


Phenolic compounds and *in vitro* antioxidant activity of *Moringa stenopetala* grown in South Ethiopia

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ABSTRACT

Moringa stenopetala is a traditional medicinal and nutritional plant in Ethiopia. The aim of this work was to carry out a chemical analysis focusing on secondary metabolites, particularly phenolic compounds, and antioxidant activity of aqueous and methanol extracts of *Moringa stenopetala* leaf. The phenolic compounds were analyzed by high-performance liquid chromatography diode array detector (HPLC-DAD). Hydroxycinnamic acid ($538 \pm 6 \mu\text{g/g}$), 3-Hydroxybenzoic acid ($31 \pm 6 \mu\text{g/g}$), and quercetin-3-O-rutinoside ($1155 \pm 65 \mu\text{g/g}$) were the major components in methanol extract. Whereas, syringic ($84 \pm 13 \mu\text{g/g}$), chlorogenic ($165 \pm 19 \mu\text{g/g}$), succinic ($1811 \pm 105 \mu\text{g/g}$), and fumaric ($1582 \pm 65 \mu\text{g/g}$) acids were the major organic acids in the aqueous extract. Methanol extract had higher total flavonoid ($11 \pm 2 \text{ mg}$ of catechin equivalent per gram of dried extract) and total phenolic ($39 \pm 3 \text{ mg}$ of gallic acid equivalent per gram of dried extract) contents. This extract showed stronger DPPH scavenging ($\text{EC}_{50} = 78 \pm 6 \mu\text{g/mL}$) and ferrous chelating ($\text{EC}_{50} = 239 \pm 12 \mu\text{g/mL}$) activities. Due to the abundance sources of bioactive compounds and antioxidant activity, the dried leaf of *M. stenopetala* could be used for the development of nutraceuticals or incorporated into functional foods.

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INTRODUCTION

Moringa stenopetala is one of the 14 *Moringa* species that belongs to the family of Moringaceae.^[1] The plant is native to South Ethiopia, North Kenya, and Eastern Somalia.^[2] It is a vital source of living and a cultural heritage in South Ethiopia. The plant is commonly called Shiferaw in Amharic, cabbage tree in English and it has different names like Shelagda, Halako, and Aleko in the various areas of Southern Ethiopia.^[3] *M. stenopetala* is a multi-purpose plant with unique draught-resistant characteristics. In South Ethiopia, people use the leaf for human consumption and animal feed.^[4] In the region, the plant becomes the most preferred and marketable leafy vegetables. Having plenty of *Moringa* trees in the garden or farmland is an indication of pride in society. Particularly, in the Arba Minch and Wollayta areas, the local people cook the leaves with flour of maize and sorghum to prepare a traditional food known as kurkufa.^[5] Konso and Gidole people use the leaves as food, medicine,^[4] and an ingredient for the preparation of traditional fermented beverage,^[6] locally known as *Cheka*. The powder of dried leaves has been also used as a herbal medicine and widely available in supermarkets of Ethiopia.^[2]

The leaf extract of *M. stenopetala* was found with having low cholesterol content and change the hematological composition of blood and biochemical parameters in mice models.^[7] Crude extracts and fractions lower blood glucose level^[8] and showed anti-hypertensive and anti-hyperlipidemic

effects.^[9] Different solvent extracts of the leaf showed antimicrobial activity^[10] against *different bacteria*. Tamirat *et al.*^[11] revealed that the polar leaf extract showed the anti-inflammatory and analgesic effects in the mice model.

The information regarding effect of decoction on antioxidant activity^[12] and the presence of different phenolic compounds and aliphatic organic acids in leaf extract of *M. stenopetala* is limited.^[8] In this study, the major phenolic constituents of the leaf extracts were analyzed using a high-performance liquid chromatographym diode array detector (HPLC-DAD). The antioxidant properties were also evaluated by DPPH radical scavenging ability and ferrous chelating activity.

MATERIALS AND METHODS

Collection and preparation of plant extracts

The fresh leaves of *M. stenopetala* (Figure 1A) were collected from the compound of Hawassa College of Education, Hawassa, South Ethiopia. The identity of the tree has already been established by the Department of Biology on campus, and trees were properly labeled by the same name. The leaves were air dried for 6 days. The dried sample was ground to fine powder (Figure 1B) using electric grinder (FM100 model, China). The extract was prepared by dissolving 5 g of fine powder in 50 mL of methanol. The contents were kept in an orbital shaker for 8 h at room temperature. Thereafter, the extract was filtered using Whatman no.1. After filtration under reduced pressure, the extract was evaporated to dryness under N₂. Decoction was performed by adding 20 mL of distilled water to 1 g sample, heated (heating plate, VELP scientific) and boiled for 5 min. The mixtures were left to stand for 5 min and sonicated (model 750D, VWR Intl. Ltd., Montreal, QC, Canada) for 1 min and then centrifuged (model Durafuge 300, Precision Scientific, Richmond, VA, USA) at 5000 rpm for 15 min. Then aqueous extract was freeze dried for 8 h in a freeze dryer (model 2085C0000, Kinetics Thermal Systems, Stone Ridge, NY, USA). Both dried methanol and aqueous extracts were stored in a sealed plastic container at 20°C. The extraction procedure was done in triplicate

Total phenolic content (TPC)

TPC was estimated using the Folin-Ciocalteu colorimetric assay, based on the procedures described by Yishak *et al.*^[13] To 0.1 mL of the extract (1 mg/mL), 1 mL Folin-Ciocalteu reagent (diluted ten times) was added. The mixture was left for 5 min and 1 mL (75 g/L) of sodium carbonate was added. The absorbance of the resulting blue color was measured at 765 nm with UV-visible spectrophotometer (JENWAY, 96500 model) after incubation for 90 min at room temperature. Quantification was done

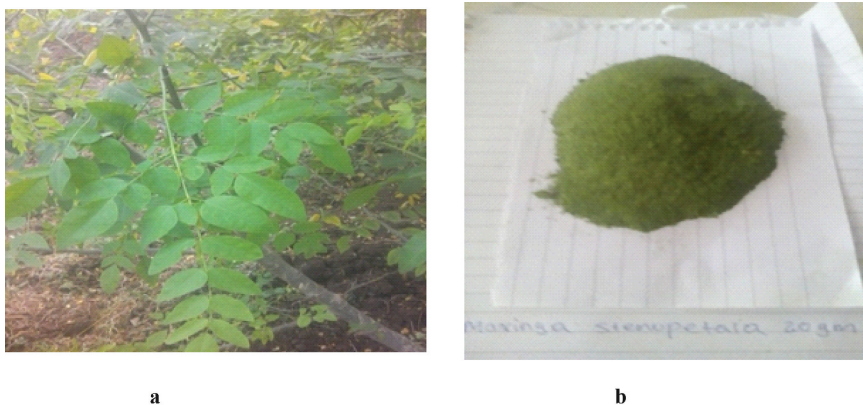


Figure 1. *Moringa stenopetala*, leaf (A), powder (B).

based on a standard curve of gallic acid. Results were expressed as milligram of gallic acid equivalent per gram of dry extract (mg GAE/g) using the equation obtained from calibration curve of gallic acid ($y = 0.073x - 0.046$, $R^2 = 0.991$). All tests were performed in triplicate.

Total flavonoid content (TFC)

TFC was determined using the aluminum chloride colorimetric method as described by Engeda *et al.*^[14] The extract (1 mL, 1 mg/mL) was diluted with 1.25 mL distilled water. At zero time, 75 μ L 5% NaNO₂ were added to the mixture. After 6 min, 150 μ L 10% AlCl₃ were added. After another 5 min, 1 mL of 1 M NaOH was added to the mixture. Immediately, the absorbance of the mixture, pink in color, was determined at 510 nm versus prepared water blank. A standard curve was prepared using catechin ($y = 0.0068x + 0.164$, $R^2 = 0.99$; $p < .001$). Results were expressed as milligram of catechin equivalents per gram of dry extract.

Phenolic compounds

Separation and identification of phenolic compounds were carried out using HPLC coupled to electrospray ionization and triple quadrupole mass spectrometry (LC-MS) as described by Rupasinghe *et al.*^[15] The analysis was performed using a Waters Alliance 2695 separation module (Waters, Milford, MA, USA) coupled with a Micromass Quattro micro API MS/MS system and controlled with Mass Lynx V4.0 data analysis system (Micromass, Cary, NC, USA). Electrospray ionization in negative ion mode was used in the multiple reaction mode of mass spectrometric analysis. The column used was an Allure biphenyl (100 mm \times 2.1 mm) (Restek Chromatography Products, Bellefonte, PA, USA). For the separation of each compound, the mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.35 mL/min. A linear gradient profile was used with the following proportions of solvent A applied at time t (min); (t , A %): (0, 94%), (2, 83.5%), (2.61, 83%), (2.72, 82.5%), 3.63, 82.5%), (4.08, 81.5%), (4.76, 80%), (6.75, 20%), (8.75, 94%), (12, 94%). The retention time of each compound in the extract (Figure 2) was compared with the retention time of the standards in different mobile phases (Figures 3 and 4). The peaks, showing the same retention time as that of the standards, were preliminary identified and were further analyzed by MS. The MS was used to get the molecular weights of the compounds by scanning from 50 to 1500 m/z .

For the quantitative analysis of phenolic compounds, a calibration curve was obtained by injection of known concentrations (2.5–100 μ g/mL) of different standard compounds (Table 1). The results were expressed in microgram per gram of dry weight (dw), as mean \pm SD of three independent analyses.

Antioxidant activity

DPPH scavenging method

The DPPH scavenging activity was determined by the method described by Engeda.^[16] Various concentrations ranging from 50 up to 1000 μ g/mL of the extracts and commercial antioxidants (BHT and ascorbic acid) were taken in different test tubes. Volume of 2 mL of freshly prepared DPPH solution (0.06% w/v) prepared in methanol was added in each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference standards were vortexed and left to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was then taken at 520 nm. Methanol was used as blank. The ability to scavenge the DPPH radical was calculated as DPPH scavenging (%) = $[(A_c - A_s)/A_c] \times 100$, where A_c is the absorbance of the control and A_s is the absorbance in presence of the sample of the extracts. The EC₅₀ value was defined as the effective concentration (in μ g/mL) of extracts

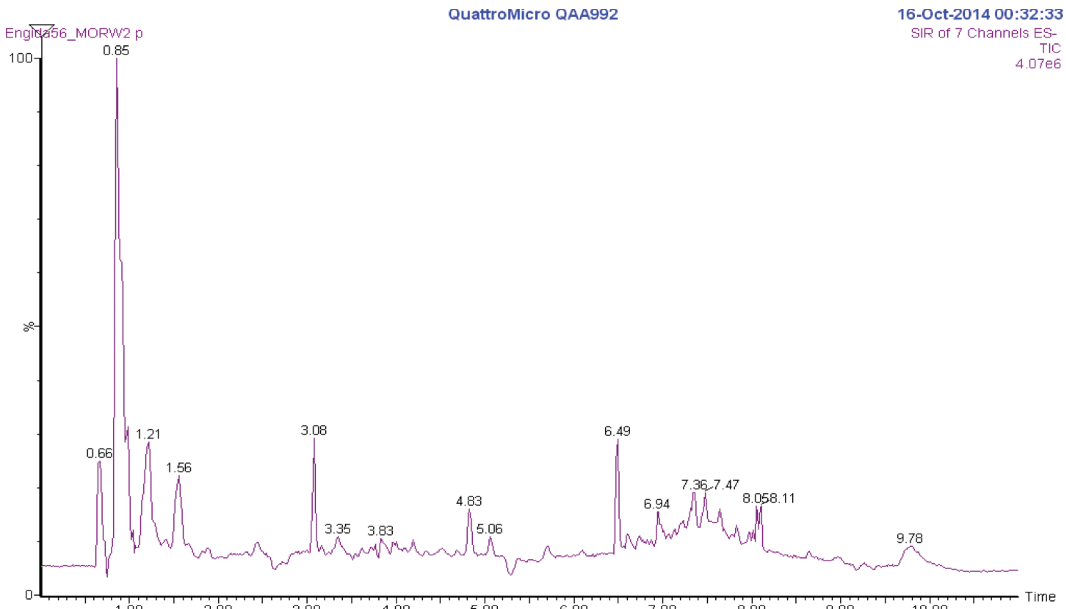


Figure 2. HPLC Chromatogram of *Moringa stenopetala*.

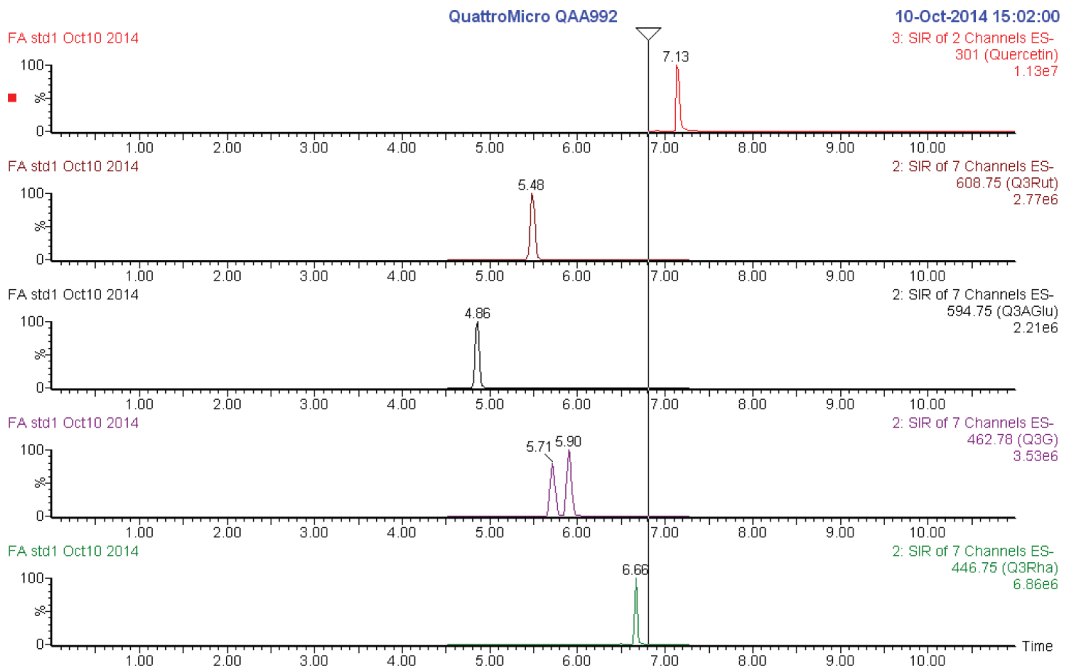


Figure 3. HPLC Chromatogram of quercetin and derivatives.

that scavenges the DPPH radical by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations. Using the dose-response curve, the EC_{50} values of commercial antioxidant (BHT and ascorbic acid) and crude extracts were calculated.

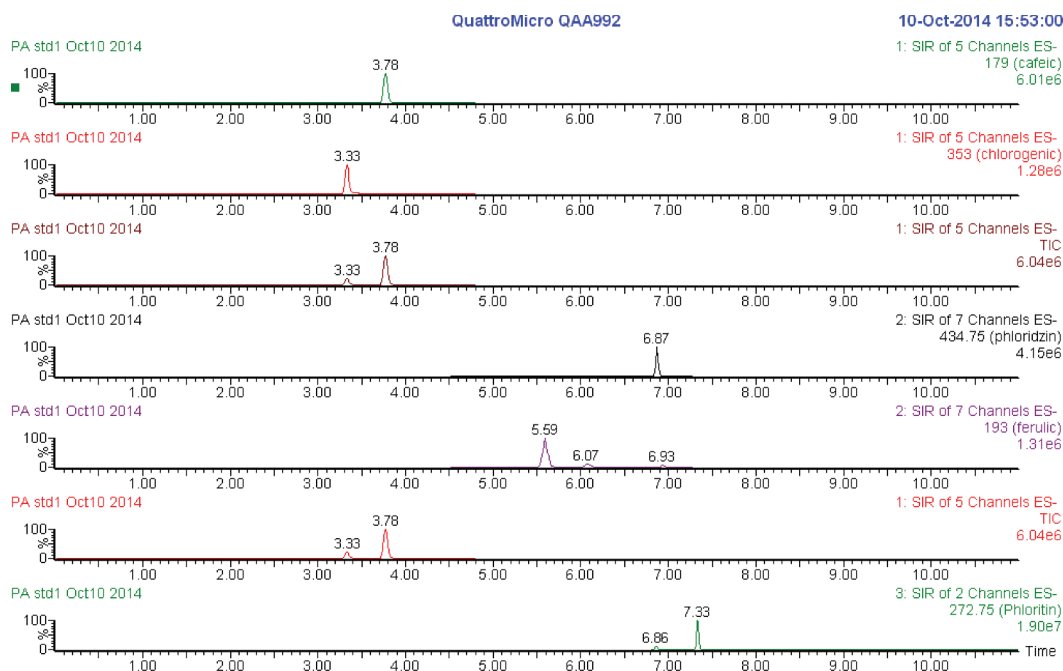


Figure 4. HPLC Chromatogram of phenolic acids.

Table 1. Regression equations of standard phenolic compounds for quantification of compounds in the extracts.

Standard chemical used	Regression equation	Correlation coefficient (R^2)
Caffeic acid	$y = 12161.281x + 15193.95$	0.999
Chlorogenic acid	$y = 2426.04x + 2982.54$	0.998
Ferulic acid	$y = 2590.53x + 4051.16$	0.995
Isoferulic acid	$y = 461.42x + 39.11$	0.999
Hydroxycinnamic acid	$y = 1534.72x + 330.12$	0.956
3-Hydroxybenzoic acid	$y = 847.02x + 161.43$	0.989
Syringic acid	$y = 1259.55x + 222.59$	0.998
Quercetin-3-O-glucoside	$y = 9649.17x + 4449.30$	0.999
Quercetin-3-O-galactoside	$y = 2853.93x + 8285.48$	0.999
Quercetin-3-O-rutinoside	$y = 7947.03x + 2168.42$	0.999
Quercetin	$y = 19925.94x + 33058.53$	0.986
Quercetin-3-O-rhamnoside	$y = 10784.83x + 8917.24$	0.995
Quercetin-3-O-arabinoglucoside	$y = 6688.89x + 1751.29$	0.999
Succinic acid	$y = 1266.91x + 151.77$	0.977
Fumaric acid	$y = 1435.88x + 234.39$	0.966

Chelating effects on ferrous ions

The ferrous ion chelating activity of extracts was determined according to the method of Mohammad *et al.*^[17] with slight modification. A volume of 3 mL of various concentrations (100–800 $\mu\text{g/mL}$) of the extracts in methanol was added to a solution of 2 mM FeCl_2 (0.05 mL). After adding 0.1 mL of 5 mM ferrozine, the mixture was shaken vigorously using a vortex mixer and left at room temperature for 10 min. Then absorbance of the solution was measured at 562 nm and the inhibition percentage of ferrozine- Fe^{2+} complex formation was calculated as a metal chelating effect (%) = $[(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$, where A_{Control} is the absorbance of control (the Fe^{++} ferrozine complex) and A_{Sample} is the absorbance of the test sample. Ethylenediamine tetraacetic acid (EDTA), ascorbic acid, quercetin, and BHT were used as a reference control.

Statistical analysis

All antioxidant activity assays and phenolic contents were carried out in triplicate and mean values were calculated and linear regression analysis was used to calculate the EC₅₀ value. The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests was used to assess differences between means. A significant difference was considered at a level of $p < .05$.

RESULT AND DISCUSSIONS

Total phenolic and flavonoid contents

There are various publications reporting the TPC of *Moringa* from different origins obtained by different extraction methodologies.^[18,19] In this study (Table 2), the methanol extract showed higher TPC than that of aqueous extract. The TPC of *M. stenoptala* decocted leaf sample found in this study was in agreement with previously report^[12] but higher than those reported from central and north western Ethiopia.^[20] Whereas, the TPC of the present study was lower than that of *M. oleifera* collected from different countries^[21,22] but greater than the TPC of the leaf collected from Chad.^[21] Similarly, the methanol extract had higher TFC than that of decocted aqueous extract (Table 2) also had higher TFC than the leaf extract of *M. oleifera* collected from different countries.^[22,23] The differences in TPC and TFC might be due to regions with different elevations, sample treatment, season of harvesting, and leaf maturity.^[24] These results suggest that *M. stenoptala* leaves may be exploited as an important source in nutraceutical and functional food industries.

LC-MS analysis of phenolic compounds

The quantifications of phenolic compounds ($\mu\text{g/g}$ of dry matter) were accomplished by comparing retention times, molecular mass and peak areas between the standards and the samples. The HPLC-DAD analysis showed that the phenolic profile was characterized by 15 compounds. As shown in Table 3, seven phenolic acids, six flavonols, and two aliphatic organic acids were identified and quantified in both extracts. This is the first study that reports the presence of phenolic acids and aliphatic organic acids in *M. stenoptala* leaf extracts. The methanol extract had higher 3-Hydroxybenzoic acid, ferulic, caffeic, and hydroxycinnamic acids than that of aqueous extract. While the aqueous extract had higher syringic, chlorogenic, and isoferulic acids. In both extracts hydroxycinnamic acid was the highest followed by chlorogenic, syringic, isoferulic, and 3-Hydroxybenzoic acids. Ferulic and caffeic acids present in lowest amount. The study conducted on *M. oleifera* collected from three different countries^[21] had higher ferulic acid content than the result of present study. Chlorogenic acid concentration was lower than amount found in *M. oleifera* leaves collected from Mexico,^[25] also lower than amounts found in different tropical fruits.^[26] Hydroxycinnamic acid from *M. oleifera* collected from Egypt^[27] was lower than that of the present study. Fumaric and succinic acids were reported for the first time and aqueous extract had higher content of these two aliphatic carboxylic acids.

Quercetin-3-O-rutinoside, commonly called rutin, has numerous biological activities such as antimicrobial,^[28] anticancer,^[29] anti-inflammatory,^[30] antioxidant,^[31] antiglycemic,^[32] antiviral,^[33] and therapy for Alzheimer's disease.^[34] Out of six identified flavonols, rutin was found as the highest

Table 2. TPC and total TFC of methanol and aqueous leaf extracts *M. stenoptala*.

Extract	TPC (mgGAE/g)* ¹ \pm SD	TFC (mgCAE/g)* ² \pm SD
Water	31 \pm 0.9	2 \pm 0.4
Methanol	39 \pm 3	11 \pm 2

Where *1 and *2 are total phenolic and total flavonoid contents expressed as gallic acid and catechin equivalents per gram of dried extract, respectively. Values are expressed as mean \pm SD ($n = 3$).

Table 3. Spectral information and concentration of phenolic acids, flavonols, and aliphatic organic acids in aqueous and methanol leaf extracts of *M. stenopetala*.

Compound	MW	Parent ion [M-H] ⁻ m/z	tR (min)	(µg/g of DW)	
				Water	Methanol
Phenolic acids					
Chlorogenic acid	354.31	353	3.33	165 ± 18	159 ± 22
3-ydroxybenzoic acid	138.12	137	4.03	8 ± 1	31 ± 6
Syringic acid	198.17	197	3.87	84 ± 13	50 ± 5
Ferulic acid	194.15	193	5.59	5 ± 0.5	14 ± 2
Isoferulic acid	194.18	193	6.07	44 ± 6	43 ± 4
Caffeic acid	180	179	3.78	5 ± 1	7 ± 0.8
Hydroxycinnamic acid	164.18	163	7.27	177 ± 9	538 ± 36
Flavonols					
Quercetin	302.24	301	7.13	1 ± 0.4	4 ± 0.5
Quercetin-3-O-glucoside	464.38	462.78	5.90	4 ± 0.7	6 ± 1
Quercetin-3-O-galactoside	464.38	462.75	5.71	5 ± 0.7	0.8 ± 0.2
Quercetin-3-O-rhamnoside	448.38	446.75	6.61	1 ± 0.3	0.9 ± 0.1
Quercetin-3-O-rutinoside	610.52	608.75	5.48	700 ± 42	1155 ± 65
Quercetin-3-O-arabinoglucoside	496.5	594.75	4.86	0.6 ± 0.3	2 ± 0.8
Aliphatic organic acids					
Fumaric acid	116.07	115	1.17	1811 ± 105	263 ± 38
Succinic acid	118.09	117	1.21	1582 ± 65	935 ± 103

DW: dried weight of plant material

and the rest present in detectable amounts (Table 3). Rutin was higher in methanol extract, whereas, the rest flavonoids were found in small amounts. The study conducted by Habtemariam.^[8] On the leaf of *M. stenopetala* sample purchased from various sources of Ethiopian herbal suppliers showed higher rutin content, but the rutin content of the present study was greater than the amount present in *M. oleifera* collected from different countries^[35,36] also higher than the amounts found in several spices, fruits, and vegetables, such as mango,^[37] apple,^[38] cabbage,^[37] and *Coriandrum sativum*,^[39] but far below the amounts found in cereals such as buckwheat.^[36] Similarly, methanol extract had higher amounts of quercetin, quercetin-3-O-glucoside, and quercetin-3-O-arabinoglucoside than that of the aqueous extract. The amount of quercetin obtained under this study was lower than the amount obtained from *M. oleifera* collected from different countries.^[31,40]

Antioxidant activity

DPPH scavenging

The DPPH radical scavenging effects of *M. stenopetala* leaf extracts are shown in Figure 5. As the concentration of sample increased, the percent inhibition of DPPH radical also increased. This suggested that dried leaf extract of *M. stenopetala* contain compounds that can donate electron/hydrogen easily and stabilizes free radicals. The EC₅₀ values for each sample was calculated to show the concentration of sample needed to reduce 50% of the DPPH in the assay. Low EC₅₀ values correspond to high antioxidant activity. The EC₅₀ values of both extracts and references were calculated from graph of percentage scavenging activity against concentration of the extracts (Table 4). Stronger scavenging activity (lower EC₅₀ values) was recorded for methanol extract, which appeared nearly two times stronger than that of the aqueous extract. This can also be related to the higher TPC obtained in methanol extract. Rutin as the principal component of the leaves of *M. stenopetala*, possessed strong DPPH scavenging activity^[41] with the EC₅₀ value of 5 µg/mL, showing comparable antioxidant potential to vitamin C. Similarly according to Beibei *et al.*^[42] quercetin-3-O-glucoside also showed comparable antioxidant potential to vitamin C. Under the present findings, the HPLC analysis of methanol extract showed higher content of rutin and quercetin-3-O-glucoside contributing for this extract to exhibit stronger DPPH scavenging activity than that of aqueous extract. The DPPH scavenging activity of L-ascorbic acid and BHT tested as references were found to be significantly ($p < .05$) stronger (lower EC₅₀) than that of methanol and the aqueous extracts. The aqueous extract of the present study showed stronger DPPH scavenging activity than that of similar extract of *M. oleifera* leaves^[8] but weaker than the result reported by Gaafar *et al.*^[40]

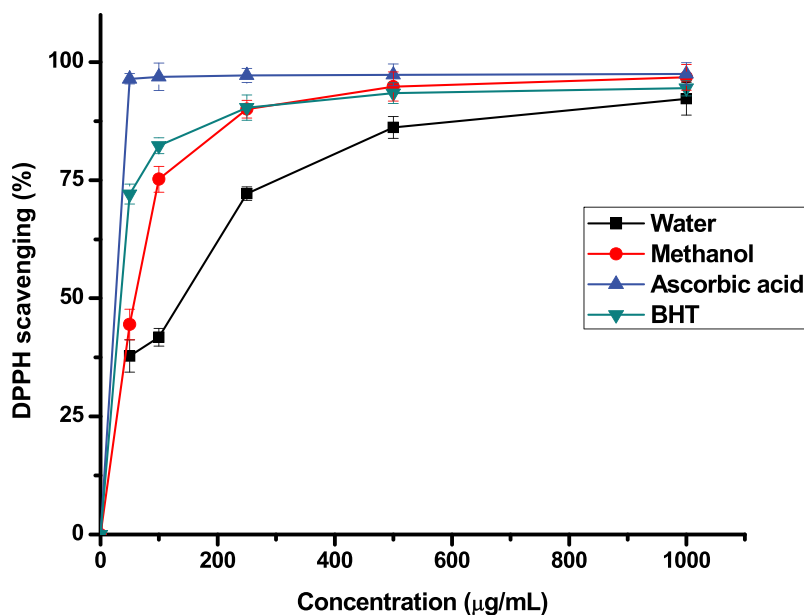


Figure 5. DPPH radical scavenging activity (%) of aqueous and methanol dried leaf extracts from *M. stenopetala* and references (L-ascorbic acid and BHT). Values are average of triplicate measurements (mean \pm SD).

Table 4. The EC₅₀ (µg/mL) values of aqueous and methanol leaf extracts of *M. stenopetala*.

Sample	DPPH scavenging	Ferrous chelating
Water	142 \pm 9.82 ^d	385 \pm 17 ^c
Methanol	78 \pm 6 ^c	239 \pm 12 ^b
BHT	31 \pm 4 ^b	> 8 \times 10 ²
Ascorbic acid	22 \pm 3 ^a	> 8.0 \times 10 ²
EDTA	-	51 \pm 3 ^a
Quercetin	-	> 8 \times 10 ²

Values are expressed as mean \pm SD from triplicate experiments; values with different letters in a column are significantly different at the level of $p < 0.05$.

The DPPH scavenging activity of methanol extract of *M. stenopetala* conducted under this study was weaker than the result of similar study conducted by Habtemariam and Varghese,^[41] on dried powdered leaf of *M. stenopetala* sample purchased from Ethiopian supermarkets but stronger than that of the identified samples collected from different parts of north and northwestern Ethiopia.^[20] Both the methanol and aqueous extracts showed stronger DPPH radical scavenging activity than that of *M. oleifera* collected from South West China^[43] and India.^[41] However, the DPPH radical scavenging activities of the present samples were weaker than that of the thirteen *M. oleifera* cultivars collected from different geographical locations in the world.^[44] All these cultivars showed a stronger DPPH radical scavenging ability than that of ascorbic acid, which was contradictory to the present result.

Ferrous chelating activity

The chelation of metals can be crucial in the prevention of radical generation and increase the oxidation stability of lipids through blocking these metal ions, and thus limiting the formation of chain initiators.^[45] Ferrozine can quantitatively form complexes with Fe²⁺. In the presence of chelating agents, the complex formation is disrupted resulting in a decrease in the pink color of the complex. The reduction of color intensity at 562 nm wavelength allows estimation of the metal chelating activity of the chelators. In this

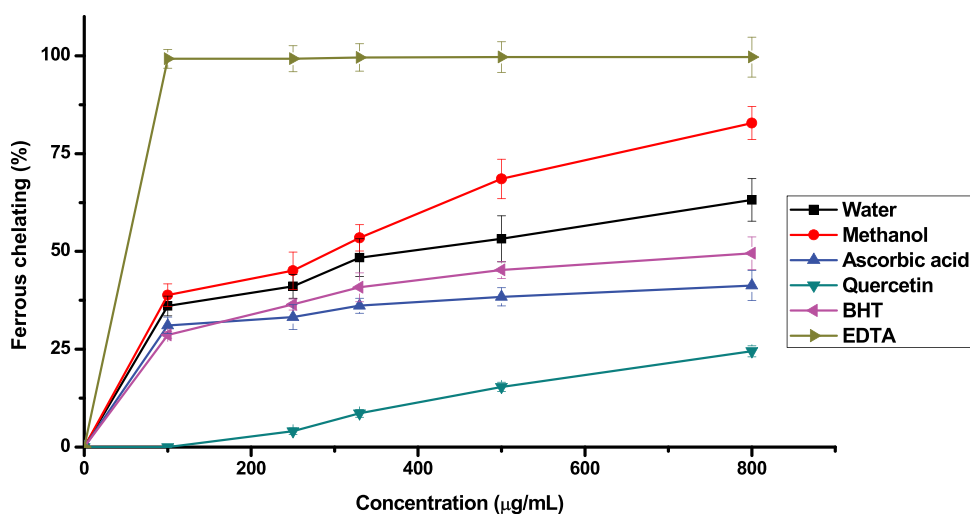


Figure 6. Ferrous ion chelating activity (%) of decoction aqueous and methanol extracts from dried leaf of *M. stenopetala* and references (ascorbic acid, BHT, quercetin, and EDTA). Values are average of triplicate measurements (mean \pm SD).

assay, the methanol extract, decoction aqueous extract and standard compounds were assessed for their ability to compete with ferrozine for Fe^{2+} in the solution. The percentage of iron chelating activities of all extracts and references were concentration dependent (Figure 6). At 800 $\mu\text{g/mL}$, the percentages of iron chelating capacity of the extracts and references decreased in the order of: EDTA ($99 \pm 0.1\%$) > methanol extract ($89 \pm 4\%$) > aqueous extract ($63 \pm 5\%$) > BHT ($50 \pm 1\%$) > ascorbic acid ($41 \pm 0.3\%$) > quercetin ($24 \pm 3\%$). Table 4 shows that the methanol extract exhibited stronger iron chelating (lower EC_{50}) activity than that of aqueous extract ($p < .05$). This may be because methanol extract has greater amounts of flavonoids, containing multiple hydroxyl and carbonyl groups, and chelate ferrous ions are important for the generation of reactive oxygen species (ROS) that may damage cell.^[46] EDTA (positive control) was an excellent chelator for ferrous ions and its chelating capacity ($\text{EC}_{50} = 51 \pm 3 \mu\text{g/mL}$) was much stronger than that of the methanol and aqueous extracts ($p < .05$). Nevertheless, in this assay, ascorbic acid, BHT, and quercetin showed weaker chelating activity of iron (II) ions ($\text{EC}_{50} > 800 \mu\text{g/mL}$) than that of decoction aqueous and methanol extracts, which is consistent with the findings of Nic'iforovic *et al.*^[47]

CONCLUSION

Chromatographic separation enabled the identification and quantification of a wide range of phenolic compounds present in the dried leaf extracts of *M. stenopetala*. Our results showed that methanol extract was rich in hydroxycinnamic acid, quercetin-3-*O*-rutinoside, and 3-Hydroxybenzoic acid constituents and demonstrated stronger antioxidant activity. Whereas, the aqueous extract had higher fumaric, succinic, chlorogenic and syringic acids. Unlike *M. oleifera*, little scientific research has been conducted on the phytochemical content and biological activities of *M. stenopetala* grown in Ethiopia. To confirm the beneficial effects of this valuable draught-resistant plant, further research could be conducted on different *M. stenopetala* cultivars grown under different environmental conditions and considering effect of season of harvesting on the phytochemical contents, which can be a source of industrial-scale production for nutraceuticals and fortification of functional foods.

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Disclosure statement

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References

- [1] Abuye, C.; Urga, K.; Knapp, H.; Selmar, D.; Omwega, A. M.; Imungi, J. K. A Compositional Study of *Moringa Stenopetala* Leaves. *East Afr Med. J.* **2003**, *80*(5), 247–252. DOI: [10.4314/eamj.v80i5.8695](https://doi.org/10.4314/eamj.v80i5.8695).
- [2] Mohammed, A. S.; Medicinal and Dietary Role of *Moringa Stenopetala* Bak. F. Cuf. In South Ethiopia: A Review. *Afri. J. Agri. Sci. Technol.* **2013**, *1*(1), 1–6.
- [3] Yalemtehay, M.; Amare, G. Documentation on the Uses of *Moringa Stenopetala* and Its Possible Antileishmanial and Antifertility Effects, SINET. *Ethiop. J. Sci.* **1998**, *21*(2), 287–295. DOI: [10.4314/sinet.v21i2.18126](https://doi.org/10.4314/sinet.v21i2.18126).
- [4] Abera, M.; Michael, B.; Holger, K. Evaluating the Nutritive Values and in Vitro Degradability Characteristics of Leaves, Seeds and Seedpods from *Moringa Stenopetala*. *J. Sci. Food Agric.* **2009**, *89*(2), 281–287. DOI: [10.1002/jsfa.3439](https://doi.org/10.1002/jsfa.3439).
- [5] Eyassu, S. Actual and Potential Applications of *Moringa Stenopetala*, Underutilized Indigenous Vegetable of Southern Ethiopia. *Int. J. Agri. Food Res.* **2014**, *3*(4), 8–19.
- [6] Adane, G. Chemical Characterization and Estimation of Chaka. *Am. J. Appl. Chem.* **2017**, *55*, 73–83. DOI: [10.11648/j.ajac.20170505.12](https://doi.org/10.11648/j.ajac.20170505.12).
- [7] Ghebreselassie, D.; Mekonnen, Y.; Gebru, G.; Ergete, W.; Huruy, K. The Effects of *Moringa Stenopetala* on Blood Parameters and Histopathology of Liver and Kidney in Mice. *Ethiop J. Health. Dev.* **2011**, *25*(1), 51–57. DOI: [10.4314/ejhd.v25i1.69850](https://doi.org/10.4314/ejhd.v25i1.69850).
- [8] Habtemariam, S.; Investigation into the Antioxidant and Antidiabetic Potential of *Moringa Stenopetala*: Identification of the Active Principles. *Nat. Prod. Commun.* **2015**, *10*, 475–478. DOI: [10.1177/1934578X1501000324](https://doi.org/10.1177/1934578X1501000324).
- [9] Bekesho, G.; Eyasu, M.; Asfaw, D.; Ashenfi, T. In Vivo Antihypertensive and Antihyperlipidemic Effects of the Crude Extracts and Fractions of *Moringa Stenopetala* (Baker F.) Cufod. Leaves in Rats. *Front. Pharmacol.* **2016**, *7*, 1–10. DOI: [10.3389/fphar.2016.00097](https://doi.org/10.3389/fphar.2016.00097).
- [10] Seleshi, S.; Kang, S. N. In Vitro Antimicrobial Activity of Different Solvent Extracts from *Moringa Stenopetala* Leaves. *Preven. Nutr. Food Sci.* **2019**, *24*(1), 70–74. DOI: [10.3746/pnf.2019.24.1.70](https://doi.org/10.3746/pnf.2019.24.1.70).
- [11] Tamirat, Y.; Nedi, T.; Assefa, S.; Teklehaymanot, T.; Shibeshi, W. Anti-inflammatory and Analgesic Activities of Solvent Fractions of the Leaves of *Moringa Stenopetala* Bak. (Moringaceae) in Mice Models. *BMC Complement. Altern. Med.* **2017**, *17*(473), 2–10. DOI: [10.1186/s12906-017-1982-y](https://doi.org/10.1186/s12906-017-1982-y).
- [12] Daniel, A.; Engeda, D.; Chetan, C. Effect of Thermal Treatment on Phenolic Content, Antioxidant, and α -amylase Inhibition Activities of *Moringa Stenopetala* Leaves. *Afr. J. Food Sci.* **2015**, *9*(9), 487–494. DOI: [10.5897/AJFS2014.1241](https://doi.org/10.5897/AJFS2014.1241).
- [13] Yishak, A.; Engeda, D.; Daniel, A.; Mitiku, A. Phytochemicals and Antioxidant Activity of Different Apple Cultivars Grown in South Ethiopia: Case of the Wolayta Zone. *Int. J. Food Prop.* **2021**, *24*(1), 354–363. DOI: [10.1080/10942912.2021.1885440](https://doi.org/10.1080/10942912.2021.1885440).
- [14] Engeda, D.; Geremew, B.; Gulelat, D. H.; Rupasinghe, V. H. P. Effect of Extraction Solvents on Total Phenolic Contents and in Vitro Antioxidant Activity of the Leaves of *Lippia Adoensis* Var. Koseret Sebsebe. *Food Sci. Qual. Manag.* **2020**, *94*, 29–37.
- [15] Rupasinghe, H. P. V.; Wang, L.; Huber, G. M.; Pitts, N. L. Effect of Baking on Dietary Fibre and Phenolics of Muffins Incorporated with Apple Skin Powder. *Food Chem.* **2008**, *107*, 1217–1224. DOI: [10.1016/j.foodchem.2007.09.057](https://doi.org/10.1016/j.foodchem.2007.09.057).
- [16] Engeda, D. In Vitro Antioxidant and α -Amylase Inhibition Activities of Spiced Red Chili Paste (Datta) from South Ethiopia. *Ethiop. Pharm. J.* **2015**, *31*, 93–106. DOI: [10.4314/epj.v31i2.2](https://doi.org/10.4314/epj.v31i2.2).
- [17] Mohammad, A. E.; Fereshteh, P.; Ahmad, R. B. Iron Chelating Activity, Phenol and Flavonoid Content of Some Medicinal Plants from Iran. *Afric. J. Biotechnol.* **2008**, *7*, 3188–3192. DOI: [10.5897/AJB08.476](https://doi.org/10.5897/AJB08.476).

- [18] Sultana, B.; Anwar, F.; Ashraf, M. Effect of Extraction Solvent/technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. *Molecules*. **2009**, *14*(6), 2167–2180. DOI: [10.3390/molecules14062167](https://doi.org/10.3390/molecules14062167).
- [19] Vongsak, B.; Sithisarn, P.; Mangmool, S.; Thongpraditchote, S.; Wongkrajang, Y.; Gritsanapan, W. Maximizing Total Phenolics, Total Flavonoids Contents and Antioxidant Activity of *Moringa Oleifera* Leaf Extract by the Appropriate Extraction Method. *Ind. Crop Prod.* **2013**, *44*, 566–571. DOI: [10.1016/j.indcrop.2012.09.021](https://doi.org/10.1016/j.indcrop.2012.09.021).
- [20] Zenebe, H.; Zenebe, T.; Yirga, B.; Gopalakrishnan, V. K.; Krishna, C. In Vitro Antioxidant Activities of the Methanolic and Aqueous Extracts of *Moringa Stenopetala* Leaves. *Drug Invent. Today* **2018**, *10*(5), 642–649.
- [21] Alessandro, L.; Giovanni, F.; Franca, C.; Stefano, R.; Laura, S.; Gelsomina, F.; Angela, S.; Alberto, B.; Alberto, S.; Federica, P.; et al. Nutritional Characterization and Phenolic Profiling of *Moringa Oleifera* Leaves Grown in Chad, Sahrawi Refugee Camps, and Haiti. *Int. J. Mol. Sci.* **2015**, *16*(8), 18923–18937. DOI: [10.3390/ijms160818923](https://doi.org/10.3390/ijms160818923).
- [22] Ganiyu, O.; Adedayo, O. A.; Tosin, A. O.; Sunday, I. O.; Aline, A. B.; Margareth, L. A. Phenolic Extract from *Moringa Oleifera* Leaves Inhibits Key Enzymes Linked to Erectile Dysfunction and Oxidative Stress in Rats' Penile Tissues. *Biochem. Res. Int.* **2015**, , 1–8. DOI: [10.1155/2015/175950](https://doi.org/10.1155/2015/175950).
- [23] Shahid, I.; Bhanger, M. I. Effect of Season and Production Location on Antioxidant Activity of *Moringa Oleifera* Leaves Grown in Pakistan. *J. Food Compos. Anal.* **2006**, *19*, 544–551. DOI: [10.1007/s13197-012-0831-8](https://doi.org/10.1007/s13197-012-0831-8).
- [24] Ukwueze, C. K.; Okogwu, O. I.; Ebem, E. C.; Nwonumara, G. N.; Nwodo, J. N. Evaluation of the Influence of Geographical Location on Phytochemical Composition of *Moringa Oleifera* Seeds. *World Appl. Sci. J.* **2019**, *37*(3), 196–201. DOI: [10.5829/idosi.wasj.2019.196.201](https://doi.org/10.5829/idosi.wasj.2019.196.201).
- [25] Mónica, A.; Valdez, S.; Verónica, Y.; Mejía, G.; Alfredo, T. V.; Guadalupe, G. A.; José, S. P.; José, J.; Alba, R.; Erick, S. C. Nutritional Content and Elemental and Phytochemical Analyses of *Moringa Oleifera* Grown in Mexico. *J. Chem.* **2015**, *2*–9.
- [26] Pricila, V. P.; Ricardo, F. A.; Moreira, L. C. T.; Carlos, D. M. The Content of Chlorogenic Acids in Tropical Fruits. *J. Sci. Food Agric.* **2002**, *82*(10), 1177–1181. DOI: [10.1002/jsfa.1163](https://doi.org/10.1002/jsfa.1163).
- [27] Sobhy, A.; Sohaimy, E.; Gamal, M. H.; Sameh, E.; Mohamed, M. H. A.; Al-H, R. R. Biochemical and Functional Properties of *Moringa Oleifera* Leaves and Their Potential as a Functional Food. *Glob. Advan. Res. J. Agri. Sci* **2015**, *4*(4), 188–199.
- [28] Corina, D.; Iulia, A. P.; Cristina, A. D.; Monica, H.; Istvan, Z.; Dan, N.; Monica, L.; Roxana, M. G.; Codruta, M. S. Antiproliferative and Antimicrobial Properties of Pure and Encapsulated Rutin. *Farmacia*. **2018**, *66*(2), 302–308.
- [29] Marcello, I.; Robert, K.; Andrea, C.; Rita, S.; Elena, M.; Varoni, A.; Azzimonti, A.; Dziedzic, L.; Rimondini, R. D. Rutin, a Quercetin Glycoside, Restores Chemosensitivity in Human Breast Cancer Cells. *Phytother. Resear.* **2017**, *31*10, 1529–1538. DOI: [10.1002/ptr.5878](https://doi.org/10.1002/ptr.5878).
- [30] Pensak, J.; Rungsinee, P.; Monika, M.; Helmut, V. Enhancement of Anti-inflammatory Activity of Polyphenolic Flavonoid Rutin by Encapsulation. *Pak. J. Pharm. Sci.* **2017**, *30*(5), 1521–1527.
- [31] Sulastri, E.; Muhammad, S. Z.; Nurafni, I. A.; Syakila, A.; Ririen, H.; Risfah, Y. A. Total Phenolic, Total Flavonoid, Quercetin Content and Antioxidant Activity of Standardized Extract of *Moringa Oleifera* Leaf from Regions with Different Elevation. *Pharmacog. J.* **2018**, *10*(6s), 104–108. DOI: [10.5530/pj.2018.6s.20](https://doi.org/10.5530/pj.2018.6s.20).
- [32] Samuel, W.; Abay, M.; Wondwossen, E.; Frehiwot, T.; Asfaw, M.; Ashenif, T.; Abiy, A.; Worku, G.; Negero, G.; Rekik, A.; et al. Evaluation of Antihyperglycemic Effect of Extract of *Moringa Stenopetala* (Baker F.) Aqueous Leaves on Alloxan-Induced Diabetic Rats, Diabetes, *Metabolic Syndrome and Obesity. Targets and Therapy.* **2021**, *14*, 185–192. DOI: [10.2147/DMSO.S266794](https://doi.org/10.2147/DMSO.S266794).
- [33] Keivan, Z.; Boon-Teong, T.; Sing-Sin, S.; Pooi-Fong, W.; Mohd, R. M.; Sazaly, A. In Vitro Antiviral Activity of Fisetin, Rutin and Naringenin against Dengue Virus Type-2. *J Med Plants Re* **2011**, *5*(23), 5534–5539.
- [34] Habtemariam, S. Rutin as a Natural Therapy for Alzheimer's Disease: Insights into Its Mechanisms of Action. *Curr. Med. Chem.* **2016**, *23*(9), 860–873. DOI: [10.2174/0929867323666160217124333](https://doi.org/10.2174/0929867323666160217124333).
- [35] Pravej, A.; Shereen, F.; Elkholy, S. A. M.; Prawez, A.; Mahmoud, A. S. E. HPLC Based Estimation and Extraction of Rutin, Quercetin and Gallic Acid in *Moringa Oleifera* Plants Grown in Saudi Arabia. *J. Chem. Pharm. Res.* **2016**, *8*(8), 1243–1246.
- [36] Habtemariam, S.; Antioxidant and Rutin Content Analysis of Leaves of the Common Buckwheat (*Fagopyrum Esculentum* Moench) Grown in the United Kingdom: A Case Study. *Antioxidants*. **2019**, *8*(6), 1–10. DOI: [10.3390/antiox8060160](https://doi.org/10.3390/antiox8060160).
- [37] Fernanda, D.; Adriana, M.; Lucas, C.; Milton, F.; Letícia, S.; Livia, C.; José, T. F.; Roger, W.; Helena, G. Determination of Rutin in Fruits and Vegetables in Natura. *J. Food Nutr. Res.* **2019**, *58*(4), 328–338.
- [38] Domingos, P. F.; Almeida, M. S.; Giãob, M. P.; Mari, A. H. G. Bioactive Phytochemicals in Apple Cultivars from the Portuguese Protected Geographical Indication “Maçã De Alcobaça:” Basis for Market Segmentation. *Int. J. Food Prop.* **2017**, *20*(10), 2206–2214. DOI: [10.1080/10942912.2016.1233431](https://doi.org/10.1080/10942912.2016.1233431).
- [39] Valentina, L.; Christova-Bagdassarian, U.; Kristine, S. B.; Maria, S. A.; Ayaz, A. Comparative Analysis of Total Phenolic and Total Flavonoid Contents, Rutin, Tannins and Antioxidant Capacity in Apiaceae and Lamiaceae Families. *Indian J. Hortic.* **2014**, *4*(3/4), 131–140.

- [40] Gaafar, A. A.; Eman, A.; Ibrahim, L.; Mohsen, S. A.; Ahmed, F. M.; Zeinab, A. S. Characterization of Polyphenols, Polysaccharides by HPLC and Their Antioxidant, Antimicrobial and Antiinflammatory Activities of Defatted Moringa (*Moringa Oleifera* L.) Meal Extract. *Int. J. Pharm Clin.* **2016**, *8*(6), 565–573.
- [41] Habtemariam, S.; Varghese, G. K. Extractability of Rutin in Herbal Tea Preparations of *Moringa Stenopetala* Leaves, *Beverages*; 169–182: **2015**, Vol. 1. DOI: [10.3390/beverages1030169](https://doi.org/10.3390/beverages1030169).
- [42] Beibei, Z.; Jiawen, D.; Hua, L.; Yaqiang, H.; Wu, T.; Haodi, D.; Zhang, G.; Zhicheng, Y. C. Optimization of Phenolic Compound Extraction from Chinese *Moringa Oleifera* Leaves and Antioxidant Activities. *J. Food Qual.* **2019**, 1–3. DOI: [10.1155/2019/5346279](https://doi.org/10.1155/2019/5346279).
- [43] Bajpai, M.; Pande, A.; Tewari, S. K.; Prakash, D. Phenolic Contents and Antioxidant Activity of Some Food and Medicinal Plants. *Int. J. Food. Sci. Nutr.* **2005**, *56*(4), 287–291. DOI: [10.1080/09637480500146606](https://doi.org/10.1080/09637480500146606).
- [44] Ashwell, R. N.; Rofhiwa, M.; Bhukumthetho, N.; Hafiz, A.; Christian, P.; Plooy, D.; Johannes, V. S.; Antioxidant, Antimicrobial and Phytochemical Variations in Thirteen *Moringa Oleifera* Lam. Cultivars. *Molecules*. **2014**, *19*(7), 10480–10494. DOI: [10.3390/molecules190710480](https://doi.org/10.3390/molecules190710480).
- [45] Kumar, S.; Pandey, A. K. Pandey, “Antioxidant, Lipo-protective and Antibacterial Activities of Phytoconstituents Present in Solanum Xanthocarpum Root” *Inter. Rev. Biophy. Chem.* **2012**, *3*(3), 42–47.
- [46] Mengfei, L.; Junjie, Z.; Xiayang, C. Bioactive Flavonoids in *Moringa Oleifera* and Their Health Promoting Activities. *J. Funct. Foods*. **2018**, *47*, 469–479. DOI: [10.1016/j.jff.2018.06.011](https://doi.org/10.1016/j.jff.2018.06.011).
- [47] Nic'iforovic', N.; Mihailovic, V.; Maškovic, P.; Solujic, S.; Stojkovic, A.; Pavlovic, D. Antioxidant Activity of Selected Plant Species; Potential New Sources of Natural Antioxidants. *Food Chem. Toxicol.* **2010**, *48*(11), 3125–3130. DOI: [10.1016/j.fct.2010.08.007](https://doi.org/10.1016/j.fct.2010.08.007).