

Identified Analytical Profile for Microelements, Trace Metals, Amino Acids and Screening of Anti-Diabetic Activity from Flower and Leaf Extracts of *Moringa oleifera* in Streptozotocin (STZ)-Induced Diabetic Rats

Hemalatha Kamurthy^{1*}, Sirajunisa Talath²

¹Department of Pharmacognosy, Acharya & BM Reddy College of Pharmacy, Bangalore, India

²Department of Pharmaceutical Chemistry, RAK College of Pharmacy, RAKMHSU, Ras Al Khaimah, UAE

Email: *hemalathak@acharya.ac.in

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Abstract

Background: *Moringa oleifera* plant is popularly known for its rich phyto-constituents and nutritional value and important medicinal values in both traditional and modern systems of medicine. We explored the present study for measurements of microelements, amino acid, phenolic content in hydro-alcoholic flower and leaf extracts of *Moringa oleifera* along with anti-diabetic activity in Streptozotocin (STZ)-induced diabetic male Wistar rats.

Methodology: The micronutrients were determined by using atomic absorption spectrophotometer at 285 nm and 422 nm for Calcium (Ca), phosphorus (P), Iron (Fe), and Zinc (Zn), etc. The trace elements were also measured by spectrophotometer. The essential amino acid was determined by using Amino acid analyser. The total phenolic content in hydro-alcoholic extracts (flower and leaf) *M. oleifera* measured the absorbance at 760 nm by UV spectrophotometer. The screening of anti-diabetic activity HAFE and HALE of *Moringa oleifera* at two different dose of 100 and 200 mg/kg b.w. for 21 days were performed by determining the changes in biochemical parameters. **Result and Discussion:** The results reveals that the presence of micronutrients, trace elements and amino acids in both flower and leaf of *M. oleifera*. The hydroalcoholic extracts of HAFE and HALE at 200 mg/kg b.w. showed significant antidiabetic activity compared with standard Glibenclamide. Whereas dose at 100 mg/kg b.w. showed moderate activity. **Conclusion:** In conclusion, the *M. oleifera* exhibits more effectiveness against STZ-induced diabetes. The HAFE and HALF extracts exhibited significant anti-diabetic

property and active components may be isolated and clinical studies is required for further evaluation. Because of the rich source of phytoconstients, nutritional elements will be helpful in processed food products as dietary supplements especially for malnutrition in children in the current era.

Keywords

Moringa oleifera, Microelements, Nutritional Values, Gallic Acid, Amino Acids, Streptozotocin, Glibenclamide

1. Introduction

Moringa oleifera (Moringaceae), commonly known as **Moringa** and also called as “Miracle Tree” or “Tree of Life” [1] due to its rich source of micronutrients, trace elements, essential amino acids and antioxidants properties [2]. The Moring plant originated from Asia. Nearly about 13 species of *Moringa* with forms ranging from small herbaceous plants to massive trees. Most abundantly found in north and north-western parts of India. Also found in Madagascar, Bangladesh, Arabian Peninsula, Pakistan and African countries. Among 13 species, the three species *Moringa oleifera*, *M. stenopetala* and *M. peregrina* are majorly cultivated [3]. This moringa plant is usually called by different vernacular names like Drum stick tree or horse radish tree, benzolive tree, moringa, the traditional folklore, different parts of Moring plant are connected with a wide range of nutritional benefits, medicinal value and miscellaneous applications [4]. The important medicinal properties such as antioxidant, diuretic, hypotensive, wound healing properties, etc., were reported in this plant [5] [6] [7]. Nutritionally, rich source of Calcium that increases milk production in lactation [8] has been reported. Traditionally in Ayurvedic system, used specifically for the treatment of cancer [9]. The leaves and buds of *M. oleifera* leaves were rubbed on the temples portion of the face to cure headache, as an anti-scorbutic. The fresh juice of leaves mixed with honey to treat eye disorders, seed part used in ophthalmic preparation, purgative, venereal affection anti-inflammatory and as tonic [10]. The phytoconstituents present in the whole Moring plant, vital mineral supplements like K, Ca, P, Fe, protein, amino acids, beta-carotene, vitamins, and rich source of phenolic content such as Gallic acid, zeatin, β -sitosterol, quercetin, kaempferol, caffeoylquinic acid, etc., [11] and high concentrations of natural anti-oxidants especially Vitamin A, C and E and phenolic compounds [12] [13] [14].

Objective

The objective of this study was to assess the microelements, trace metals, amino acids of *Moringa oleifera* (Flower and leaf parts) and to measure the anti-diabetic activity of hydro-alcoholic flower (HAFE) and (HALE) extract of *M. oleifera*.

2. Materials and Methods

2.1. Collection and Identification of Plant

The whole plant of *Moringa oleifera* was collected from local areas of Bangalore; The plant was authenticated by Dr. N.M Ganesh Babu (Assistant professor heading center for herbal gardens). Voucher specimen kept in the Acharya and BM Reddy College of Pharmacy, Bangalore, Karnataka, India.

2.2. Hydro Alcoholic Extraction of *Moringa oleifera*

The dried leaves and flower parts of *M. oleifera* were pulverized (1 kg of each) individually and subjected to hydro-alcoholic (1:1) solution containing flasks separately. Both the flaks were kept at 120 rpm on a rotary shaker for 24 h, [15]. After completion of extraction (after 24 hours), both the extracts were filtered and these collected filtrates were subjected to lyophilized to achieve hydro alcoholic leaf extract (HALE) (3 g) and hydro alcoholic flower extract (HAFE), later the yields of both extracts were calculated and extracts were stored at 2°C to 8°C till the completion of research work [16].

2.3. Phytochemical Screening

Preliminary qualitative phytochemical tests like Phenols [17] flavones, tannins, saponins, alkaloids, etc., [18] were carried out for hydro-alcoholic flower and extracts of *Moringa orelifera* as per the standard protocol [19].

2.4. Determiration of Microelements Contents in *M. Oleifera* Flowers and Leaf Parts

The report of Microelements composition such as Calcium (Ca), Magnesium (Mg), Phosphorus (P), Sodium (Na), Potassium (K) ,etc. The various mineral contents in *M. oleifera* leaves have been determined by mineralising the sample with humid voice as per the strong procedure [20].

Weight accurately 0.5 g of each samples (flower and leaves) separately and pulverised to 0.5 mm, both samples (flower and leaf) were in individual tubes and dissolved in 5 ml of extraction solution (mixture of sulphuric acid-selenium-salicylic acid: 7.2%).

Simultaneously blank solution was prepared by using the extraction solution without sample. Both test samples and blank solutions were kept aside for 2 hours. After 2 hours, these samples were heated at 100°C to 340°C temperature, allowed to cool for 24 hours with ambient temperature, diluted up to 2/3rd of tubes, further subjected to agitation followed by cooling and add 75 ml distilled water. Decant the superannuated solution after the agitation process is completed. These samples were used in further quantification process to determine the macronutrients in both flower and leaf parts of *M. olerifera*.

1) For determination of Magnesium (Mg) and Calcium (Ca) after dilution in the Lanthane [(La (NO₃)₃ 6H₂O)] respectively by using by using an atomic absorption spectrophotometer (model PERKIN ELMER A100) at 285 nm and 422

nm respectively.

2) The determination of total phosphorus (P) content by using auto-sensor (model SKALAR 1000) at 880 nm, ammonium molybdate as an indicator.

3) Whereas the determination of other two contents such as Sodium (Na) and Potassium (K) by using flame photometer (model CORNING 400).

The standard solutions of different ranges have been prepared for micronutrients dosages accordingly. The different ranges are mentioned as follows

Composition of Magnesium (Mg) and Calcium (Ca): standards of both the solutions Magnesium (Mn) and Calcium (Ca) (1000 ppm) allowed to accomplish the permitted to prepare concentration range for calcium and magnesium likes in between 5 and 30 ppm and 0.5 and 3 ppm respectively.

Composition of phosphor (P): Potassium hydrogen phosphate (K_2HPO_4) solution (300 ppm) allow to accomplish the concentration range lies in between 3 to 15 ppm.

Composition of Sodium (Na) and Potassium (K): Sodium-potassium standard solution (100 ppm), allowed to prepare the concentration range in between 0 to 10 ppm.

2.5. Determination of Zinc (Zn) and Iron (Fe) Content in Flower and Leaf

Weigh accurately 0.5 g of samples flower and leaves, subjected to pulverised to 0.5 mm, dissolved the samples by adding the extraction solution 5 ml containing solutions of Nitric acid (HNO_3 ; 65%) and sulphuric acid (H_2SO_4 ; 96%) mixture and perchloric acid ($HClO_4$ 70%) each tube. Simultaneously blank solution was prepared by using the 5ml of extraction solution without sample. Both test samples (Flower and leaf) and blank solutions were kept aside for 2 hours. After completion of 2 hours, these samples were subjected to heating by maintaining the temperature in between $70^\circ C$ - $240^\circ C$, then these samples mixtures were allowed to cooled for 24 hours with ambient temperature diluted up to 2/3rd of tubes, further subjected for agitation followed by cooling and add 75ml distilled water. Once agitation process is completed, these solutions are decanted. These solutions were used for quantification process to analyse the Iron (Fe) and Zinc (Zn) content in both flower and leaf parts of *M. olerifera*. Both Iron (Fe) and Zinc (Zn) were measured at 219 nm and 248 nm respectively in atomic absorption. The standard concentration range lies in between 6 to 36 ppm for Iron (Fe) and 1 to 6 ppm for zinc (Zn).

2.6. Determination of Trace Metals Contents in *M. Oleifera* Flowers and Leaf Parts

Dried Flowers and Leaves of *Moringa oleifera* were powdered by using warming blender, weigh accurately 2.5 g grounded samples (flower and Leaf) were taken in to two different conical flasks of capacity 250 ml each, concentrated HNO_3 2.5 ml was added gradually with constant shaking in both the flask. These sample mixture were heated on a hot plate until the production of brown fumes stopped

[21]. The contents present in both the beakers were allowed to cooled and mixed with the 10 ml of 70% HClO₄. Once again both the beakers were heated gently until the solutions turns to colourless and allowed them in order to condense the small quantity by evaporation. Add distilled water after cooling the solutions, filtered through Whatman filter paper and finally make up the volume 100 ml with double distilled water. The total concentrations of trace metals like Cd, Pb [22] Ni, Mo, Sn, Se, Li, Hg, As and Cr, etc., were investigated by using (Model: Perkin Elmer Analyst 100) atomic absorption spectroscopy [23].

2.7. Determination of Essential Amino Acids in Flower and Leaf of *Moringa oleifera*

About 5 g of both in hydro-alcoholic extracts (flower and leaf) were taken and add 10 ml NaCl (10%) for both the extracts, one hour extracts were stirred continuously; after one hour, add the 10 ml of trichloro acetic and filtrated [24]. These filtrates were centrifuged further to collect the precipitate, wash the precipitate, dried in desiccator. The 20 mg of protein is dissolved in 10 ml of 6 N HCl and refluxed for 20 hours, the acid portion is removed by evaporation under reduced pressure. Finally, obtained residues were allowed to mix with isopropanol 10% for the determination of amino acids by using Amino acid analyser (Ependorf-Germany Lc 3000).

2.8. Determination of Total Phenolic Content in *M. oleifera*: By UV Spectrometer

Analysis of Total Phenolic Content (TPC) from flower and leaf extracts:

The total phenolic contents of both leaves and flowers of extracts samples were determined by [25] spectroscopic/calorimetric estimation. The determination of total phenolic content of hydro alcoholic leaf extract (HALE) (3 g) and hydro alcoholic flower extract (HAFE) of *Moringa oleifera* was determined using Folin-Ciocalteu method. Both the extracts were weighed accurately 50 mg of each and mixed with 200 ml of Ciocalteu reagent along with 3.16 ml. The mixtures remained on in beaker for 30 seconds to 8 minutes. Then added 20% Na₂CO₃ in it and left it for 2 hours at 20°C UV spectrophotometer was used for its absorbance at 760 nm [26]. Marker Gallic acid (Sigma-Aldrich) was used as a reference and standard solution were prepared in the concentration range 1 - 50 µg/mL and measured the absorbance at 760 nm [27] to get the calibration curve. The data analysis was reported in mg Gallic acid/ml of each extract.

2.9. The Anti-Diabetic Activity

2.9.1. Experimental Animals

The body weight of Male Wistar rats in between 150 - 200 g were selected for diabetic study. Good laboratory conditions were maintained for animals till the end of activity by maintaining the temperature at 22°C ± 2°C, humidity maintained at 45°C ± 5°C (12-hour day and 12-hour night cycle). All animals received a standard laboratory diet and water ad libitum. Assessment of an-

ti-diabetic activity was carried out after the approval of the Institutional Animal Ethical Committee as per the Institutional Ethical Guidelines for the care of laboratory animals and maintaining the same while performing the activity.

2.9.2. Chemicals

The assessment of various parameter for anti-diabetic study was performed with the kits procured from Primal Healthcare Limited, Lab Diagnostic Division, Mumbai, India and diabetic induced drug (Streptozocin) was Purchased from Himedia Laboratories, Mumbai, India.

2.9.3. Anti-Diabetic Study

The induction of Diabetes: The diabetes was induced by using Streptozocin injection freshly prepared (STZ—65 mg/kg; i.p.) by using citrate buffer; pH maintained was 4.5, after 15 minutes of administration of standard anti-diabetic drug (Glibenclamide (5 mg/Kg. b.w.) in rats (overnight-fasted) [28]. After induction of diabetes after 72 hours, the blood glucose levels were measured with the glucose meter (Glucocard 01-mini, Arkray Factory Inc., Japan). Till 14 days, the diabetic rats were maintained in good laboratory facility to stabilize the blood glucose levels. The glucose levels were measured on 14th day in diabetic rats was showing blood glucose > 200 mg/dL were chosen to determine the anti-diabetic activity for both hydro-alcoholic flower extract (HAFE) and hydro-alcoholic Leaf extract (HALE) of *Moringa oleifera*.

2.9.4. Study Design and Doses

To assess the anti-diabetic activity HAFE and HALE of *Moringa oleifera*, the following groups were segregated as follows, six animals of each groups.

Group I: Treated as control rats received 0.2% Carboxy Methyl Cellulose (CMC; 5 mL/kg).

Group II: Treated as Diabetic induced rats (Streptozotocin 50 mg/Kg b.w.), given 0.2% CMC (5 mL/kg).

Group III: Diabetic rat received, treated with hydro-alcoholic flower extract (HAFE) *M. olerifea* (100 mg/kg b.w.).

Group IV: Diabetic rat received, treated with hydro-alcoholic flower extract (HAFE) *M. olerifea* (200 mg/kg b.w.).

Group V: Diabetic rat received, treated with hydro-alcoholic leaf extract (HALE) *M. olerifea* (100 mg/kg b.w.).

Group VI: Diabetic rat received, treated with hydro-alcoholic leaf extract (HALE) *M. olerifea* (200 mg/kg b.w.).

Group VII: Treated as diabetic treated rats with standard drug Glibenclamide (5 mg/kg b.w.) (dissolved in 0.5 mL distilled water) [29].

HAFE and HALE doses were selected from our previous data per the literature study [30] and all the samples such as a vehicle, HAFE, HALE, and Glibenclamide were given through oral route with the help of rat oral needle to all the eight groups up to 28 days. The test samples (HAFE and HALE) and standard Glibenclamide in 0.2% CMC, were freshly prepared before oral administration

every day. The fasting glucose level in blood and also body weight were measured at two days on 14th and 28th days after the treatments. On 28th day, Glibenclamide, HAFE, HALE and vehicle, were given to the Overnight-fasted rats; after 1 hour, the all the treated animals were anaesthetized with ketamine (100 mg/kg, i.p.). Blood samples were collected by puncturing the retro-orbital plexus and finally stored in disodium ethylene diamine tetra acetate to measure the various biochemical parameters.

Both HAFE and HALE of *Moringa oleifera* and Glibenclamide were administered orally in rats up to 28 days. Sodium citrate buffer, HAFE and HALF extracts and Glibenclamide were administered on 28th day in rats (overnight-fasted). On the same day after 1 hour, treated rats were anesthetized with ketamine (100 mg/kg, i.p.) and blood samples were collected from the retro-orbital plexus by puncturing, finally stored the container containing disodium ethylene diamine tetra acetate solution to measure the various biochemical parameters.

2.9.5. Measurement of Biochemical Parameters

By using the kits, the blood glucose level, Hb, and HbA1c were identified using whole blood, whereas the serum triglycerides (TG), total protein (TP), total cholesterol (TC), high-density lipoprotein (HDL) were measured by using semiauto-analyzer which are commercially available. (Photometer 5010V5+, Germany). By radioimmunoassay method, the serum insulin was measured.

3. Result and Discussion

3.1. Hydro-Alcoholic Extract

The hydro-alcoholic extracts (flower and leaf) *Moringa oleifera* after lyophilisation, the yield was 3.1 g and 1.4 g from flower and leaf extracts respectively.

3.2. Phytochemical Screening

Hydro-alcoholic extracts of *M. oleifera* reveals that, presence of phytoconstituents such as Alkaloids, flavonoids, phenolic, triterpenoids, tannins, saponins, carbohydrates, glycosides, etc.

3.3. Microelements Composition *M. oleifera*

The composition of rich source microelements of *M. oleifera* (Flower and Leaf) is shown in **Table 1** and **Table 2**.

The proximate composition of microelements (flower part) such as Calcium 1689.12 mg, Magnesium 320.15 mg, Phosphorus 286.05 mg, Sodium 580.14 mg, Potassium 393.57 mg, iron 32.5 mg, Zinc 94.25 mg, whereas the other part (leaf part) the composition of microelements showed the presence of Calcium 1897.34 mg, Magnesium 437.2 mg, Phosphorus 286.05, Sodium 223.04 mg, Potassium 1468.23 mg, iron 28.13 mg, Zinc 72.11 mg respectively. These obtained results were assured by the previous studies' results [31] [32].

The dried Moringa leaves contain high amounts of heavy metals than the flowers. The dried flowers and leaves, Iron content were found to be 28.13 mg

Table 1. Results of mineral composition (mg/100g) measurement in *Moringa oleifera* flower powder.

Microelements		Trace metals	
Element	mg/100g	Element	mg/100g
Calcium (Ca)	1689.12	Cadmium (Cd)	0.05
Magnesium (Mg)	320.15	Lead (Pb)	0.0005
Phosphorus (P)	286.05	Nickle (Ni)	0.13
Sodium (Na)	580.14	Molybdenum (Mo)	0.033
Potassium (K)	393.57	Tin (Sn)	0.0051
Iron (Fe)	28.13	Selenium (Se)	0.162
Zinc (Zn)	94.25	Lithium (Li)	0.008
		Mercury (Hg)	ND
		Arsenic (As)	ND
		Chromium (Cr)	0.00013

ND = Not Detected.

Table 2. Results of mineral composition (mg/100 g) measurement in *Moringa oleifera* Leaf powder.

Microelements		Trace metals	
Element	mg/100g	Element	mg/100g
Calcium (Ca)	1897.34	Cadmium (Cd)	0.02
Magnesium (Mg)	473.2	Lead (Pb)	0.0011
Phosphorus (P)	286.05	Nickle (Ni)	0.24
Sodium (Na)	223.4	Molybdenum (Mo)	0.033
Potassium (K)	1468.23	Tin (Sn)	0.002
Iron (Fe)	37.5	Selenium (Se)	0.223
Zinc (Zn)	72.11	Lithium (Li)	0.068
		Mercury (Hg)	ND
		Arsenic (As)	ND
		Chromium (Cr)	0.00092

ND = Not Detected.

and 37.5 mg respectively, resulting that the leaves may be a possible rich source of Fe supplement for humans and livestock. It is one of the vital trace element for CNS system functioning and in the oxidation of proteins, carbohydrates and fats [33].

The zinc metal is very essential in the diet of humans and as well as animals. The proximate values of zinc content in flower and leaf of *Moringa* exhibited 94.25 mg, 72.11 mg respectively [34].

It is very much essential for the synthesis of RNA, DNA, RNA, and the func-

tion of insulin and other several enzymes functions and is also needed for the reproduction of cell and also growth of sperm cells [35].

3.4. Determination of Trace Metals Contents

The trace elements (Cd, Pb, Ni, Mo, Cr, Sn, Se, etc.) are necessary with few quantities by humans. The concentrations of trace metals of both flower and leaf of *Moringa* are shown in **Table 1** and **Table 2** respectively. Overall, the greater content of heavy metals were in leaf part compared to *Moringa* flowers.

3.5. Determination of Trace Metals Contents

The proximate composition of Amino acid (mg/ml) in hydro-alcoholic leaf and flower extracts of *Moringa oleifera* (**Table 3**). The composition in flower and leaf, Threonine 35.17 and 42.98, Valine 22.1, and 26.42, Methionine 0.13 and 0.43, Leucine 24.62 and 22.17, Isoleucine 35.8 and 44.97, Phenylalanine 3.44 and 3.21, Histidine 29.62 and 27.64, Lysine 25.47 and 2.07, Arginine 9.81 and 7.68 respectively [36].

3.6. Total Phenolic Content (TPC)

According to TPC reports on hydro-alcoholic (flower and leaf) extracts *M. oleifera*, reveals that the highest content TPC 119.4 ± 0.95 mg GAE/g and 137.87 ± 1.9 mg GAE/g of flower and leaf extracts respectively (**Table 4**) [37].

Table 3. Results of Amino acid composition (mg/ml) measurement in *Moringa oleifera* flower and leaf powder.

Sl. No	<i>Moringa oleifera</i> flower	<i>Moringa oleifera</i> leaf
Threonine	35.17	42.98
valine	22.2	26.42
Methionine	0.31	0.43
Leucine	24.62	22.17
Isoleucine	35.8	44.97
Phenylalanine	3.44	3.21
Histidine	29.62	27.64
Lysine	25.47	20.07
Arginine	9.81	7.68

Table 4. Total Phenolic Content (TPC): by UV spectrometer.

Extracts	Total phenolic content (mg GAE*/g of extract)
Hydro-alcoholic flower extract (HAFE)	119.4 ± 0.95
Hydro-alcoholic Leaf extract (HALE)	137.87 ± 1.9

3.7. The Anti-Diabetic Activity

Blood Glucose level variation in Diabetic Rats. Administration of STZ-NIC was significantly ($P < 0.001$) high in the blood glucose level compared to normal rats in the control group. A significant ($P < 0.001$) decrease in blood glucose level was observed in diabetic rats after the oral administration of both flower and leaf extracts of HAFE and HALE at 200 mg/kg b.w. dose and also Glibenclamide (Standard), 5 mg/kg, b.w., diabetic control rats (Table 5) [38].

Serum Insulin, Hb, HbA1c, and TP Levels Changes were measured in Diabetic Rats. STZ-NIC-mediated diabetes induction in rats increases HbA1c levels and reduces serum insulin, Hb, and TP significantly ($P < 0.001$) when compared to the normal control group (Table 6). Oral administration of both doses of HAFE and HALE and Glibenclamide (standard) exhibited a significant ($P < 0.001$) decrease in HbA1c levels and an increase in Hb, TP, and slightly increased serum insulin levels than the diabetic control rats. At 200 mg/kg b.w. dose, both HAFE and HALE showed significant ($P < 0.001$ and $P < 0.01$) higher efficacy than that of 100 mg/kg dose on normalization of Hb and HbA1c levels [39].

The present study reveals that the presence of phytoconstituents especially bioflavonoids, terpenoids may be plays vital role in the glucose uptake stimulation especially in peripheral tissues and regulates the enzymatic activity, which is involved in carbohydrate metabolism [40].

The proximate values of lipid profile in diabetic rats exhibited significant ($P < 0.001$) higher level of TC and TG and decreased levels of HDL were measured after STZ-NIC induced diabetic rats than normal control rats (Table 7).

The lipid profile in HAFE and HALE extracts, was significantly ($P < 0.001$) reduction in TC, TG Levels, whereas HDL levels were found to be moderately higher significantly ($P < 0.05$) compared to diabetic rats treated with vehicle. Administration of HAFE and HALE extracts at 200 mg/kg dose showed significant

Table 5. Blood glucose lowering effect of HAFE and HALF of *M. oleifera* in diabetic rats.

Groups	Dose (mg/kg)	Before diabetes induction (0 day)	Blood Glucose Level (mg/dl) (After diabetes induction (14 th day)	14 th Day	28 th Day
Control	Vehicle	59.50 ± 2.09	65.33 ± 1.76	68.33 ± 2.03	60.50 ± 1.87
Diabetic control	Vehicle	81.78 ± 2.01 ^a	251.50 ± 1.29 ^a	272.00 ± 3.13 ^a	304.50 ± 2.04 ^a
HAFE	100	70.50 ± 1.89	264.13 ± 1.70 ^a	157.00 ± 3.22 ^b	92.67 ± 2.41 ^b
HAFE	200	62.50 ± 1.19	242.18 ± 5.02 ^a	155.17 ± 4.54 ^b	85.33 ± 3.51 ^{b,c}
HALE	100	71.50 ± 2.09	264.13 ± 1.70 ^a	173.00 ± 3.22 ^b	96.17 ± 1.42 ^b
HALE	200	63.50 ± 1.02	257.33 ± 1.04 ^a	163.17 ± 4.54 ^b	88.14 ± 3.51 ^{b,c}
Glibenclamide (Standard)	5	61.00 ± 2.12	252.67 ± 8.71 ^a	142.83 ± 3.22 ^b	73.16 ± 1.57 ^{b,c}

All data are expressed as mean ± SEM ($n = 6$). Vehicle: 0.2% CMC (5 mL/kg). ^a $P < 0.001$ diabetic control compared with control. ^b $P < 0.001$ HAFE,HALE 100, 200 mg/kg and Glibenclamide 5 mg/kg compared with diabetic control. ^c $P < 0.05$ Glibenclamide 5 mg/kg compared with HAFE and HALE 200 mg/kg.

Table 6. Effect of HAFE and HALF of *M. oleifera* on haemoglobin, glycosylated haemoglobin, serum insulin, and total protein in diabetic rats.

Groups	Dose (mg/kg)	Hb (g/dL)	HbA1c (%)	Serum insulin (μ IU/mL)	TP (g/dL)
Control	Vehicle	13.74 \pm 1.03	6.12 \pm 0.12	9.15 \pm 0.15	7.22 \pm 0.24
Diabetic control	Vehicle	7.62 \pm 1.24 ^a	12.05 \pm 0.33 ^a	4.67 \pm 0.15 ^a	4.99 \pm 0.19 ^a
HAFE	100	10.98 \pm 0.28 ^b	7.32 \pm 0.15 ^b	6.64 \pm 0.17 ^b	5.82 \pm 0.52 ^b
HAFE	200	11.67 \pm 0.33 ^b	8.23 \pm 0.17 ^b	7.59 \pm 0.33 ^b	6.88 \pm 0.73 ^c
HALE	100	10.53 \pm 0.42 ^b	7.54 \pm 0.16 ^b	6.84 \pm 0.71 ^b	5.89 \pm 0.31 ^b
HALE	200	12.13 \pm 0.18 ^b	8.43 \pm 0.22 ^b	7.72 \pm 0.22	6.72 \pm 0.22 ^c
Glibenclamide (Standard)	5	12.94 \pm 0.23 ^b	6.75 \pm 0.16 ^b	8.15 \pm 0.54	6.55 \pm 0.41 ^c

All data are expressed as mean \pm SEM ($n = 6$). Vehicle: 0.2% CMC (5 mL/kg). ^a $P < 0.001$ diabetic control compared with control. ^b $P < 0.001$ HAFE and HALE 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control. ^c $P < 0.001$ Glibenclamide 5 mg/kg compared with HAFE and HALE 200 mg/kg.

Table 7. Effect of HAFE and HALE on lipid profiles in diabetic rats.

Groups	Dose (mg/kg)	Serum lipid levels (mg/dL)		
		TC	TG	HDL
Control	Vehicle	75.32 \pm 2.51	88.36 \pm 2.34	63.71 \pm 1.34
Diabetic control	Vehicle	129.31 \pm 3.21 ^a	147.27 \pm 3.39 ^a	41.50 \pm 1.67 ^a
HAFE	100	80.36 \pm 0.77 ^b	94.88 \pm 3.84 ^b	60.71 \pm 2.69 ^b
HAFE	200	74.37 \pm 2.84 ^b	82.74 \pm 0.34 ^b	52.18 \pm 1.64 ^c
HALE	100	75.37 \pm 2.11 ^b	87.63 \pm 1.81 ^b	56.76 \pm 1.56 ^b
HALE	200	73.75 \pm 0.14 ^b	81.28 \pm 1.84 ^b	51.33 \pm 3.33 ^c
Glibenclamide (Standard)	5	72.46 \pm 1.26 ^b	74.54 \pm 3.17 ^b	60.81 \pm 2.19 ^b

All data are expressed as mean \pm SEM ($n = 6$). Vehicle: 0.2% CMC (5 mL/kg). ^a $P < 0.001$ diabetic control compared with control. ^b $P < 0.001$ HAFE and HALE 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control. ^c $P < 0.001$ Glibenclamide 5 mg/kg compared with HAFE and HALE 200 mg/kg.

deduction in TC, TG levels and moderately increase in HDL level than HAFE and HALE extracts 100 mg/kg dose did not show statistical significance (**Table 7**).

Concentrations of total cholesterol (TC), TG, HDL ratio, which are indicators of susceptibility to cardiovascular diseases (CVDs). The results shows in STZ-induced diabetic rats, administered standard (Glibenclamide) and extracts (HAFE & HALF) of *M. oleifera* extracts to decrease the risk of cardiac complaints by decreasing the HDL [41].

The results reveals that there were significantly raised levels of triglycerides in

the diabetic rats. Reduction in the triglycerides with hydro-alcoholic flower and leaf extracts of *M. oleifera*, therefore by defeating the diabetes—induced boost the levels of lipids of both HAFE and HALE may be benefits in reducing the risk of cardiovascular problems of diabetics.

4. Conclusions

The analytical profile was identified from both the flower and leaf part of *Moringa oleifera* recognized the presence of secondary metabolites like alkaloids, glycosides, tannins, flavonols, phenols, etc., the micronutrients, trace elements have greater nutritional values especially in food products which will be benefited for those who are suffering from malnutrition. Apart from this, the anti-diabetic study report reveals that the hydroalcoholic extracts of both flower and leaf of *M. oleifers* influenced anti-hyperglycemic activity in streptozocin induced male rats, due to the presence of phytoconstitents which may help for further investigation and illumination.

Hence, this reported paper reviewing the literature on the phytochemistry and pharmacology screening of the plant reveals that the more attention is focused on research work, which is essential to investigate the exact phytoconstituents and biological properties on a wider range to elucidate the exact mechanism of action. This aspect will be vital usage in nutraceutical, food industries, phytopharmaceutical and also in cosmetic preparations.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Okereke, E.K., *et al.* (2014) Safe African Medicinal Plants for Clinical Studies. In: Kuete, V., Ed., *Toxicological Survey of African Medicinal Plants*, Elsevier, Amsterdam, 535-555. <https://doi.org/10.1016/B978-0-12-800018-2.00018-2>
- [2] Julia, P., Coppin, H., *et al.* (2015) Chapter 17. Variations in Polyphenols and Lipid Soluble Vitamins in *Moringa oleifera*. In: Preedy, V., Ed., *Processing and Impact on Active Components in Food*, Elsevier, Amsterdam, 655-663. <https://doi.org/10.1016/B978-0-12-404699-3.00079-2>
- [3] Mallenakuppe, R., Homabalegowda, H., Gouri, M.D., Basavaraju, P.S. and Chandrashekharaiah, U.B. (2019) History, Taxonomy and Propagation of *Moringa oleifera*—A Review. *SSR Institute of International Journal of Life Science*, **5**, 2322-2327. <https://doi.org/10.21276/SSR-IJLS.2019.5.3.7>
- [4] Singh, D., Arya, P.V., Aggarwal, V.P. and Gupta, R.S. (2014) Evaluation of Antioxidant and Hepatoprotective Activities of *Moringa oleifera* Leaves in Car-

- bon-Tetrachloride Intoxicated Rats. *Antioxidants*, **3**, 569-591.
<https://doi.org/10.3390/antiox3030569>
- [5] Santos, A.F., Argolo, A.C., Paiva, P.M. and Coelho, L.C. (2012) Antioxidant Activity of *Moringa oleifera* Tissue Extracts. *Phytotherapy Research*, **26**, 1366-1370.
<https://doi.org/10.1002/ptr.4591>
- [6] Faizi, S., Siddiqui, B.S., Saleem, R., Siddiqui, S., Aftab, K. and Gilani, A.H. (1995) Fully Carbamate and Hypotensive Thiocarbamate Glycosides from *Moringa oleifera*. *Phytochemistry*, **38**, 957-963. [https://doi.org/10.1016/0031-9422\(94\)00729-D](https://doi.org/10.1016/0031-9422(94)00729-D)
- [7] Guevara, A.P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K. and Maoko, T. (1999) An Antitumor Promoter from *Moringa oleifera*. *Mutation Research*, **440**, 181-188. [https://doi.org/10.1016/S1383-5718\(99\)00025-X](https://doi.org/10.1016/S1383-5718(99)00025-X)
- [8] Iqbal, S. and Bhangar, M.I. (2006) Effect of Season and Production Location on Antioxidant Activity of *Moringa oleifera* Leaves Grown in Pakistan. *Journal of Food Consumption Analysis*, **19**, 544-551. <https://doi.org/10.1016/j.jfca.2005.05.001>
- [9] Fahey, J.R. (2016) Microbiological Monitoring of Laboratory Mice. In: Sundberg, J.P. and Ichiki, T., Eds., *Genetically Engineered Mice Handbook*, CRC Press, Boca Raton, 157-164.
- [10] Fahey, J.W. (2017) *Moringa oleifera*: A Review of the Medicinal Potential. *Acta Horticulturae*, **1158**, 209-224. <https://doi.org/10.17660/ActaHortic.2017.1158.25>
- [11] Anwar, F., Latif, S., Ashraf, M. and Gilani, A.H. (2007) *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. *Phytotherapy Research*, **21**, 17-25.
<https://doi.org/10.1002/ptr.2023>
- [12] Kumar, P.S., Mishra, D., Ghosh, G. and Panda, G.S. (2010) Medicinal Uses and Pharmacological Properties of *Moringa oleifera*. *International Journal of Phytomedicine*, **2**, 210-216.
- [13] Amaglo, N.K., Bennett, R.N., Lo Curto, R.B. and Rosa, E.A.S. (2010) Profiling Selected Phytochemicals and Nutrients in Different Tissues of the Multipurpose Tree *Moringa oleifera* L., Grown in Ghana. *Food Chemistry*, **122**, 1047-1054.
<https://doi.org/10.1016/j.foodchem.2010.03.073>
- [14] Gowrishankar, R., Kumar, M. and Menon, V. (2010) Trace Element Studies on *Tinospora cordifolia* (Menispermaceae), *Ocimum sanctum* (Lamiaceae), *Moringa oleifera* (Moringaceae), and *Phyllanthus niruri* (Euphorbiaceae) Using PIXE. *Biological Trace Elemental Research*, **133**, 357-363.
<https://doi.org/10.1007/s12011-009-8439-1>
- [15] Parekh, J. and Chanda, S. (2007) *In-Vitro* Antibacterial Activity of the Crude Methanol Extract of *Woodfordia fruticosa* Kurz. Flower Lythraceae. *Brazilian Journal of Microbiology*, **38**, 204-207. <https://doi.org/10.1590/S1517-83822007000200004>
- [16] Barbosa, E.A., et al. (2022) Chemical Characterization of Flowers and Leaf Extracts Obtained from *Turnera subulata* and Their Immunomodulatory Effect on LPS-Activated RAW 264.7 Macrophages. *Molecules*, **27**, Article 1084.
<https://doi.org/10.3390/molecules27031084>
- [17] Evans, W.C., Evans, D. and Edward, G. (2002) Trease and Evans' Pharmacognosy. WB Saunders, New York, 553-557.
- [18] Ogbaba, J., Iruolaje, F. and Dogo, B. (2017) Antimicrobial Efficacy of *Guiera senegalensis* and *Prosopis africana* Leave Extract on Some Bacterial Pathogens. *European Journal of Biology and Medical Science Research*, **5**, 27-36.
- [19] Thilagavathi, T., et al. (2015) Preliminary Phytochemical Screening of Different Solvent Mediated Medicinal Plant Extracts Evaluated. *International Research Jour-*

- nal of Pharmacy*, **6**, 246-248. <https://doi.org/10.7897/2230-8407.06455>
- [20] Houba, V.W., Van Vark, W. and Vander Lee, J.J. (1989) Plant Analysis Procedure. Department of Soil Sciences and Plant Analysis. Plant Analysis (Part 7, Chapter 2.3). Wageningen, The Netherlands.
- [21] Gupta, J. and Gupta, A. (2013) Studies of Trace Metals in the Leaves of *Moringa oleifera*. *Journal Chemtracks*, **15**, 63-66.
- [22] Aissi, A.K., *et al.* (2014) Evaluation of Toxicological Risk Related to Presence of Lead and Cadmium in *Moringa oleifera* Lam. Leaves Powders Marketed in Cotonou (Benin). *Food and Nutrition Sciences*, **5**, 770-778. <https://doi.org/10.4236/fns.2014.59087>
- [23] Novozamsky, I., Houba, V.J.G., van Eck, R. and van Vark, W. (1983) A Novel Digestion Technique for Multi-Element Plant Analysis. *Communication in Soil Science and Plant Analysis*, **14**, 239-248. <https://doi.org/10.1080/00103628309367359>
- [24] Helrich, K. and Official Methods of Analysis of AOAC (1995) Official Method of Analysis of the Association of Official Analytical Chemist's. 15th Edition, Washington DC.
- [25] Singleton, V.L. and Rossi, J.A. (1965) Colorimetry of Total Phenolics wi Phosphomolybdic-Phosphotungstic Acid Reagents. *The American Journal of Enology and Viticulture*, **16**, 144-158.
- [26] Senguttuvan, J., Paulsamy, S. and Karthika, K. (2014) Phytochemical Analysis and Evaluation of Leaf and Root Parts of the Medicinal Herb, *Hypochoeris radicata* L. for *In-Vitro* Antioxidant Activities. *Asian Pacific Journal of Tropical Biomedicine*, **1**, 359-367. <https://doi.org/10.12980/APJTB.4.2014C1030>
- [27] Leone, A., Fiorillo, G., Criscuoli, F., *et al.* (2015) Nutritional Characterization and Phenolic Profiling of *Moringa oleifera* Leaves Grown in Chad, Sahrawi Refugee Camps, and Haiti. *International Journal of Molecular Sciences*, **16**, 18923-18937. <https://doi.org/10.3390/ijms160818923>
- [28] Dewanjee, A.K.S., Sahu, D.R. and Gangopadhyay, M. (2009) Antidiabetic Activity of *Diospyros peregrina* Fruit: Effect on Hyperglycemia, Hyperlipidemia and Augmented Oxidative Stress in Experimental Type 2 Diabetes. *Food and Chemical Toxicology*, **47**, 2679-2685. <https://doi.org/10.1016/j.fct.2009.07.038>
- [29] Kumar, E.K.D. and Janardhana, G.R. (2011) Antidiabetic Activity of Alcoholic Stem Extract of *Nervilia plicata* in Streptozotocin and Nicotinamide Induced Type 2 Diabetic Rats. *Journal of Ethnopharmacology*, **133**, 480-483. <https://doi.org/10.1016/j.jep.2010.10.025>
- [30] Okumu, M.O., Mbaria, J.M., Kanja, L.W., Gakuya, D.W., Kiama, S.G., Ochola, F.O. and Okumu, P.O. (2016) Acute Toxicity of the Aqueous-Methanolic *Moringa oleifera* (Lam) Leaf Extract on Female Wistar Albino Rats. *International Journal of Basic & Clinical Pharmacology*, **5**, 1-7. <https://doi.org/10.18203/2319-2003.ijbcp20163153>
- [31] Arise, A.K., Arise, R.O., Sanusi, M.O., Esan, O.T. and Oyeyinka, S.A. (2014) Effect of *Moringa oleifera* Flower Fortification on the Nutritional Quality and Sensory Properties of Weaning Food Croat. *Journal of Food Science and Technology*, **6**, 65-71. <https://doi.org/10.17508/CJFST.2014.6.2.01>
- [32] Mukunzi, D., Nsor-Atindana, J., Xiaoming, Z. and Gahungu, A. (2011) Comparison of Volatile Profile of *Moringa oleifera* Leaves from Rwanda and China Using HS-SPME. *Pakistan Journal of Nutrition*, **10**, 602-608. <https://doi.org/10.3923/pjn.2011.602.608>

- [33] Serum, K.S. (2007) T3 and T4 Concentrations in Lambs with Nutritional Myodegeneration. *Journal of Veterinary Internal Medicine*, **21**, 1135-1137. <https://doi.org/10.1111/j.1939-1676.2007.tb03078.x>
- [34] Brisibe, E.A., Umoren, U.E., Brisibe, F., Magalhaes, P.M., Ferrerira, J.F.S., Luthria, D., Wu, X. and Prior, R.L. (2009) Nutritional Characterization and Antioxidant Capacity of Different Tissues of *Artemisia annua* L. *Food Chemistry*, **115**, 1240-1246. <https://doi.org/10.1016/j.foodchem.2009.01.033>
- [35] Mutayoba, S.K., Dierenfeld, E., Mercedes, V.A., Frances, Y. and Knight, C.D. (2011) Determination and Anti-Nutritive Components for Tanzanian Locally Available Poultry Feed Ingredients. *International Journal of Poultry Science*, **10**, 350-357. <https://doi.org/10.3923/ijps.2011.350.357>
- [36] Harborne, J. (1973) *Phytochemical Methods: A Guide to Modern Techniques of Plants Analysis*. Chapman and Hall Ltd., London.
- [37] EL-Massry, Fatma, H.M., Mossa, M.E.M. and Youssef, S.M. (2013) *Moringa oleifera* Plant Value and Utilization in Food Processing. *Egyptian Journal of Agricultural Research*, **91**, 1597-1609. <https://doi.org/10.21608/ejar.2013.166383>
- [38] Daisy, P., Azhagu, S.B. and Rajathi, M. (2009) Antihyperglycemic Effect of Phyllanthus Extracts in Alloxan-Induced Diabetic Rats. *Int J Ph Sci*, **1**, 261-264.
- [39] Ramachandran, S., Rajasekaran, A. and Adhirajan, N. (2013) *In Vivo* and *in Vitro* Antidiabetic Activity of *Terminalia paniculata* Bark: An Evaluation of Possible Phytoconstituents and Mechanisms for Blood Glucose Control in Diabetes. *ISRN Pharmacology*, **2013**, Article ID: 484675. <https://doi.org/10.1155/2013/484675>
- [40] Gupta, R., Sharma, A.K., Sharma, M.C., Dobhal, M.P. and Gupta, R.S. (2011) Anti-diabetic and Antioxidant Potential of β -Sitosterol in Streptozotocin-Induced Experimental Hyperglycemia. *Journal of Diabetes*, **3**, 29-37. <https://doi.org/10.1111/j.1753-0407.2010.00107.x>
- [41] Lamarche, B., Tchernof, A., Moorjani, S., *et al.* (1997) Small, Dense, Low-Density Lipoprotein Particles as a Predictor of the Risk of Ischemic Heart Disease in Men: Prospective Results from the Quebec Cardiovascular Study. *Circulation*, **95**, 69-75. <https://doi.org/10.1161/01.CIR.95.1.69>