SHORT COMMUNICATION article

Analysis of hepatoprotective and antioxidant activities, and determination of the phenolic profile of *Moringa oleifera*

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Abstracts: *Moringa oleifera*, known as the tree of life, is classified as an herbal plant due to its vast medicinal benefits. Usually, the plant is used to cure diseases. This study investigated the antioxidants and hepatoprotective properties of *Moringa oleifera* grown in different regions of Pakistan. Using assays such as DPPH radical scavenging, iron chelation, and phosphomolybdenum reducing activity, the extract revealed strong antioxidant potential and protection against lipid peroxidation in phospholipid homogenates. It also exhibited significant alpha-amylase inhibition and hepatoprotective effect against paracetamol-induced toxicity, confirmed by biochemical and histopathological analysis. Phytochemical profiling of the leaf extract revealed the presence of multiple phenolic and flavonoid compounds, including various syringic acid, 3-caffeoylquinic acid, 5-p-coumaroylquinic acid, 5-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-di-caffeoylquinic acid, 3-feruloyl-4-caffeoylquinic acid, dihydromyricetin, apigenin-7-glucoside, qurecetin-3-galactoside, quercetin-3-glucoside, quercetine-3-rutinoside-7-glucoside, Isorhamnetin-3-hydroxyferuloyl glucoside-7-glucoside, Quercetin 3-(methoxycaffeoyldiglucoside)-7-glucoside, and quercetin-3-(caffeoyl diglucoside)-7-glucoside. Data suggest that *Moringa oleifera* from Pakistan is rich in bioactive compounds contributing to its antioxidant and liver-protective activities.

Introduction

Free radicals are generated in the oxidation process. These radicals have a damaging effect and cause different diseases. The chain of these reactions causes cell spoilage. The antioxidants neutralize these free radicals and protect against oxidative stress [1]. Excessive Reactive oxygen species (ROS) leads to oxidative cells, tissues, and organs damage, which has been observed to warning many biological functions [2]. Antioxidants prevent oxidation by various processes, mostly by scavenging free radicals from the body like superoxide anion, hydrogen peroxide, nitric oxide, and hydroxyl radical [3]. Several plant species have therapeutic effects due to the presence of phenolic compounds [4, 5]. *Moringa oleifera* (*M. oleifera)* is a plant with many reports on its pharmacological and health benefits. *M. oleifera* is found in India but widely distributed in other parts of the world, including Africa, Europe, and Asia. Moringa is known as 'horseradish tree' or 'drumstick tree' [6]. The most abundant compounds found in *M. oleifera* are Quercetin and kaempferol glycosides [7]. The traditional and nontraditional use of Moringa, its pharmacological effects and their phytopharmaceutical formulations, clinical studies, toxicity profile, and various other uses have recently recognized [8]. The search of literature has shown that there are few studies on the antioxidant and hepatoprotective potential of *M. oleifera* from Pakistan. The overall aim of this study was to determine the in vitro antioxidant activities, hepatoprotective activities, and phenolic profile of aqueous extract being used in traditional formulations.

Materials and methods

Collection of plant: The leaves of *M. oleifera* were collected from Sialkot, Pakistan during 2025, and its identification was done in the Department of Botany, University of Poonch, Rawalakot, AJK, Pakistan.

Antioxidant activities: The DPPH radical activity by *M. oleifera* leaves was checked using the method [9]. The ability to chelate iron was studied following the method [10]. The reduction potential of *M. oleifera* leaves was determined following phosphomolybdenum method [11]. The lipid peroxidation was studied in egg yolk phospholipid by the method reported [3].

Alpha amylase inhibitory activity: The starch iodine method was used for antidiabetic assay with slight modifications [12].

In-vivo hepatoprotective activity: Male albino mice (25-35 g) and aged 2.5 months from the breeding center in CEMB University of Panjab were used for *in vivo* studies. The animals were kept in separate cages with access to food and water under controlled conditions. The mice were randomly divided into four groups, each consisting of five animals, and were fasted overnight before the initiation of the experiment. **Group I** (normal control) given normal saline, and **Group II** (hepatotoxic control) was given paracetamol (70.0 mg/kg). **Groups III & IV** (treated groups) were administered oral doses of aqueous extract of *M. oleifera*, 100 mg/kg and 250 mg/kg, respectively. The treatments were continued for seven days, and the animals were fed orally. In the plant group, the extract was fed to mice after three hours of paracetamol dose. On 8^{th} day, all mice were sacrificed and blood was drawn by heart puncture and level of ALT and AST activities were determined by commercial kits. The livers were collected for biochemical and histopathological analysis. Liver was prepared in normal saline (0.9%), and the level of TBARS, NPSH, and Catalase activities was determined. The TBARS was determined by the method of [13]. Catalase was determined by the Aebi method [14]. While non-protein thiol was determined by using the method [15].

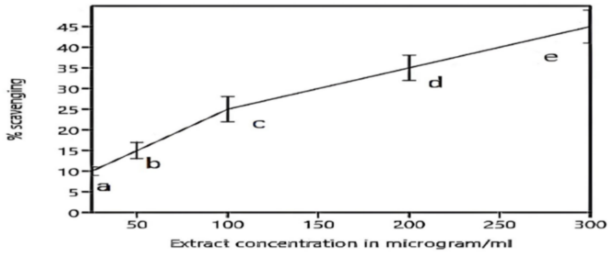
HPLC analysis of phenolics and flavonoids: The dried aqueous extract of *M. oleifera* was filtered and subjected to analysis HPLC system by the method [16]. The unknown compounds were identified from the absorption spectra of compounds in the literature [17].

Ethical approval: All animal procedures were in strict accordance with the NIH guide for the Care and Use of Laboratory Animals and were approved by the University of Poonch Rawal Akot Ethical Committee (UPRAEC-2025).

Statistical analysis: The results were expressed as means ± SD. Different means were compared by LSD test for the significance of the data.

Results and discussion

Radical scavenging activity: The antioxidant potential of aqueous extracts of *M. oleifera* was evaluated using the DPPH free radical scavenging assay at different concentrations. DPPH radical is the most common radical used to measure antioxidant activities [18-20]. DPPH is violet in color and changes its color to yellow when the extract was added. DPPH is reduced by reaction with extract which provides hydrogen atoms. Normally, phenolics and flavonoids have high reducing activities [21-23]. **Figure 1** shows that the scavenging percentage as obtained by the extract was slightly less than that of standard ascorbic acid. These results are in line with previous reports [5, 24].



Figures 1: DPPH radical scavenging activity of *Moringa oleifera*

The values with different superscripts are statistically significant

Iron chelation assay: When Fe $^{2+}$ reacts with 1,10-phenanthroline, a red colored complex is formed. The addition of extract of natural products to this complex fades its color, and it becomes colorless. This shows the chelating capacity of plant extracts [25]. It was hence concluded that by increasing the concentration of the extract, the chelating activity was also increased. In **Figure 2**, At maximum concentration of extract i.e. 300 μg/ml, the highest chelating activity was observed by the leaves extract 30.0% and it was comparable with that of ascorbic acid, 78.0%. **Figure 2** shows that there is a high significant difference among different tested concentrations for metal chelation activities ($p<0.01$).

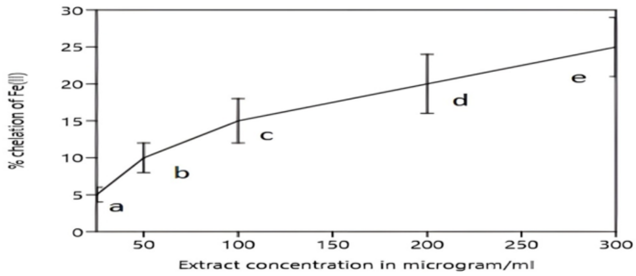


Figure 2: Metal chelating activity of *Moringa oleifera*

The values with different superscripts are statistically significant

Reducing activity: The reduction potential of plant extracts as evaluated by the phosphomolydenum assay involves the formation of a green colored complex as a result of a decrease in oxidation state from VI to V [26]. A direct estimate of the reduction potential of *M. oleifera* leaves extract was obtained by measuring absorbance at 695 nm. **Figure 3** shows that there is a high significant difference among different tested concentrations for reducing activities ($p<0.01$). **Figure 3** shows that maximum reducing ability was shown by *M. oleifera* leaves extract as the absorbance increases with increasing concentration of the extract. Actually, the molybdenum ion is reduced from Mo (V) to Mo (VI), changing the color of the solution from yellowish to green complex.

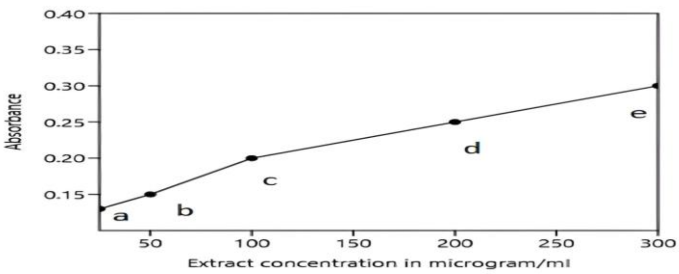


Figure 3: Reducing the activity of *Moringa oleifera*

The values with different superscripts are statistically significant

Lipid peroxidation in egg yolk: Egg yolk phospholipid was induced with lipid peroxidation using iron sulphate, and the anti-lipid peroxidative property of the extract was determined. The extracts reduced the lipid peroxidation in dose dependent manner (**Figure 4**). The 2-thiobarbituric acid reacts with lipid peroxides and generate a pink color chromogen which is estimated at 532 nm.

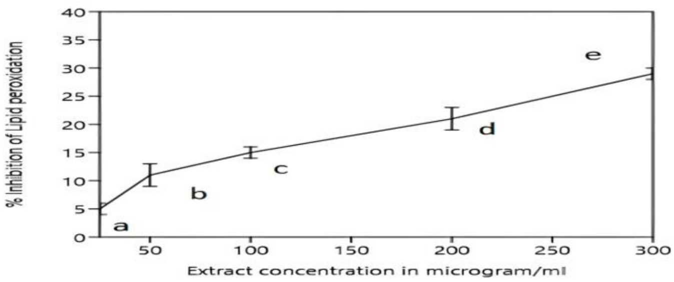


Figure 4: Percentage inhibition of lipid peroxidation by *c* aqueous extract

Alpha amylase inhibition assay: The present data confirmed a dose-dependent α-amylase inhibitory effect of plant extract, supporting a clear dose-response relationship. *M. oleifera* leaf extract demonstrated inhibitory effects statistically significantly tested concentrations, suggesting limited efficacy in this model **(Figure 5)**. Plant-based enzyme inhibitors are increasingly favored for their safety and added antioxidant benefits over synthetic drugs [27]. The findings highlight *M. oleifera* as the most promising natural α-amylase inhibitors, with potential therapeutic or nutraceutical applications in managing T2DM [28]. However, more pharmacological validation of these species is recommended to support their development as plant-based antidiabetic agents. The glucose-lowering effect of the extract may have been produced by the flavonoids, saponins, and tannins [22, 29, 30].

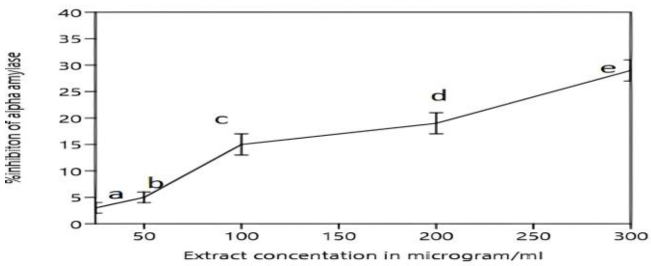


Figure 5: Alpha amylase inhibitory effect of *Moringa oleifera*

The values with different superscripts are statistically different

In-vivo hepatoprotective activity of *M. oleifera*: Paracetamol administration at a dose of 250 mg/kg resulted in liver damage as shown by biochemical parameters and histopathology (**Table 1,** and **Figure 6**). The treatment with aqueous extracts of all plants at dose of 100 and 250 mg/kg after paracetamol treatment reversed these changes (**Table 1**). Paracetamol is a popular analgesic drug, which does not cause toxicity in therapeutic doses but can result in lethal hepatic necrosis in experimental animals and humans and is known hepatotoxic agent. When the liver is damaged, it causes the leakage of cellular enzymes into the plasma, which are markers of hepatotoxicity [31, 32]. These enzymes are measured in serum to know the extent of hepatocellular damage. As the level of these enzymes were increased on paracetamol treatment, it shows possible toxicity (**Table 1**). Administration of *M. oleifera* aqueous extracts (100 mg/kg and 250 mg/kg, p.o.) brought back the altered levels of ALT and AST showing hepatoprotection. The level of TBARS was significantly increased by treatment with paracetamol, which was decreased by plant extracts (**Table 1**). The plant was not only found effective in decreasing TBARS *in vitro* but also *in vivo*. Catalase activity was significantly decreased in the paracetamol treatment group ($p<0.05$); however, it was restored to control levels with extract treatment. Glutathione release increases from the liver with paracetamol intoxication [33].

However, treatment with extracts of *M. oleifera* restored the levels of NPSH (**Table 1**). The current data are supported by the previous findings [34]. The findings of histopathology revealed that the control group shows hepatic lobules with typical architecture, central veins, intact hepatocyte cords, and minimal cytoplasmic vacuolation. The treated group showed severe foci of hepatocellular necrosis, ballooning degeneration, sinusoidal dilation, and inflammatory cell infiltration- classical signs of acute hepatotoxicity [35]. However, moderately protective sections of *M. oleifera* treated groups exhibited less necrosis, less severe cytoplasmic vacuolation, and partial lobular reconstruction (**Figure 6**).

Table 1: Phenolic profile of *Moringa oleifera* leaves aqueous extract

Rt (min)	Identity	Absorption spectra (nm)	Mean	STD
1.1	Syringic acid	276	69.1	0.34
4.2	3-caffeoylquinic acid	324, 232	285.1	0.69
5.8	5-p-Coumaroylquinic acid	310, 230	119.0	1.0
6.7	3-Caffeoylquinic Acid	326, 298sh	34.0	0.5
7.3	5-Caffeoylquinic acid	326, 298sh	127.5	0.4
8.3	4-Caffeoylquinic acid	326, 298sh	36.8	1.0
9.3	3,4-Di-caffeoylquinic acid	325, 242	61.0	1.3
10.4	3-Feruloyl-4-caffeoylquinic acid	324, 246	35.9	1.2
12.4	Dihydromyricetin	335, 260	9.11	0.1
14.4	Apigenin-7-glucoside	336	14.9	0.3
15.8	Qurecetin-3-galactoside	354, 256	62.5	0.4
16.7	Quercetin-3-glucoside	356, 256	59.8	0.3
18.3	Quercetine-3-rutinoside-7-glucoside	342, 254	6.70	0.3
19.7	Isorhamnetin-3-hydroxyferuloylglucoside-7-glucoside	342, 248	7.57	0.1
24.9	Quercetin 3-(methoxycaffeoyldiglucoside)-7-glucoside	334, 250	1.72	0.02
27.7	Quercetin-3-(caffeoyldiglucoside)-7-glucoside	335, 253	2.54	0.02

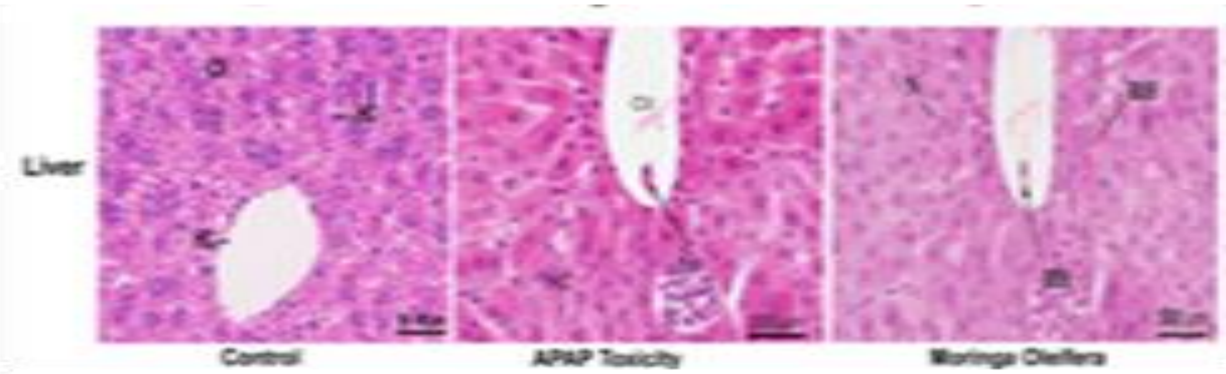


Figure 6: Histopathology showing hepatoprotective action of *Moringa oleifera* aqueous extract

High Performance Liquid Chromatography (HPLC) analysis: Herein, we report the phenolic profile of *M. oleifera* leaves aqueous extract (**Figure 7**). The phenolic profile showed many phenol containing compounds identified by their retention times. By HPLC analysis syringic acid, 3-caffeoylquinic acid, 5-p-coumaroylquinic acid, 5-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-di-caffeoylquinic acid, 3-feruloyl-4-caffeoylquinic acid, dihydromyricetin, apigenin-7-glucoside, qurecetin-3-galactoside, quercetin-3-glucoside, quercetine-3-rutino-side-7-glucoside, isorhamnetin-3-hydroxyferuloylglucoside-7-glucoside, quercetin 3-

(methoxycaffeoyldiglucoside)-7-glucoside and quercetin-3-(caffeoyldiglucoside)-7-glucosidewere identified in the leaves of *M. oleifera*. The retention times and amounts of these compounds are shown in **Table 1**. The earlier studies have reported that leaves contained free phenolics: flavonols (mainly quercetin and Kaempferol glycoside), hydroxycinnamic acid derivatives, and a small amount of lignan (isolariciresinol isomers) [36]. This study reported some new compounds in the extract of *M. oleifera,* highlighting the importance of the study.

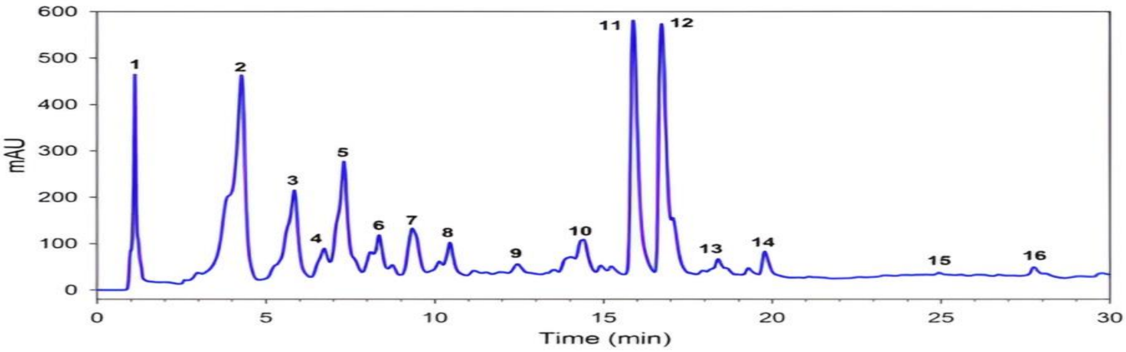


Figure 7: HPLC fingerprint of *Moringa oleifera* aqueous extract obtained from leaves showing the presence of different phenolics

Conclusion: Moringa oleifera leaves contain important phytochemicals and valuable antioxidant activities. The hepatoprotective activity of *Moringa oleifera* against paracetamol justifies the popular use of the plant against liver disease. This makes the *Moringa oleifera* a suitable candidate for its use in food and pharmaceuticals.

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Author's contribution: ZUN, SMS, MA conceived and designed the study. ZUN, SMS, ZA & IU collected data. ZUN, SMS, IH & MA contributed to data analysis. ZUN, SMS & ZA performed data analysis and data interpretation. ZUN, SMS, IZ & MA drafted the manuscript drafting. All authors read and approved the final manuscript.
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Author declarations: The authors confirm that they have followed all relevant ethical guidelines and obtained any necessary IRB and/or ethics committee approvals.
Generative AI disclosure: No Generative AI was used in the preparation of this manuscript.

تحليل الخصائص الوقائية للكبد والمضادة للأكسدة، وتحديد التركيب الفينولي لنبات المورينجا أوليفيرا

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4 قسم الصيدلة، جامعة هزارة، مانسهرا، خيبر بختونخوا، باكستان
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الملخص: تُصنف المورينغا أوليفيرا، المعروفة بشجرة الحياة، ضمن النباتات الطبية نظرًا لفوائدها العلاجية العديدة. وعادةً ما تُستخدم هذه النبتة لعلاج الأمراض. هدفت هذه الدراسة إلى بحث خصائص مضادات الأكسدة وحماية الكبد في المورينغا أوليفيرا المزروعة في مناطق مختلفة من باكستان. وباستخدام اختبارات مثل إزالة جذور DPPH الحرة، واستخلاب الحديد، ونشاط اختزال الفوسفوموليبدينوم، أظهر المستخلص قدرةً قويةً كمضاد للأكسدة وحمايةً من بيروكسدة الدهون في متجانسات الفوسفوليبيد. كما أظهر تثبيطًا ملحوظًا لإنزيم ألفا-أميليز وتأثيرًا وقائيًا للكبد ضد السمية الناجمة عن الباراسيتامول، وهو ما أكدته التحليلات البيوكيميائية والنسيجية المرضية. كشف التحليل الكيميائي النباتي لمستخلص الأوراق عن وجود مركبات فينولية وفلافونويدية متعددة، بما في ذلك حمض السيرينجيك، وحمض 3-كافويل كينيك، وحمض 5-بارا-كوماريل كينيك، وحمض 5-كافويل كينيك، وحمض 4-كافويل كينيك، وحمض 3،4-ثنائي كافويل كينيك، وحمض 3-فيروليل-4-كافويل كينيك، وثنائي هيدروميريسيتين، وأبيجينين-7-جلوكوزيد، وكيرسيتين-3-جالاكتوزيد، وكيرسيتين-3-جلوكوزيد، وكيرسيتين-3-روتينوزيد-7-جلوكوزيد، وإيزورامنيتين-3-هيدروكسي فيروليل جلوكوزيد-7-جلوكوزيد، وكيرسيتين 3-(ميثوكسي كافويل ثنائي جلوكوزيد)-7-جلوكوزيد، وكيرسيتين-3-(كافويل (ديغلوكوزيد)-7-غلوكوزيد. تشير البيانات إلى أن نبات المورينجا أوليفيرا من باكستان غني بالمركبات النشطة بيولوجيًا التي تساهم في خصائصه المضادة للأكسدة والواقية للكبد.