



Unlocking the Therapeutic Potential of *Moringa oleifera* Leaves: Evaluating the Impact of Its Nutritional Properties

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ABSTRACT

The research investigated the composition of *Moringa oleifera* leaves in terms of their nutritional and phytochemical properties, while also exploring the impact of solvent selection on the extraction of phenolic compounds. The study involved analyzing the leaves for essential nutrients like proteins, carbohydrates, fiber, and fats, as well as screening for various compounds such as phenols, flavonoids, alkaloids, saponins, and tannins. Phenolic compounds were extracted using methanol and hexane solvents, and the resulting extracts were examined for phlorotannins, flavonoids, and phenols. Additionally, the study evaluated the plant's ability to scavenge DPPH and ferric (Fe³⁺) ion radicals, inhibit bovine serum albumin denaturation, and stabilize the human red blood cell membrane (IC₅₀). The findings revealed that *Moringa oleifera* leaves are abundant in essential nutrients, phenols, and flavonoids, and moderately rich in alkaloids, saponins, and tannins, while lacking phlorotannins. The methanol extracts demonstrated higher concentrations of phlorotannins, flavonoids, and phenols compared to the hexane extract, indicating its potential for the development of novel therapeutic applications. The choice of solvent had a significant impact on the types and concentrations of phenolic compounds extracted from the plant material. Furthermore, the plant exhibited notable scavenging activity against DPPH and ferric (Fe³⁺) ion radicals. The methanol extract displayed stronger antioxidant and anti-inflammatory potential than the hexane extract, suggesting that *Moringa oleifera* leaves could serve as a natural source of bioactive compounds and antioxidants in the food and pharmaceutical industries. Overall, the study emphasizes the health benefits associated with *Moringa oleifera* leaves and underscores the importance of solvent selection when extracting phenolic compounds for therapeutic purposes.

Keywords: *Moringa oleifera*, Solvent extraction, Anti-inflammatory, Antioxidant potential, phytochemical analysis, Nutritional composition.

INTRODUCTION

Moringa oleifera, also known as the drumstick tree or horseradish tree, has a long history of use in traditional medicine and is now gaining recognition as a functional food [1]. This plant is highly nutritious, containing vitamins, minerals, amino acids, and a variety of phytochemicals such as flavonoids, tannins,

and alkaloids, which offer numerous health benefits [1, 2]. The leaves, in particular, are rich in antioxidants that play a vital role in protecting cells from oxidative stress [3, 4].

Studies have highlighted the therapeutic properties of *Moringa oleifera* leaves, including their anti-inflammatory, antimicrobial, antidiabetic, and

hepatoprotective activities [5]. The abundance of antioxidants in the leaves may also contribute to the prevention and treatment of conditions related to oxidative stress, such as cardiovascular disease, cancer, and neurodegenerative disorders [6, 7]. Moreover, the anti-inflammatory properties of *Moringa oleifera* leaves have been observed, suggesting their potential as a natural remedy for various inflammatory conditions [8]. Phytochemicals like flavonoids found in *Moringa oleifera* are believed to contribute to its anti-inflammatory effects by reducing inflammation in the body [9].

Apart from its therapeutic potential, *Moringa oleifera* is gaining attention as a functional food. The leaves and pods are rich in protein, making them a valuable source of vegetable protein, particularly in areas where animal protein is scarce. Additionally, the seeds of *Moringa oleifera* are oil-rich, which can be utilized for cooking or as an ingredient in cosmetic products [10].

Ongoing research continues to explore the properties of *Moringa oleifera* and its potential therapeutic benefits [11, 12, 13, 14]. The current study aims to investigate the potential therapeutic benefits of *Moringa oleifera* leaves by examining the effects of different solvents on the extraction of phenolic compounds. It further seeks to evaluate the nutritional composition of the leaves, identify and quantify the phytochemical constituents present, assess their antioxidant activity, and explore their potential as an anti-inflammatory agent. Through this research, a more comprehensive understanding of the therapeutic potential of *Moringa oleifera* leaves can be obtained, which may contribute to the development of new treatments or functional food products.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of *Moringa oleifera* were collected from Ilishan town, Ogun state in November 2012. The leaves were authenticated at the herbarium of the Department of Botany, University of Ibadan, and a voucher specimen (Voucher number: UIH-22770) was deposited for reference. To prepare the leaves for analysis, they were air-dried at room temperature for two weeks and then ground into a coarse powder using an electric blender (15a). The dried leaves were further pulverized into a uniform powder and stored in an airtight container for subsequent analysis.

Various equipments, such as test tubes, spatulas, spectrophotometer, and pH meter. As well as reagents like distilled water, methanol, n-hexane, and hydrochloric acid, were employed in the study. For the extraction and partitioning of plant materials, 100 grams of the powdered leaves were soaked in 500ml of methanol at room temperature for 48 hours. The methanol and hexane fractions obtained were concentrated using a rotary evaporator with the water bath set at 50°C.

Proximate analysis of the crude plant sample was conducted using the methods recommended by the Association of Official Analytical Chemists [15b]. This analysis involved determining the moisture content, crude protein, crude fiber, crude fat, ash content, and total soluble carbohydrates. The Kjeldahl method was employed for determining crude protein, which involved estimating the nitrogen content by titration of the ammonium borate with 0.1N sulfuric acid. The calculation of total soluble carbohydrate content was done by subtracting the sum of protein, fat, fiber, moisture, and ash content from 100%.

The study encompassed two methods of analysis, namely qualitative and quantitative analyses of the methanol and hexane fractions of *M. oleifera* leaves. Additionally, phytochemical screening was performed to identify and quantify the presence of various phytochemical compounds.

Qualitative Analysis

Phytochemical screening of the methanol and hexane fractions of the dry powdered leaf extract was carried out using standard procedures [16, 17 and 18] to identify the presence of various compounds, including saponins, phenols, alkaloids, flavonoids, tannins, phlobatannins, steroids, carbohydrates, and proteins. The study also determined the total phenolic composition, total flavonoids, and total proanthocyanidins using the Folin Ciocalteu reagent, aluminium chloride colorimetric method, and the procedure of Sun et al. [19], respectively. The concentrations of flavonoids and proanthocyanidins were expressed in mg quercetin equivalents/mg.

Furthermore, the study investigated the phytochemical composition and in vitro antioxidant activity of *Moringa oleifera* leaf extracts. Quantitative phytochemical analysis of the *M. oleifera* leaf extracts was conducted using various methods described in the study [20]. The total phenolic composition was determined using the modified Folin-Ciocalteu reagent method [21, 22]. Total flavonoids were estimated using the aluminium chloride colorimetric method according to Pourmorad et al. [23], while total proanthocyanidin concentrations were determined based on the procedure of Sun et al. [24]. Tannin content was determined using the Folin-Ciocalteu method and Van Burden and Robinson [25], and the total alkaloid concentration was determined using the

alkaline precipitation gravimetric method described by Harborne [26].

In vitro antioxidant screening was conducted using the DPPH radical scavenging activity method. The quantitative DPPH antioxidant assay involved adding test solutions at different concentrations (100 µg/ml - 500 µg/ml) to 1 ml of freshly prepared DPPH solution, followed by incubation in the dark at room temperature for 20 minutes. The absorbance was measured at 517 nm using a spectrophotometer, with methanol as the reference. Gallic acid was used as a standard, and the percentage inhibition was calculated using the formula: % inhibition = [(Absorbance of control - Absorbance of the test sample)/Absorbance of control] x 100 [29].

Ferric-Reducing Power Assay

The reducing power of the extract was assessed following the method described by Oyaizu [30]. A mixture containing 2.5 mL of 0.2M phosphate buffer (pH 6.6) and 2.5 mL of K₃Fe(CN)₆ (1% w/v) was added to 1.0 mL of the extract (0.1 mg/ml - 0.5 mg/ml) dissolved in distilled water. The resulting mixture was incubated at 50 °C for 20 minutes, followed by the addition of 2.5 mL of trichloroacetic acid (TCA, 10% w/v). After centrifugation at 3000 rpm for 10 minutes, the upper layer of the solution (2.5 mL) was collected and mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ (0.1%, w/v). The absorbance was measured at 700 nm against the blank sample. Ascorbic acid was used as a positive control [31 and 32].

In vitro anti-inflammatory assays were conducted to evaluate the human red blood cell membrane stabilizing potentials of *M. oleifera* extracts. The assay utilized a 10% (v/v) human red blood cell suspension, with aspirin and diclofenac sodium as standard drugs. The assay mixtures consisted of 2 mL

of hyposaline (0.36% w/v) sodium chloride, 1.0 mL of phosphate buffer (pH 7.4, 0.15 M), 0.5 mL of (10% v/v) human red blood cell suspension, and 0.5 mL of plant extracts or standard drugs at various concentrations (50, 100, 250, 500 $\mu\text{g}/\text{ml}$). The mixtures were incubated at 37°C for 30 minutes and then centrifuged at 4000 RPM for 10 minutes. The haemoglobin content in the suspension was measured using a spectrophotometer at 560 nm. The percentage of membrane stabilization was calculated as follows: % membrane stabilization = $100 - [(\text{Optical density of Test sample} / \text{Optical density of Control}) \times 100]$ [33 and 34].

The BSA denaturation inhibition assay was performed according to Mizushima and Kobayashi [35], as modified by Anyasor et al., [36]. For this assay, 50 μL of different concentrations of the test samples (100, 250, 500 $\mu\text{g}/\text{ml}$) were added to 450 μL of 5% BSA solution. The pH was adjusted to 6.3 using 1N HCl, and the solution was incubated at 37°C for 20 minutes and then heated to 57°C for 30 minutes. After cooling, 2.5 mL of phosphate buffer saline was added, and the absorbance was measured at 600 nm. Diclofenac sodium and aspirin were used as standards, and the control did not contain the extract. The percentage inhibition was calculated as $(\text{Absorbance of control} - \text{Absorbance of test sample}) / \text{Absorbance of control} \times 100$ [37, 38].

Statistical analysis was performed, and the results are presented as means \pm standard deviation (SD). Significant differences were determined using unpaired Student's t-test, with a p-value < 0.05 considered statistically significant.

RESULTS

The findings of this study have significant implications for understanding the potential health benefits of *Moringa oleifera* and lay the

groundwork for further research in this field. The data presented in Tables 1, 2, and 3 shed light on the nutritional and medicinal value of *Moringa oleifera* leaves, which can be utilized in the development of functional foods and nutraceuticals.

Table 1 provides an overview of the proximate composition of *Moringa oleifera* leaves, illustrating the percentage composition of various components in the plant. The table reveals that *Moringa oleifera* leaves are particularly abundant in proteins (31.92 ± 0.22), with a substantial carbohydrate content (37.95 ± 1.73) and moderate levels of ash (10.21 ± 1.61), fiber (7.45 ± 0.25), fats (6.77 ± 3.29), and moisture (5.70 ± 0.10).

Table 2 presents the results of the qualitative phytochemical screening of *Moringa oleifera* leaves using methanol and hexane solvents. The table indicates that *Moringa oleifera* leaves are notably rich in phenols and flavonoids, moderately rich in alkaloids, saponins, and tannins, and devoid of phlobatannins.

Table 1: Proximate Composition of *Moringa oleifera* leaves

Parameter (%)	Mean Composition \pm SD
Moisture	5.70 ± 0.10
Fat	6.77 ± 3.29
Protein	$31.92 \pm 0.22^{**}$
Ash	$10.21 \pm 1.61^*$
Fibre	7.45 ± 0.25
Carbohydrate	$37.95 \pm 1.73^{**}$

The values are expressed as mean \pm standard deviation. Letters represent the level of significance among the group. Groups with different letters differ significantly, while groups with the same letters are not significantly different. Mean \pm S.D, n= 3

Table 2:Qualitative Phytochemical Screening

Phytochemical	Methanol	Hexane
Phenols	+++	-
Alkaloids	++	-
Flavonoids	+++	+
Saponins	++	+
Tannins	++	+
Phlobatannins	-	-
Steroids	+	++
Proteins	+++	++
Carbohydrates	++	+

+++ = Highly present, ++ = Moderately present, + = Present in trace amounts, - = Absent

Table 3 quantitatively assesses the phytochemical content of *Moringa oleifera* leaves using methanol and hexane solvents. The table demonstrates that the methanol

extract exhibits higher concentrations of phlobatannins (mgGAE/mg) ($74.7 \pm 4.95a$), flavonoids (mgGAE/mg) (30.3 ± 0.57), and phenols (mgGAE/mg) (9.2 ± 0.28) compared to the hexane extract. Furthermore, the table indicates that the crude plant sample contains a moderate amount of alkaloids and tannins.

These findings provide valuable insights into the composition and phytochemical profile of *Moringa oleifera* leaves, emphasizing their potential as a source of beneficial compounds. The information presented in these tables contributes to the understanding of *Moringa oleifera*'s nutritional and medicinal value and serves as a basis for future research and the development of functional food products and nutraceuticals.

Table 3:Quantitative Phytochemical Evaluation of *M. oleifera* Leaves

Phytochemical	Methanol	Hexane
Phenols (mg GAE/mg)	9.2 ± 0.28^a	7.3 ± 0.141^b
Flavonoids (mg QUE/mg)	30.3 ± 0.57^a	26.2 ± 0.919^b
Proanthocyanidins (mgQUE/mg)	74.7 ± 4.95^a	78.1 ± 3.25^b

Phytochemical	Crude plant sample Mean \pm S.D
Alkaloids (%)	3.00 ± 0.11
Tannins (mg QUE/mg)	2.24 ± 0.03

The values presented in the tables are expressed as mean \pm standard deviation. The letters used in the tables represent the level of significance among the groups. Groups with different letters indicate significant differences, while groups with the same letters are not significantly different. The data presented in the tables are based on a sample size of three (n=3).

Table 4 provides the results of the DPPH radical inhibition assay, which measures the ability of the test samples to scavenge the DPPH free radical. The table demonstrates that the methanol extract exhibits a higher percentage inhibition of the DPPH radical compared to the hexane extract. At a concentration of 500 $\mu\text{g/ml}$, the methanol extract shows a percentage inhibition of 77.85%, while the hexane extract exhibits a percentage inhibition of 30.7%. The IC50 values, representing the concentration of the sample required to scavenge 50% of the DPPH radical, are also provided. The IC50

value for the methanol extract is 267.38 $\mu\text{g/ml}$, whereas the hexane extract has an IC50 value of 980.39 $\mu\text{g/ml}$. These findings indicate that the methanol extract possesses stronger antioxidant activity compared to the hexane extract.

The values presented in the tables are expressed as mean \pm standard deviation. The letters used in the tables represent the level of significance among the groups. Groups with different letters indicate significant differences, while groups with the same letters are not significantly different. The data

presented in the tables are based on a sample size of three (n=3).

Table 4: Effect of increasing concentrations of Test Samples on DPPH Radical Inhibition

Concentration (µg/ml)	Methanol	Hexane
	Mean inhibition ±SD	% Mean %inhibition ±SD
100	31.49 ± 0.49a	4.84 ± 3.91b
200	62.63 ± 0.97a	5.36 ± 0.73b
300	68.17 ± 3.42a	7.61 ± 3.97b
400	69.89 ± 2.94a	21.63 ± 1.71b
500	77.85 ± 1.47a	30.7 ± 0.24b
IC50 (µg/ml)	267.38 a	980.39 b

Table 5 displays the results of the ferric-reducing power assay, which assesses the ability of the test samples to reduce ferric ions (Fe³⁺) to ferrous ions (Fe²⁺), indicating their antioxidant potential. The table reveals that the methanol extract exhibits higher ferric-reducing power compared to the hexane extract. At a concentration of 0.5 mg/ml, the methanol extract shows an absorbance value of 0.595, while the hexane extract exhibits an absorbance value of 0.484. These results suggest that the methanol extract possesses stronger reducing power, indicating its higher antioxidant activity. The letters used in the table represent the level of significance among the groups, with different letters denoting significant differences between groups.

Table 5: Ferric Reducing Power Assay

Concentration (mg/ml)	Methanol	Hexane
	Mean Absorbance ±SD	Mean Absorbance ±SD
0.1	0.255 ± 0.006a	0.182 ± 0.002b
0.2	0.368 ± 0.003a	0.269 ± 0.009b
0.3	0.460 ± 0.011a	0.341 ± 0.004b
0.4	0.521 ± 0.013a	0.419 ± 0.005b
0.5	0.595 ± 0.023a	0.484 ± 0.003b

Table 6 presents the effect of increasing concentrations of the test samples on the percentage inhibition of protein denaturation. The table provides the concentrations of the

test samples in µg/ml and the mean percentage inhibition of protein denaturation for both the methanol and hexane fractions. The IC₅₀ values for each fraction are also included. The IC₅₀ value represents the concentration of the test sample required to inhibit 50% of protein denaturation. The table demonstrates that the methanol fraction exhibits significantly higher percentage inhibition of protein denaturation compared to the hexane fraction at all concentrations. The mean percentage inhibition for the methanol fraction increases with increasing concentration of the test sample, whereas the hexane fraction shows a lower percentage inhibition that increases slowly with increasing concentration. The IC₅₀ values for the methanol and hexane fractions are 462.96 µg/ml and 892.86 µg/ml, respectively. These findings indicate that the methanol fraction is more effective in inhibiting protein denaturation compared to the hexane fraction.

Table 6: Effect of Increasing Concentrations of Test Samples On % Inhibition Of Protein Denaturation

Concentration (µg/ml)	Methanol	Hexane
	Mean inhibition ±SD	% Mean %inhibition on ±SD
100	9.84 ± 1.86 ^a	5.74 ± 3.07 ^b
250	32.56 ± 1.53 ^a	14.88 ± 3.95 ^b
500	51.39 ± 3.62 ^a	27.67 ± 1.43 ^b
IC ₅₀ (µg/ml)	462.96 ^a	892.86 ^b

The values presented in the tables are expressed as mean ± standard deviation. The letters used in the tables represent the level of significance among the groups. Groups with different letters indicate significant differences, while groups with the same letters are not significantly different. The data presented in the tables are based on a sample size of three (n=3).

Figure 1 provides valuable information about the antioxidant potential of the test samples. The graph illustrates the effect of increasing concentrations of the test samples on DPPH

radical inhibition. The x-axis represents the concentration of the test samples in micrograms per milliliter ($\mu\text{g/ml}$), while the y-axis represents the percentage of DPPH radical inhibition. The graph shows that as the concentration of the test samples increases, the percentage of DPPH radical inhibition also increases. The methanol fraction demonstrates higher DPPH radical inhibition compared to the hexane fraction. The IC_{50} values, representing the concentration of the test sample required to scavenge 50% of the DPPH radical, are $267.38 \mu\text{g/ml}$ for the methanol fraction and $980.39 \mu\text{g/ml}$ for the hexane fraction. These findings indicate that the methanol fraction exhibits stronger antioxidant potential than the hexane fraction.

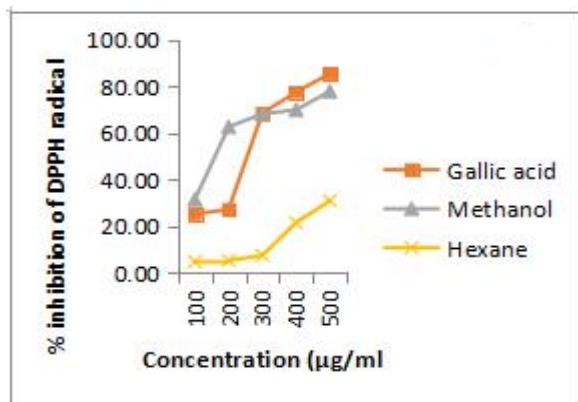


Figure 1: Graph of Test sample concentration against % inhibition of DPPH Radical

Figure 2 illustrates the ferric-reducing power of the test samples, including ascorbic acid, the methanol fraction, and the hexane fraction. The x-axis of the graph represents the concentration of the test samples in milligrams per milliliter (mg/ml), while the y-axis represents the absorbance values. The graph demonstrates that as the concentration of the test samples increases, the absorbance values also increase, indicating higher reducing power. The methanol fraction exhibits higher reducing power compared to the hexane fraction. Notably, ascorbic acid shows the highest reducing power among the

tested samples. These results suggest that the methanol fraction possesses moderate reducing power, likely attributed to its high content of phytochemicals such as phenols, flavonoids, and proanthocyanidins. These findings have implications for the development of natural antioxidants and functional foods.

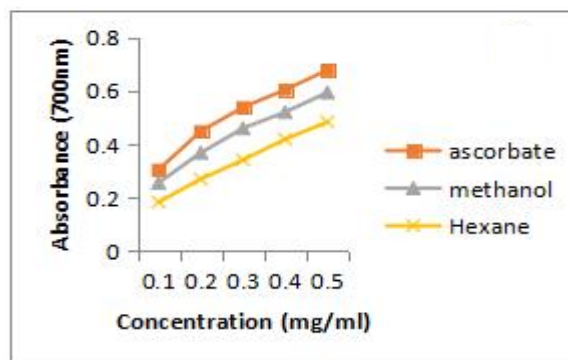


Figure 2: Graphical representation of the ferric-reducing power of Ascorbic acid, methanol and hexane fractions.

Figure 3 depicts the relationship between test sample concentration and the percentage of inhibition of protein denaturation. The graph shows a similar trend as presented in the table, with the methanol fraction exhibiting higher percentage inhibition of protein denaturation compared to the hexane fraction at all concentrations. The graph visually supports the data from the table, indicating the superior inhibitory activity of the methanol fraction.

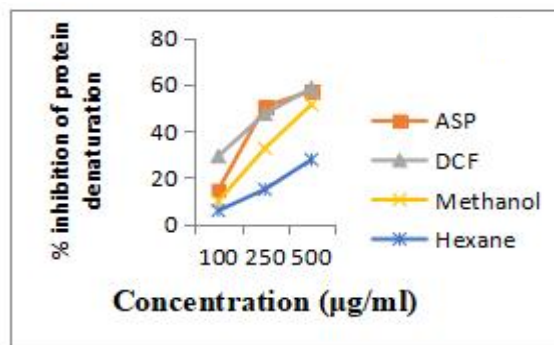


Figure 3: Graph of Test sample concentration against % inhibition of Protein denaturation

Figure 4 displays the graph of test sample concentration against the percentage of membrane stabilization. The graph demonstrates a similar trend as described in the table, with the methanol fraction showing higher percentage inhibition of hemolysis compared to the hexane fraction at all concentrations. However, it is important to note that the difference in membrane stabilization between the two fractions is insignificant at the concentration of 50 µg/ml.

These figures provide visual representations of the data, reinforcing the findings described in the tables and further supporting the antioxidant potential and reducing power of the methanol fraction compared to the hexane fraction.

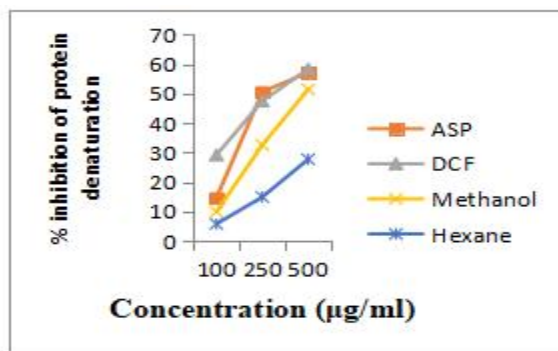


Figure 4: Graph of Test sample concentration against % membrane stabilization.

Table 7 provides information on the effect of increasing concentrations of test samples on the percentage inhibition of hypotonicity-induced hemolysis. The table presents the concentration of the test samples in micrograms per milliliter (µg/ml) and the

Table 7: Effect of increasing concentrations of test samples on % inhibition of hypotonicity-induced hemolysis

Concentration (µg/ml)	Methanol Mean % inhibition ±SD	Hexane Mean %inhibition ±SD
50	66.33 ± 7.22 ^a	59.86 ± 1.56 ^b
100	72.45 ± 8.56 ^a	66.33 ± 2.04 ^b
250	80.10 ± 3.86 ^a	73.81 ± 1.56 ^b
500	85.71 ± 1.44 ^a	79.59 ± 2.04 ^b
IC ₅₀ (µg/ml)	221.24 ^a	239.23 ^b

mean percentage inhibition of hemolysis for the methanol and hexane fractions. The values are expressed as mean ± standard deviation. The table also includes the IC₅₀ values for both fractions.

The data in the table indicates that the methanol fraction exhibits a higher percentage inhibition of hemolysis compared to the hexane fraction at all concentrations tested. However, the difference in inhibition between the two fractions is not statistically significant at the concentration of 50 µg/ml. The mean percentage inhibition of hemolysis for the methanol fraction increases as the concentration of the test sample increases. On the other hand, the hexane fraction shows a lower percentage inhibition of hemolysis that gradually increases with increasing concentration. The IC₅₀ values for the methanol and hexane fractions are 221.24 µg/ml and 239.23 µg/ml, respectively. These results indicate that the methanol fraction exhibits slightly greater effectiveness in inhibiting hypotonicity-induced hemolysis compared to the hexane fraction.

The data in the table is presented as mean ± standard deviation. The letters used in the table represent the level of significance among the different groups. Groups with different letters indicate significant differences between them, while groups with the same letters are not significantly different. The values in the table are expressed as mean ± standard deviation, and the data was obtained from three replicates (n=3).

The data in the table is presented as mean \pm standard deviation. The letters used in the table represent the level of significance among the different groups. Groups with different letters indicate significant differences between them, while groups with the same letters are not significantly different. The values in the table are expressed as mean \pm standard deviation, and the data was obtained from three replicates ($n=3$).

DISCUSSION

Moringa oleifera is a plant that has gained attention for its nutritional and medicinal properties. The proximate analysis of *Moringa oleifera* leaves reveals that they are rich in proteins and carbohydrates, with moderate amounts of ash, fiber, fats, and moisture [39]. This composition makes *Moringa oleifera* leaves a good source of essential nutrients needed for proper bodily functions.

Qualitative phytochemical screening using methanol and hexane solvents indicates the presence of phenols, flavonoids, alkaloids, saponins, and tannins in *Moringa oleifera* leaves, while phlorotannins are absent. These compounds possess antioxidant and anti-inflammatory properties, making *Moringa oleifera* leaves a potential source of essential nutrients and valuable for functional foods and nutraceutical development [40, 41].

In this study, the use of methanol as a solvent for extraction resulted in higher concentrations of phlorotannins, flavonoids, and phenols compared to hexane. Additionally, moderate amounts of alkaloids and tannins were found in the crude plant sample. These compounds have been shown to offer various health benefits such as reducing cholesterol levels, regulating blood sugar levels, and promoting wound healing. These findings align with previous studies emphasizing the significant impact of solvent

selection on the concentration and types of phenolic compounds extracted from plant materials [42].

Numerous studies have been conducted on the nutritional and medicinal properties of *Moringa oleifera* leaves. For instance, research has shown that these leaves possess antioxidant and anti-inflammatory properties, which can help reduce the risk of chronic diseases. They have also been found to aid in regulating blood sugar levels, making them a potential treatment for diabetes [43].

Based on the results of this study, it is evident that *Moringa oleifera* leaves hold significant nutritional and medicinal value that can be utilized in the development of functional foods and nutraceuticals [44]. The research underscores the importance of selecting appropriate solvents for extracting specific phenolic compounds from plant materials. This information is beneficial for researchers studying the bioactivity of these compounds and industries interested in utilizing plant extracts as natural sources of antioxidants and other bioactive compounds [45].

Phenolic compounds, known for their antioxidant properties, have been extensively studied for their potential health benefits. The results of this study, which observed higher concentrations of phenolic compounds in methanol extracts compared to hexane extracts, are consistent with previous research [46]. The polarity of the solvent used for extraction affects the solubility of different phytochemicals, with methanol being a more polar solvent than hexane [47]. This could explain the higher concentration of phenolic compounds observed in the methanol fraction.

The study also demonstrated that the methanol extract of the test samples exhibited stronger antioxidant activity compared to the hexane extract. These findings are in line with

previous studies that have reported higher antioxidant potential in methanol extracts [48,49]. The protein denaturation assay used in the study is a well-established method for evaluating the anti-inflammatory and anti-oxidative potential of natural products. The results indicate that the methanol extract has a greater ability to prevent protein damage caused by oxidative stress, highlighting its anti-inflammatory potential.

Overall, this study provides valuable insights into the bioactive potential of *Moringa oleifera* leaves and emphasizes the importance of selecting appropriate solvents for extracting specific phenolic compounds. These findings are particularly valuable for researchers and industries interested in utilizing plant extracts as natural sources of antioxidants and other bioactive compounds [50-51].

Furthermore, the study highlights the antioxidant potential and reducing power of *Moringa* leaves, consistent with previous research emphasizing the benefits of plant-based products as antioxidants. Specifically, the methanol fraction of *Moringa* leaves exhibited stronger antioxidant potential than the hexane fraction, which is in line with studies indicating higher antioxidant potential in methanol extracts. The obtained IC₅₀ values also suggest that the methanol fraction has a stronger antioxidant potential than the hexane fraction, similar to a study on the antioxidant activity of different solvent extracts of *Capparis spinosa* L. leaves [52].

Additionally, the study demonstrates that the methanol fraction possesses moderate reducing power, while ascorbic acid exhibited the highest reduction, consistent with other studies highlighting the strong antioxidant potential of ascorbic acid. The higher reducing power of the methanol fraction could be attributed to its high content of

phytochemicals, including phenols, flavonoids, and proanthocyanidins, known for their antioxidant potential [53-55].

Moreover, the results indicate that the methanol fraction has a higher % inhibition of protein denaturation and hemolysis compared to the hexane fraction, consistent with studies emphasizing the cytoprotective effects of plant-based extracts. The dose-dependent inhibitory effect on hypotonicity-induced hemolysis in human erythrocytes aligns with previous studies demonstrating the potential of natural plant extracts in inhibiting hemolysis [56, 57].

In conclusion, this study provides significant insights into the bioactive potential of *Moringa oleifera* leaves and highlights the importance of selecting appropriate solvents for extracting specific phenolic compounds. The findings contribute to the development of natural antioxidants and functional foods with potential health benefits.

CONCLUSION

The study indicates that *Moringa oleifera* leaves hold promise as a potential source of bioactive substances with both medicinal and nutritional significance. It highlights the antioxidant and anti-inflammatory potential of the plant extracts, with the methanol extract exhibiting stronger activity compared to the hexane extract. These findings suggest that *Moringa oleifera* leaves can be further explored for the development of functional foods and nutraceuticals. Moreover, the study underscores the importance of selecting appropriate solvents for extracting specific phenolic compounds to effectively investigate their bioactive properties. The results emphasize the value of utilizing plant extracts as natural reservoirs of antioxidants and other bioactive compounds.

Recommendations

The study presents important insights into the phytochemical composition, antioxidant, and anti-inflammatory properties of *Moringa oleifera* leaves' methanol and hexane extracts. However, additional research is required to delve into the possibilities offered by other solvents and fractions. Further investigations are needed to explore the bioavailability and potential toxicity of the bioactive compounds. It is crucial to evaluate the effects of these extracts in animal models and conduct human clinical trials to assess their potential benefits and risks. Additionally, exploring the potential of other plant parts could lead to the development of novel functional foods and nutraceuticals.

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