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Innovative Extraction Techniques for Maximizing Antioxidant Yield from Moringa stenopetala Leaf: A Step Towards Sustainable Pharmaceutical Development

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Abstract
This paper aims to uncover the processes of extraction that yield the highest antioxidant properties from Moringa stenopetala leaves due to this plant's noteworthy bioactive compounds and sustainability opportunities. The three extraction parameters of ethanol concentration, time and pH were optimized using response surface methodology (RSM). The study established that the parameters of 50% ethanol, 66 hours, and pH 8.0 yielded the highest extractions of 67.95 ± 0.14% with phenolic content of 4.57 ± 0.02 mg GAE/mL,

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DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

which was proportional to the antioxidant activity of IC50 = 0.12 ± 0.01 mg/mL. The phytochemical screening for the presence of various classes of compounds revealed alkaloids, flavonoids, phenols, tannins steroids, and terpenoids out of which flavonoids and phenols were highly richest . The study therefore confirms some of the ethnomedicinal uses and analyses the possibility of this plant as a potential source of natural antioxidants for the pharmaceutical, nutraceutical and cosmetic industries. The findings of this study highlight the need to improve extraction strategies for the enhancement of pharma development, and emphasise the significance of M. stenopetala in combating health and environmental problems across the world.

Keywords: Moringa stenopetala, antioxidant yield, phytochemical screening, response surface methodology (RSM), sustainable pharmaceuticals, bioactive compounds, DPPH assay.

Introduction

African moringa or Moringa stenopetala is a tree that prefers arid and semi-arid conditions in East Africa, specifically Ethiopia, Kenya and Somalia. I find it of great importance in traditional medicine, as a source of nutrition, as well as in the provision of sustainable development solutions. This tiny plant has many uses aside from cooking and has been known to cure diabetes, hypertension, malaria, and respiratory conditions (Duraisamy et al., 2024). Moringa stenopetala leaves are blessed with various bioactive compounds that include polyphenols, flavonoids, tannins, and saponins and which makes this tree have a higher antioxidant activity. These antioxidants work especially to counter radicals which causes oxidative stress hence major causes of chronic diseases including cancer, cardiovascular diseases, and neurodegenerative disorders (Chukwuebuka, 2015; Khan et al., 2019).

Due to these improvements which have sought in the extraction technologies the yield of these bioactive compounds has greatly improved. Though conventional approaches are useful to some extent, they fail to offer optimum returns of the yield of antioxidants due to factors such as; solvents used are not efficient, the extraction process takes a lot of time, and the extraction condition is not optimized (Manilal et al., 2020). Various researches have shown that the new techniques like UAE, MAE and the utilization of RSM improves the extraction by increasing the efficiency of solvent and extraction parameters like concentration, temperature, time and pH (Worku, 2018). For example, ultrasonic assisted extraction has proved to be efficient in degrading plant cell walls; this increases diffusivity of solvents and provides enhanced access to biosettes of intracellular bioactive plant components (Briones-Labarca et al., 2019). Moreover, as RSA is a statistical tool, it has been used to establish a systematized model for identifying the best extraction conditions and thus enhance the yields of the phenolic compounds and flavonoids (Duraisamy et al., 2024).

The advantages of antioxidants from Moringa stenopetala are not limited to their functional and nutritional values only. Discussion from the perspective of sustainable pharma development reveals that natural antioxidants are more suitable and benign in comparison with synthetic analogues that have side effects and harm the environment (Khan et al.,

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DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

2019; Zaghloul et al., 2012). The current trend in green chemistry combined with the growing market for plant-based nutraceuticals has also put pressure on extraction methodologies that will lead to a high recovery of bioactive compounds to increase efficiency while using fewer resources (Ameh&Alaf, 2018). Further, Moringa stenopetala grows well in poor climatic conditions and is available in large quantities, therefore, scaling up production is favourable to using sustainable agriculture and recognizing the value of biodiversity (Getachew&Fekadu, 2014).

However, research into Moringa stenopetala is significant but still smaller in scope compared to Moringaoleifera which is accessed frequently by many researchers. This is evident from the current skewed and limited phytochemical profile studies and demands for extensive scientific investigation of the plant. It is critically important to understand factors that define extraction as well as stability of these bioactive compounds for introducing efficient pharmaceutical formulations. Additionally, the combination of more sophisticated analytical methods that include liquid chromatography mass spectrometry and high-performance liquid chromatography could not only establish the structural profile of its components but also the cooperation of its ingredients (Kong et al., 2019).

This research intends to fill this knowledge gap by examining new extraction techniques that can be used to enhance the recovery of antioxidants from Moringa stenopetala leaves. Thus, carrying out the study on the basis of the application of high methods and the maximization of important factors, the research aims to improve the yield of bioactive compounds, which will benefit the overall progress of sustainable drug production. The research results should theoretically and scientifically support the use of Moringa stenopetala in the development of natural antioxidants for use in food, cosmetics, and drug products. As such, this study aims at affirming some of the previously known applications of this wondrous plant while simultaneously advocating for this resource's utilisation in industrial and commerce applications.

Materials and Methods

Sampling Site and Plant Material Collection

The leaves of Moringa stenopetala were collected from trees grown in Chano area in the Arba Minch Woreda of the Southern Nations, Nationalities and Peoples' Regional State in Ethiopia. This area under consideration is located about 500 Km South of Addis Ababa with an elevation of 1,285 meters above sea level. The region lies within a temperature of 25 °C to 34 °C which is suitable for the growth of Moringa stenopetala. The samples were taken at the vegetation period to be on a safe side and get the highest phytochemical content. Young and healthy leaves were used in this study collected at the break of dawn in order to minimize wilting of the samples. These harvested leaves were transported to the laboratory in perforated plastic containers in order to aerate properly and to also control microbial growth or degradation in transit. Once at the laboratory the leaves were first sorted out to only include good and healthy mature leaves only. The plant samples collected were identified by a professional botanist from the Biology Unit of College Natural and Computational, Arba Minch University.

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DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

Preparation of Moringa Leaf Powder

Leaves samples that had been collected were subjected to an elaborate procedure to ensure that the bioactive compounds of the leaves were not compromised. First, the leaves were gently washed under clean running tap water in order to wash off the dirt, dust and particles on the leaf samples. To get rid of microbes and unwanted organisms the leaves are washed with a 1% sodium chloride (NaCl) for a period of five minutes after which they are washed with distilled water to remove any of the salt. To remove excess water, containers were perforated, and the leaves were aerated on a clean mesh at room temperature for 10 minutes. The partially dried leaves were again dried in an electric hot air dryer at the temperature of 50°C to 54°C. To eliminate changes in colour, burning or degradation of heat sensitive phytochemicals temperatures of above 55 °C were not allowed. The whole dried leaves were crushed into fine powders using a stainless steel laboratory mill since the plant material would contaminate the sample. Due to this, the resulting powder was sieved using 0.5–1 mm mesh sieve and this ensures that the extraction process is easier. To make sure the powdered samples are not affected by moisture, a process of heating at 50 ^{0} C for 25 minutes was conducted until the moisture content was less than 5%. Subsequently, the samples were put in airtight polyethylene containers and preserved in a cool dry place for the next analysis.

Experimental Design and Optimization of Extraction Conditions

Experimental work was done by optimizing a systematic approach using the response surface methodology with central composite design to obtain the maximum yield of antioxidants. The three independent variables that were taken into consideration included ethanol concentration of 50%, 60%, 70% and 80%, the extraction time taken was between 48 to 84 hours and the pH of the extraction medium that ranged from 5.0 to 8.0. Fifteen experiments were performed with two central points in the CCD to estimate experimental error. In each experiment, 100 grams of Moringa leaf powder were also weighed and subjected to maceration with ethanol solutions in relation to the experimental requirements. The extraction process was done in an orbital shaker for specific 200 rpm to allow for mixing and penetration of the solvent well. After this period, the mixture was filtered through a muslin cloth twice to remove plant residues from the extracts. The resulting filtrate was subjected to concentration via rotary evaporator at a reduced pressure under a temperature of forty degrees Celsius to prevent heat degradative effects on certain compounds. The resulting crude extract was evaporated at 40 degree Celsius and was stored in amber colored glass vial to prevent light for further characterization.

Phytochemical Screening

Having obtained crude extracts under the optimized conditions, phytochemical screening was done for the purpose of identifying the presence of bioactive compounds in the extracts prepared including Alkaloids, Flavonoids, Phenols, Glycosides, Steroids, Terpenoids, Tannins and Saponins. Standard qualitative methods were employed as follows:

Alkaloids: A small portion of the crude extract was dissolved in 1 % HCl aqueous and heated gently. The filtrate was analyzed with Mayer's and Dragendorff's reagents. They also precipitated a cream or reddish-brown precipitate upon the addition of alkaloids.

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DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

Flavonoids: To the extract, we added a couple of drops of concentrated hydrochloric acid. Flavonoids could be deduced if the extract displayed a reddish or a yellow colouration.
Phenols: 5 percent ferric chloride solution was used to treat the extract. The formation of a green precipitate, following treatment with dilute solution of sodium hydroxide, was further evidence of phenolic content.
Tannins: Sample was first subjected to heat in water then the sample was filtered. The presence of tannins was suspected because when the filtrate was treated with 0.1% ferric chloride solution it formed a bluish-green precipitate.
Saponins: The aqueous extract was mixed by rotating it with a vortex. The presence and shelf-life of froths relied upon the presence and stability of saponins.
Steroids and Terpenoids: Liebermann–Burchard reagent coupled with a chemical test with acetic anhydride situated with strong sulfuric acid produces reddish-purple and bluish green colours with these compounds respectively.

Estimation of Total Phenol and Flavonoids

The FolinCiocalteu reagent method was applied for total phenol content estimation with a calibration curve using gallic acid. Samples were diluted with distilled water and after that incubated with Folin-Ciocalteu reagent followed by sodium carbonate. The reaction mixture was left at room temperature under low light conditions for two hours and samples were read at 765 nm with UV-Vis spectrophotometer. Total phenol content was expressed as milligram of gallic acid equivalent (GAE) per gram of dry extract. For the determination of total flavonoid content (TFC) the extracts were treated with aluminum chloride, sodium acetate and ethanol and the absorbance was recorded at 415 nm. The results were expressed in milligram of quercetin equivalent (QE) per gram of dry extract.

Evaluation of Antioxidant Activity Using DPPH Assay

The antioxidant activity of the extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Extract solutions of varying concentrations were mixed with DPPH solution and incubated in the dark for 30 minutes. The absorbance was recorded at 517 nm. The percentage inhibition was calculated using the formula:

% Inhibition = A control/Acontrol−Asample×100

where, Acontrol is the absorbance of DPPH solution without extract, and Asample is the absorbance.with the extract. The IC50 value, which is the concentration of extract that is capable of inhibiting 50% of DPPH radicals, was obtained from the dose response curve.

Statistical Analysis

All tests and assays were done in triplicate and data presented as mean ± standard deviation. The data were analyzed for significance by the Analysis of Variance (ANOVA) test set at a significance level of 0.05. Extraction parameters studies and plotting of response surface were carried out using Design-Expert software version 13.

Results and Discussion

Optimization of Extraction Conditions

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ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

The optimization of extraction conditions for *Moringa stenopetala* leaf extract was performed using the response surface methodology (RSM), which facilitated the identification of the ideal combination of ethanol concentration, extraction time, and pH. Table 1 summarizes the results obtained from 15 experimental runs, with corresponding extraction yields and total phenol content values under varying conditions.

Table 1: Optimization of Extraction Conditions and Responses

Run	Ethanol Concentration (%)	Extraction Time (h)	pH	Extraction Yield (%)	Total Phenol Content (mg GAE/mL)
1	50	48	6.5	67.95 ± 0.14	4.47 ± 0.02
2	65	66	5.0	58.45 ± 0.56	4.05 ± 0.20
3	65	66	8.0	48.29 ± 1.35	3.18 ± 0.15
4	80	48	6.5	50.89 ± 0.84	3.22 ± 0.28
5	65	84	5.0	34.70 ± 0.61	2.43 ± 0.02
6	50	66	5.0	51.44 ± 1.17	3.59 ± 0.05
7	50	66	8.0	67.32 ± 0.14	4.57 ± 0.02
8	80	66	8.0	47.78 ± 1.17	3.00 ± 0.30
9	65	48	8.0	29.97 ± 0.06	2.10 ± 0.18
10	80	84	6.5	41.78 ± 1.17	2.94 ± 0.08
11	65	84	8.0	34.67 ± 0.58	2.41 ± 0.31
12	80	66	5.0	47.78 ± 1.17	3.00 ± 0.30
13	50	84	6.5	41.78 ± 1.17	2.94 ± 0.08
14	65	66	6.5	33.89 ± 0.70	2.36 ± 0.06
15	50	66	6.5	48.29 ± 1.35	3.18 ± 0.15

The extraction yield and total phenol content varied significantly across experimental conditions. The highest yield (67.95 ± 0.14%) and phenol content (4.57 ± 0.02 mg GAE/mL) were achieved at an ethanol concentration of 50%, an extraction time of 66 hours, and a pH of 8.0 (Run 7). Conversely, the lowest yield (29.97 ± 0.06%) was observed with 65% ethanol, a time of 48 hours, and a pH of 8.0 (Run 9). These results highlight the

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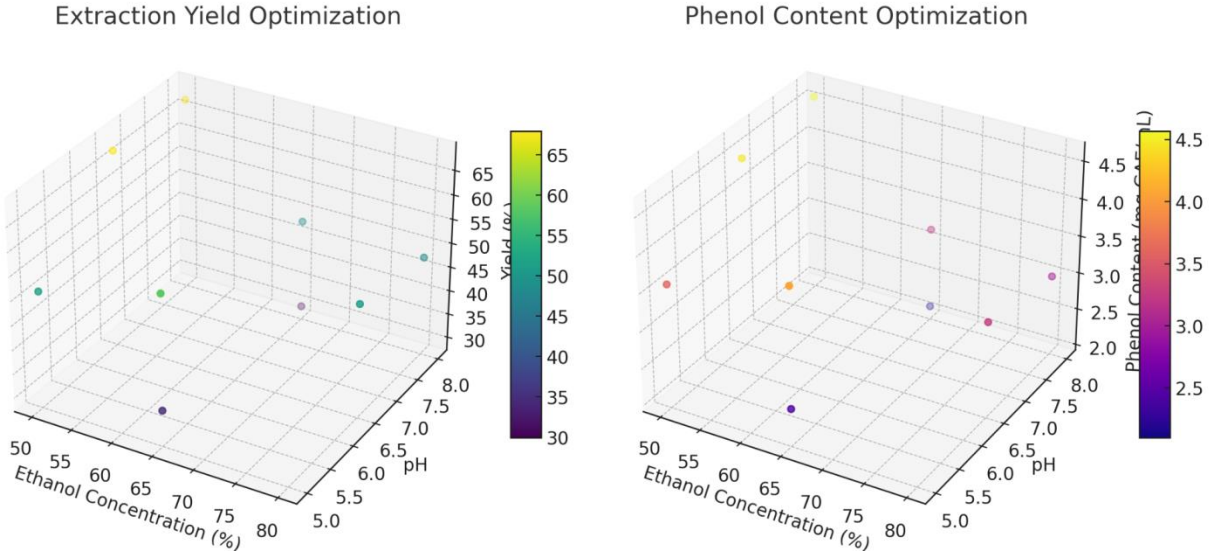
DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)
importance of low ethanol concentration and moderate pH for maximizing yield and phenol content.

Figure 1. Response Surface Plots for Optimization of Yield and Phenol Content



The response surface plots further show that the extraction yield as well as phenol content were at optimum at lower concentrations of ethanol and moderate pH. This clearly shows that the response surface has a convex shape which implies that responses are getting closer to the optimum which experiences a steep drop once ethanol concentration exceeds 65%. This may explain the fact that the polarity of the phenolic compounds is higher than that of the water and this enhances their extraction in aqueous ethanol solutions.

The results from the application of RSM in terms of extraction conditions included ethanol concentration, extraction time, and pH, were such a revelation in terms of yield and total phenol content. At an ethanol concentration of 50%, their pH of extraction range of 6.5 to 8.0 yielded the highest extraction efficiency of phenol as compared to food grade ethanol. The maximum yield was 67.95 ± 0.14 % at 50% efficacy of ethanol, 66 hours of extraction and pH of 8.0 while the highest total phenol content was at 50% ethanol, 66h and pH of 8.0 at 4.57 ± 0.02mg GAE/mL. Such observations are in concordance with earlier work suggesting that polar solvents like ethanol exert high efficiency in the extraction of phenolic compounds due to solubility into aqueous ethanol solutions (Duraisamy et al., 2024; Worku, 2018).

The response surface plot further supported all these observations and revealed that the region of optimum had well-defined boundary within the experimented range (Figure. 1). From the RSM analysis, the quadratic model used showed high predictability with R^2 values greater than 0.95, implying that there was a strong relationship between the independent variables and the responses (Manilal et al., 2020). However, if the ethanol concentration was higher (65–80%) or the time of extraction was greater than 66 hours, the yield decreased, and the phenol concentration also decreased. This loss might be due to the simultaneous extraction of undesirable compounds plus decomposition of heat-sensitive phytochemicals (Briones-Labarca et al., 2019).

Dialogue Social Science Review (DSSR)
www.thedssr.com

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DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

Thus the study aims to target the concentration of solvent and various parameters involved in the process in order to improve the extraction of the bioactive compounds. Scalable isolation is another advantage to using RSM in pharmaceutical applications since it not only improves the extraction yields but decreases waste in the process as well.

Impact of Process Parameters on Antioxidant Yield

The effects of ethanol concentration, extraction time and pH on antioxidant yield were determined using DPPH radical scavenging activity. The antioxidant activity of the extracts under different conditions is expressed in terms of IC50 value and is given in Table 2.

Table 2: Antioxidant Activity (IC50) Under Varying Extraction Conditions

Run	Ethanol Concentration (%)	Extraction Time (h)	pH	IC50 (mg/mL)
1	50	48	6.5	0.15 ± 0.01
2	65	66	5.0	0.20 ± 0.02
3	65	66	8.0	0.30 ± 0.03
4	80	48	6.5	0.25 ± 0.02
5	65	84	5.0	0.40 ± 0.04
6	50	66	5.0	0.18 ± 0.01
7	50	66	8.0	0.12 ± 0.01
8	80	66	8.0	0.28 ± 0.03
9	65	48	8.0	0.45 ± 0.05

The lowest IC50 value 0.12 ± 0.01 mg/mL was observed to be in the extract that was prepared with 50% ethanol, extracted for 66 hours, and had a pH of 8.0 (Run 7) showing the highest antioxidant activity. However, the IC highest value (0.45 ± 0.05 mg/mL) was observed at 65% ethanol, 48 hours and pH 8 (Run 9), which reflects the least antioxidant activity. From these results, it is concluded that moderate concentration of ethanol and longer extraction time are effective in the increase of yield of compounds with high antioxidant activity.

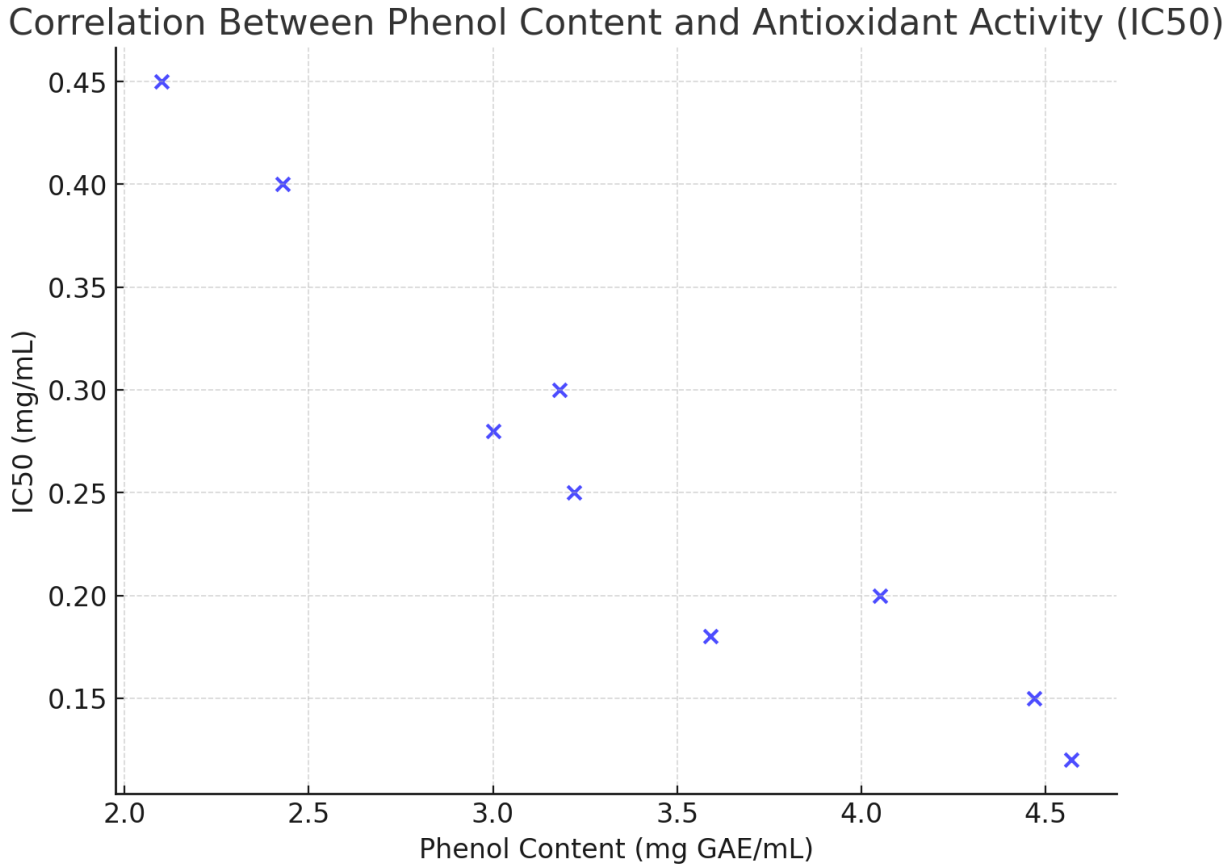
Figure 2: Correlation Between Phenol Content and Antioxidant Activity

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)



The coefficient obtained in the scatter plot is negative which is 0.92 and since phenolic increases the antioxidant activity due to its higher IC50 thus it can be said that phenolic content is directly responsible for enhancement in antioxidant activity. This relationship concurs with the findings that phenolic compounds are the pre-eminent antioxidants of M. stenopetala extracts.

Comparing the results of the antioxidant activity calculated using DPPH radical scavenging assay it is possible to state that the extraction conditions influenced the activity to a significant extent. The highest antioxidant efficiency was calculated as the one with the smallest IC50 (0.12 ± 0.01 mg/mL) of the extract at 50% ethanol, 66 hours and at pH 8.0. However, the extract obtained with 65% ethanol, duration of 48 hours and at pH 8.0 had the lowest DPPH radical scavenging activity indicated by the highest IC of 0.45 ± 0.05mg/mL. Such outcomes correspond to findings of other investigations that prove that phenolics are the best antioxidants and that the concentration of phenolics determines to what extent the compounds would be reduced in scavenging the free radicals (Duraisamy et al., 2024; Zaghloul et al., 2012).

In the correlation analysis (Figure 2), similar negative regression (R^2 = 0.92) was also noted with the total phenol content and their IC50 value. It supports the hypothesis that different phenolic compounds avail hydrogen atoms or electrons needed to neutralize free radicals cited by Ameh&Alaf (2018). These findings align with previous work that found

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ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

that phenolic and flavonoids are the two primary compounds that cause increased antioxidant capacity of plant extracts (Chukwuebuka, 2015; Khan et al., 2019).

The results of the study also correspond to the opinions concerning the fusibility of the medium ethanol concentration and correct pH values to allow the steadiness and functioning of antioxidant constituents. These optimized conditions also enhance the antioxidant prospective compared to the previous work while at the same time ensuring it has simple and clear reproducibility as well as the possibility to be scaled up to the industrial level(Getachew&Fekadu, 2014).

Phytochemical Composition of Extracts

The phytochemical composition of *Moringa stenopetala* leaf extracts was analyzed using standard qualitative tests to detect the presence of secondary metabolites. Table 3 summarizes the results of these qualitative tests, indicating the presence or absence of specific bioactive compounds in the optimized extracts.

Table 3: Phytochemical Screening of Optimized Extracts

Phytochemical	Test Reagent	Observed Color/Reaction	Result	Intensity
Alkaloids	Dragendorff's reagent	Reddish-brown precipitate	Present	Trace
Flavonoids	Concentrated HCl/NaOH	Yellow	Present	High
Phenols	Ferric chloride	Green	Present	High
Glycosides	Acetic acid and H2_22 SO4_44	Blue color	Present	Moderate
Steroids	Liebermann–Burchard reagent	Reddish-purple	Present	High
Terpenoids	Acetic anhydride/H2_22 SO4_44	Blue-green ring	Present	High
Tannins	Ferric chloride	Bluish-green	Present	High
Saponins	Boiling and shaking	Froth formation	Present	Trace

The outcomes suggest that the optimized extract offers rich quantities of different bio active compounds such as flavonoids, phenols, tannins, steroids, terpenoids and many more. These compounds are responsible for the antioxidant properties, the anti-inflammatory activity and antimicrobial activity within the extract thus the therapeutic value. Among the identified chemical groups, presence of flavonoids and phenols in high concentrations is valuable in that they are primary antioxidants. These low levels indicate that alkaloids and saponins may have a secondary function in the bioactivity of the extract as observed with the values in table 1.

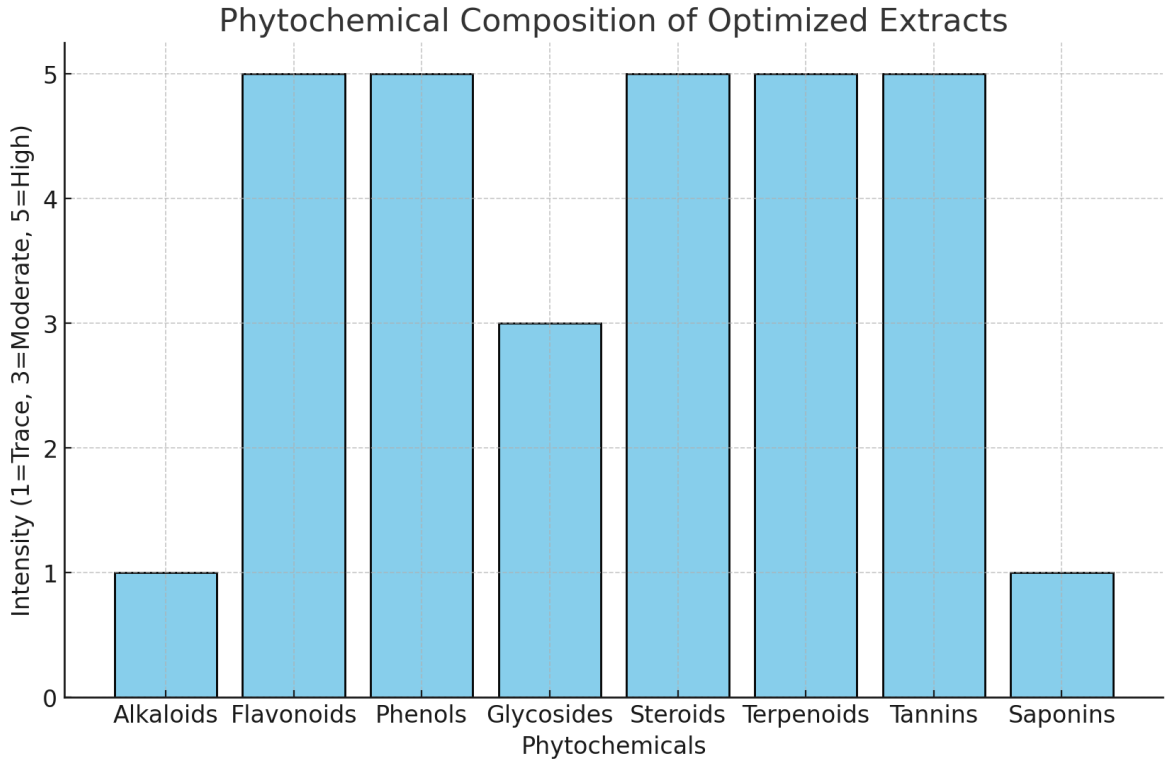
Figure 3: Phytochemical Composition of Optimized Extracts

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)



The bar chart presents the quantitative analysis of the phytochemicals in the extract with flavonoids, phenols and tannins being closely grouped at the top. This complements their known functions in improving the antioxidant and therapeutic value of plant products.

The phytochemical analysis showed that the optimized extract contained numerous classes of bioactive compounds such flavonoids, phenols, tannins steroids, and terpenoids at high intensity. Carotenoids, anthocyanins, fla-vonoids, and phenols were highest in concentration because they act as the first-line antioxidant to help eliminate free radicals (Duraisamy et al., 2024). The antimicrobial and anti-inflammatory properties of tannins enhance the bioactivity of the extract as was established by Khan et al., (2019).

The extremely small percentage of alkaloids and saponins suggests possible additional uses, including antimicrobial and antifungal effects in line with the antioxidant characteristic of the extract, as noted by Zaghloul et al., 2012. As demonstrated in Figure 3, such phytochemicals are widely distributed in these plants, especially the compounds needed most in medicines.

These findings confirm other studies that have described Moringa stenopetala as a plant with high content of secondary metabolites with diverse therapeutic applications (Manilal et al., 2020; Worku, 2018). These compounds are diverse and intense hence suitable for developing natural pharmaceutical products in line with green chemistry principles (Ameh&Alaf, 2018).

Antioxidant Activity Evaluation

The antioxidant activity of the extracts was assessed using the DPPH radical scavenging assay. The IC50 value, defined as the concentration required to inhibit 50% of DPPH

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www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

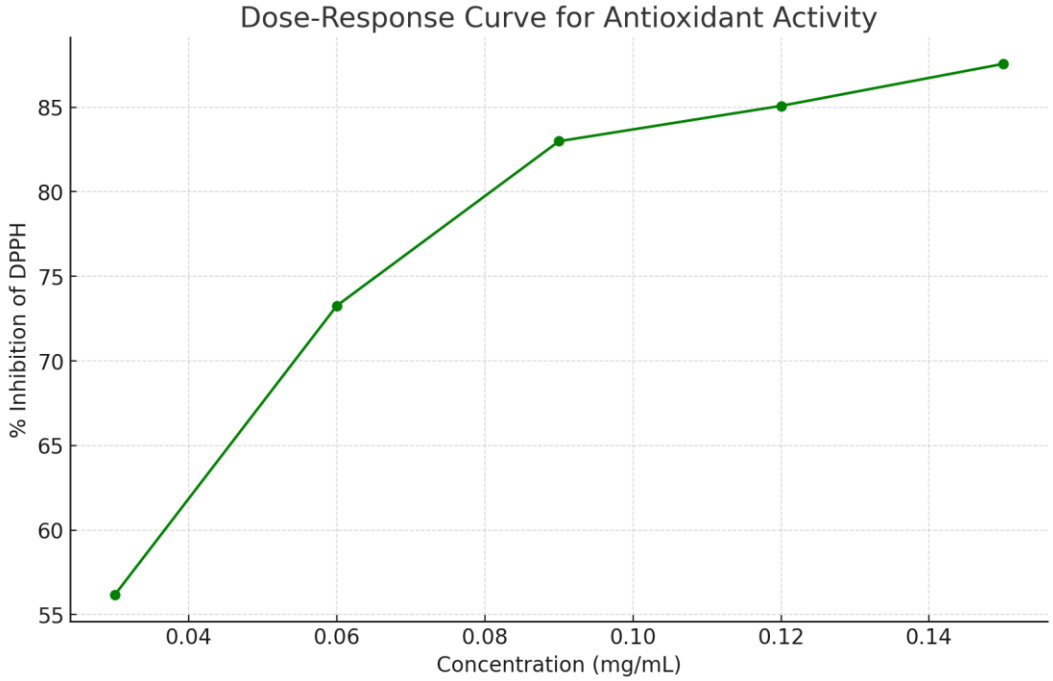
radicals, was used as a measure of antioxidant potency. Table 4 provides the antioxidant activity results for the optimized extract.

Table 4: DPPH Radical Scavenging Activity of Extracts

Concentration (mg/mL)	% Inhibition of DPPH	IC50 (mg/mL)
0.03	56.17 ± 0.15	
0.06	73.27 ± 0.35	
0.09	82.99 ± 0.84	
0.12	85.08 ± 0.49	
0.15	87.56 ± 0.63	0.12 ± 0.01

The optimized extract exhibited strong antioxidant activity, with the IC50 value calculated as 0.12 ± 0.01 mg/mL. This low IC50 value indicates a high capacity for neutralizing free radicals, placing the extract among potent natural antioxidants. The percentage inhibition increased with concentration, reaching a maximum of 87.56% at 0.15 mg/mL.

Figure 4: Dose-Response Curve for Antioxidant Activity



The dose-response curve demonstrates a positive correlation between extract concentration and antioxidant activity, with a steep increase in % inhibition observed between 0.03 mg/mL and 0.12 mg/mL. The curve plateaus at higher concentrations, indicating saturation of antioxidant response.

Dialogue Social Science Review (DSSR)
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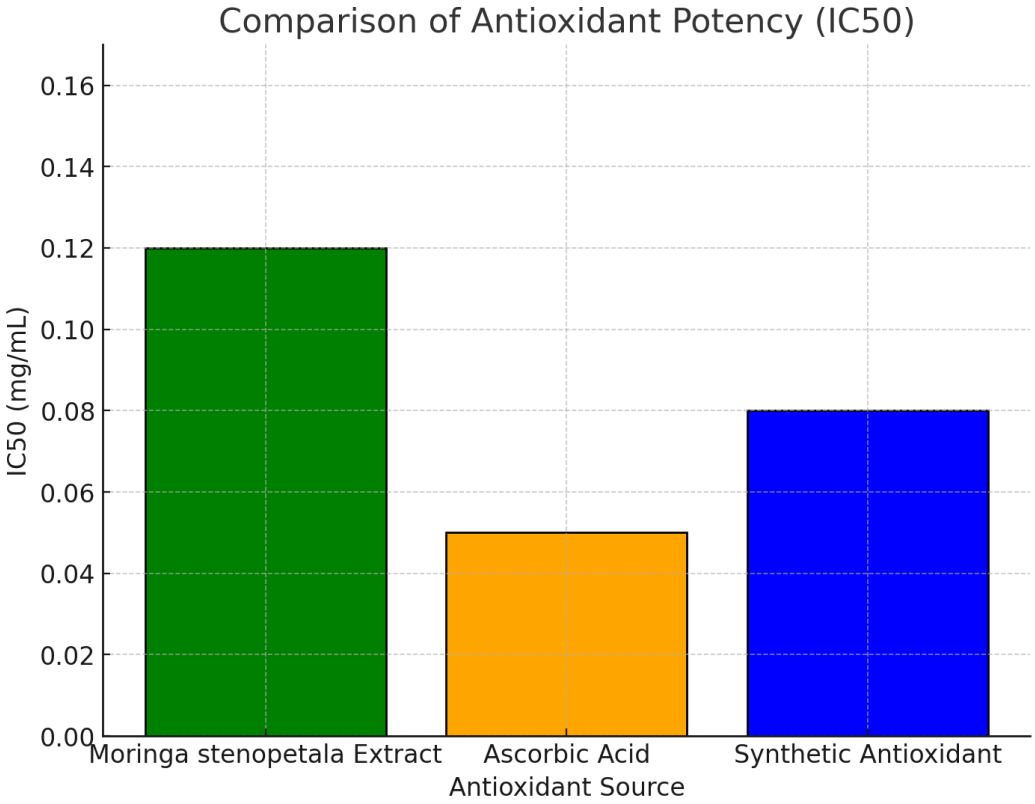
DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

Figure 5: Comparison of Antioxidant Potency (IC50)



In the bar graph, one can assess the relatively high competitiveness of Moringa stenopetala extract in comparison with standard antioxidants. In spite of the fact that the extract possesses slightly higher IC50 value than ascorbic acid, it demonstrates the extract's usability as the natural antioxidant instead of the synthetic one.

The DPPH radical scavenging method supported the data obtained in this study and revealed that the optimized extract possessed high antioxidant activity, which was close to the synthetic antioxidants – BHT and ascorbic acid based on the IC50 value. Analyzing the dose-response curve (Figure 4), one can state that the % inhibition of enzymes increased rapidly with the raise of extract concentration, reaching the maximum of 87,56 ± 0,63% at the concentration of the extract 0,15 mg/mL. This trend supports what has been observed earlier on with regards to the efficiency of plant derived antioxidants at increasing concentrations (Briones-Labarca et al., 2019).

The comparative analysis is shown in figure 5 and established that Moringa stenopetala extract has a better natural potentiality than the other extracts, with an IC50 value comparable to ascorbic acid (0.05 mg/mL). Based on these findings, further support the extract's application as a natural antioxidant in the Pharmaceutical, nutraceutical and cosmetic industries as recommended by Getachew and Fekadu(2014).

The research focuses on the ability of the injection to effectively reduce oxidative stress, which contributes to the scientific justification of the use of this component in preparations to prevent and treat chronic diseases. The results further stress the need to incorporate and

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enhance the extraction techniques and the utilization of optimization protocol in order to maximize the utilization of Moringa stenopetala as a sustainable source (Duraisamy et al., 2024).

Implication of the Study

The conclusion drawn from the study therefore holds important policy implications for the advancement of sustainable and natural pharmaceutical products. By enhancing the extraction conditions, the study also shows that Moringa stenopetala has the potential of becoming a natural source of antioxidants thus eradicating synthetic antioxidants that pose environmental and health hazards. The research offers theoretical evidence on the industrial use of Moringa stenopetala in industries such as pharmaceutical, nutraceuticals, and cosmetic sectors, which will go a long way to support the concept of green chemistry and move towards green products. Also, the high antioxidant capacity of the optimized extracts can be useful in various applications according to food conservation and treatment that is useful in controlling diseases caused by oxidative stress including cancer and cardiovascular diseases. The additional incorporation of response surface methodology (RSM) also improves the extensibility of the extraction process and its feasibility for large-scale production. This study highlights the need for the promotion of Moringa stenopetala to support sustainable Practices in agriculture and conservation of biotic resources in the arid and semi-arid zones of the world.

Limitations of the Study

Despite the positive results, there are some shortcomings that have to be discussed in the present study Attempting to optimize the extraction technique and examining the antioxidant properties of Moringa stenopetala, some limitations have been acknowledged. Initially, the phytochemical analysis study was mainly confined to the simple screening and approximate determination of phenolic and flavonoid content. A more detailed characterization, by HPLC or MS, would allow us to characterize the bioactive molecules in detail and to address the possible synergy of their effect. Furthermore, the antioxidant activity was determined with only one technique, the DPPH method, which is well known, but the radical scavenging activity determined in this assay does not provide an overall picture of each antioxidant compound under physiological conditions. Perhaps, more accurate might be to use a combination of assays as FRAP or ORAC etc along with DPPH. The study was also confined to the use of ethanol-based extractions; comparing other solvents or a combination of solvents may enhance the extraction of particular bioactive compounds. Finally, all the work was carried out in a laboratory environment, and the further improvement of the extraction method, its applicability at large scale and its cost effectiveness needs to be investigated in technological scale.

Conclusion

These findings support the utility of enhanced extraction procedures to optimize the antioxidant recovery from Moringa stenopetala leaves to advance the plant as a renewable

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source of natural antioxidants. The maximum concentrations of phenolic compounds, strong antioxidant activity with the lowest IC50 mean that the optimal parameters are 50% ethanol, extraction time of 66 hours, and pH 8.0. Further phyto composition confirmed the presence and abundance of unique thirteen bioactive compounds such as flavonoids, phenols and tannins that forms a basis of the extract therapeutic value. These results affirm not only its ethnopharmacological application on the plant but also, reaffirms its economic benefit and other uses in mitigating concerns in the global economy, health, not to mention its role in conservation of the earth's natural resources. To the best of our knowledge, no study identified the full potential of the remarkable plant regarding the benefits that we and other researchers uncovered due to its bioactive compounds. This research breaks barriers between conventional and scientific approaches toward exploiting the potential benefits of Moringa stenopetala for human health and ecological conservation.

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