

Research Article

Phenolic Content and Antioxidant and Antimicrobial Activities of *Malva sylvestris* L., *Malva oxyloba* Boiss., *Malva parviflora* L., and *Malva aegyptia* L. Leaves Extract

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Background. The plants brought by Arabs were of real therapeutic values. Ibn Al-Baitar, an Islamic scholar (pharmacist, botanist, and physician), in his encyclopedia wrote the detailed characterization of more than one thousand herbs describing their medicinal value, methods of preparation, and their route of administration. **Objectives.** The current investigation points towards the quantitative characterization of the phenolic contents among the four edible *Malva* plants species (*Malva sylvestris* L., *Malva oxyloba* Boiss., *Malva parviflora* L., and *Malva aegyptia* L.) and also towards assessing their antibacterial activity against one Gram-positive isolate (*Staphylococcus aureus*) and four Gram-negative strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonnei*, and *Proteus vulgaris*. It also aimed to evaluate the free radical scavenging activity of hexane, methanolic, aqueous, and acetone extracts of four *Malva* species. **Methods.** By utilizing the Folin–Ciocalteu procedure and gallic acid as a reference molecule, the phenolic contents were estimated. In addition, the broth microdilution method was used to evaluate four plants' 16 extracts, and the DPPH (2,2-diphenyl-1-picrylhydrazyl) method was utilized to assess the abovementioned extracts against oxidative stress. **Results.** The results showed that the methanolic extract of *M. oxyloba* has the highest contents of phenols (191.54 ± 2.84 mg of GAE/g) and has the best antioxidant capacity with an IC₅₀ value of 1.94 ± 1.84 µg/ml, which is very close to Trolox. Regarding the ferrous ion chelating activity of the extract, the methanolic extract of *M. sylvestris* exhibits appreciable activity with IC₅₀ values of 52.7 ± 1.8 µg/ml. In addition, the plant extract and acetone extract of *M. sylvestris* showed a strong antibacterial activity with a MIC value of 0.0078 mg/ml. **Conclusion.** The methanolic extract of *M. oxyloba* has a pharmacological potential as a valuable natural product that can be utilized as a main ingredient in the design and development of new therapeutic formulations. It exerts multiple inhibitory properties against oxidative stress and bacterial growth. As such, it is emerging as a promising therapeutic agent for the treatment of various neurodegenerative diseases and many types of human infectious diseases.

1. Introduction

The history of phytotherapy started with our ancestors, who had the knowledge of the herbs and knew that the consumption of certain kinds of herb resulted in the soothing effect of several types of diseases. Recently, herbal remedies have become a controversial issue all over the world as large numbers of herbals are utilized in large quantities in cosmetics, as food preservatives and as nutritional supplements

and as natural ingredient in large number of drugs in many of the pharmaceutical industries. In fact, people around the world are more moving towards or prefer the herbal medicines due to the belief that these phytotherapeutic products are natural and safer than synthetic medicines [1]. In recent years, the term oxidative stress has been gaining interest among many research scientists, and this has led to the development of numerous techniques in the prevention of diseases caused by the action of the harmful free radicals that

cause oxidative stress. In addition, they studied the possible roles of antioxidants in protecting the body from many dangerous diseases caused due to oxidative stress. Common free radical such as hydroxyl (OH[•]), superoxide (O²⁻), and nitric monoxide (NO[•]) are considered as causative agents for many chronic diseases such as cancer, diabetes, cardiovascular diseases, rheumatoid arthritis, and other oxidative diseases. At the same time, there are many antioxidant compounds, mostly probably derived from plants, such as vitamin E, vitamin C, creatinine, and other phenolic compounds have the ability to protect the body against the diseases caused by free radicals [2].

It has been observed that the bacterial strains are being increasingly resistant to the antibiotics that are available in the market due to their overuse. The available reports suggest that the overuse of the antiseptic in house and hospitals also contributed to the increase in the resistance of bacteria. However, if the patients with bacterial infection, that is, resistance to antibiotics, did not receive effective treatment may lead to long-term infections that may result in high mortality as was observed in the patients with pneumococcal infections and typhoid fever [3].

Malva (Malvaceae family) is a very common plant growing in wildly in the Middle Eastern countries and commonly known as mallow and has been used by Greek and Romans as an emollient and laxative; also, it has been used to treat some diseases associated with alimentary canal and respiratory disorders. In Asian countries, it is used to treat injuries and inflammations. People use *Malva* species in different ways; many use it in their food; they eat it raw mixed in a salad or cooked as a vegetable. Other people use its flowers for medicinal purpose to treat injuries and different skin disorders [4]. The intensive use of *Malva* species among Arabian population urges us to study and identify the medical and chemical characteristics of *Malva sylvestris* L., *Malva oxyloba* Boiss., *Malva parviflora* L., and *Malva aegyptia* L.

Malva sylvestris is used as a folkloric food and has traditional medicine particularly in the treatment of dermatitis, peptic ulcer, cough, diabetes, and sore throat [5]. It contains tannins, vitamins (A, C, and E), folic acid, niacin, polyphenols (anthocyanins), scopoletin, malvone A (2-methyl-3-methoxy-5,6-dihydroxy-1,4-naphthoquinone) malvaline, malvidin 3-(6''-malonyl -glucoside)-5-glucoside, malvin, flavonoids (three 8-hydroxyflavonoids, hypolaetin 3'-sulphate, and gossypetin 3-sulphate-8-O-β-D-glucoside) polysaccharides (mucilage) and coumarins [6].

In a study established by Tabaraki et al., they identified the bioactive molecule from *Malva sylvestris* using GC-MS technique and found that the major compound in the methanolic extract was 2-methoxy-4-vinylphenol [7]. Moreover, *M. sylvestris* anthocyanins contents reduced plasma total triglycerides and cholesterol and also protected rats from ethanol-induced gastritis due to the high contents of mucilage [8–10].

Hot poultice of *Malva parviflora* used in traditional medicine to treat wounds, swelling, sores, bruises, and broken limbs. It contains tannin, flavonoid, polyphenol, saponin, resin, and alkaloid. Several investigations showed that *M. parviflora* has antiulcerogenic, analgesic, antidiabetic, anti-inflammatory, antibacterial, and neuroprotective activities [11]. Throughout the literature, no previous studies were carried out to

investigate the constituents and the pharmacological activities of *M. oxyloba* and *M. aegyptia*. This prompted us to search for new source of safer and inexpensive antioxidants from four edible species of *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* plants. The extracts were prepared in aqueous solution, hexane, acetone, and methanol solvent; these extracts were tested for their antioxidant activity using DPPH method, and to estimate their total phenol contents using the Folin–Ciocalteu assay, the relationship between antioxidant activity and total phenol contents was correlated; their antimicrobial properties were also studied.

2. Materials and Methods

2.1. Chemicals. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma Aldrich company (Denmark), DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin–Ciocalteu, and Ferrozine reagent were brought from Sigma Aldrich (Germany), while methanol, hexane, and acetone were obtained from Loba Chemie (India). Moreover, dimethyl sulfoxide (DMSO) was brought from Riedel-de Haën (Germany); Mueller–Hinton (Dickinson, USA) and nutrient broth were purchased from HiMedia (India).

2.2. Instruments. Shaker device (Memmert, Germany), rotavap (Heidolph-OB2000, Germany), UV-Visible spectrophotometer (Jenway-7315, England), UV-Visible spectrophotometer (Shimadzu, Japan) Balance (Radwag-AS 220/c/2, Poland, and Shimadzu, Japan), grinder (Molineux N-1, China), filter papers (Macherey-Nagel-MN 617 and Whatman no.1, USA), micropipettes (Finnpipette, Finland), incubator (Nuve-Model 002, Turkey), syringe filter 0.45 μm pore size (Microlab, China), 96-well plates (Greiner Bio-One, North America), and multichannel micropipette (Thermo Fisher Scientific, DE, Germany).

2.3. Collection and Preparation of Plants. The leaves of *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* plants were collected in March 2018 during the flowering time from Tulkarm plains and mountains in the West Bank area of Palestine. The four *Malva* species were identified in the pharmacognosy and herbs laboratory, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. The herbarium voucher specimen stored in the Pharmacognosy Laboratory under the following codes: Pharm-PCT-1507, Pharm-PCT-1505, Pharm-PCT-1506, and Pharm-PCT-1502.

The leaves of each of the *Malva* species were washed with distilled water and then dried separately at room temperature. The drying took about 2-3 weeks until all the four plants species materials were well dried. By using the grinder, the dried parts for each type were grounded well, and the material powders were kept under dry condition ready for further use.

2.4. Extraction. The exhaustive maceration was used to extract *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* plants materials by soaking 150 g of powdered

samples sequentially into 500 ml of distilled water, *n*-hexane, ethyl acetate, and methanol in an enclosed glass bottle with occasional shaking at room temperature for 72 hrs.

Thereafter, the macerated materials were then filtered through a Whatman No. 1 filter paper and then evaporated under reduced pressure in a rotary evaporator set at 35°C in a water bath until the solvents completely evaporated, while the aqueous extracts were freeze-dried. The final dried extracts were stored at 4°C in a refrigerator.

2.5. Total Phenol Content. The total phenol contents of *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* plants four extracts were estimated calorimetrically utilizing the Folin–Ciocalteu procedure and gallic acid as a reference molecule [12–14]. Briefly, from each plant fraction, 1 mg/ml aqueous solution was prepared for this analysis, while the reaction mixtures were prepared by taking 0.5 ml of the aqueous solution from each plant fraction, 2.5 ml of 10% Folin–Ciocalteu's testing agent dissolved in water, and 2.5 ml of 7.5% NaHCO₃ in aqueous solution. Thereafter, the produced mixtures were kept in a thermostat at 45°C for 45 min. The absorbance was estimated utilizing a spectrophotometer at a wavelength of 765 nm. All of the studied samples were tested in triplicate for each plant fraction, and the mean values of absorbance obtained were reported. The same method was repeated for the standard molecule gallic acid, and then the required calibration curve was constructed. Based on the measured absorbance, the concentration of gallic acid equivalent was determined in mg of GAEq/g for each working plant sample.

2.6. Free Radical Scavenging DPPH Method. According to the method described by Jothy et al. [15] with some modifications, the antioxidant activity was determined using DPPH scavenging assay. A stock solution of Trolox and 16 plant extracts in methanol was prepared with a concentration of 1 mg/mL; from the stock solution by serial dilution method, various concentrations were prepared. Then, 0.002% DPPH was prepared. A concentration ratio (1:1:1) of the above prepared solution, DPPH, and methanol were arranged, respectively. These solutions were incubated in a dark place for 30 min, while methanol was utilized to zero the UV-Vis-spectrophotometer. Then, the absorbance readings were measured at 517 nm. Antioxidant activity is given as percent DPPH scavenging that is calculated as

$$\% \text{DPPH scavenging} = \frac{M - S}{M} \times 100\%, \quad (1)$$

where *M* is the absorbance of the control sample and *S* is the absorbance of the tested extracts. The results are documented as an IC₅₀ value which is the inhibitory concentration required to decrease 50% of the activity.

2.7. Ferrous Ion-Chelating Activity. The samples were estimated for the ferrous ion-chelating activity as described by Chua et al. [16] Briefly, 750 μL of the sample in test material (2–500 μg/mL) or standard substance (1–50 μg/ml) were

incubated with 50 μL ferrous chloride solution (FeCl₂) (2 mM) for 5 min. The reaction was initiated by the addition of 50 μL ferrozine solution (5 mM) into the mixture and allowed to stand for 10 minutes in dark. The absorbance of the reaction mixture was measured at 562 nm. All experiments were performed at least in triplicate. The chelating effect was calculated as a percentage using the following equation. EDTA was used as a positive control in this assay.

$$\% \text{chelating activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%, \quad (2)$$

where *A*_{control} is the absorbance of the control reaction mixture without the test compounds, and *A*_{sample} is the absorbance of the test compounds. IC₅₀ values, which represented the concentration of the extract that caused 50% of Fe²⁺ ion chelation, were calculated from the plot of chelating percentage against concentration.

2.8. Microbial Isolates. The bacterial isolates were obtained from American-type culture collection (ATCC). The isolates included one Gram-positive strains: *Staphylococcus aureus* (ATCC-25923), in addition to four Gram-negative strains: *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853), *Shigella sonnei* (ATCC-25931), and *Proteus vulgaris* (ATCC-838).

2.9. Antimicrobial Test. For determination, the antimicrobial activity for four plants' 16 extracts, a micro broth dilution method was employed. Some colonies from the used bacterial pathogens were planted in agar broth for 24 hrs. A little amount of the bacteria was added inside a 5 ml of Mueller–Hinton tube and was mixed. Then, the turbidity was compared with McFarland standard tube 0.5 (1.5 × 10⁸ cfu/ml). Then, 2 ml of the bacterial suspension was diluted with 4 ml broth. The four *Malva* species extracts were prepared by dissolving 50 mg of each in 1 ml DMSO (100%) with a 50 mg/ml concentration. Then, 100 μl from Mueller–Hinton was placed in a sterile 96-well microplate. By using a micropipette, a volume of 100 μl of plant extracts was added into the first well and mixed. Serial dilution was done by transferring 100 μl from well to next well up to well 11 from which 100 μl was discharged after mixing process; well 12 had only broth without plants extracts, so bacterial growth will be noticed. However, a serial dilution was established under sterile conditions. After that, a 10 μl of bacterial suspension was employed into all wells but not in well 11. While well 12 must be turbid, if not we should know that something wrong happened with our media or bacteria. Then, the used microplate was incubated for 24 h. Each plant extract was examined in duplicate. MIC is defined as the minimum concentration of the antibacterial agents that resulted in nonvisible turbidity compared with a control well.

2.10. Statistical Analysis. The extraction yields, total phenols, and antioxidant results of the four studied plant species were expressed as means ± SD standard deviation. Statistics were compared utilizing unpaired *t*-tests.

TABLE 1: Extractions yields percentages of four *Malva* species in water, hexane, methanol, and acetone.

Extracts	<i>M. sylvestris</i> , % (\pm SD)	<i>M. oxlyoba</i> , % (\pm SD)	<i>M. parviflora</i> , % (\pm SD)	<i>M. aegyptia</i> , % (\pm SD)
Aqueous	3.25 \pm 0.75	3.18 \pm 0.61	2.91 \pm 0.55	3.21 \pm 0.41
Hexane	1.01 \pm 0.11	0.97 \pm 0.15	0.89 \pm 0.09	0.74 \pm 0.05
Methanol	4.31 \pm 0.97	5.58 \pm 1.1	3.88 \pm 0.89	5.15 \pm 0.94
Acetone	1.22 \pm 0.12	0.98 \pm 0.09	2.1 \pm 0.047	1.74 \pm 0.65

3. Results

3.1. Plants Extract Yields. The obtained yields of *M. sylvestris*, *M. oxlyoba*, *M. parviflora*, and *M. aegyptia* plants aqueous, hexane, methanol, and acetone solvents were calculated and performed in Table 1. The results showed that methanolic extracts of *M. sylvestris*, *M. oxlyoba*, *M. parviflora*, and *M. aegyptia* have the highest extraction yields, which were 4.31 \pm 0.97, 5.58 \pm 1.1, 3.88 \pm 0.89, and 5.15 \pm 0.94%, respectively.

3.2. Phenolic Content. For the evaluation of total phenol content, the absorption values of several concentrations of the gallic acid standard are listed in Table 2. From the calibration curve of gallic acid (Figure 1), the following equation was used to estimate total phenol content in *M. sylvestris*, *M. oxlyoba*, *M. parviflora*, and *M. aegyptia* plants aqueous, hexane, methanol, and acetone extracts [14].

$$y = 0.0112x + 0.0176, \quad (3)$$

$$r^2 = 0.9956,$$

where y is the absorbance at 765 nm and x is the total phenol content of the four *Malva* plants species various extracts.

Obviously, Table 3 indicates that acetone extract of *M. sylvestris*, the methanolic extract of *M. oxlyoba*, and hexane extracts of *M. aegyptia* and *M. parviflora* plants have the highest total phenols among the other screened solvents.

3.3. Antioxidant Activity. The antioxidant activities of hexane, methanol, acetone, and aqueous *M. sylvestris*, *M. oxlyoba*, *M. parviflora*, and *M. aegyptia* plants extracts were evaluated using DPPH free radical method. In addition, in the current study, Trolox, the powerful antioxidant compound, was used as a reference positive control. The DPPH inhibition effects for each studied *Malva* plant species are calculated in Tables 4–7 and shown in Figures 2–5. From Figures 2–5, the IC₅₀ values of hexane, methanol, acetone, and aqueous *M. sylvestris*, *M. oxlyoba*, *M. parviflora*, and *M. aegyptia* plants extracts were calculated in addition to the Trolox standard (IC₅₀ value of 1.86 \pm 0.8 μ g/ml). These results are demonstrated in Table 8, which showed that among *M. sylvestris* extracts, the acetone extract has the highest IC₅₀ value of 4.07 \pm 1.04 μ g/ml, among *M. oxlyoba* extracts, the methanolic extract was the best one with IC₅₀ value of 1.94 \pm 1.84 μ g/ml, and among *M. aegyptia* and *M. parviflora* extracts, the hexane extracts were the best with IC₅₀ values of 6.9 \pm 1.42 μ g/ml and 22.9 \pm 1.47 μ g/ml, respectively.

The ferrous ion chelating activity of the different extracts is mentioned in Table 9. It is interesting to mention that the aqueous extract showed better activity than the other

TABLE 2: Absorption values of several concentrations of the standard gallic acid [14].

The concentration of gallic acid (μ g/ml)	Absorption at $\lambda_{max} = 765$ nm
0.0	0.00
10	0.14
40	0.49
50	0.55
70	0.79

extracts; the IC₅₀ values were ranged from 52.7 \pm 1.8 to 95.5 \pm 3.4 μ g/ml. The hexane extract of different plants was inactive; this might be due to the absence of polar chemical compound which is able to form chelate with the ferrous ion. The acetone extract of the plant exhibited weak activity. The IC₅₀ values ranged from 315.5 \pm 7.0 to 425.5 \pm 10.2 μ g/ml. The ferrous ion chelating activity of *M. sylvestris* was better than the other extracts.

3.4. Antimicrobial Capacities. Most of *M. sylvestris*, *M. oxlyoba*, *M. parviflora*, and *M. aegyptia* plants hexane, methanol, acetone, and aqueous extracts exhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonnei*, and *Proteus vulgaris* at various levels as presented in Table 10.

4. Discussion

Over thousands of years, the human orientations towards herbal products have been increasing significantly, for food purposes or for the medicinal use. Millions of people around the world believe in the efficacy and safety of the plants as being a natural resource in many areas of their lives. Many valuable medicines have been derived from plant source such as digoxin, vincristine, pilocarpine, atropine, and many others [17].

In recent years, some groups of natural compounds have received remarkable attention including polyphenols and simple phenols, which are an essential group of secondary metabolites with a wide diversity in structures and are biosynthesized in different parts of the plants for various functions such as protection, pigmentation, and pollination [18]. Various investigations have linked a high phenolic diet to the prevention of several cardiovascular, metabolic, infectious, and cancer diseases due to their pharmacological and biological activities [19].

Total phenolic content of all the plant extracts of all the four species of *Malva* were estimated using the Folin–Ciocalteu assay.

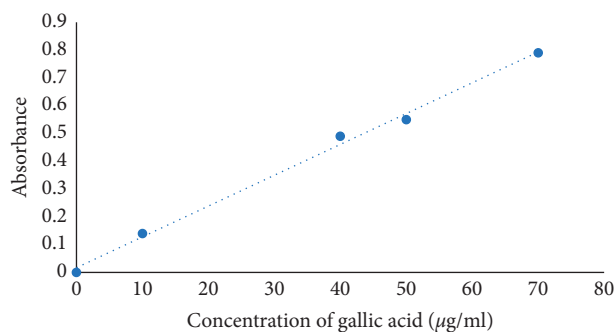


FIGURE 1: Standard calibration curve of gallic acid for the calculation of total phenols [14].

TABLE 3: Quantitative phenol content of the hexane, acetone, methanol, and aqueous extracts of *M. oxyloba*, *M. aegyptia*, *M. sylvestris*, and *M. parviflora*.

Extracts	Total phenol contents, mg of GAE/g of <i>M. oxyloba</i> , \pm SD	Total phenol contents, mg of GAE/g of <i>M. aegyptia</i> , \pm SD	Total phenol contents, mg of GAE/g of <i>M. sylvestris</i> , \pm SD	Total phenol contents, mg of GAE/g of <i>M. parviflora</i> , \pm SD
Acetone	12.35 \pm 0.73	25.28 \pm 2.99	104.01 \pm 2.04	27.54 \pm 2.66
Methanol	191.54 \pm 2.84	77.58 \pm 2.44	20.47 \pm 1.48	42.44 \pm 2.36
Hexane	88.19 \pm 2.79	106.9 \pm 2.22	69.49 \pm 1.15	132.19 \pm 1.97
Aqueous	95.5 \pm 2.67	17.11 \pm 2.15	23.35 \pm 0.76	32.95 \pm 1.67

TABLE 4: DPPH scavenging activity of Trolox and four *M. parviflora* extracts.

Conc.	Trolox	\pm SD	Acetone extract	\pm SD	Methanolic extract	\pm SD	Hexane extract	\pm SD	Aqueous extract	\pm SD
0	0	0	0	0	0	0	0	0	0	0
1	50.72	1.06	29.85	0.63	27.3	1.13	32.8	0.42	10.3	0.44
2	51.0	0.49	36.35	1.34	32.7	0.28	32.8	0.42	31.5	0.45
3	70.2	0.35	36.35	1.34	33.05	1.06	37	0.81	32.1	0.85
5	70.2	0.35	36.9	1.69	35.8	0.35	37.27	1.22	36.5	1.25
7	83.95	0.21	43.1	2.2	37.7	0.98	37.27	2.4	40.0	2.75
10	95.99	1.69	43.1	1.69	37.7	0.99	37.27	2.1	40.1	2.55
20	99.71	1.06	43.1	1.69	39.65	1.2	39.44	1.13	41.0	1.44
30	99.71	0.49	49.2	1.55	43.1	1.99	49.95	4.32	43.2	2.15
40	99.71	0.49	55.0	1.83	44.1	2.4	54.7	0.35	52.1	0.65
50	99.71	0.35	55.0	2.7	47.0	1.97	61.0	2.2	51.5	1.8
80	99.71	2.25	55.0	1.64	47.8	2.6	67.5	0.85	58.1	2.15

TABLE 5: DPPH scavenging activity of Trolox and four *M. oxyloba* extracts.

Conc.	Trolox	\pm SD	Acetone extract	\pm SD	Methanolic extract	\pm SD	Hexane extract	\pm SD	Aqueous extract	\pm SD
0	0	0	0	0	0	0	0	0	0	0
1	50.72	1.06	10.23	0.34	40.7	2.22	10.23	0.37	44	1.7
2	51	0.49	32.92	0.57	52.15	2.82	32.92	0.38	43.15	0.58
3	70.2	0.35	32.9	1.12	62.25	1.5	32.9	1.5	43.35	1.5
5	70.2	0.35	35.65	0.45	85.25	1.34	35.65	0.35	51.13	1.13
7	83.95	0.21	40.86	0.45	95.35	0.85	40.86	0.35	52.13	1.12
10	95.99	1.69	40.86	0.39	95.35	2.22	40.86	0.52	53.13	2.22
20	99.71	1.06	42.09	0.21	96.25	1.75	51.22	1.8	52.45	0.72
30	99.71	0.49	44.1	0.63	96.65	1.99	54.75	0.45	53.42	1.99
40	99.71	0.49	51.18	1.45	96.65	2.11	54.75	0.63	54.41	1.62
50	99.71	0.35	51.18	0.63	96.65	2.1	65.35	0.45	55.5	1.97
80	99.71	2.25	59.17	1.8	99.5	1.34	67	1.88	56.64	1.34

Total phenolic content of *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* plants hexane, methanol, acetone, and aqueous extracts were estimated using the

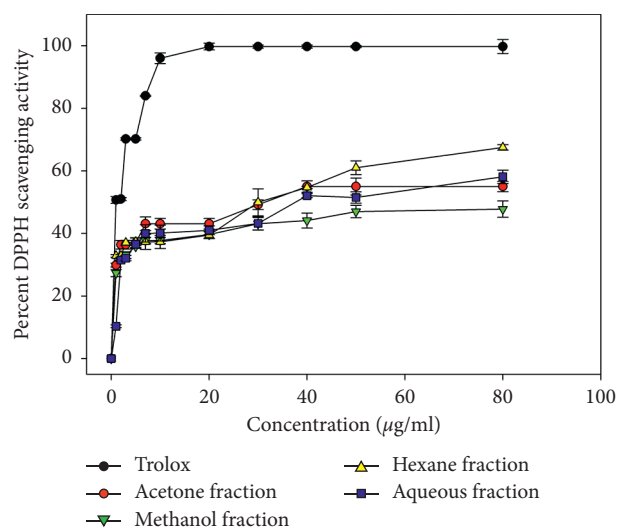
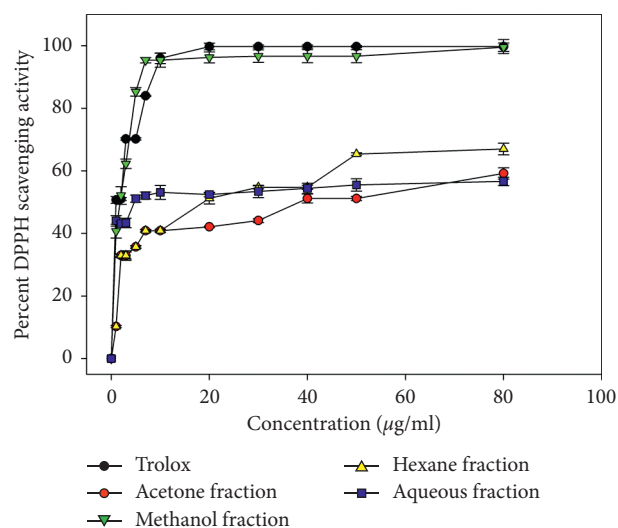
Folin-Ciocalteu assay. However, the results revealed that the total phenolic contents of these herbal species ranged from 12.35 \pm 0.73 to 191.54 \pm 2.84 mg GAE/g. In fact, the methanolic

TABLE 6: DPPH scavenging activity of Trolox and four *M. aegyptia* extracts.

Conc.	Trolox	±SD	Acetone extract	±SD	Methanolic extract	±SD	Hexane extract	±SD	Aqueous extract	±SD
0	0	0	0	0	0	0	0	0	0	0
1	50.72	1.06	43	0.7	39.15	1.48	41.78	1.01	51.78	0.37
2	51.21	0.49	43.25	1.2	38.8	0.98	41.78	1.38	61.78	0.38
3	70.22	0.35	43.25	1.2	44.08	1.3	41.78	1.5	71.78	1.5
5	70.2	0.35	50.13	0.94	49.22	1.1	43.25	0.83	73.25	0.35
7	83.95	0.21	50.13	0.94	49.22	1.1	46.71	0.83	76.71	0.35
10	95.99	1.69	50.13	0.39	49.22	2.22	46.71	0.85	76.71	0.52
20	99.71	1.06	51.4	0.21	63.2	0.7	46.71	1.13	76.71	1.8
30	99.71	0.49	54.4	0.84	63.85	1.99	63.5	1.27	73.5	0.45
40	99.71	0.49	54.4	0.65	64.1	1.62	77.2	1.87	77.2	0.63
50	99.71	0.35	55	2.2	80.5	1.97	94	2.2	84	0.45
80	99.71	2.25	56.84	1.66	82.45	1.34	94.32	2.71	84.32	1.88

TABLE 7: DPPH scavenging activity of Trolox and four *M. sylvestris* extracts.

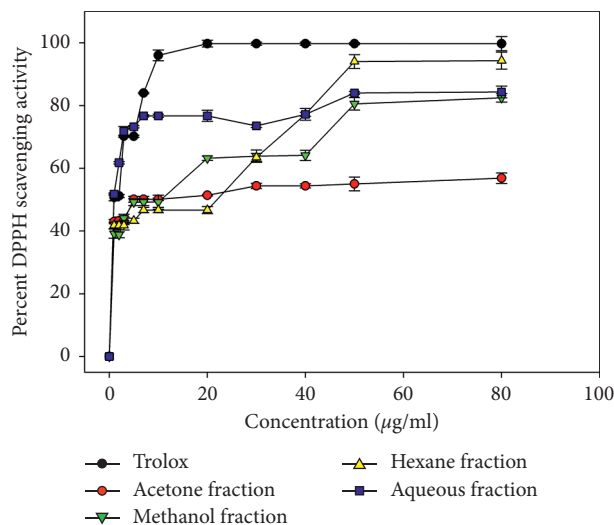
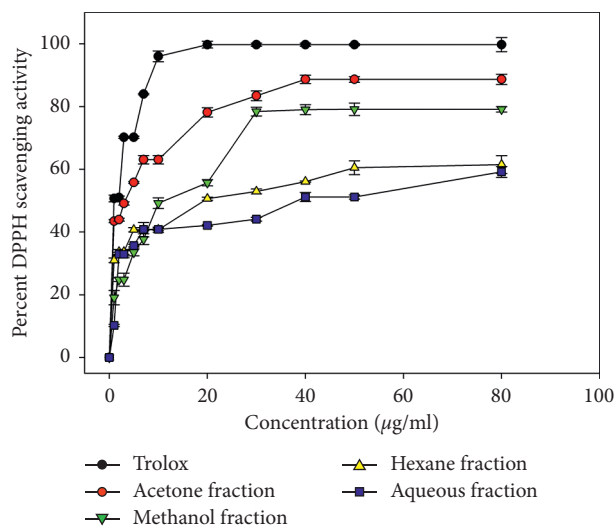
Conc.	Trolox	±SD	Acetone extract	±SD	Methanolic extract	±SD	Hexane extract	±SD	Aqueous extract	±SD
0	0	0	0	0	0	0	0	0	0	0
1	50.72	1.06	43.45	0.49	19.1	2.3	30.97	0.74	10.23	0.34
2	51	0.49	43.95	0.49	24.8	0.57	33.65	0.77	32.92	0.57
3	70.2	0.35	49.13	0.52	24.8	2.1	33.65	0.77	32.9	1.12
5	70.2	0.35	55.8	0.28	33.5	1.1	40.71	0.68	35.65	0.45
7	83.95	0.21	63.05	1.34	37.7	1.7	40.71	2.3	40.86	0.45
10	95.99	1.69	63.05	1.34	49.2	1.7	40.71	0.85	40.86	0.39
20	99.71	1.06	78.15	1.48	55.7	0.99	50.65	0.49	42.09	0.21
30	99.71	0.49	83.4	1.55	78.4	1.4	52.95	0.77	44.1	0.63
40	99.71	0.49	88.65	1.34	79	1.62	56.05	0.21	51.18	1.45
50	99.71	0.35	88.65	0.91	79.12	1.97	60.5	2.2	51.18	0.63
80	99.71	2.25	88.65	1.66	79.12	0.88	61.5	2.83	59.17	1.71

FIGURE 2: DPPH scavenging activity of Trolox (reference compound) and four *M. parviflora* extract.FIGURE 3: DPPH scavenging activity of Trolox (reference compound) and four *M. oxyloba* extracts.

extract of *M. oxyloba* showed the highest phenolic content (191.54 ± 2.84 mg GAE/g), followed by the hexane extracts of *M. parviflora* and *M. aegyptia* plants (106.9 ± 2.22 mg GAE/g) and (132.19 ± 1.97 mg GAE/g), respectively, and *M. sylvestris* (104.01 ± 2.04 mg GAE/g), whereas *M. oxyloba* acetone extract

showed the lowest phenolic content (12.35 ± 0.73 mg GAE/g) among all the extract of these plants.

In a study conducted by Afolayan et al., the total phenolic content in the methanolic extract of *M. parviflora* was 2.9 ± 0.03 mg GAE/g [20]. Another study performed by

FIGURE 4: DPPH scavenging activity of Trolox (reference compound) and four *M. aegyptia* extracts.FIGURE 5: DPPH scavenging activity of Trolox (reference compound) and four *M. sylvestris* extracts.TABLE 8: DPPH Free radical scavenging antioxidant activity (IC_{50}) values.

Extracts	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of <i>M. oxyloba</i>	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of <i>M. aegyptia</i>	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of <i>M. sylvestris</i>	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of <i>M. parviflora</i>	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of Trolox
Acetone	32.35 ± 0.73	15.48 ± 0.99	4.07 ± 1.04	27.54 ± 1.66	
Methanol	1.94 ± 1.84	7.58 ± 1.44	10.47 ± 1.48	72.44 ± 1.36	
Hexane	18.19 ± 0.79	6.9 ± 1.42	19.49 ± 1.15	22.9 ± 1.47	1.86 ± 0.80
Aqueous	15.5 ± 0.67	7.11 ± 2.15	33.35 ± 0.76	32.95 ± 0.67	

$n = 3$ individual experiments.

TABLE 9: Ferrous ion chelating activity of extracts (IC_{50}) values.

Extracts	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of <i>M. oxyloba</i>	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of <i>M. aegyptia</i>	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of <i>M. sylvestris</i>	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ of <i>M. parviflora</i>	IC_{50} values ($\mu\text{g/ml}$), $\pm\text{SD}$ EDTA
Acetone	425.5 ± 10.2	405.1 ± 3.2	315.5 ± 7.0	350.0 ± 6.2	
Methanol	269.1 ± 6.3	295.2 ± 5.1	225.2 ± 4.5	260.2 ± 8.2	
Hexane	—	—	—	—	12.1 ± 1.1
Aqueous	95.5 ± 3.4	86.1 ± 4.7	52.7 ± 1.8	75.8 ± 7.4	

$n = 3$ individual experiments.

TABLE 10: Antimicrobial activities (MICs) of the acetone, hexane, and methanolic fractions of *Malva* species.

Microorganisms	MIC, mg/ml acetone extract				MIC, mg/ml hexane extract				MIC, mg/ml methanolic extract				MIC, mg/ml aqueous extract			
	MS	MO	MA	MP	MS	MO	MA	MP	MS	MO	MA	MP	MS	MO	MA	MP
<i>S. aureus</i>	0.125	3.125	6.25	25	—	—	12.5	25	12.5	3.125	6.25	50	12.5	6.25	12.5	3.125
<i>S. sonnei</i>	3.125	25	6.25	—	25	25	0.125	12.5	12.5	3.125	—	6.25	6.25	—	—	12.5
<i>E. coli</i>	12.5	12.5	3.125	6.25	25	—	12.5	12.5	6.25	0.078	3.125	25	50	25	12.5	—
<i>P. aeruginosa</i>	3.125	25	6.25	12.5	—	6.25	6.25	6.25	6.25	0.78	6.25	6.25	—	1.56	—	3.125
<i>P. vulgaris</i>	0.078	6.25	6.25	12.5	—	12.5	12.5	6.25	12.5	6.25	25	12.5	12.5	—	6.25	3.125

—: no bacterial inhibition, MO: *Malva oxyloba*, MS: *Malva sylvestris*, MA: *Malva aegyptia*, and MP: *Malva parviflora*.

Tabaraki et al. found that the total phenolic content of *M. sylvestris* methanolic extract was 15.11 ± 0.28 mg GAE/g [7]. However, another study established by Beghdad et al. showed that the total phenolic content of the *M. sylvestris* leaves extract was 24.12 ± 0.72 mg GAE/g [21].

In the last two decades, the health benefits of antioxidant molecules obtained from various species of vegetables and plants have become the major focus of the researchers due to the fact that many of these dangerous diseases such as cancer, diabetes, atherosclerosis, and many other stress-related diseases can be prevented by the appropriate use of these products [22].

Several reports suggest that, due to the side effects, adverse reactions, and possible carcinogenic effects of using chemically synthesized antioxidants, there is a universal agreement that the chemical antioxidants need to be replaced with natural ones. Therefore, the present study aims to explore the antioxidant capacities of the *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* hexane, methanol, acetone, and aqueous extracts. Due to the side effect, adverse reaction, and the possible carcinogenic effect that come along the continuous use of chemically synthesized antioxidants, several reports suggest that there is a universal agreement on the replacement of the chemically synthesized antioxidant with the natural ones. Therefore, the present study was aimed to explore the antioxidant capabilities of the *Malva* species with various extracts.

In the current study, the antioxidant capacities were estimated using the DPPH scavenging method and comparing the obtained results with a positive control compound Trolox that has IC_{50} 1.86 μ g/ml. The plants, in general, showed high antioxidant activity ranging from 1.94 ± 1.84 to 72.44 ± 1.36 μ g/ml. Particularly, the methanolic extract of *M. oxyloba* showed the highest antioxidant capacity with IC_{50} value of 1.94 ± 1.84 μ g/ml among the other methanolic extracts studied; this was followed by the acetone extract of *M. sylvestris* (4.07 ± 1.04 μ g/ml), which had the highest antioxidant activity among the other acetone extracts studied. In addition, the hexane extract of *M. aegyptia* showed the highest antioxidant activity (6.9 ± 1.42 μ g/ml) among the other hexane extracts, and finally, the aqueous extracts of *M. aegyptia* showed the highest antioxidant (7.11 ± 2.15 μ g/ml) among the other aqueous extracts. The lowest antioxidant activity was observed in all the four extracts of *M. parviflora*. This study shows that methanolic extract of *M. oxyloba* has the IC_{50} value almost similar to the value of Trolox (1.86 μ g/ml),

which suggests that this extract can be used as a natural alternative therapy for the prevention and treatment of various degenerative diseases and disorders.

Afolayan and his coworkers reported the IC_{50} value of *M. parviflora* to be 94.3 ± 0.03 μ g/ml [20]. In another study, Beghdad et al. showed that *M. sylvestris* leaves extract had an antioxidant IC_{50} value of 16.691 ± 0.2 μ g/ml [21]. This suggests that the results obtained in the present investigation have the highest IC_{50} values when compared to other studies indicating that the plant species has the highest free radical scavenging activity which may be due to their total phenolic content, and in the present studies, the total phenolic content was the highest among all the reported literatures.

Bacterial resistance to many of the recently used modern antibiotics has become a common phenomenon. This has prompted many researchers to look for new chemical from natural origin or plant origin which is safer and effective in overcoming the resistance issue; to study the antimicrobial activity from plant origin, many experts went to study herbal antimicrobial effect trying to solve bacterial resistance problem and to develop new drugs [23].

In the present study, all the four extracts of the *Malva* species were used to explore the antimicrobial potentials against the five bacterial strains using the broth microdilution method.

In the present study, the antimicrobial activity of *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* species hexane, methanol, acetone, and aqueous extracts was conducted against five bacterial strains using the broth microdilution method. The results showed that *M. sylvestris* acetone extracts inhibited the bacterial growth with the highest MIC values of 0.125 mg/ml against *S. aureus* and 0.078 mg/ml against *P. vulgaris* among the other acetone extracts. In addition, the hexane extract of *M. aegyptia* inhibited the highest growth of bacterial strains among hexane extracts of the other plants with MIC value of 0.125 mg/ml against *S. sonnei*. Moreover, the methanolic extract of *M. oxyloba* showed the highest antibacterial activity among the others methanolic extracts with MIC values of 0.078 and 0.78 mg/ml against *E. coli* and *P. aeruginosa*, respectively, while the aqueous extracts of *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* showed weak antibacterial activity in comparison with the other plants extracts. In fact, *M. sylvestris* acetone extract exhibited strong activity against *P. vulgaris* with MIC value of 0.078 mg/ml, while *M. oxyloba* methanolic extract showed

appreciable activity against *E. coli* with MIC value of 0.078 mg/ml.

In a study conducted by Razavi et al. [10], they found that the methanolic extract of *M. sylvestris* inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* with MIC values of 32 and 36 mg/ml, respectively [10]. At the same time, in the present study, the methanolic extract of *M. sylvestris* inhibited the growth of the *Escherichia coli* and *Staphylococcus aureus* with MIC values of 6.25 and 12.5 mg/ml, respectively.

In fact, due to the high phenolic contents of *M. sylvestris*, *M. oxyloba*, *M. parviflora*, and *M. aegyptia* species, the hexane, methanolic, acetone, and aqueous extracts of these species showed strong antibacterial activities, and several prior researches generally confirm that the phenolic compounds affect strongly the microbial growth [24].

These finding indicated that the acetone extract of *M. sylvestris* and the methanolic extract of *M. oxyloba* can be used as a suitable candidate in the manufacturing of natural phytoalexins and as a natural alternative to synthetic antibiotics. In line with previous studies, the current study found a strong positive correlation between antioxidant activities of plants extracts and total phenolic content, which implies that the studied plant's phenols are the major contributor to the antioxidant activity in these extracts. Naturally, occurring phenols have an antioxidant activity as these molecules have the ability to scavenge free radicals by a single-electron transfer mechanism which decreases the oxidative stress in life cells and tissues [7].

5. Conclusion

In view of the abovementioned results, the methanolic extract of *M. oxyloba* is a pharmacologically valuable natural product that can be utilized as the backbone for the design and development of new therapeutic formulations. It exerts multiple inhibitory properties against oxidative stress and bacterial growth. As such, it represented promising therapeutic agents for the treatment of various neurodegenerative diseases and many types of human infectious diseases.

It may be concluded from our studies that the methanolic extract of the *M. oxyloba* is of great pharmacological value, as this natural product can be utilized in the design and development of the new therapeutic formulations as the main ingredient. Due to its inhibitory properties against oxidative stress and bacterial growth, it may be one of the promising therapeutic agents to treat various neurodegenerative diseases and many of the infectious diseases in human.

Data Availability

The data used to support the findings of this study are available within the manuscript.

Conflicts of Interest

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

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