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Antioxidant and *α*-amylase/*α*-glucosidase Inhibitory Activities 0f Leaf, Bark, and Seed Extracts of *Moringa oleifera* Grown in Buon Ma Thuot, Vietnam

Pham B. Phuong[1]* and Nguyen T. Thanh[2],

[1]*Faculty of Agriculture and Forestry, Thai Nguyen University of Agriculture and Forestry (TUAF), Thai Nguyen, Vietnam*
[2]*Department of Biology, Faculty of Natural Sciences and Technology, Tay Nguyen University, Dak Lak, Vietnam*

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ABSTRACT

Buon Ma Thuot in Vietnam's Central Highlands features basalt-rich soil and a mild highland climate that may favor the accumulation of bioactive phytochemicals. This study assessed the antioxidant and *α*-amylase/*α*-glucosidase inhibitory activities of *Moringa oleifera* leaf, bark, and seed extracts collected from this region to identify the most bioactive plant part and isolate functional fractions. Sequential solvent fractionation (hexane, chloroform, diethyl ether, ethyl acetate, water) was applied to the ethanol-extracted leaf sample, which exhibited the highest initial activity, to enrich bioactive compounds. The Buon Ma Thuot *Moringa* leaf extract exhibited significantly higher phenolic and flavonoid contents than those reported for *M. oleifera* from other regions, correlating with enhanced antioxidant and enzyme-inhibitory effects. Among the fractions, the chloroform-soluble layer showed the strongest DPPH radical scavenging activity ($IC_{50} \approx 117$ µg/mL), the diethyl ether fraction had the highest *α*-amylase inhibition ($IC_{50} \approx 11.5$ mg/mL), and the ethyl acetate fraction provided the most potent *α*-glucosidase inhibition ($IC_{50} \approx 2.1$ mg/mL). The latter value is notably more potent than most previously reported values (e.g., >5 mg/mL) for *Moringa* extracts, highlighting the phytochemical richness of leaves grown in this ecological zone. These findings suggest that *M. oleifera* leaves from Buon Ma Thuot are promising natural sources of antioxidants and mild enzyme inhibitors, with potential applications in functional foods or adjunct therapy for type 2 diabetes.

Keywords: *Moringa oleifera*, antioxidant, *α*-amylase inhibition, *α*-glucosidase inhibition, phenolics, Buon Ma Thuot

Introduction

Moringa oleifera originates from the foothills of the southern Himalayas and has been used for over 4,000 years. Today, it is cultivated widely across Africa, Central and South America, and Southeast Asia. [1–3] In Vietnam, it is the only species of its genus and has been found growing wild in many areas (e.g., Thanh Hoa, Ninh Thuan, Binh Thuan, An Giang, Phu Quoc), though it was historically underutilized. Only in recent decades, after seeds were introduced from abroad, has *M. oleifera* been intentionally cultivated when its special uses were recognized. [1,4] *M. oleifera* is highly valued for its exceptional nutritional content, with almost all parts of the plant being usable. It provides a rich array of bioactive compounds, including phenolics, flavonoids, glucosinolates, and alkaloids, which have been reported to exhibit antioxidant and enzyme inhibitory properties.[5] Recent studies report that *M. oleifera* contains around 90 nutrients, including seven vitamins, six minerals, 18 amino acids, and 46 antioxidant compounds. [2] Tthese constituents exhibit strong anti-inflammatory, antibiotic, and detoxifying properties that help treat fibroids, prostate tumors, lower blood pressure, protect the liver, and reduce cholesterol levels. The stems and leaves are the parts with the highest nutrient content. [2].

*Corresponding author; Email: phambangphuong@tuaf.edu.vn
Tel: +84-987809070

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Key bioactive compounds in *M. oleifera* (such as glucosinolates, isothiocyanates, phenolic acids, and flavonoids) have been linked to antioxidant, anticancer, and anti-diabetic effects. [6–7]. Because of these attributes, *M. oleifera* is often referred to as the "miracle tree" or "tree of life" in folk medicine. [6–7] Several studies worldwide have examined the biological activities of *M. oleifera*. For instance, extracts from the leaves have shown significant antioxidant activity due to their high phenolic content. [8] Leaf extracts have also shown inhibitory effects on carbohydrate-digesting enzymes, supporting their potential for glycemic control and diabetes management. [9] However, the potency of these effects can vary widely depending on factors such as the plant's origin, growing conditions, and the extraction method employed. [10]. In particular, the unique environmental conditions of cultivation can influence phytochemical accumulation. Buon Ma Thuot, located in Vietnam's Central Highlands, has a distinct tropical savanna climate with fertile basaltic soil and an elevation of ~500 m. These conditions may yield *M. oleifera* with a phytochemical profile different from those grown elsewhere, as has been observed for other crops in this region (e.g., its renowned coffee). To date, there is limited information on *M. oleifera* grown in the Central Highlands of Vietnam, and it remains unclear how its bioactive properties compare to those reported from other countries.

This study investigates the antioxidant properties and *α*-amylase and *α*-glucosidase inhibitory activities of *M. oleifera* extracts from leaves, bark, and seeds cultivated in Buon Ma Thuot. The aim is to identify the plant part with the highest potential activity and to evaluate the efficacy of different solvent fractions of the leaf extract as a basis for developing natural anti-diabetic agents.

Materials and Methods

Chemicals and Reagents:
Gallic acid (≥98%), quercetin (≥95%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), porcine pancreatic *α*-amylase, and bacterial *α*-glucosidase from *Bacillus stearothermophilus* (≥10 U/mg) were purchased from Sigma-Aldrich (USA). *p*-Nitrophenyl-*α*-D-glucopyranoside (pNPG) and iodine reagents were obtained from Merck. Acarbose was used as a positive control for enzyme inhibition assays. All other chemicals and solvents were of analytical grade (Xilong or Merck).

Plant Material:
Fresh leaves, bark, and seeds of *Moringa oleifera* were collected from Buon Ma Thuot city (Dak Lak province, Vietnam) in August 2022. The geographic coordinates of the collection site were 12.679649, 108.009871. The plant was authenticated by a botanist at Tay Nguyen University, and a voucher specimen was deposited in the university herbarium (TTN-MO-2022-01). The samples were washed, air-dried in the shade for 7 days, and then ground into a fine powder.

Extraction and Fractionation:
Dried powder (50 g for each plant part) was macerated in 95% ethanol (Xilong Scientific, China; analytical grade) at room temperature for 48 hours. The mixture was filtered, and the solvent was evaporated under reduced pressure to obtain crude ethanol extracts of the leaf, bark, and seed. The yield (% w/w) of each extract was recorded. The crude leaf extract (which showed the highest activity in preliminary tests) was further fractionated by sequential solvent partitioning. The leaf extract (10 g) was suspended in 100 mL of water and partitioned with n-hexane, chloroform, diethyl ether, and ethyl acetate (100 mL x 4; Merck, Germany; ≥99% purity) in succession. This yielded five fractions: hexane, chloroform, diethyl ether, ethyl acetate, and water (remaining aqueous layer). Each fraction was evaporated to dryness. All extracts and fractions were kept at –20°C.

DPPH Radical Scavenging Assay:
The antioxidant activity of extracts and fractions was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. [11]. A 0.1 mM solution of DPPH in methanol was prepared fresh daily. Then, 0.1 mL of DPPH solution was mixed with 0.1 mL of each extract or fraction at different concentrations (dissolved in methanol). The reaction mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). L-ascorbic acid (Sigma-Aldric, USA) was used as the positive control. The radical scavenging activity was calculated as a percentage inhibition of DPPH using the following equation number 1:

$$\text{Inhibition } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance of the control (DPPH solution without sample) and A_1 is the absorbance in the presence of the sample. The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was calculated using linear regression in Microsoft Excel.

α-Amylase Inhibitory Assay:
The *α*-amylase inhibitory activity was evaluated using a modified starch–iodine colorimetric method. [13]. The reaction mixture consisted of 25 µL of sample solution, 50 µL of *α*-amylase enzyme (0.5 U/mL, from porcine pancreas, Sigma-Aldrich, USA), and 50 µL of 1% soluble starch (prepared in 20 mM phosphate buffer, pH 6.9) was used as substrate. The mixture was incubated at 37°C for 10 minutes. The reaction was terminated by adding 25 µL of 1 M HCl, followed by 100 µL of iodide reagent (prepared by dissolving 5 mM I_2 and 5 mM KI in distilled water). Absorbance was measured at 620 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Acarbose (Bayer AG, Germany) was used as the positive control. Percentage inhibition was calculated using the following equation number 2:

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the blank (without inhibitor) and A_{sample} is the absorbance with the extract. IC_{50} values were determined from the inhibition curves using Microsoft Excel.

α-Glucosidase Inhibitory Assay:
The *α*-glucosidase inhibitory activity was assessed using *p*-nitrophenyl-*α*-D-glucopyranoside (pNPG, Sigma-Aldrich, USA) as substrate, following a modified method described by Gopalakrishnan *et al*. [9] The reaction mixture contained 50 µL of sample solutions, 100 µL of *α*-Glucosidase enzyme (0.25 U/mL, from *B. stearothermophilus*, Sigma-Aldrich, USA), and 50 µL of 4 mM pNPG (prepared in phosphate buffer, pH 6.8). The mixture was incubated at 37°C for 15 minutes. The reaction was terminated by adding 50 µL of 0.2 M sodium carbonate (Na_2CO_3). The amount of released *p*-nitrophenol (PNP) was quantified by measuring absorbance at 405 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Acarbose (Bayer AG, Germany) was used as a positive control. Inhibition percentage was calculated using the equation number 3:

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the blank (without inhibitor) and A_{sample} is the absorbance with the extract. IC_{50} values were determined from the inhibition curves using Microsoft Excel.

Statistical Analysis:
All experiments were conducted in triplicate (n = 3). Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's post-hoc test were used to determine significant differences at a 95% confidence level ($p < 0.05$) among samples or concentrations where applicable. Linear regression was used to calculate IC_{50} values for radical scavenging and enzyme inhibition assays.

Results and Discussion

Phytochemical Contents (Total Phenolics and Flavonoids):
The extraction yields of crude ethanol extracts differed among plant parts. The leaf extract yielded the highest amount (37.67%), followed by seed (18.59%) and bark (7.92%). This indicates that leaves contain more ethanol-soluble bioactive compounds than seeds or bark. The crude leaf extract was further fractionated by solvents of varying polarity. The recovery yeilds of fractions (based on dry weight of the original leaf extract) were: water (43.75%), hexane (20.58%), ethyl acetate (3.85%), diethyl ether (2.00%), and the lowest in chloroform (1.55%) (Table 1). These yield differences suggest that the leaf extract contains substantial amounts of both polar and non-polar constituents (as reflected by the predominance of water and hexane fractions).
The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts are presented in Table 1. Among the crude extracts of different plant parts, the leaf extract showed by far the highest TPC and TFC (62.77 ± 0.00 mg GAE/g and 59.69 ± 0.00 mg QE/g, respectively). In contrast, the seed and bark extracts had much lower phenolic and flavonoid levels (TPC 27.42 and 24.63 mg GAE/g; TFC 21.42 and 28.82 mg QE/g, respectively). The higher levels of polyphenols and flavonoid content in leaves are consistent with the fact that leaves are the primary photosynthetic and metabolic organs, where secondary metabolites tend to accumulate.
Fractionation of the leaf extract revealed considerable variation in phytochemical content between fractions (Table 1). The chloroform fraction contained the highest phenolic concentration (94.32 ± 0.87 mg GAE/g), whereas the hexane fraction contained the highest flavonoid concentration (130.37 ± 2.45 mg QE/g). The water fraction also showed a relatively high phenolic content (65.23 ± 1.96 mg GAE/g) but very low flavonoid content (2.63 ± 0.87 mg QE/g), indicating that most

Table 1: Total phenolic content (TPC) and total flavonoid content (TFC) of *M. oleifera* extracts (mean ± SD, n = 3)

Sample	TPC (mg GAE/g)	TFC (mg QE/g)
Crude extracts		
Leaf	62.77 ± 0.00^{c}	59.69 ± 0.00^{c}
Seed	27.42 ± 0.00^{b}	21.42 ± 0.00^{a}
Bark	24.63 ± 0.00^{a}	28.82 ± 0.00^{b}
Leaf fractions		
Hexane	21.26 ± 1.07^{a}	130.37 ± 2.45^{e}
Chloroform	94.32 ± 0.87^{d}	81.42 ± 1.68^{c}
Diethyl ether	33.40 ± 1.04^{b}	23.19 ± 0.34^{b}
Ethyl acetate	29.05 ± 2.81^{b}	96.78 ± 5.78^{d}
Water	65.23 ± 1.96^{c}	2.63 ± 0.87^{a}

Note: GAE = gallic acid equivalents; QE = quercetin equivalents. Different superscript letters in a column indicate significant differences at $p < 0.05$.

Table 2: DPPH radical scavenging (%) of *M. oleifera* crude extracts at various concentrations (mean ± SD, n = 3)

Sample	78 µg/mL	156 µg/mL	312 µg/mL	625 µg/mL	1250 µg/mL	2500 µg/mL
Leaf extract	25.25 ± 0.90 ^{a}	30.26 ± 1.49 ^{b}	49.35 ± 0.37 ^{c}	70.65 ± 1.07 ^{d}	91.15 ± 0.53 ^{e}	–
Bark extract	6.42 ± 0.14 ^{a}	10.05 ± 0.03 ^{b}	17.84 ± 0.28 ^{c}	25.58 ± 0.77 ^{d}	40.26 ± 0.52 ^{e}	–
Seed extract	–	4.28 ± 0.53 ^{a}	9.68 ± 0.18 ^{b}	15.73 ± 0.40 ^{c}	24.55 ± 0.21 ^{d}	37.82 ± 0.53 ^{e}

Note: "–" indicates that is was not detected or not measured. Within each row, values with different letters are significantly different ($p < 0.05$). Vitamin C (standard) achieved 90.10 ± 0.41% inhibition at 50 µg/mL (data not shown).

Table 3: DPPH radical scavenging (%) of *M. oleifera* leaf extract fractions at various concentrations (mean ± SD, n = 3)

Fraction	62 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
Hexane	24.84 ± 0.60 ^{a}	33.35 ± 0.69 ^{b}	49.90 ± 0.66 ^{c}	74.32 ± 0.32 ^{d}	91.65 ± 0.57 ^{e}
Chloroform	43.49 ± 0.60 ^{a}	46.06 ± 0.83 ^{b}	62.76 ± 1.24 ^{c}	76.20 ± 0.58 ^{d}	96.16 ± 1.19 ^{e}
Diethyl ether	30.21 ± 0.39 ^{a}	38.48 ± 1.15 ^{b}	51.44 ± 0.47 ^{c}	65.28 ± 0.60 ^{d}	80.00 ± 0.58 ^{e}
Ethyl acetate	30.58 ± 1.00 ^{a}	43.01 ± 0.80 ^{b}	53.07 ± 0.61 ^{c}	70.56 ± 1.49 ^{d}	87.05 ± 0.38 ^{e}
Water	35.09 ± 0.34 ^{a}	47.02 ± 1.02 ^{b}	62.31 ± 0.78 ^{c}	73.42 ± 0.51 ^{d}	90.43 ± 1.24 ^{e}

Note: Within each row, values followed by different letters differ significantly ($p < 0.05$).

Table 4: α-Amylase inhibition (%) by *M. oleifera* crude extracts at various concentrations (mean ± SD, n = 3).

Sample	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	1560 µg/mL	3120 µg/mL	6250 µg/mL	12500 µg/mL	25000 µg/mL
Acarbose (control)	22.81 ± 0.56 ^{a}	28.25 ± 0.42 ^{b}	32.33 ± 0.57 ^{c}	47.95 ± 0.66 ^{d}	77.81 ± 0.58 ^{e}	–	–	–	–	–
Leaf extract	–	–	–	–	–	1.59 ± 0.40 ^{a}	4.58 ± 0.48 ^{b}	7.14 ± 0.39 ^{c}	11.73 ± 0.47 ^{d}	28.11 ± 0.30 ^{e}
Seed extract	–	–	–	–	–	–	–	–	–	–
Bark extract	–	–	–	–	–	–	–	–	–	–

"–" indicates no detectable inhibition. Different letters in a row indicate significant differences between concentrations for that sample ($p < 0.05$).
Note: "–" indicates no detectable inhibition. Each row's values with different letters are significantly different ($p < 0.05$).

Table 5: *α*-Amylase inhibition (%) by *M. oleifera* leaf extract fractions at various concentrations (mean ± SD, n = 3). "–" indicates no inhibition

Fraction	625 µg/mL	1250 µg/mL	2500 µg/mL	5000 µg/mL	10000 µg/mL
Hexane	–	–	–	–	–
Chloroform	2.09 ± 0.48 [a]	7.38 ± 0.55 [b]	9.24 ± 0.36 [c]	13.17 ± 0.88 [d]	33.02 ± 0.82 [e]
Diethyl ether	2.09 ± 0.72 [a]	16.32 ± 0.21 [b]	21.13 ± 0.65 [c]	26.93 ± 0.36 [d]	42.89 ± 0.96 [e]
Ethyl acetate	4.21 ± 0.06 [a]	7.35 ± 0.55 [b]	10.80 ± 0.63 [c]	15.11 ± 0.36 [d]	31.60 ± 0.50 [e]
Water	–	–	–	–	–

Note: Only the chloroform, diethyl ether, and ethyl acetate fractions showed *α*-amylase inhibition (IC_{50} ≈ 11.46 mg/mL, 15.77 mg/mL, and 16.80 mg/mL, respectively). Hexane and water fractions exhibited no activity (–). Different letters in each active fraction's row indicate significant differences ($p < 0.05$).

Table 6: *α*-Glucosidase inhibition (%) by *M. oleifera* crude extracts at various concentrations (mean ± SD, n = 3)."–" indicates no inhibition observed.

Sample	62.5 µg/mL	125 µg/mL	250 µg/mL	312 µg/mL	500 µg/mL	625 µg/mL	1000 µg/mL	1250 µg/mL	2500 µg/mL	5000 µg/mL
Acarbose (control)	18.35 ± 0.49 [a]	33.40 ± 0.75 [b]	58.14 ± 0.93 [c]	–	75.87 ± 0.62 [d]	–	87.62 ± 0.65 [e]	–	–	–
Leaf extract	–	–	–	5.21 ± 1.15 [a]	–	5.69 ± 0.49 [a]	–	12.38 ± 0.26 [b]	25.94 ± 0.95 [c]	43.24 ± 0.54 [d]
Seed extract	–	–	–	–	–	–	–	–	–	–
Bark extract	–	–	–	–	–	–	–	–	–	–

Note: "–" indicates no inhibition detected. Different letters in the leaf extract row indicate significant differences ($p < 0.05$).

Table 7: *α*-Glucosidase inhibition (%) by *M. oleifera* leaf extract fractions at various concentrations (mean ± SD, n = 3). "–" indicates no inhibition observed.

Fraction	312 µg/mL	625 µg/mL	1250 µg/mL	2500 µg/mL	5000 µg/mL
Hexane	–	–	–	–	–
Chloroform	–	–	–	–	–
Water	–	–	–	–	–
Diethyl ether	4.30 ± 0.28 [a]	8.67 ± 0.11 [b]	18.11 ± 0.44 [c]	28.26 ± 0.91 [d]	50.33 ± 0.73 [e]
Ethyl acetate	7.08 ± 0.10 [a]	11.89 ± 0.22 [b]	35.27 ± 0.70 [c]	83.40 ± 1.08 [d]	95.47 ± 0.59 [e]

Note: Only the diethyl ether and ethyl acetate fractions showed *α*-glucosidase inhibition (IC_{50} ≈ 4.86 mg/mL and 2.11 mg/mL, respectively). Hexane, chloroform, and water fractions had no activity (–). Different letters in each active fraction's row indicate significant differences ($p < 0.05$).

flavonoids were extracted into less polar fractions. Overall, the distribution of phenolics and flavonoids among fractions suggests that the phenolic compounds in *M. oleifera* leaf cover a wide polarity range, while the majority of flavonoids are relatively non-polar (and thus enriched in the hexane and chloroform fractions). These results align with previous reports. For example, Abdulkadir *et al.* found that methanolic extracts of *M. oleifera* leaves had a very high flavonoid content (~98.7 mg QE/g), much higher than in seeds (~3.0 mg QE/g) or bark (~28.3 mg QE/g). [13]. Adisakwattana and Chanathong reported a total flavonoid content of 15.39 ± 0.58 mg QE/g in *M. oleifera* leaf extract, [14] which is lower than observed here, possibly due to differences
in extraction solvent or plant origin. Our findings indicate that the ethanol extract and its fractions from Buon Ma Thuot *M. oleifera* are rich in phenolics and flavonoids, which are well-established contributors to antioxidant activity.

Antioxidant Activity (DPPH Radical Scavenging and UV Absorption)

The DPPH radical scavenging assay was used to assess the antioxidant capacity of the extracts. The percentage of DPPH inhibition for the crude extracts at various concentrations is presented in Table 2. All measurements were carried out in triplicate, and values were expressed as mean ± SD. All extracts exhibited dose-dependent DPPH scavenging activity, which increased with concentration (Table 2). Among the three parts, the leaf extract showed the highest antioxidant effect, achieving ~91% radical scavenging at 1.25 mg/mL, whereas the bark and seed extracts reached only ~40% and ~38% inhibition, even at 1.25–2.5 mg/mL, respectively. Calculated IC_{50} values confirmed the leaf extract as the most potent (IC_{50} ≈ 425.43 ± 2.77 µg/mL), followed by bark (IC_{50} ≈ 1555.06 ± 8.25 µg/mL) and seed (IC_{50} ≈ 3296.82 ± 6.42 µg/mL). All were significantly less active than the standard vitamin C (IC_{50} = 25.81 ± 0.56 µg/mL). These results

indicate that the leaf extract has substantially greater antioxidant constituents, which is in agreement with its higher polyphenol content, whereas seed and bark extracts contribute relatively minor antioxidant activity.

Fractions of the leaf extract were also tested for DPPH scavenging capacity. As shown in Table 3, all fractions exhibited increasing radical scavenging with higher concentrations. The hexane and chloroform fractions showed the strongest DPPH scavenging among the fractions, each reaching ~91–96% inhibition at 1.0 mg/mL. At 1.0 mg/mL, the chloroform fraction scavenged 96.16 ± 0.53% of DPPH – the highest among fractions – while the hexane fraction was similar (91.65 ± 0.49%). The ethyl acetate and water fractions also achieved high inhibition (~87–90% at 1.0 mg/mL). The diethyl ether fraction was the weakest, reaching ~80% at 1.0 mg/mL. The IC_{50} values for the fractions (in ascending order of potency) were: chloroform (117.52 ± 2.61 µg/mL) < water (170.67 ± 4.11 µg/mL) < ethyl acetate (265.75 ± 6.88 µg/mL) < hexane (321.70 ± 3.79 µg/mL) < diethyl ether (327.18 ± 5.12 µg/mL). All fractions were less potent antioxidants than vitamin C (IC_{50} = 25.81 µg/mL). The chloroform fraction's superior antioxidant activity is consistent with its highest phenolic content (Table 1), reinforcing the correlation between phenolic content and radical scavenging ability. In general, our results agree with the trend observed by Oyeyinka et al. (2023), which is that antioxidant capacity increases with total polyphenol content. [5] Indeed, among both crude extracts and fractions, those with higher polyphenol levels (leaf extract and chloroform fraction) demonstrated better DPPH scavenging performance.In addition to the DPPH assay, the UV-Vis absorption spectra of the extracts were examined to evaluate their potential as UV absorbers (which may relate to antioxidant or photoprotective properties). Figure 1 shows the UV spectra (150–280 nm) of the crude leaf, bark, and seed extracts at 150 µg/mL. All extracts exhibited a strong absorption band in the ultraviolet range around 190–210 nm, with a peak at ~210 nm. Notably, the seed extract showed the highest absorbance in this UV range, exceeding that of the leaf and bark extracts at the same concentration. Figure 2 displays the absorption spectra of the various leaf extract fractions (hexane, chloroform, diethyl ether, ethyl acetate, water) at 150 µg/mL. All fractions similarly had high absorption below 240 nm with maxima around 210–220 nm. Among fractions, the chloroform fraction demonstrated the highest absorbance, followed by the ethyl acetate and hexane fractions, whereas the water and diethyl

ether fractions showed comparatively lower absorbance around 210 nm. These data suggest that certain constituents of the seed extract and chloroform/ethyl acetate leaf fractions strongly absorb UV-C and UV-B wavelengths (below 280 nm). While these wavelengths are largely filtered by the atmosphere and not the main focus for sunscreens (which target UV-A/B), the high absorbance indicates a dense presence of conjugated compounds. The seed extract and chloroform fraction, in particular, may contain chromophores (possibly polyphenols or other UV-absorbing metabolites) that could be explored for photoprotective applications. However, further analysis is required to determine their efficacy in the UV-A/B range relevant to skin protection.

In summary, the antioxidant assays confirm that *M. oleifera* leaves possess significant antioxidant activity, attributable to their rich phenolic content. The crude leaf extract and especially its chloroform-soluble fraction were the most effective in scavenging free radicals. Although less potent than standard vitamin C, these natural extracts could still be beneficial as dietary antioxidants or as part of an antioxidant phytotherapeutic regimen.

α-Amylase Inhibitory Activity

The crude *M. oleifera* extracts were tested for their ability to inhibit α-amylase (the enzyme responsible for starch breakdown). The results are summarized in Table 4. The leaf extract exhibited slight α-amylase inhibition that increased with concentration, reaching about 28.1% inhibition at the highest tested concentration of 25,000 µg/mL. In contrast, the seed and bark extracts showed no measurable α-amylase inhibitory activity up to 25,000 µg/mL (indicated by "–" in Table 4). The leaf extract's IC_{50} for α-amylase was estimated at a very high 45,236 µg/mL (approximately 45.2 mg/mL), indicating weak inhibitory potency. By comparison, the positive control, acarbose achieved 77.8% inhibition at just 400 µg/mL, with an IC_{50} of about 206 µg/mL (0.206 mg/mL). Thus, the leaf extract is roughly 200 times less potent than acarbose in inhibiting α-amylase. This finding suggests that, while *M. oleifera* leaves contain some α-amylase inhibitors (perhaps flavonoids or other polyphenols known to inhibit amylase), the activity is relatively low in the crude extract. Neither bark nor seed contributed any significant amylase inhibition.

Given the minimal activity of crude extracts, we next examined the leaf extract fractions for any improved α-amylase inhibition. Table 5 presents the α-amylase inhibition by various leaf fractions. Interestingly, only three fractions showed any activity: the chloroform, diethyl ether, and ethyl acetate fractions. The hexane and water fractions exhibited no inhibition at concentrations up to 10,000 µg/mL. Among the active fractions, the diethyl ether fraction demonstrated the highest inhibition, reaching 42.9% at 10,000 µg/mL, followed by the ethyl acetate fraction (31.6%) and the chloroform fraction (33.0%). The diethyl ether fraction's IC_{50} was calculated to be 11,462 µg/mL (11.46 mg/mL), which was the lowest (best) among the fractions. The chloroform and ethyl acetate fractions had IC_{50} values of 15,773 µg/mL and 16,797 µg/mL, respectively. These values, while somewhat better than the crude leaf extract, are still much higher than that of acarbose. The data suggest that the compounds responsible for the modest α-amylase inhibition reside mainly in the moderately polar fractions (chloroform, ethyl acetate, diethyl ether), with the diethyl ether-soluble constituents being the most effective inhibitors of this enzyme. The lack of activity in hexane (non-polar) and water (very polar) fractions further narrows down the polarity range of potential amylase-inhibiting compounds (likely mid-polarity phenolics or alkaloids, etc.). Magaji *et al*. similarly reported that among various *M. oleifera* extracts, the leaf extract and its fractions showed the strongest α-amylase inhibition compared to extracts from other plant parts. [15]. In our study, although the inhibition is weak, the trend is consistent: leaf-derived samples > seed or bark, and certain fractions concentrating inhibitory compounds perform better than the crude extract. Overall, the α-amylase inhibition results indicate that *M. oleifera* leaf extracts have limited ability to inhibit starch digestion. The activity is concentrated in certain solvent fractions but remains weak relative to pharmaceutical inhibitors. Nevertheless, even modest inhibition of α-amylase could contribute to a mild reduction in postprandial glucose spikes if such extracts are consumed, potentially supporting diabetic management in combination with other therapies. Further purification of the active fractions might yield specific compounds with higher inhibitory potency.

α-Glucosidase Inhibitory Activity

The crude extracts were next evaluated for α-glucosidase inhibitory activity, which is relevant for slowing down disaccharide breakdown and glucose absorption. The leaf extract again was the only crude extract to exhibit measurable α-glucosidase inhibition, whereas the seed and bark extracts showed no activity (Table 6). The leaf extract caused 43.24% inhibition at 5,000 µg/mL, with an IC_{50} of 5,696 ± 1.08 µg/mL (about 5.7 mg/mL). In comparison, acarbose was much more potent, achieving ~87.6% inhibition at 1,000 µg/mL and an IC_{50} of ~316.6 µg/mL. Thus, similar to the amylase results, the leaf extracts glucosidase inhibition is weak (roughly 18 times less potent than acarbose on a weight basis). Nonetheless, there was a dose-dependent increase in inhibition with rising concentrations of the leaf extract (Table 6). No inhibition was detected for bark or seed even at 5,000 µg/mL (those rows remain all "–"), indicating that active α-glucosidase inhibitors are essentially absent in those parts or below detectable levels.

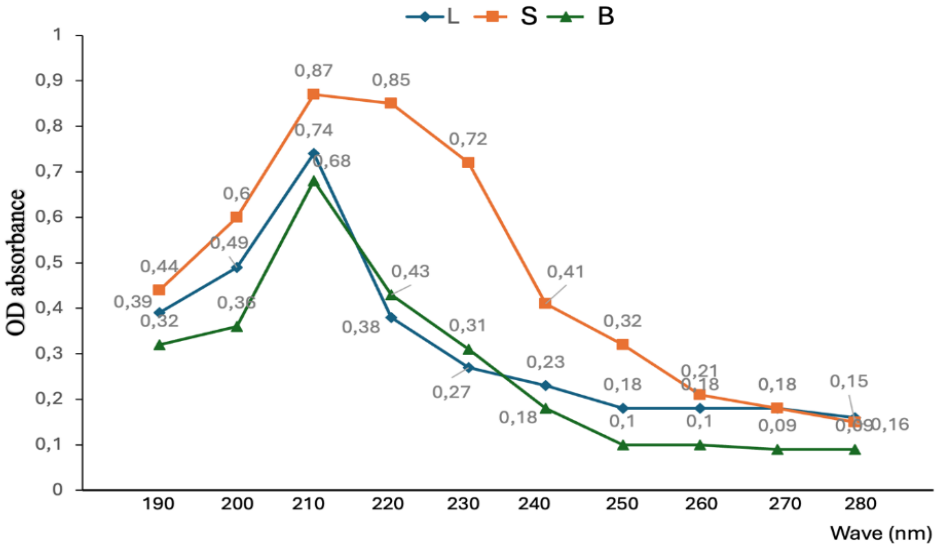

Figure 1: Absorption spectrum of extracts from *Moringa oleifera* at a concentration of 150 µg/ml (150–280 nm). L: Leaf extract of *Moringa oleifera*; S: Seed extract of *Moringa oleifera*; B: Stem bark extract of *Moringa oleifera*.

The fractionated leaf extracts yielded more pronounced *α*-glucosidase inhibition in certain fractions (Table 7). Similar to the amylase case, only two fractions—diethyl ether and ethyl acetate—exhibited significant *α*-glucosidase inhibitory activity, whereas the hexane, chloroform, and water fractions showed no activity (Table 7). The ethyl acetate fraction was notably the most potent, reaching 95.47% inhibition at 5,000 µg/mL. In fact, at 2,500 µg/mL, the ethyl acetate fraction already achieved over 83% inhibition, far surpassing the diethyl ether fraction (28.26% at 2,500 µg/mL). The diethyl ether fraction showed a more modest effect, about 50.33% inhibition at 5,000 µg/mL.

(The diethyl ether fraction reached ~18% and ~28% inhibition at 1,250 and 2,500 µg/mL, respectively, and ~50% at 5,000 µg/mL.) The IC_{50} of

the ethyl acetate fraction was calculated as 2,107.3 µg/mL (~2.11 mg/mL), while that of the diethyl ether fraction was 4,855.7 µg/mL (~4.86 mg/mL). These values indicate that the ethyl acetate fraction contains the most effective *α*-glucosidase inhibitors among the fractions, being roughly twice as potent as the diethyl ether fraction and about 2–3 times more potent than the crude leaf extract. Notably, the ethyl acetate fraction's IC_{50} (~2.1 mg/mL) is even slightly better than what Adisakwattana and Chanathong (2011) observed for a methanolic *M. oleifera* leaf extract (IC_{50} > 5 mg/mL for *α*-glucosidase from porcine pancreas). [14]. This suggests that partitioning the leaf extract enriched certain compounds (likely mid-polar flavonoids or other glycosidase inhibitors) in the ethyl acetate layer.

In summary, the *α*-glucosidase inhibition results highlight the ethyl acetate fraction of *M. oleifera* leaf as the most promising for anti-diabetic activity, with an IC_{50} of ~2.1 mg/mL, which, while still higher than acarbose (0.317 mg/mL), is relatively strong for a crude plant-derived fraction. The diethyl ether fraction also contains inhibitors, but at a lower concentration or efficacy. The absence of activity in hexane and water fractions suggests that highly non-polar and highly polar constituents are not contributing, and the active principles are likely moderately polar compounds (possibly flavonoid glycosides or other phenolics). These compounds could be similar to known natural *α*-glucosidase inhibitors found in other plants. The data corroborate other studies that identified *M. oleifera* leaves as a source of *α*-glucosidase inhibitors, although potency can vary with extraction solvent. [14] Further purification of the ethyl acetate fraction could isolate specific inhibitor molecules. Notably, even partial inhibition of *α*-glucosidase can be beneficial in controlling postprandial hyperglycemia by slowing glucose release from complex carbohydrates. Therefore, *M. oleifera* leaf extracts or their refined fractions have potential as supplementary therapy for type 2 diabetes, though their effectiveness in vivo and safety would need thorough evaluation.

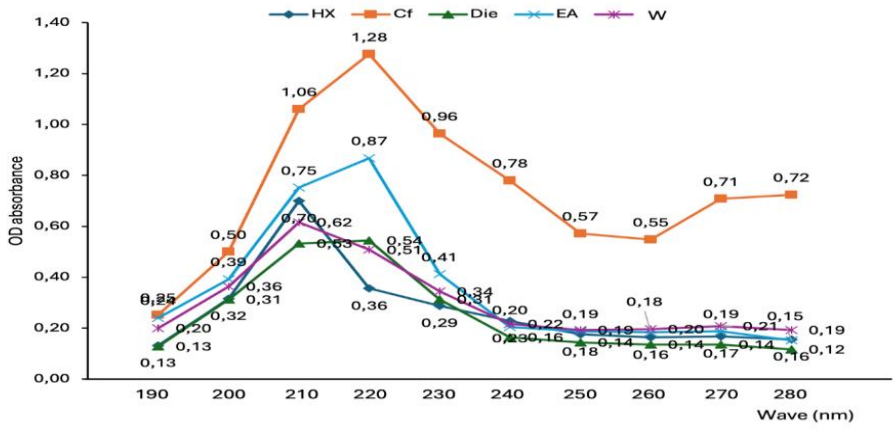

Figure 2: Absorption spectrum of fractionated extracts from Moringa oleifera leaf extract at a concentration of 150 µg/ml (190–280 nm). HX: hexane fraction; Cf: chloroform fraction; Die: diethyl ether fraction; EA: ethyl acetate fraction; W: aqueous fraction

Comparison with Previous Studies:
Compared to prior international studies on *M. oleifera*, our results show several notable distinctions and novel insights. Firstly, the plant material in this study was sourced from Buon Ma Thuot, a unique ecological region (the Central Highlands of Vietnam) characterized by fertile red basalt soil and a distinct climate. This provenance may contribute to the exceptionally high phytochemical levels observed. For example, Abdulkadir *et al.*(2015) reported a methanolic *M. oleifera* leaf extract DPPH IC_{50} of ~720 µg/mL, [13] times higher than the ~425 µg/mL IC_{50} of the Buon Ma Thuot leaf extract in our study. This suggests the Buon Ma Thuot leaves possess stronger antioxidant capacity, potentially due to their higher phenolic content or different compound profile. Likewise, Adisakwattana and Chanathong (2011) observed an *α*-glucosidase IC_{50} greater than 5 mg/mL for a crude *M. oleifera* leaf extract, [14] whereas our ethyl acetate leaf fraction achieved an IC_{50} of ~2.1 mg/mL. In other words, by partitioning the Buon Ma Thuot leaf extract, we obtained a fraction with roughly twice the glucosidase inhibitory potency of that prior extract. Similar findings were reported by Siddhuraju and Becker, [16] who observed considerable variation in antioxidant activity and phenolic content of M. oleifera leaves collected from different agro-climatic regions, emphasizing the strong environmental influence on phytochemical accumulation. The Central Highlands environment (with its mild climate and rich soil) may enhance the accumulation of bioactive compounds in the leaves. Additionally, our use of solvent fractionation is a novel aspect that allowed concentration of the active constituents into specific fractions, yielding higher measured activities than many unfractionated extracts reported in the literature. While numerous studies have examined *M. oleifera* from various regions, this work is among the first to analyze material from Buon Ma Thuot and to demonstrate improved enzyme inhibition through targeted fractionation. These findings underscore the novelty of our study and provide new, region-specific data on *M.oleifera*'s antioxidant and anti-diabetic potential compared to previously published results.

Conclusion

This study demonstrated that *Moringa oleifera* leaves from Buon Ma Thuot, Vietnam, are a rich source of natural antioxidants and possess mild inhibitory activities against carbohydrate-digesting enzymes. The leaf ethanol extract showed the highest total polyphenol and flavonoid contents among the tested plant parts, correlating with its superior DPPH radical scavenging capacity. Solvent fractionation of the leaf extract revealed that the chloroform and hexane fractions concentrated most of the antioxidant compounds, whereas the ethyl acetate and diethyl ether fractions concentrated the compounds responsible for enzyme inhibition. The chloroform fraction exhibited the strongest antioxidant effect (DPPH IC_{50} ~117.5 µg/mL), and the ethyl acetate fraction showed the most potent *α*-glucosidase inhibition (IC_{50} ~2107 µg/mL). Although the *α*-amylase and *α*-glucosidase inhibitory effects of the crude leaf extract were relatively weak (IC_{50} in the milligram per mL range), the activities were noticeably enhanced in specific fractions. The bark and seed extracts contained significantly lower levels of bioactive compounds and showed negligible activity in the assays.
In conclusion, the leaves of *M. oleifera* cultivated in the Central Highlands of Vietnam are the most promising part of the plant for antioxidant and anti-diabetic applications. The potent antioxidant activity supports the use of *Moringa* leaves as a dietary supplement to combat oxidative stress. Meanwhile, the enzyme inhibition results suggest that leaf extracts (especially certain refined fractions) could help modulate postprandial blood glucose levels, although they are not as potent as standard drugs like acarbose. Furthermore, compared to similar studies on *M. oleifera* from other countries, the Buon Ma Thuot leaves exhibited higher levels of phenolics and stronger bioactivities, highlighting the influence of the unique regional growing conditions and the value of fractionation in enhancing activity. This work provides a novel contribution by linking a specific cultivation environment to improved phytochemical potency. Further studies, including in vivo evaluations and compound isolation, are warranted to fully elucidate the therapeutic potential of *M. oleifera* leaf constituents in the management of oxidative stress and type 2 diabetes.

List of Abbreviations

DPPH: 2,2-Diphenyl-1-picrylhydrazyl
GAE: Gallic acid equivalents (unit for total phenolics)
QE: Quercetin equivalents (unit for total flavonoids)
IC_{50}: Concentration required to achieve 50% inhibition (of DPPH or enzyme activity)
pNPG: *p*-Nitrophenyl-*α*-D-glucopyranoside (*α*-glucosidase substrate)
UI: Enzyme activity unit (One unit = amount of enzyme that catalyzes the conversion of 1 µmol substrate per minute)

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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