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Research Article

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Phytochemical Screening, and Antioxidant Activity of Methanolic Extract and Solvent Fractions of the Stem Bark of *Moringa Stenopetala Bak. Cuf.*

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Abstract

Background: *Moringa stenopetala Bak. Cuf. is a medicinal plant that has been used extensively in Traditional Medicine in both Ethiopia and East Africa. Many studies have been conducted regarding the pharmacological properties of the leaves of this plant. However, very little research has been done on the bioactive potential of the stem bark of M. stenopetala.*

Objectives: *The main objective of this study was to determine the phytochemical make-up and antioxidant activity of the methanolic extract and solvent fractions of the stem bark of M. stenopetala.*

Methods: *The extraction of the dried stem bark with absolute methanol, further extractions were performed using fractions of the extract with solvents of chloroform, acetone and water. The preliminary phytochemical screening was done to the extract and solvent fractions using standard methods. The antioxidant activity of all three solvent fractions of the stem bark were evaluated using the DPPH radical scavenging assay.*

Results: *The preliminary phytochemical screening of the solvent fractions revealed that each fraction contained a variety of secondary metabolites, including flavonoids, phenols, tannins, saponins, terpenoids, alkaloids, phytosterols and cardiac glycosides; the quantity of these compounds varied between fractions. The DPPH assay indicate the aqueous and the acetone fractions at a concentration of 100µg/mL showed a strong percentage inhibition of 73.63% and 72.53%, respectively. The chloroform fraction showed moderate activity of 69.23%. When compared to the results of ascorbic acid as a reference standard, which showed a 98.59% percentage inhibition at the same concentration. The antioxidant activity of the crude extract and both solvent fractions is likely due to the presence of phenolic and flavonoid compounds.*

Conclusion: *In conclusion, the results obtained in this study support the traditional use of the stem bark of M. stenopetala as a natural source of antioxidant phytochemicals, as well as providing the necessary justification for future quantitative and bioactivity-guided studies on M. stenopetala.*

Keywords: Antioxidant Activities, DPPH Assay, Medicinal Plants, *Moringa Stenopetala*, Phytochemicals

1. Introduction

Medicinal plants have been an important part of all primary healthcare systems around the globe for centuries, and today. This is especially true for developing nations where these plants are advantageous due to their accessibility, cultural acceptability and high quantity [1,2]. Moreover, diversity of bioactive secondary metabolites concentrated in (or derived from) them, could serve as lead compounds in developing modern medicines [2,3]. In rural Africa, traditional medicine continues to form an important part of local healthcare and provides an alternative method of treating patients. It is valued for its holistic approach and cost-effective methods for delivering therapeutic care [4,5].

The global incidence of diseases associated with oxidative stress is steadily increasing. These diseases include cardiovascular disorders, cancers, diabetes, and chronic inflammatory conditions [6]. Studies have established that oxidative stress results from an imbalance between free radicals and the body's antioxidant defence mechanisms. This imbalance plays a significant role in the development of many diseases [6,7]. Consequently, there is growing interest within the scientific community in identifying natural sources of antioxidants. Medicinal plants, in particular, are being explored for their potential role in disease prevention and the promotion of human health.

The Moringaceae family is represented by one genus: Moringa, made up of thirteen species native to Northeast Africa, Madagascar, Southwest Asia, and parts of Africa [8,9]. Out of these thirteen species, *Moringa oleifera*, *Moringa stenopetala*, *Moringa concanensis*, and *Moringa peregrina* have been particularly attractive for study due mainly to their potential to provide both food nutrients and medicine [10,11]. However, numerous species from this genus still need to be researched, especially those found in Northeast Africa and Madagascar.

Moringa stenopetala Bak. Cuf. is found in Ethiopia, Kenya, and Somalia, where it is abundantly found throughout southern Ethiopia [12,13]. Locally, people refer to it by different names; among them are 'Shiferaw' in Amharic, 'Haleko' in Gofa and Wolayta, and 'Shelagta' in Konso [14]. Different parts of the plant have been said to treat a variety of conditions, such as headaches, stomach pain, malaria, high blood pressure, diabetes, asthma, and digestive problems [15]. People will typically eat the leaves as a vegetable, but the roots and barks can also be boiled into a tea for medicinal uses. Some of the traditional uses for *M. stenopetala* have recently been corroborated by scientific evidence, such as *in vivo* research demonstrating the analgesic and anti-inflammatory effects of leaf extracts of *M. stenopetala* [16]. Analyses of the chemical composition of *M. stenopetala* leaves using chromatographic techniques have indicated that they possess high concentrations of phenolic compounds and flavonoids [1,14].

Several bioactive metabolites, such as isothiocyanates, fatty acids, sterols, glucosinolates, and glycerides, have also been isolated from various tissues of *M. stenopetala*, including the seed, leaf, and root tissues. [17]. While there is a significant amount of ethnomedicinal evidence for *M. stenopetala* and an increasing body of pharmacological evidence supporting its various uses, little research has been conducted examining the phytochemical composition and antioxidant activity of the stem bark of *M. stenopetala* [18]. The stem bark may contain numerous important but underutilized bioactive secondary metabolites. This study aimed to examine the phytochemical composition and assess the *in vitro* antioxidant activity of the methanol extract and solvent fractions of the stem bark of *M. stenopetala* using standard phytochemical techniques and DPPH radical scavenging assays [19].

2. Materials and Methods

2.1. Chemicals and Apparatus

All chemicals utilized in the experiment were of analytical grade and used without further purification. Methanol (99.99%), ethanol (100%), chloroform (98%), acetone (99.8%), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Fine Chemical PLC, Addis Ababa, Ethiopia, and Sigma-Aldrich. Ascorbic acid was used as a reference standard. Reagents used for phytochemical screening included Wagner's reagent (iodine in potassium iodide), ferric chloride, sodium hydroxide, potassium hydroxide, ammonia solution, hydrochloric acid, sulfuric acid, and glacial acetic acid, purchased from Alchem Private Trading Limited Company, Addis Ababa, Ethiopia. Major laboratory equipment used included an electric grinder, orbital shaker, rotary evaporator, vacuum filtration unit, water bath, oven, lyophilizer (Alpha 1-2 LD plus, Martin Christ Co. Ltd., Germany), UV–Visible spectrophotometer (Jenway 6858, England), analytical balance, and standard glassware.

2.2. Plant Material

The fresh and healthy stem bark samples of *moringa stenopetala were* collected from Harar, eastern Ethiopia. A botanist identified the plant, and a voucher specimen AbHu0126 was deposited at the Herbarium of the College of Natural and Computational Sciences, Haramaya University, for future reference. The harvested plant materials were carefully packed into plastic-coated sacs and transported to the laboratory room at school of pharmacy, College of Health and Medical Science, Haramaya University. The fresh barks were washed using tap water and then dried at room temperature under shade for three weeks. Once dried, the barks were milled into a coarse powder using an electric blender. The powdered material was accurately weighed and stored until the extraction begins.

2.3. Preparation of the 80 % Methanol Extract

The powdered material (150g) was macerated with absolute methanol (600 ml) at a solid-to-solvent ration of 1:4 (w/v) in a

conical flask with intermittent shaking at 230 rpm using an orbital shaker for 72 hours. The extract was filtered through a funnel plunged with nylon cloth, and the filtrate was passed through Whatman filter paper (No. 1). The procedure was performed two more times, each time with a different fresh solvent added to the leftover residue or marc. The resulting combined extract after successive filtration was concentrated using a rotor evaporator at 40 °C. Then, solid crystals were obtained and percent yields were determined and then powdered, transferred into a tight container, labelled, and stored in a refrigerator at -4 °C until used for fractionation and further experimental procedures.

2.4. Preparations of Solvent Fractions

Liquid–liquid partitioning of the crude methanolic extract was carried out following a modified method described by Khan et al [20]. Fourteen grams (14 g) of the concentrated methanolic extract were suspended in 50 ml distilled water to form an aqueous solution and successively partitioned with an equal volume of chloroform and acetone successively based on solvent polarity using a separatory funnel (Figure 1). Each fraction was collected separately and concentrated using a rotary evaporator at 40 °C. The aqueous fraction was concentrated by lyophilization. All fractions were stored at 4 °C until further use.

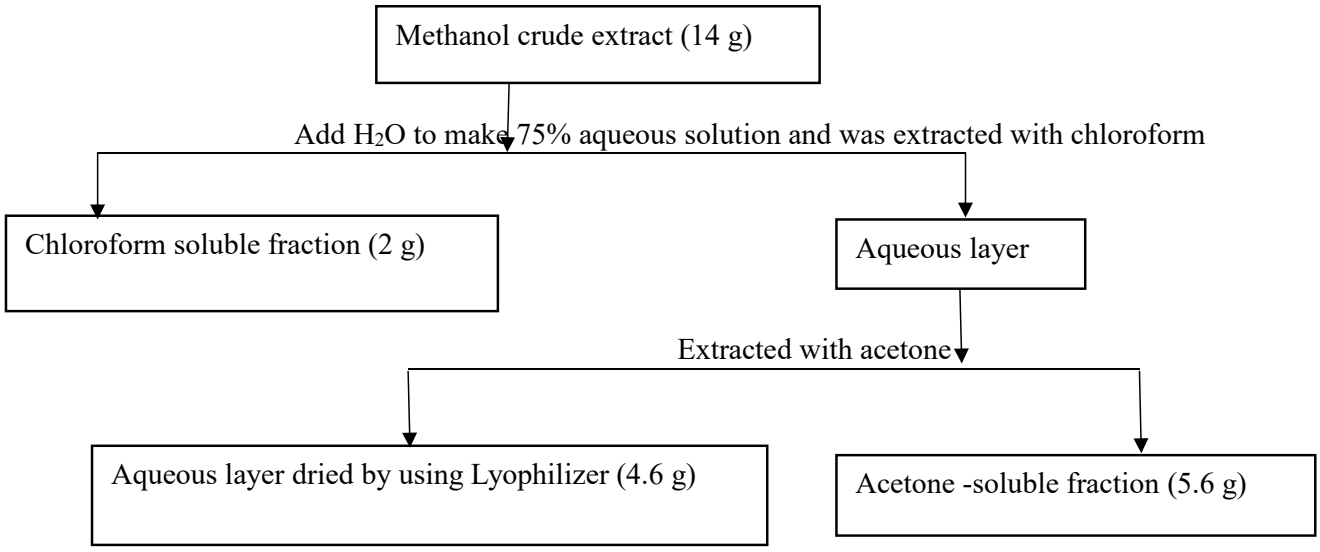

Figure 1: Schematic Representation of Liquid-Liquid Partitioning of M. Stenopetala Bark Methanolic Extract

2.5. Phytochemical Screening

Qualitative phytochemical screening of the crude methanolic extract and solvent fractions was conducted using standard procedures to detect major classes of secondary metabolites, including flavonoids, saponins, phenols, tannins, terpenoids, steroids, phytosterols, cardiac glycosides, alkaloids, anthocyanins, and quinones [21-26].

2.6. Evaluation of Antioxidant Activity

2.6.1. DPPH Radical Scavenging Assay

The antioxidant activity of the crude methanolic extract and solvent fractions was evaluated using the DPPH radical scavenging assay as described in the literature [26]. Stock solutions of the extract and fractions were prepared in methanol at a concentration of 1 mg/mL and serially diluted to obtain concentrations of 500, 250, 125, and 62.5 µg/mL. An aliquot of 1 mL of each dilution was mixed with 4 mL of freshly prepared DPPH solution (0.04% w/v in methanol) to yield final concentrations of 100, 50, 25, and 12.5 µg/mL. The reaction mixtures were incubated at 37 °C for 30 min in the dark, and absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Methanol was used as a blank, DPPH solution as a control, and L-ascorbic acid as a positive reference standard.

The percentage radical scavenging activity was calculated using the equation:

$$\%\text{RSA} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation.

3. Results

3.1. Extraction Yield

The percentage yields of the crude methanolic extract of *Moringa stenopetala* stem bark and its solvent fractions are presented in Table 1. From 150 g of dried stem bark powder, 20.37 g of crude methanolic extract was obtained, corresponding to a yield of 13.58%. Among the fractions, the aqueous (71.9%) and acetone (46,7%) fractions showed higher yields compared to the chloroform (14.3%) fraction.

Extract and fractions	Weight of extract/fraction	Nature of extracts	Yield in (%) *
Methanolic extract	20.37 g	Oily blue-green	13.58
Chloroform fraction	2.0 g	Black jelly	14.3
Acetone fraction	5.6 g	Greasy red	46.7
Aqueous fraction	4.6 g	Honey jelly	71.9
*%Yield = [Weight of crude/Weight of sample] x100			

Table 1: Percentage Yield of the Methanolic Crude Extract and its Solvent Fractions of *M. Stenopetala* Stem Bark

3.2. Phytochemical Screening

Qualitative phytochemical screening of the crude methanolic extract and solvent fractions of *M. stenopetala* stem bark revealed the presence of various secondary metabolites (Table 2). Flavonoids were detected in the crude extract, chloroform fraction, and aqueous fraction but were absent in the acetone fraction. Saponins were present in the crude extract, chloroform fraction, and aqueous fraction, whereas phenolic compounds were detected in all tested samples. Tannins were observed in the chloroform and aqueous fractions but were absent in the crude extract and acetone fraction. Terpenoids were present in the crude extract, acetone fraction, and aqueous fraction. Steroids and anthocyanins were not detected in any of the extracts or fractions analyzed. Phytosterols, cardiac glycosides, and alkaloids were detected in all samples. Quinones were present in the chloroform and aqueous fractions but absent in the crude extract and acetone fraction.

Phytochemical screening	Test type	Methanolic extract	Chloroform fraction	Acetone fraction	Aqueous fraction
Flavonoids	Alkaline test	+	+	-	+
Saponins	Foam test	+	+	-	+
Phenols	Alkaline test	+	+	+	+
Tannins	Ferric chloride test	-	+	-	+
Terpenoids	Salkowski test	+	-	+	+
Steroids	-	-	-	-	-
Phytosterols	Salkowski test	+	+	+	+
Cardiac glycosides	Keller-Killiani test	+	+	+	+
Alkaloids	Wagner's test	+	+	+	+
Anthocyanin	-	-	-	-	-
Quinones	-	+	-	+	-
+ = the presence of phytochemical constituents - = the absence of phytochemical constituents					

Table 2: Phytochemical Screening of Crude Extract and Different Solvents Fractions of *M. Stenopetala* Stem Bark

3.3. DPPH Radical Scavenging Assay

The antioxidant activity of the crude methanolic extract and solvent fractions of *M. stenopetala* stem bark was evaluated using the DPPH radical scavenging assay. The results are summarized in Table 3 and illustrated in Figure 2. All tested samples exhibited concentration-dependent radical scavenging activity. At the lowest tested concentration (12.5 µg/mL), percentage inhibition ranged from 58.24% for the crude methanolic extract to 64.84% for the aqueous fraction. Increasing the concentration resulted in increased scavenging activity across all samples. At 100 µg/mL, the aqueous fraction demonstrated the highest radical scavenging activity (73.63%), followed by the acetone fraction (72.53%), chloroform fraction (69.23%), and crude methanolic extract (63.74%). The reference standard, L-ascorbic acid, exhibited significantly higher radical scavenging activity, with 98.67% inhibition at the same concentration. The absorbance values of the samples decreased progressively with increasing concentration, indicating effective hydrogen-donating ability of the extract and fractions. All assays were performed in triplicate, and results are expressed as mean ± standard deviation.

Conc.(µg/ml)	Absorbance					DPPH % inhibition				
	Methanol extract	CHCl3 Fraction	Acetone Fraction	Aqueous fraction	AA	Methanol extract	CHCl3 Fraction	Acetone fraction	Aqueous fraction	AA
12.5	0.38±0.05	0.34±0.15	0.33±0.17	0.32±0.02	0.02±0.01	58.24	62.64	63.74	64.84	98.24
25	0.37±0.023	0.32±0.22	0.31±0.13	0.3±0.20	0.01±0.02	59.34	64.83	65.93	67.03	98.35
50	0.35+0.14	0.30±0.11	0.27±0.32	0.29±0.14	0.013±0.1	61.54	67.03	70.34	68.13	98.50
100	0.33±0.25	0.28±0.25	0.25±0.02	0.24±0.31	0.012±0.01	63.74	69.23	72.53	73.63	98.67
Control			**0.910**							
Where, $CHCl_3$ = Chloroform, AA stands for ascorbic acid and the results were expressed in mean ±standard deviation and the test was done in triplicates.										

Table 3: % Radical Scavenging Activity of Methanolic Extract and Solvent Fractions of *M. Stenopetala* Stem Bark

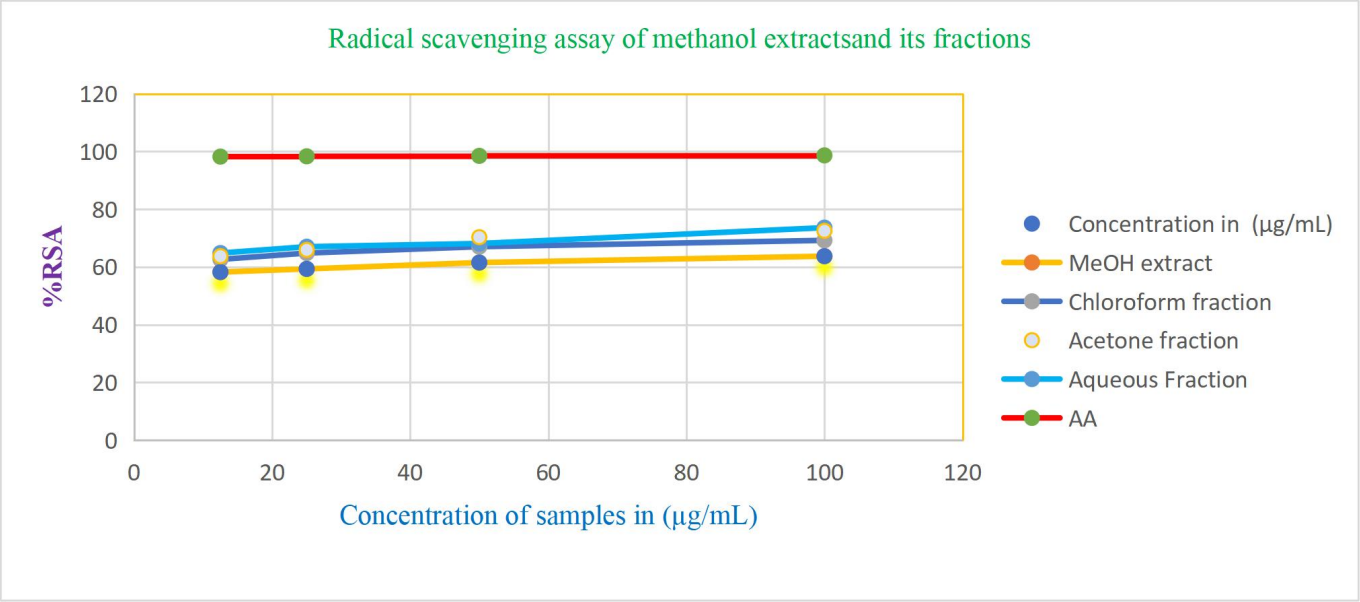

Figure 2: Radical Scavenging Activity (RSA) of Methanolic Extract and Solvent Fractions of M. Stenopetala Stem Bark and the Positive Reference L-Ascorbic Acid (AA)

4. Discussion

The present study investigated the phytochemical composition and *in vitro* antioxidant activity of the methanolic extract and solvent fractions of the stem bark of *Moringa stenopetala*. Although various parts of this plant, particularly the leaves and seeds, have been extensively studied, information regarding the bioactive potential of the stem bark remains limited. The findings of this study therefore provide new insight into the chemical and antioxidant profile of this underexplored plant part.

The extraction yield results indicated that the aqueous and acetone fractions constituted a higher proportion of the crude methanolic extract compared to the chloroform fraction. This pattern suggests that the stem bark of *M. stenopetala* is rich in polar and semi-polar constituents. Similar observations have been reported for other parts of the plant, where polar solvents were more efficient in extracting bioactive metabolites such as phenolic compounds, flavonoids, and glycosides [21,27]. The higher recovery of polar fractions may reflect the abundance of hydroxyl-rich secondary metabolites commonly associated with antioxidant activity [27,28].

Qualitative phytochemical screening revealed the presence of several classes of secondary metabolites, including flavonoids, phenols, tannins, saponins, terpenoids, alkaloids, phytosterols, and cardiac glycosides, with variation among the solvent fractions. The absence of steroids and anthocyanins across all samples may be attributed to their low abundance in the stem bark or limitations associated with qualitative detection methods. The widespread presence of phenolic compounds in all fractions is noteworthy, as phenols are well recognized for their ability to donate hydrogen or electrons and neutralize free radicals, thereby contributing to antioxidant activity [29].

The antioxidant potential of the extracts and fractions was evaluated using the DPPH radical scavenging assay, a widely employed method for assessing hydrogen-donating capacity [29,30]. All samples exhibited concentration-dependent radical scavenging activity, indicating their ability to quench free radicals. Among the tested samples, the aqueous and acetone fractions demonstrated relatively higher antioxidant activity compared to the chloroform fraction and crude methanolic extract. This observation is consistent with the phytochemical results, which showed a broader distribution of phenolic and flavonoid compounds in the more polar fractions [27].

Although the radical scavenging activity of the stem bark extracts was lower than that of the reference standard ascorbic acid, the observed inhibition percentages indicate moderate antioxidant potential. Comparable levels of antioxidant activity have been reported for extracts obtained from the leaves, seeds, and roots of *M. stenopetala* and other *Moringa* species, supporting the notion that antioxidant constituents are distributed across different plant parts [18]. The differences in antioxidant activity among fractions may be explained by variations in the concentration and structural characteristics of bioactive compounds extracted by solvents of differing polarity [27].

The antioxidant activity observed in this study may have pharmacological relevance, as oxidative stress plays a key role in the pathogenesis of various chronic diseases [31]. The presence of multiple phytochemical classes suggests that the antioxidant effects may result from synergistic interactions among compounds rather than the action of a single constituent [32]. However, it is important to note that the current study employed only an *in vitro* chemical assay, which does not directly translate to biological efficacy *in vivo*.

5. Conclusion

The current findings support the traditional use of *M. stenopetala* and highlight the stem bark as a potential source of antioxidant phytochemicals. Nevertheless, further studies are warranted to quantify total phenolic and flavonoid contents, determine IC_{50} values, isolate and characterize individual bioactive compounds, and evaluate their biological activities using cellular and in vivo models. Such investigations would provide a more comprehensive understanding of the therapeutic potential of *M. stenopetala* stem bark and facilitate its possible application in drug discovery and natural antioxidant development.

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