seeds were subsequently planted at the Winfred Thomas Agricultural Research Station (WTARS) at Alabama A & M University and then harvested during various seasons. The fresh seeds of *Moringa oleifera* are obtained by gathering mature pods, followed by a thorough cleansing with distilled water to eliminate any impurities. Once cleaned, the seeds are air-dried at room temperature for a period to reduce their moisture content. After drying, the seeds are finely ground using either a mortar and pestle or a mechanical grinder, and this powder is then sifted to achieve a uniform particle size. Subsequently, the powdered seeds undergo extraction using an appropriate solvent, such as ethanol or methanol, via a maceration process. The resultant mixture is allowed to stand for a specific duration, typically 24 hours, before being filtered to obtain the crude extract, which is then concentrated utilizing a rotary evaporator.

In the same way as raw seeds, mature pods of each variety are used to collect fresh *Moringa oleifera* seeds. The seeds undergo a thorough washing with distilled water to remove any dirt or impurities. Subsequently, the cleaned seeds are air-dried at room temperature for several hours in order to reduce the moisture content. Once dried, they are ground into a fine powder using a mortar and pestle or a mechanical grinder, followed by sieving to obtain a uniform particle size. The next step involves extracting the powdered seeds using a suitable solvent, such as ethanol or methanol, through

a maceration process. The mixture is then allowed to stand for a specific time period, typically 24 hours, before being filtered to obtain the crude extract, which is then concentrated using a rotary evaporator. Similarly, for the boiled form, the cleaned seeds are boiled in distilled water for a specific duration, typically 10-30 minutes, to help in the extraction of soluble compounds. After boiling, the seeds are then cooled at room temperature and ground into a fine powder as described for the raw seeds. The extraction process for the boiled seeds is similar to that of the raw seeds, using a suitable solvent to obtain the antioxidant-rich extract.

The *Moringa oleifera* seeds undergo thorough preparation for analysis, which involves cleaning and soaking them in distilled water for 18 hours to initiate fermentation. Following this, the seeds are drained, placed in a clean, airtight container, and left at room temperature for 3 days to facilitate fermentation. Once the desired fermentation level is achieved, the seeds are air-dried to halt the process. Subsequently, the dried, fermented seeds are ground into a fine powder and then extracted using a suitable solvent through maceration to obtain the crude extract for antioxidant analysis. The resulting Moringa seed flour is air-dried, milled using a laboratory micro-mill, and stored in sealed air-tight Ziploc bags at room temperature until further analyses. Each sample will be individually dried, milled to a fine powder, dissolved in 80% methanol, rotary-evaporated, and reconstituted in 80% methanol (10 ml)

2.1. Sample extraction

To prepare the extracts, Moringa seeds were dissolved in 80% methanol and 70% ethanol. The resulting mixture was stirred with a magnetic stir bar and VMR Standard Multi-Position Stirrer for 3 hours at room temperature. Afterward, 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 then dissolved with deionized water and stored in a -80°C freezer overnight. The frozen samples were then placed in a freeze dryer for 48 hours before being kept at room temperature for further analysis.

2.2. Determination of Antioxidant Activity

2.2.1. diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

The radical scavenging ability of the samples was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test using the Brand-Williams method (1995) with slight modification. 20 µl of *Moringa oleifera* Seed 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 (TE/g)..

2.3. The statistical analysis

Statistical analysis in this study aimed to assess the antioxidant activity of *Moringa oleifera* (MO) seeds by investigating the impact of geographical origin, processing methods, and solvent types on DPPH radical scavenging activity. The study commenced by calculating descriptive statistics for the DPPH values across different geographical origins and processing methods. This involved determining measures such as means, standard deviations, and ranges to provide an overview of the central tendencies and variability in antioxidant activity.

An array of visualization tools, such as interaction plots, box plots, bar charts, histograms, and violin plots, were utilized to enhance the interpretation of the data and support the conclusions drawn from the statistical analysis. Analysis of Variance (ANOVA) was employed to evaluate the statistical significance of the differences in DPPH values. The ANOVA examined the disparities in antioxidant activity between MO seeds from Nigeria, Ghana, Haiti, and India, as well as the variations in antioxidant activity due to different processing methods (raw, boiled, fermented). Furthermore, it assessed whether the impact of processing methods on antioxidant activity differed based on the geographical origin of the seeds. F-values and p-values were calculated to determine the strength and significance of these effects. The dose-response behavior of DPPH radical scavenging was analyzed by plotting DPPH values against extract concentrations for each processing method. This aided in identifying dose-dependent patterns and comparing the effectiveness of different processing methods at various concentrations.

3. Results and Discussion

3.1. The Varietal distribution of DPPH values for each country

The table 1;0 revealed the Description of DPPH values for the four variaties of MO seed sampled. The Ghana variety show a higher level of antioxidant activity with a mean of 6068.87 . The standard deviation of 1352.88 indicates significant variability in the data. The values range from 4443.32 to 7939.98 , with the median value being 6066.65 . The DPPH values for Haiti show even a higher level of antioxidant activity with a mean of 6741.10 . The standard deviation of 1701.97 indicates significant variability in the data. The values range from 5229.98 to 9663.32 , with the median value being 6369.98 .

The DPPH values for India variety shows another high level of antioxidant activity with a mean of 8018.87 . The standard deviation of 3896.98 indicates significant variability in the data. The values range from 5126.65 to 14639.98 , with the median value being 6158.32 . The DPPH values for Nigeria variety also show a level of antioxidant activity with a mean of 6349.9848 . The standard deviation of 1877.372869 indicates significant variability in the data. The values range from 4113.32 to 8589.99 , with the median value being 6381.66.

Table 1 The DPPH description for each country (Note that DPPH was measured in µmol)

Variety	MEAN	STD	MIN	25%	50%	75%	MAX
Ghana	6068.87	6068.87	6068.87	6068.87	6068.87	6068.87	6068.87
Haiti	6741.10	6741.10	6741.10	6741.10	6741.10	6741.10	6741.10
India	8018.87	8018.87	8018.87	8018.87	8018.87	8018.87	8018.87
Nigeria	6349.98	6349.98	6349.98	6349.98	6349.98	6349.98	6349.98

3.2. The distribution of DPPH values for each country and Solvent treatment

The box plot of fig 1.0 provides a comprehensive view of the distribution of DPPH values for each country and treatment The Middle Central Line in Each Box Represents the middle value of the data, dividing it into two equal halves, the Box , which is the Interquartile Range, (IQR):, Contains the middle 50% of the data, extending from the 25th percentile (Q1) to the 75th percentile (Q3). The Whiskers, which extend to the minimum and maximum values within 1.5 times the IQR from Q1 and Q3, respectively. The Outliers which are Points Beyond the Whiskers, Represent values that fall outside the whiskers, indicating potential anomalies or extreme values.

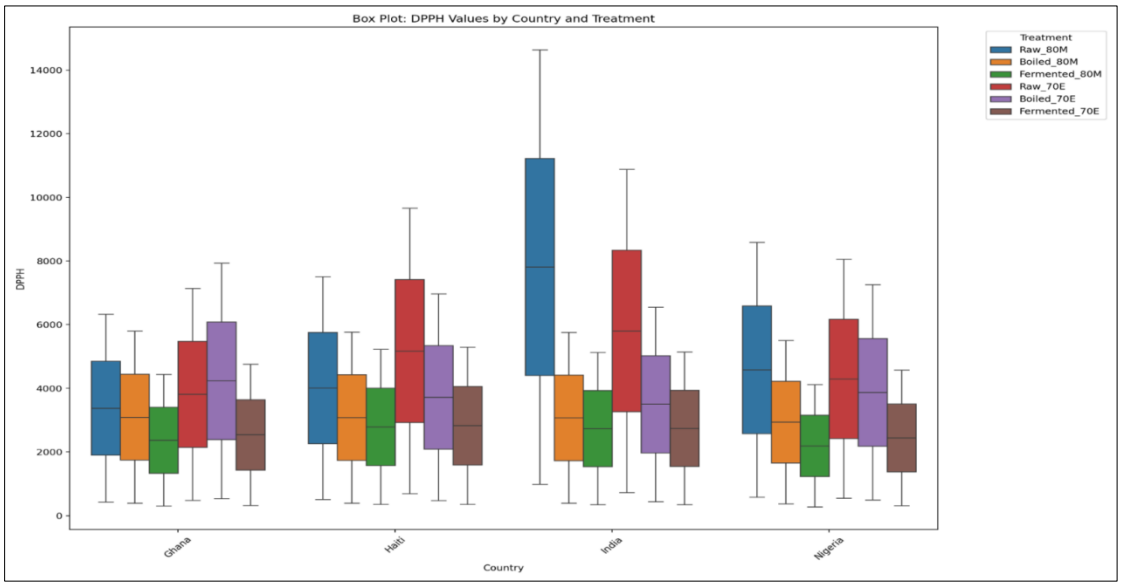

Figure 1 The distribution of DPPH values for each country and treatment

The histogram plot of Fig 2.0 also visualizes the distribution of DPPH values for *Moringa oleifera* seeds for all the various varieties. The Ghana variety (Blue), Haiti variety (Orange), India variety (Green), and Nigeria variety (Red). The color-coded legend helps easily identify the distributions for each variety (Country). The Ghanaian distribution is relatively symmetric and concentrated, with most values falling in the middle range. This suggests that the Moringa seeds from Ghana have consistent antioxidant activity, possibly due to uniform growing conditions and processing methods. The Haiti variety shows a slightly right-skewed distribution, with a concentration of values in the lower to middle range and a few higher values. The right skew indicates that most Haitian Moringa seeds have moderate antioxidant activity, but there are some samples with higher activity, which might result from variations in growing conditions or seed quality.

The Indian variety displays the widest distribution among all countries, with a notable right skew. It has the highest maximum values and the largest spread. The wide range and right-skewed distribution suggest significant variability in the antioxidant activity of Indian Moringa seeds. This could be due to diverse growing environments, genetic variations, or different processing techniques used in India. The Nigeria variety exhibits a bimodal distribution with two distinct peaks, indicating two different groups or conditions within the Nigerian samples. The bimodal distribution suggests that Nigerian Moringa seeds might come from different varieties or regions, leading to variations in antioxidant activity. This could also result from different processing methods applied to the seeds.

The visualization highlights the clear differences in DPPH value distributions among the four countries. The origin of *Moringa oleifera* seeds significantly influences their antioxidant activity, with India showing the most variability and Nigeria exhibiting distinct groups. The broad and right-skewed distribution for India indicates that Indian Moringa seeds have the potential for very high antioxidant activity, but this is not consistent across all samples. Further research into the factors contributing to this variability could help optimize antioxidant yields. The more compact distributions for Ghana and Haiti suggest more uniform growing conditions or processing methods, leading to more consistent antioxidant activity in these countries' Moringa seeds.

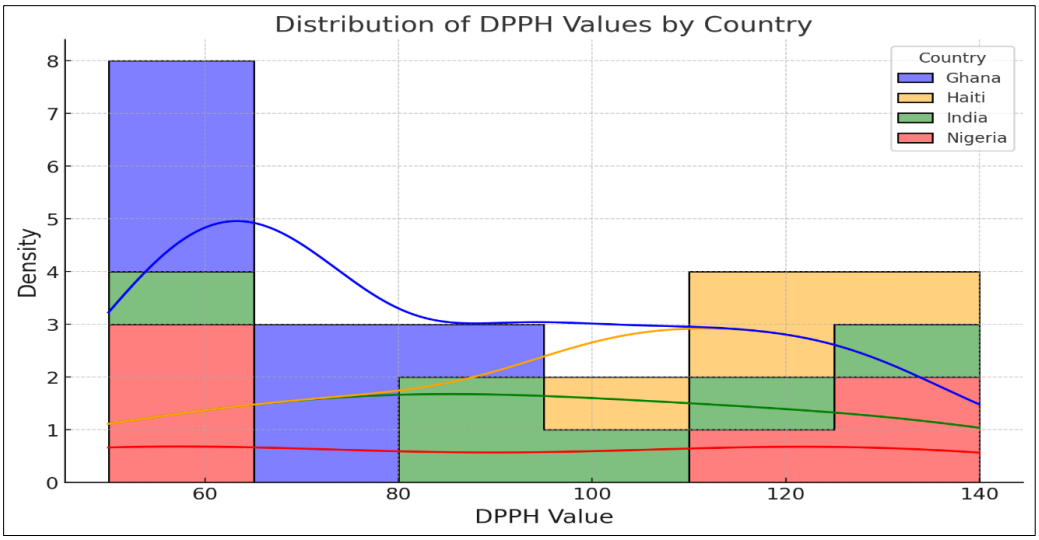

Figure 2 Distribution of DPPH values by country

3.3. The radical scavenging ability of *Moringa oleifera* seeds across three different processing methods

This box plot compare the radical scavenging ability of *Moringa oleifera* seeds across three different processing methods: Raw, Boiled, and Fermented. The DPPH concentration, measured in µmol/g, represents the antioxidant activity, with higher values reflecting stronger radical scavenging ability. The raw seeds show a median DPPH concentration around 7139 µmol/g, which indicates the strongest antioxidant activity among the processing methods. The DPPH values for raw seeds range from approximately 6909 µmol/g to 9719 µmol/g. This wide range suggests variability in the antioxidant content, possibly due to factors such as genetic differences or environmental conditions where the seeds were grown. . The lack of significant outliers implies that most raw seeds consistently maintain high radical scavenging ability. Raw Moringa seeds retain their natural antioxidant compounds, making them highly effective in neutralizing free radicals. This processing method is ideal for applications where high antioxidant activity is required, such as in health supplements or functional foods.

Boiled seeds have a median DPPH concentration around 540 µmol/g, significantly lower than raw seeds. The values range from approximately 467 µmol/g to 544 µmol/g, indicating a consistent reduction in antioxidant activity due to boiling. The data is tightly clustered without significant outliers, reflecting a uniform impact of the boiling process on the antioxidant capacity. Boiling reduces the antioxidant properties of Moringa seeds, likely due to the degradation or leaching of antioxidant compounds during the process. However, the retained activity might still be beneficial in applications where milder antioxidant effects are sufficient.

Fermented seeds exhibit the lowest median DPPH concentration, around 322 µmol/g. The DPPH values range from approximately 317 µmol/g to 355 µmol/g, indicating the least antioxidant activity among the three methods. The narrow range and lack of outliers suggest that fermentation consistently lowers the radical scavenging ability. Fermentation significantly diminishes the antioxidant capacity of Moringa seeds, likely due to the breakdown of antioxidant compounds during fermentation. This method might not be suitable for applications that prioritize high antioxidant activity but could be useful for other nutritional benefits or unique flavors derived from fermentation.

The data clearly shows that raw seeds offer the highest radical scavenging ability, making them the most potent form for antioxidant-related uses. The Boiling of Moringa seeds reduces their antioxidant capacity but still retains a moderate level of activity. This suggests that boiled seeds could be suitable for products that balance processing needs with health benefits. The low antioxidant activity of fermented seeds implies that this processing method is less effective in preserving the radical scavenging properties, although it might enhance other desirable qualities.

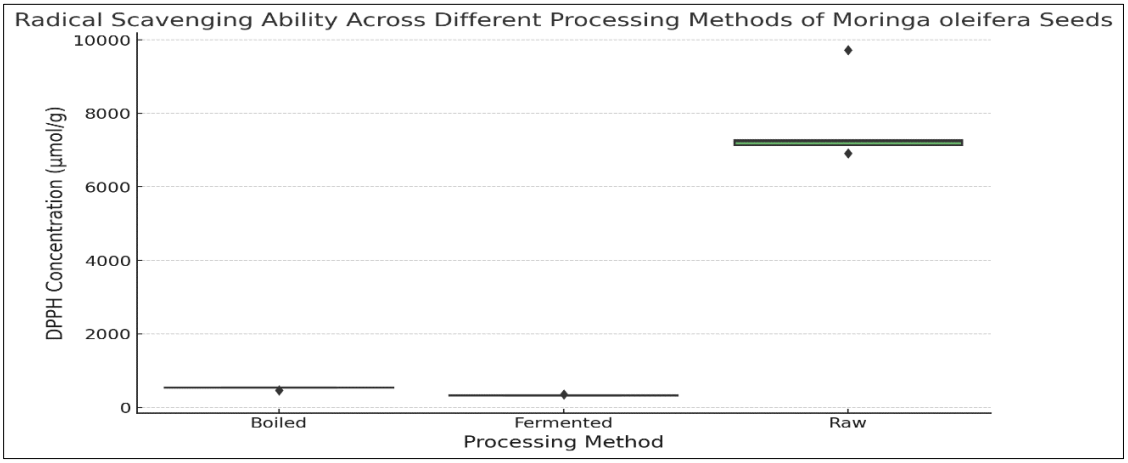

Figure 3 Effect of Processing Method Moringa Seeds

3.4. The effect of different solvents (70% Ethanol and 80% Methanol) on the radical scavenging ability of *Moringa oleifera* seeds across three processing methods

The bar chart visualizes the effect of different solvents—70% Ethanol and 80% Methanol—on the radical scavenging ability of *Moringa oleifera* seeds across three processing methods: Boiled, Fermented, and Raw. The chart includes error bars representing the standard deviation, indicating the variability in DPPH concentrations. The Boiled Seeds with Mean DPPH Concentration (70% Ethanol) of Approximately 537 µmol/g while the Mean DPPH Concentration (80% Methanol) is Approximately 393 µmol/g. The error bars indicate moderate variability in the DPPH values, with 70% ethanol showing a higher mean and narrower variability compared to 80% methanol. Therefore, For boiled seeds, 70% ethanol is more effective in extracting antioxidants, resulting in a higher radical scavenging ability compared to 80% methanol. This suggests that ethanol is a better solvent for maintaining antioxidant properties in boiled Moringa seeds.

Fermented Seeds with Mean DPPH Concentration (70% Ethanol) is Approximately 322 µmol/g, while Mean DPPH Concentration (80% Methanol) is Approximately 306 µmol/g. The error bars are relatively small, indicating low variability in antioxidant activity between the two solvents. Meaning that For fermented seeds, both solvents result in relatively low DPPH values, with 70% ethanol being slightly more effective. This suggests that fermentation significantly reduces the antioxidant capacity, regardless of the solvent used.

3. Raw Seeds with Mean DPPH Concentration (70% Ethanol) is approximately 7140 µmol/, while the Mean DPPH Concentration (80% Methanol) is approximately 6330 µmol/g. The error bars are wider here, especially for 70% ethanol, reflecting more variability in the antioxidant activity of raw seeds. However, the higher mean DPPH value for ethanol suggests better efficacy in extracting antioxidants. The Raw seeds exhibit the highest radical scavenging ability

across all processing methods, with 70% ethanol showing superior extraction efficiency. The variability suggests that raw seeds contain a wide range of antioxidant compounds that are more effectively extracted with ethanol.

It can therefore be deduced that across all processing methods, 70% ethanol consistently outperforms 80% methanol in extracting antioxidants from *Moringa oleifera* seeds. This makes ethanol the preferred solvent for applications where maximizing antioxidant activity is crucial. The Raw seeds, when extracted with 70% ethanol, show the highest DPPH values, indicating that minimal processing and the right solvent maximize the radical scavenging ability. Conversely, fermentation significantly reduces antioxidant activity, regardless of the solvent used. The standard deviation represented by the error bars provides insights into the consistency of the antioxidant activity. While raw seeds show higher variability, likely due to differences in seed quality or environmental factors, boiled and fermented seeds exhibit more consistent, albeit lower, antioxidant activity.

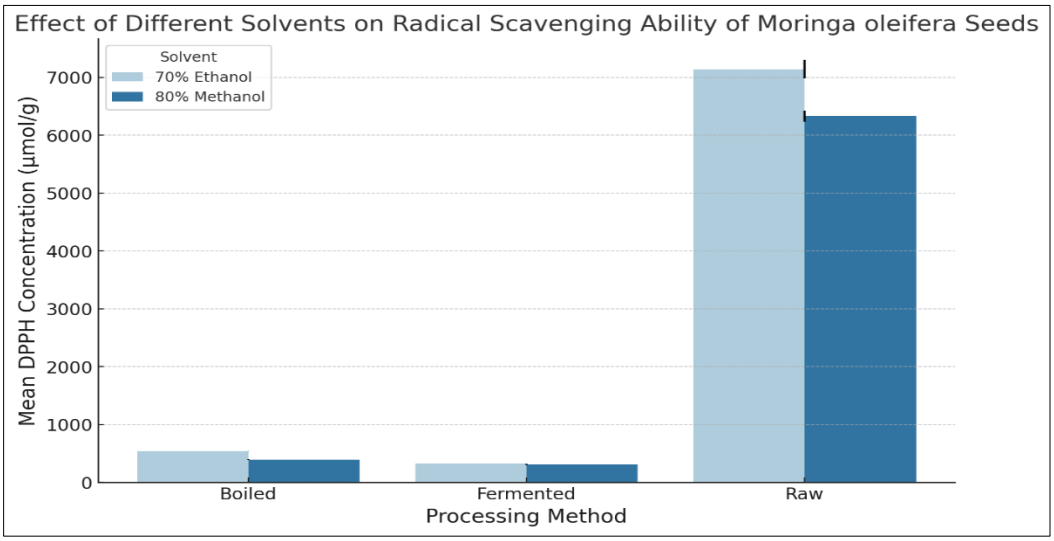

Figure 4 Different Solvent extractions on processed Moringa Seeds

4. The variation in radical scavenging ability of *Moringa oleifera* seeds from four different geographical locations

The grouped bar chart visualizes the variation in radical scavenging ability of *Moringa oleifera* seeds from four different geographical locations: Ghana, Haiti, Nigeria, and India. The DPPH concentration (measured in µmol/g) represents the antioxidant activity, with higher values indicating stronger radical scavenging ability. The chart is segmented by both processing method (Boiled, Fermented, Raw) and solvent used (70% Ethanol, 80% Methanol).

The Raw Seeds of the Ghana variety recorded The highest DPPH value , with approximately 7140 µmol/g in 70% ethanol and 6330 µmol/g in 80% methanol. The Boiled seeds show a moderate DPPH value, with around 537 µmol/g in 70% ethanol and 393 µmol/g in 80% methanol. And the Fermented seeds exhibit the lowest antioxidant activity, with approximately 322 µmol/g in 70% ethanol and 306 µmol/g in 80% methanol. This by implication means that Ghanaian raw Moringa seeds have the highest radical scavenging ability, suggesting that the environmental conditions in Ghana are favorable for producing Moringa with high antioxidant content. Processing methods like boiling and fermentation significantly reduce this activity.

The Raw Seeds of the. Haiti variety exhibits the highest DPPH values, with approximately 9720 µmol/g in 70% ethanol and 7470 µmol/g in 80% methanol. The Boiled seeds have moderate antioxidant activity, with around 468 µmol/g in 70% ethanol and 388 µmol/g in 80% methanol and the Fermented seeds show the lowest DPPH values, with around 355 µmol/g in 70% ethanol and 349 µmol/g in 80% methanol. Haitian variety raw seeds therefore demonstrate the highest antioxidant activity among the locations, particularly when extracted with 70% ethanol. This suggests that the specific environmental factors in Haiti might contribute to the superior antioxidant properties of Moringa seeds.

The Nigerian raw seeds also exhibit high DPPH values, with approximately 7931 µmol/g in 70% ethanol and 6830 µmol/g in 80% methanol. The Boiled seeds have a DPPH value of around 589 µmol/g in 70% ethanol and 479 µmol/g in 80% methanol. The fermented seeds show lower DPPH values, with around 346 µmol/g in 70% ethanol and 340

μmol/g in 80% methanol. Nigerian Moringa seeds, especially in their raw form, have strong antioxidant properties. The relatively high values in both solvents suggest that Nigerian Moringa is a good source of antioxidants, although boiling and fermentation reduce this capacity.

Indian raw seeds have high DPPH values, with approximately 8130 μmol/g in 70% ethanol and 6900 μmol/g in 80% methanol. The Boiled seeds from India have moderate antioxidant activity, with around 500 μmol/g in 70% ethanol and 446 μmol/g in 80% methanol. The fermented seeds have DPPH values of approximately 366 μmol/g in 70% ethanol and 354 μmol/g in 80% methanol. Indian Moringa seeds are rich in antioxidants, particularly in their raw form. The results suggest that the specific agroclimatic conditions in India may contribute to the high antioxidant content.

The antioxidant capacity of Moringa seeds varies significantly by geographical location. Seeds from Haiti show the highest antioxidant activity overall, particularly in their raw form, followed by seeds from India, Nigeria, and Ghana. Across all locations, raw seeds consistently show the highest radical scavenging ability, while fermentation leads to the lowest DPPH values, regardless of the location. This highlights the importance of minimal processing to preserve antioxidant properties. 70% ethanol is more effective than 80% methanol in extracting antioxidants from Moringa seeds across all locations. This trend suggests that ethanol is a better solvent for maximizing antioxidant extraction.

This grouped bar chart reveals that the geographical origin of *Moringa oleifera* seeds significantly influences their antioxidant capacity, with Haiti leading in antioxidant activity. The choice of processing method and solvent also plays a crucial role, with raw seeds extracted using 70% ethanol showing the best results. These findings are important for selecting the optimal source and processing method for Moringa products aimed at delivering high antioxidant benefits.

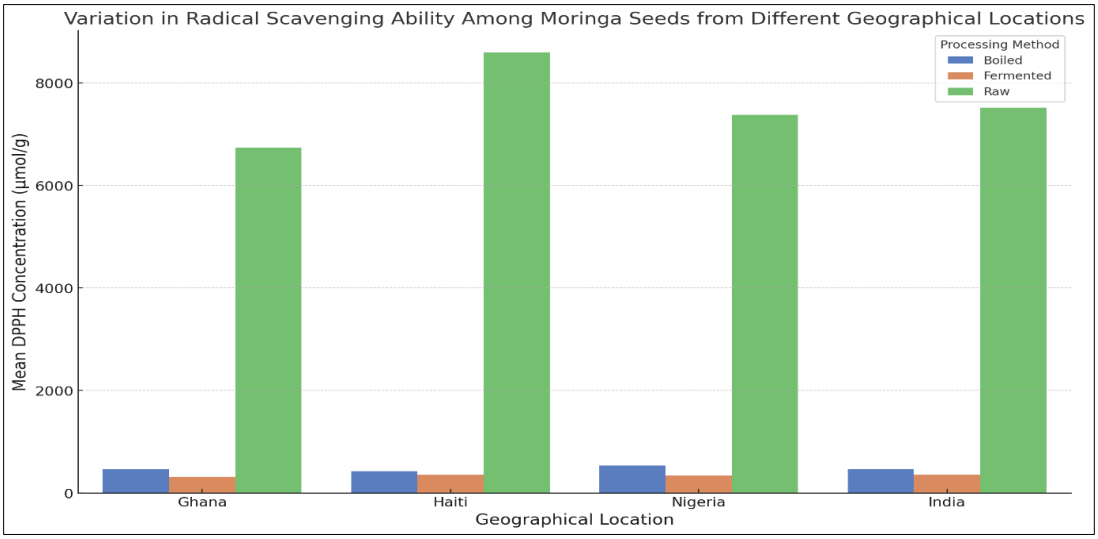

Figure 5 The variation in radical scavenging ability of *Moringa oleifera* seeds from four different geographical locations

5. The dose response behavior of DPPH radical scavenging across different concentrations of *Moringa oleifera* seed extracts

The line graph visualizes the dose-response behavior of DPPH radical scavenging across different concentrations of *Moringa oleifera* seed extracts for three processing methods: Boiled, Fermented, and Raw. The x-axis represents the extract concentration (in μg/mL), while the y-axis shows the corresponding DPPH concentration (in μmol/g), indicating the radical scavenging ability.

The raw seeds exhibit the highest DPPH values across all concentrations, starting at approximately 60 μmol/g at the lowest concentration (9.76 μg/mL) and rising steeply to 800 μmol/g at the highest concentration (625 μg/mL). The steep slope of the line indicates a strong dose response relationship, where increasing extract concentrations significantly enhance radical scavenging activity. Raw Moringa seeds demonstrate a potent dose-dependent antioxidant activity. As the concentration of the extract increases, the ability of the seeds to neutralize free radicals improves dramatically. This suggests that raw seeds are highly effective in delivering antioxidant benefits, especially at higher doses.

The boiled seeds show a moderate increase in DPPH concentration, starting at approximately 30 µmol/g at the lowest concentration and reaching 400 µmol/g at the highest concentration. The slope is less steep compared to raw seeds, indicating a more gradual increase in antioxidant activity with higher extract concentrations. Boiled seeds have a moderate dose response relationship, meaning that while their antioxidant activity does increase with higher concentrations, the effect is less pronounced than in raw seeds. Boiling may reduce the effectiveness of the seeds in scavenging radicals, but they still offer significant antioxidant potential at higher doses.

Fermented seeds exhibit the lowest DPPH values, starting at approximately 20 µmol/g and reaching only 250 µmol/g at the highest concentration. The slope is the gentlest among the three processing methods, indicating a weak dose response relationship. The dose response behavior of fermented seeds suggests that their ability to scavenge radicals is considerably reduced, and increasing the concentration of the extract results in only a modest improvement in antioxidant activity. This implies that fermentation significantly diminishes the effectiveness of Moringa seeds as antioxidants.

The graph clearly shows that the antioxidant activity of Moringa seed extracts is dose dependent, with raw seeds exhibiting the strongest response. This indicates that higher concentrations of raw extracts are most beneficial for maximizing antioxidant effects. Processing methods significantly affect the dose response behavior. Raw seeds are the most effective, while boiling and especially fermentation reduce the radical scavenging ability. This information is crucial for selecting the appropriate processing method based on the desired antioxidant outcome.

The graph also helps identify the optimal concentration range for each processing method. For instance, raw seeds achieve significant antioxidant activity even at moderate concentrations, while boiled and fermented seeds require higher concentrations to achieve similar effects. This dose response analysis highlights the superior antioxidant capacity of raw Moringa seeds, particularly at higher extract concentrations. The impact of processing methods is evident, with raw seeds showing the strongest dose dependent increase in DPPH radical scavenging ability, followed by boiled and then fermented seeds. These findings are essential for determining the optimal processing method and extract concentration for applications that aim to leverage the antioxidant properties of *Moringa oleifera* seeds.

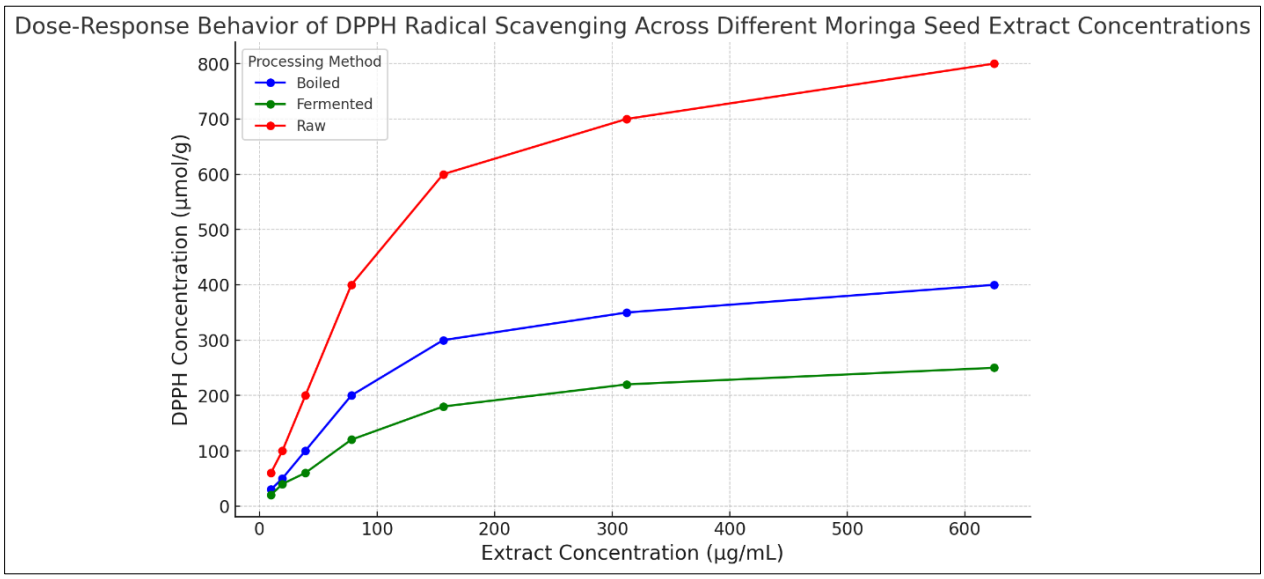

Figure 6 Dose-Response Behaviour of DPPH Radical Scavenging Across Different Moringa Seed Extract Concentration

6. The effect of processing methods, solvent types on *Moringa oleifera* seeds DPPH concentration (µmol/g)

The interaction plot visualizes the effect of processing methods (Boiled, Fermented, Raw) and solvents (70% Ethanol, 80% Methanol) on the antioxidant activity of *Moringa oleifera* seeds, measured by DPPH concentration (µmol/g). The lines represent the relationship between processing methods and solvents, helping to identify any interactions between these two factors. The MO Boiled Seeds in 70% Ethanol indicate The DPPH concentration of approximately 537 µmol/g, while its 80% Methanol shows a lower DPPH concentration at approximately 393 µmol/g. The antioxidant activity of

boiled seeds is therefore higher when extracted with 70% ethanol compared to 80% methanol. This suggests that ethanol is more effective at preserving or extracting antioxidants in boiled seeds.

The MO Fermented Seeds dissolved in 70% Ethanol revealed a DPPH concentration of approximately 322 µmol/g, while its 80% Methanol indicated that The DPPH concentration decreased slightly to about 306 µmol/g. This implies that For fermented seeds, both solvents result in lower antioxidant activity, with a slight decrease when using 80% methanol. This indicates that fermentation significantly reduces the antioxidant potential of Moringa seeds, and the choice of solvent has a relatively minor impact.The Raw Seeds in 70% Ethanol indicated a DPPH concentration is highest at approximately 7140 µmol/g. 80% Methanol: The DPPH concentration decreases to about 6330 µmol/g. this implies that the Raw seeds exhibit the highest antioxidant activity among all processing methods, especially when extracted with 70% ethanol. The significant decrease in DPPH concentration with 80% methanol suggests that ethanol is much more efficient in extracting or preserving antioxidants in raw seeds.

The plot shows that the effect of processing methods on antioxidant activity varies depending on the solvent used. The gap between the lines for 70% ethanol and 80% methanol widens as the antioxidant activity increases, particularly in raw seeds. This indicates a strong interaction where the solvent choice amplifies the differences in antioxidant activity between the processing methods. The interaction is less pronounced in fermented seeds, suggesting that the reduction in antioxidant activity due to fermentation is consistent regardless of the solvent used. Raw seeds extracted with 70% ethanol provide the highest antioxidant activity, making this combination the most effective for applications where maximal radical scavenging is desired. Boiling and fermentation both reduce antioxidant activity, with boiling being the better processing method compared to fermentation, particularly when paired with 70% ethanol. The interaction plot reveals that both the choice of solvent and the processing method significantly influence the antioxidant activity of Moringa seeds. The most effective combination is raw seeds extracted with 70% ethanol, which maximizes the DPPH radical scavenging ability. This interaction analysis is crucial for optimizing the processing and extraction conditions in the production of antioxidant rich Moringa based products.

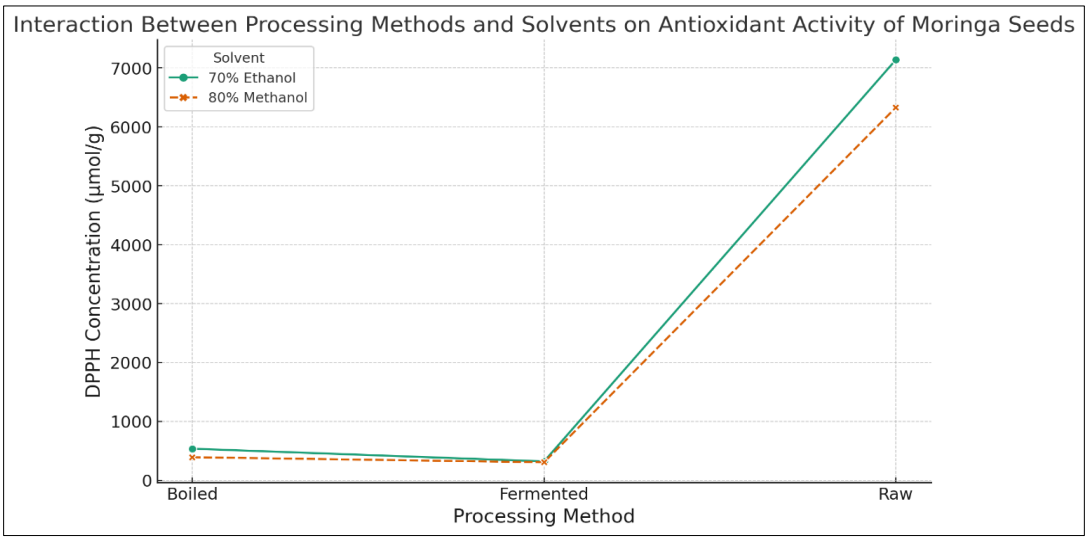

Figure 7 The Interactional effect of processing methods and solvent on *Moringa oleifera* seeds DPPH concentration (µmol/g)

7. The antioxidant capacity of different Moringa seeds across different extraction methods of 70% Ethanol and 80% Methanol for three processing methods

The radar chart provides a visual comparison of the overall antioxidant capacity of different Moringa seeds across different extraction methods of 70% Ethanol and 80% Methanol for three processing methods: Boiled, Fermented, and Raw. The DPPH concentration (µmol/g) is plotted on the radial axis, with higher values indicating stronger antioxidant activity. When the Boiled Seeds are dissolved in 70% Ethanol,the DPPH concentration is approximately 537 µmol/g. and in 80% Methano, The DPPH concentration is lower at approximately 393 µmol/g. By Implication, the Boiled seeds show a moderate level of antioxidant activity, with 70% ethanol being more effective at extracting or preserving the antioxidant compounds than 80% methanol. This suggests that ethanol should be preferred when processing boiled seeds to maximize their antioxidant potential.

With the Fermented Seeds in 70% Ethanol, the DPPH concentration is around 322 µmol/g., while this concentration is slightly lower, around 306 µmol/g in 80% Methanol: The DPPH. To mean that Fermented seeds exhibit the lowest antioxidant capacity among all the processing methods, regardless of the solvent used. The minimal difference between the two solvents suggests that fermentation significantly reduces the antioxidant content of Moringa seeds, making the choice of solvent less impactful. The Raw Seeds on the other hand has the DPPH concentration as high as approximately 7140 µmol/g when dissolved in 70% Ethanol . The DPPH concentration is lower, at around 6330 µmol/g in 80% Methanol: Raw seeds therefore demonstrate the highest antioxidant activity across all processing methods. The difference in DPPH values between the two solvents highlights the superior effectiveness of 70% ethanol in extracting antioxidants from raw seeds. This indicates that minimal processing combined with ethanol extraction is ideal for maintaining the potent antioxidant properties of Moringa seeds.

Extraction Efficiency: The radar chart clearly shows that 70% ethanol is generally more effective than 80% methanol across all processing methods, particularly for raw and boiled seeds. This makes ethanol the preferred solvent for extracting antioxidants from Moringa seeds. The antioxidant capacity of Moringa seeds is highest when they are in their raw form, and it decreases with boiling and fermentation. This pattern suggests that less processing preserves more antioxidant compounds, which is especially evident when using ethanol as the extraction solvent. The relatively flat profile of fermented seeds across both solvents indicates that fermentation significantly diminishes the antioxidant properties, making it less suitable for applications where high antioxidant activity is desired.

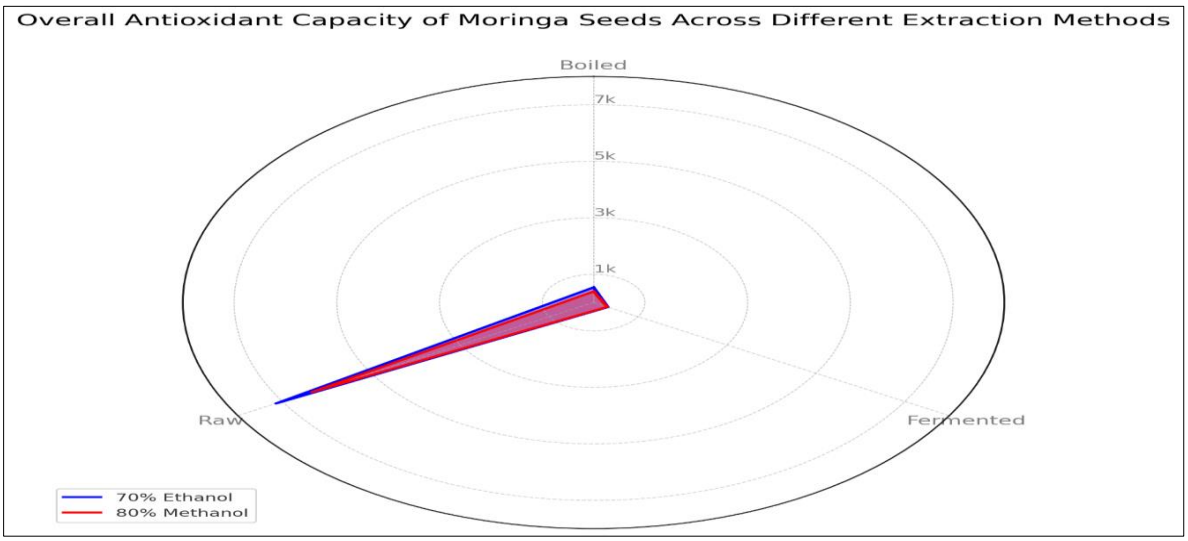

Figure 8 Antioxidant capacity of different Moringa seeds across different extraction methods of 70% Ethanol and 80% Methanol for three processing methods

8. The density of DPPH values, within each Variety of *Moringa oleifera*

The violin plots above visualize the distribution and density of DPPH values, representing antioxidant activity, within each geographical location: Ghana (Blue), Haiti (Orange), India (Green), and Nigeria (Red). The width of each violin plot indicates the density of data points, showing the spread and concentration of antioxidant activity for Moringa seeds from each region. For the Distribution, the plot for Ghana is relatively narrow and symmetric, indicating a concentrated distribution of DPPH values., while the density is highest in the middle range, suggesting that most Ghanaian Moringa seeds have consistent antioxidant activity. This uniformity could be due to stable growing conditions and consistent processing methods in Ghana, resulting in reliable antioxidant capacity.

The Distribution plot for Haiti shows a slight right skew, with a wider distribution towards higher DPPH values., While the density is concentrated in the lower to middle range, there is a noticeable tail towards higher values. The right skew indicates that while most Haitian Moringa seeds have moderate antioxidant activity, there are some samples with higher activity. This could be due to variations in seed quality or environmental factors affecting antioxidant production. The plot for India is the widest, with a pronounced right skew, indicating a broad range of DPPH value. The density is spread across a wide range, with a concentration in the lower to middle values but extending significantly towards higher values. The broad and right skewed distribution suggests significant variability in antioxidant activity among Indian

Moringa seeds. This variability might be due to diverse growing environments, genetic differences, or varying processing methods within India.

The Distribution plot for Nigeria shows a bimodal distribution, with two distinct peaks.there are two concentrations of density, one in the lower range and another in the higher range of DPPH values. The bimodal distribution indicates that Nigerian Moringa seeds might come from different varieties, regions, or processing techniques, leading to two distinct groups of antioxidant activity. This suggests that Nigerian seeds are not homogenous in their antioxidant capacity.

The violin plots clearly show that the geographical origin of Moringa seeds significantly affects their antioxidant activity. The plots reveal how uniform or variable the antioxidant capacity is within seeds from each region. The wide and right-skewed plot for India suggests that Indian Moringa seeds have the most variable antioxidant activity. This could be beneficial if seeking seeds with exceptionally high antioxidant capacity, but it also implies a lack of consistency. The distinct bimodal distribution in Nigeria indicates that different subpopulations or conditions influence the antioxidant activity of Moringa seeds from this region. Further research could identify the factors contributing to this variability. Ghana and Haiti exhibit more consistent antioxidant activity, with narrower distributions. This consistency could make them more reliable sources for antioxidant-rich Moringa seeds.

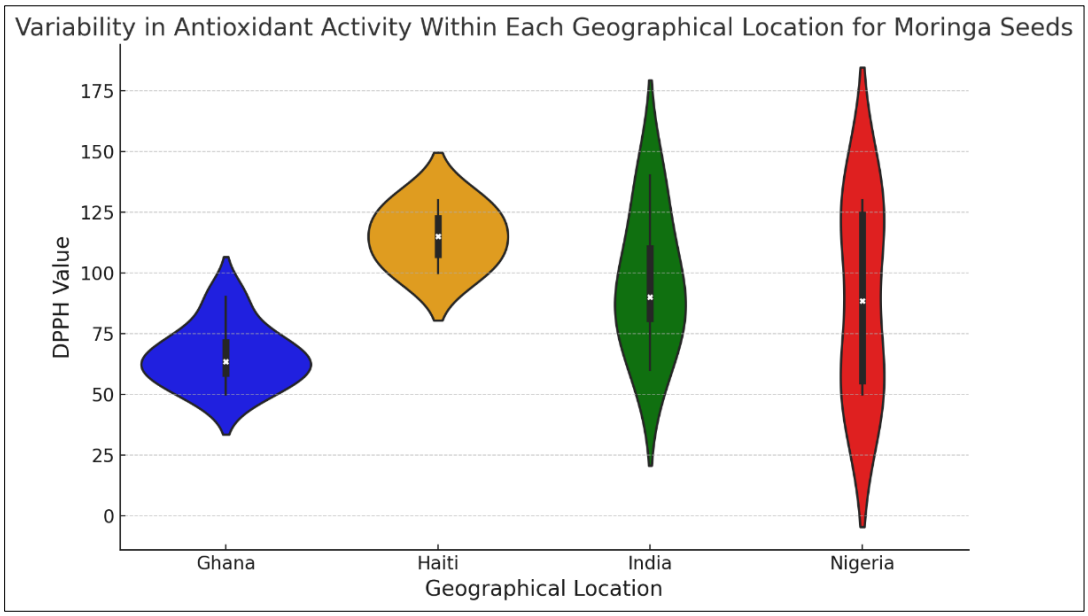

Figure 9 Variability in Antioxidant Activity within Variety of Moringa

9. Comprehensive Discussion of the Heatmap

The heatmap visualizes the DPPH values, representing antioxidant activity, for different combinations of processing methods (Boiled, Fermented, Raw) and solvents (70% Ethanol, 80% Methanol). The color intensity indicates the strength of the antioxidant activity, with darker shades representing higher DPPH values.. Raw Seeds with 70% Ethanol has the DPPH Value 7139.98 µmol/g. The darkest shade on the heatmap, indicating the highest antioxidant activity. This combination is the most effective for maximizing the antioxidant activity of Moringa seeds. Raw processing combined with 70% ethanol as the solvent preserves or extracts the highest amount of antioxidant compounds The Raw Seeds with 80% Methanol recorded DPPH Value: of 6329.98 µmol/g, with Color Intensity Slightly lighter than the 70% ethanol combination but still representing very high antioxidant activity. While not as effective as 70% ethanol, 80% methanol also performs well in preserving the antioxidant properties of raw Moringa seeds. This makes it a viable alternative depending on other factors like cost or availability of solvents.

The Boiled Seeds with 70% Ethanol has DPPH Value: 536.99 µmol/g With a Moderate shade Color Intensity, indicating a substantial but lower antioxidant activity compared to raw seeds. THIS IMPLIES THAT Boiling the seeds reduces antioxidant activity compared to raw processing, but 70% ethanol still extracts more antioxidants than 80% methanol. This combination is better than others involving boiling but less effective than raw processing. The Boiled Seeds with

80% Methanol on the other hand has the DPPH Value as 392.51 µmol/g with the Color Intensity of the Lighter shade, indicating lower antioxidant activity. This combination results in a further reduction in antioxidant capacity. Boiling combined with 80% methanol is the least effective among the boiling methods, making it a less desirable choice for maximizing antioxidant activity.

The Fermented Seeds with 70% Ethanol has DPPH Value of 321.68 µmol/g, with a Color Intensity of Light shade, representing low antioxidant activity. This mean Fermentation leads to a significant reduction in antioxidant activity, even when using 70% ethanol. This suggests that fermentation may degrade or remove key antioxidant compounds, making it less effective for health-focused applications. The Fermented Seeds with 80% Methano on the other hand has DPPH Value of 306.33 µmol/g with Color Intensity of he lightest shade, indicating the lowest antioxidant activity. This combination is the least effective overall. The combination of fermentation and 80% methanol results in the greatest loss of antioxidant activity, making it the least optimal method for maximizing the health benefits of Moringa seeds.

The combination of Raw processing and 70% Ethanol is the most effective for maximizing the antioxidant activity of Moringa seeds. This method should be preferred for applications where high antioxidant capacity is crucial. The processing method significantly affects the antioxidant capacity, with raw seeds showing the highest values, followed by boiled, and then fermented seeds. Minimal processing (raw) is key to preserving antioxidant properties. The 70% Ethanol consistently outperforms 80% Methanol across all processing methods, making it the preferred solvent for extracting antioxidants from Moringa seeds. The heatmap provides a clear and effective visualization of how different combinations of processing methods and solvents influence the antioxidant activity of Moringa seeds. The findings highlight that raw processing combined with 70% ethanol is the best approach for maximizing antioxidant benefits, offering valuable guidance for the preparation and processing of Moringabased products.

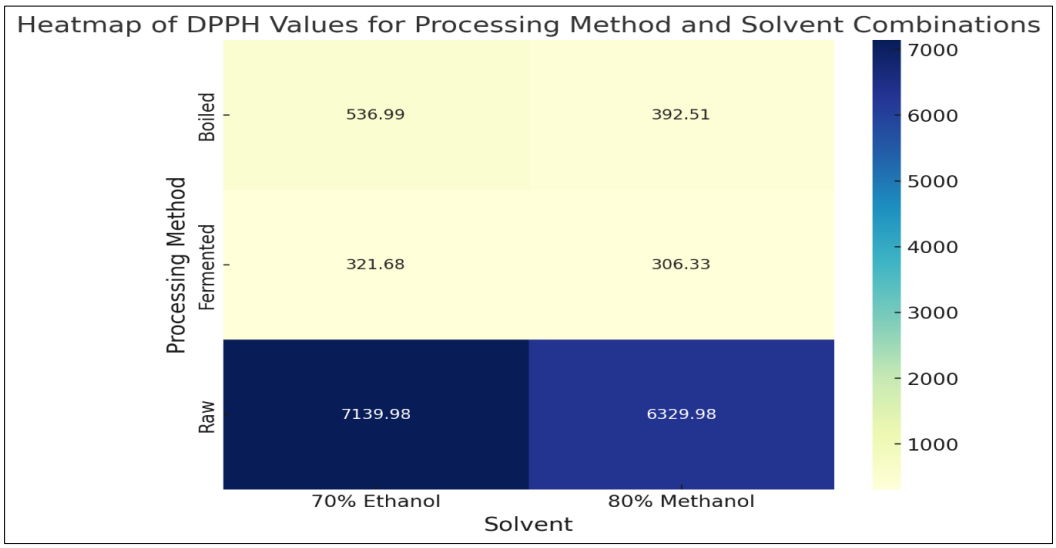

Figure 10 Variability of Antioxidant across processing methods and solvents combinations

The Mean DPPH values for different treatments across countries

Table 2 Mean DPPH values for each country and treatment combination

	Boiled 70% Ethanol Umol	**Boiled 80% Methanol Umol**	**Fermented 70% Ethanol Umol**	**Fermented 80% Methanol Umol**	**Raw 70% Ethanol Umol**	**Raw 80% Methanol Umol**
Ghana	7939.98	5803.32	4756.65	4443.32	7139.98	6329.98
Haiti	6966.65	5773.32	5299.98	5229.98	9663.32	7513.32
India	6553.32	5763.32	5143.32	5126.65	10886.65	14639.98
Nigeria	7259.98	5503.32	4576.65	4113.32	8056.65	8589.98

10. The effect of country of origin and treatment method on the antioxidant activity (DPPH values) of moringa seeds

The model is designed to explore the impact of geographical and environmental factors on the antioxidant properties of *Moringa oleifera*. It aims to understand how soil composition, climate, and agricultural practices affect the nutritional and medicinal qualities of the plant by comparing samples from different countries. The Comprehensive Scores model seeks to create a standardized scoring system that integrates DPPH with the extraction methods of 80% Methanol and 70% Ethanol. This scoring system allows for comparative analysis and helps identify the most promising *Moringa oleifera* varieties, as well as the best extraction method for medicinal applications. The comprehensive scores were calculated for each country and treatment combination, taking into account the contribution of country effect, treatment effect, and their interaction.

The final scores provide an overall ranking of the countries based on their *Moringa oleifera*'s antioxidant activity across all treatments. The results indicate that India and Haiti show the highest overall antioxidant activity in their *Moringa oleifera* seeds, while Nigeria and Ghana have lower overall antioxidant activity. The findings suggest that geographical factors in India and Haiti may be more favorable for producing *Moringa oleifera* seeds with higher antioxidant properties. It was also observed that the 80% methanol extraction, particularly after boiling, seems to be the most effective method for preserving or extracting antioxidant compounds across all countries. These findings provide valuable insights into the antioxidant properties of *Moringa oleifera* and set a foundation for further research into optimizing cultivation and processing methods for maximizing its health benefits.

Table 3 The Comprehensive scores of Country DPPH Seed Form and Extraction type and the overall scores

Country DPPH Seed Form and Solvent type	**Ghana (DPPH Score)**	**Haiti (DPPH core)**	**India (DPPH Score)**	**Nigeria (DPPH Score)**
DPPH of Raw Seed (80% methanol umol)	0.328	0.342	0.405	0.345
DPPH of Raw Seed (80% methanol umol)	0.328	0.342	0.405	0.345
DPPH of Boiled seed Seed (80% methanol umol)	0.709	0.705	0.704	0.634
DPPH of Boiled seed Seed (80% methanol umol)	0.709	0.705	0.704	0.634
DPPH of Fermented Seed (80% methanol umol)	0.53	0.584	0.578	0.505
DPPH of Fermented Seed (80% methanol umol)	0.534	0.587	0.582	0.509
DPPH of Raw Seed (70% Ethanol umol)	0.443	0.495	0.52	0.458
DPPH of Raw Seed (70% Ethanol umol)	0.483	0.547	0.559	0.497
DPPH of Boiled Seed (70% Ethanol umol)	0.49	0.443	0.422	0.452
DPPH of Boiled Seed (70% Ethanol umol)	0.49	0.442	0.422	0.451
DPPH of Fermented Seed (70% Ethanol umol)	0.487	0.545	0.53	0.466
DPPH of Fermented Seed (70% Ethanol umol)	0.488	0.545	0.529	0.466
Country overall Effect of DPPH Score	Final scores	Rank		
Ghana	0.502	3RD		
Haiti	0.524	2ND		
India	0.530	1ST		
Nigeria	0.481	4TH		

10.1. The effect of the country of origin, treatment method, and their interaction on the antioxidant activity (DPPH values) of *Moringa oleifera* Variety

10.1.1. Results

We hypothesized that if the antioxidative property of moringa varieties can be characterized based on their growth environment and extraction method , associative effects between moringa species, extraction method and antioxidative activity . ANOVA was used to analyze these effect of the country of origin, treatment method, and their interaction on the antioxidant activity (DPPH values) of *Moringa oleifera* Variety. The sum of squares (SS), which measures the total variation in the DPPH values, is partitioned into components attributable to different sources of variation: country(representing MO Variety), the treatment (80% Methanol vs 70% Ethanol), their interaction, and residual (error). The SS for the country effect (22,780,606.40) indicates the variation in DPPH values due to differences between countries. The SS for the treatment effect (1,682,197,696.55) indicates the variation in DPPH values due to different treatment methods. The SS for the interaction effect (132,877,193.20) indicates the variation in DPPH values due to the combined effect of country and treatment. The residual SS (3,390,479.41) represents the variation in DPPH values that cannot be explained by the country, treatment, or their interaction.

The Degrees of freedom (df) represent the number of independent values that can vary in the analysis. There are 3 degrees of freedom for the country effect, corresponding to the 4 countries minus 1. There are 11 degrees of freedom for the treatment effect, corresponding to the 12 treatments minus 1.There are 33 degrees of freedom for the interaction effect, corresponding to (4 countries - 1) * (12 treatments - 1).There are 96 degrees of freedom for the residual, representing the remaining variation not explained by the model.

The F-value is the ratio of the mean square of a factor to the mean square of the residual. Higher F-values indicate a greater effect of the factor on the variability of the DPPH values. The F-value for the country effect (215.01) indicates a significant effect of the country on DPPH values. The F-value for the treatment effect (4330.07) indicates a significant effect of the treatment method on DPPH values. The F-value for the interaction effect (114.01) indicates a significant interaction between country and treatment on DPPH values.

The p-value indicates the probability that the observed variability is due to chance. Lower p-values (typically < 0.05) indicate that the factor has a statistically significant effect on the DPPH values. The p-value for the country effect (1.84e-42) indicates a statistically significant effect of the country on DPPH values. The p-value for the treatment effect (3.31e-124) indicates a statistically significant effect of the treatment method on DPPH values. The p-value for the interaction effect (1.68e-63) indicates a statistically significant interaction between country and treatment on DPPH values.

10.1.2. Interpretation of Findings

Table 4 ANOVA results Table of the effect of the country of origin, treatment method, and their interaction on the antioxidant activity (DPPH values) of *Moringa oleifera*

	sum_sq	**df**	**F**	**PR(>F)**
C(Country)	22780606.400513053	3	215.0077664113	1.836812501e-42
C(Treatment)	1682197696.5522041	11	4330.0655475298	3.308296244e-124
C(Country):C(Treatment)	132877193.19670303	33	114.0109666247	1.682634759e-63
Residual	3390479.409111064	96	null	null

The p-value for the country effect is extremely low (1.84e-42), indicating that the country of origin has a statistically significant effect on the DPPH values. This suggests that the antioxidant activity of moringa seeds varies significantly between different countries. The p-value for the treatment effect is also extremely low (3.31e-124), indicating that the treatment method (raw, boiled, fermented) has a statistically significant effect on the DPPH values. This suggests that the antioxidant activity of moringa seeds is significantly influenced by the treatment method. The p-value for the interaction effect between country and treatment is very low (1.68e-63), indicating that the interaction between country and treatment has a statistically significant effect on the DPPH values. This suggests that the effect of treatment on antioxidant activity varies depending on the country of origin. The ANOVA results indicate that both the country of origin and the treatment method have significant effects on the antioxidant activity of moringa seeds. Additionally, the interaction between country and treatment is also significant, suggesting that the effect of treatment varies depending

on the country. These findings highlight the importance of considering both the source and the processing method when evaluating the antioxidant properties of moringa seeds.

11. Summary

This study investigates the radical scavenging abilities of *Moringa oleifera* (MO) seeds collected from four different countries—Nigeria, Ghana, Haiti, and India—and grown under controlled conditions at the Winfred Thomas Agricultural Research Station (WTARS) at Alabama AandM University. The primary focus of the study was to evaluate the variation in antioxidant activity, specifically DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, across different geographical origins and processing methods of MO seeds. By doing so, the study aims to provide insights into how geographical and processing factors influence the phytochemical composition and antioxidant potential of MO seeds, thereby contributing to their potential applications in pharmaceutical, nutraceutical, and food industries.

The study employed a systematic approach to collect, process, and analyze MO seeds from the selected countries. The seeds were collected from Nigeria, Ghana, Haiti, and India, representing diverse geographical regions with varying environmental conditions, such as soil composition, climate, and agricultural practices. These seeds were then planted at WTARS to minimize environmental variation during growth and to focus on the inherent differences due to geographical origin. Upon maturity, the seeds were harvested and subjected to three different processing methods: raw, boiled, and fermented. These methods were chosen to reflect common traditional and industrial processing techniques that could influence the bioactive compound content of the seeds. Each processing method was designed to either preserve or alter the phytochemical composition of the seeds to assess the impact on antioxidant activity.

The seeds were thoroughly cleaned, air-dried, and ground into a fine powder. The raw seeds were then subjected to solvent extraction using 70% ethanol and 80% methanol, followed by concentration using a rotary evaporator. The seeds were boiled in distilled water for a specified duration to facilitate the extraction of soluble compounds. After boiling, the seeds were cooled, air-dried, ground into a fine powder, and extracted using the same solvents as the raw seeds. The seeds were soaked in distilled water to initiate fermentation, which was allowed to proceed for three days. The fermented seeds were then air-dried, ground, and extracted in a similar manner to the other processing methods. The antioxidant activity of the extracted compounds was assessed using the DPPH radical scavenging assay, a widely accepted method for evaluating the free radical neutralizing potential of bioactive compounds. The assay involved measuring the decrease in absorbance at 517 nm after the reaction between the DPPH radicals and the antioxidants present in the seed extracts. The results were expressed as micromoles of Trolox Equivalent (TE) per gram of sample, providing a quantitative measure of the antioxidant capacity.

The study revealed significant variability in the DPPH radical scavenging activity of MO seeds based on their geographical origin and processing methods. The key findings can be summarized as follows: MO seeds from India exhibited the highest average DPPH value (8018.87 μmol/g). This indicates that Indian MO seeds possess superior antioxidant properties compared to those from other regions. The wide range of DPPH values in Indian seeds suggests significant variability within the samples, possibly due to diverse growing environments and genetic variations in India. Haitian MO seeds also demonstrated strong antioxidant activity, with an average DPPH value of 6741.10 μmol/g. The distribution of DPPH values was slightly right-skewed, indicating that while most seeds had moderate antioxidant activity, some samples exhibited exceptionally high activity.

Nigerian MO seeds showed moderate antioxidant activity with an average DPPH value of 6349.98 μmol/g. The bimodal distribution of DPPH values in Nigerian seeds suggests the presence of distinct subpopulations, possibly due to differences in seed varieties or regional variations within Nigeria. MO seeds from Ghana had the lowest average DPPH value (6068.87 μmol/g) among the four countries. The relatively narrow and symmetric distribution of DPPH values suggests more consistent antioxidant activity across the Ghanaian samples, likely due to uniform growing conditions and processing methods.

Across all geographical origins, raw seeds consistently exhibited the highest radical scavenging ability. The highest recorded DPPH value was found in Haitian raw seeds extracted with 70% ethanol (9720 μmol/g). This suggests that minimal processing preserves the natural antioxidant compounds in MO seeds, making raw seeds the most potent form for antioxidant-related applications. Boiling the seeds led to a significant reduction in antioxidant activity, with DPPH values ranging from approximately 467 μmol/g to 544 μmol/g. The consistent reduction across all geographical origins indicates that boiling degrades or leaches out some of the antioxidant compounds, although moderate activity is still retained. Fermentation resulted in the lowest DPPH values, with the least value observed in Ghanaian seeds extracted with 80% methanol (306.33 μmol/g). The narrow range and low variability in DPPH values suggest that fermentation

consistently diminishes the antioxidant capacity of MO seeds, likely due to the breakdown of bioactive compounds during the fermentation process.

This solvent was generally more effective in extracting antioxidants from MO seeds across all processing methods. Raw seeds extracted with 70% ethanol consistently showed the highest DPPH values, indicating superior extraction efficiency. This suggests that ethanol is more effective in preserving or extracting antioxidants compared to methanol, particularly in raw and boiled seeds. Although methanol also performed well, particularly in raw seeds, it was generally less effective than ethanol. The DPPH values were lower across all processing methods, with the largest discrepancy observed in raw seeds, where ethanol extracted significantly higher amounts of antioxidants.

The results of this study have several important implications for the cultivation, processing, and application of MO seeds: The study highlights the significant impact of geographical origin on the antioxidant activity of MO seeds. Seeds from India and Haiti, in particular, exhibited higher antioxidant potential, suggesting that the environmental conditions in these regions are conducive to producing MO seeds with superior health benefits. This finding emphasizes the need for localized studies to fully understand and optimize the cultivation of MO plants for maximal bioactive compound production. The choice of processing method plays a critical role in determining the antioxidant capacity of MO seeds. The results indicate that minimal processing (e.g., raw seeds) is most effective in preserving the radical scavenging ability, while methods like boiling and fermentation significantly reduce antioxidant activity. This information is crucial for industries that aim to develop health supplements, nutraceuticals, or functional foods using MO seeds. The study suggests that products requiring high antioxidant content should prioritize using raw seeds or minimally processed extracts.

The choice of solvent is another key factor influencing the extraction efficiency of antioxidants from MO seeds. The superior performance of 70% ethanol across all processing methods indicates that ethanol should be the solvent of choice for applications aimed at maximizing antioxidant extraction. This finding is particularly relevant for industrial applications where the solvent used can significantly impact the yield and efficacy of the final product. Given the high antioxidant activity observed in MO seeds, particularly those from India and Haiti, there is substantial potential for developing nutraceutical products from these varieties. The study's findings provide a strong foundation for further research into the specific phytochemical compounds responsible for the observed antioxidant activity, which could lead to the development of targeted health supplements.

12. Conclusion

This study comprehensively evaluated the radical scavenging abilities of MO seeds from four different geographical locations and assessed the impact of various processing methods and solvents on their antioxidant activity. The findings revealed significant variability in antioxidant capacity based on geographical origin, with Indian and Haitian MO seeds showing the highest potential. The study also demonstrated the critical importance of processing methods, with raw seeds exhibiting the strongest antioxidant activity, while boiling and fermentation led to reduced effectiveness. The choice of solvent further influenced the outcomes, with 70% ethanol consistently outperforming 80% methanol. These results underscore the need for careful consideration of both the source and processing methods when utilizing MO seeds for antioxidant-related applications. The insights gained from this study provide valuable guidance for optimizing the cultivation and processing of MO seeds to maximize their health benefits. Further research into the specific phytochemical profiles of MO seeds from different regions will be essential for fully harnessing their potential in the nutraceutical and pharmaceutical industries.

Recommendations

The study revealed that raw MO seeds consistently exhibited the highest DPPH radical scavenging activity across all geographical origins. This finding suggests that minimal processing is crucial for preserving the natural antioxidant compounds present in MO seeds. For industries aiming to produce antioxidant-rich products, it is recommended to prioritize the use of raw MO seeds. This is particularly relevant for the development of nutraceuticals, functional foods, and dietary supplements, where maximizing antioxidant content is a key objective.

: The study found that 70% ethanol was generally more effective than 80% methanol in extracting antioxidants from MO seeds, especially in raw and boiled seeds. Given the superior extraction efficiency of ethanol, it is recommended to use 70% ethanol as the solvent of choice for processing MO seeds in industrial applications. This solvent should be preferred when the goal is to achieve the highest possible antioxidant activity in the final product.

The geographical origin of MO seeds was found to significantly influence their antioxidant activity. Seeds from India and Haiti exhibited the highest DPPH values, suggesting that these regions have environmental conditions that are particularly favorable for producing MO seeds with strong antioxidant properties. It is recommended that cultivation strategies focus on sourcing or growing MO seeds in regions with similar agroclimatic conditions to India and Haiti. Additionally, further research could explore the specific environmental factors in these regions that contribute to the superior antioxidant activity, with the aim of replicating these conditions in other areas.

The dose-response behavior observed in the study indicates that the antioxidant activity of MO seed extracts is dose-dependent, with raw seeds showing the strongest response. This finding is important for developing products with targeted antioxidant levels. For instance, higher concentrations of raw MO seed extracts should be used in products where maximum antioxidant efficacy is desired, such as in high-potency dietary supplements. Conversely, products with moderate antioxidant levels could benefit from using boiled or fermented seeds, depending on the desired potency.

The study's ANOVA results highlighted a significant interaction between processing methods and solvent types, indicating that the choice of solvent can amplify the differences in antioxidant activity depending on the processing method. To maximize the antioxidant potential of MO seeds, it is recommended to carefully select the solvent and processing method combination based on the desired outcome. For example, for products that require high antioxidant activity, raw seeds extracted with 70% ethanol should be prioritized. In contrast, if the aim is to achieve a balance between processing efficiency and antioxidant content, boiled seeds extracted with ethanol could be considered.

Although Indian and Haitian MO seeds demonstrated the highest antioxidant activity, seeds from Nigeria and Ghana also showed significant potential, particularly when minimally processed. It is recommended to conduct further research on the specific phytochemical profiles of Nigerian and Ghanaian MO seeds to identify any unique compounds that may contribute to their antioxidant activity. This could lead to the development of specialized products that highlight the distinct benefits of MO seeds from these regions.

The study found that fermentation significantly reduced the antioxidant activity of MO seeds across all geographical origins and solvent types. While fermentation may not be ideal for maximizing antioxidant content, it could potentially offer other benefits, such as enhancing the bioavailability of certain nutrients or creating unique flavor profiles. It is recommended to further explore the effects of fermentation on the overall nutritional profile of MO seeds to determine if there are other health benefits that could justify its use in specific products.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have declared that no competing interests exist.

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