



Comparative Extraction, Preliminary Phytochemical Screening, and Antioxidant Activity of *Mirabilis jalapa* L. Tuberos Roots Found in Sri Lanka

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Abstract: *Mirabilis jalapa*, or the Four O'clock plant, is an ornamental plant that has medicinal value due to its various phytochemical compositions. This study aims to examine the *M. jalapa* roots for their phytochemical spectrum and determine their in vitro antioxidant capacity. The plant was collected and authenticated, and then maceration, sonication, and Soxhlet extraction techniques were employed for aqueous, methanol, dichloromethane (DCM), and hexane extracts. Thin-layer chromatography (TLC) coupled with spray reagents was used to screen bioactives. Total Phenolic Content (TPC), DPPH (2,2-diphenyl-1-picrylhydrazyl), and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical-scavenging activity assays were performed to investigate the antioxidant potential. TLC screening revealed that maceration and Soxhlet extraction separated most compounds. The TPC values of methanol and aqueous macerates were the highest among all extracts. In the DPPH scavenging assay, these extracts showed a significantly higher activity, with IC₅₀ values (half-maximal inhibitory concentration) below the lowest concentration tested (<0.03125 mg/mL). The ABTS assay identified the same two extracts as the most active, but scavenging remained below 50% (maximum 43.30% at 1 mg/mL); therefore, IC₅₀ could not be determined. Overall, methanol and aqueous extracts of *M. jalapa* roots showed significantly higher antioxidant activity, perhaps due to their variety of bioactive compounds that justified their incorporation in traditional medicine.

Keywords: *Mirabilis jalapa*; phytochemicals; thin layer chromatography; total phenolic content; antioxidant activity

1. Introduction

Medicinal plants are becoming the most popular in responding to viral and chronic illnesses in contrast to other conventional medicines, which reduce the impact of side effects because of their pharmacological activities [1]. *Mirabilis jalapa* belongs to the family Nyctaginaceae, a plant used in folk medicine. These are multibranched, leafy perennials that resemble shrubs with tuberous roots [2]. It is widely used in traditional medicine for treating dysentery, diarrhea, abdominal colic, inflammation, skin conditions, muscle pain, gastrointestinal disorders, and various other illnesses, which is supported by its rich bioactive composition [3–5]. The plant exhibits diverse pharmacological activities including antimicrobial, antiviral, antifungal, antidiabetic, antispasmodic, antinociceptive, dermatological, anti-corrosion, and anthelmintic properties [5–8]. Strong antioxidant potential arises from



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phenolics and polyphenols showing greater radical-scavenging activity, and methanolic extracts demonstrating links to flavonoids, tannins, and phenolics [8–12].

Preliminary phytochemical screening combined with bioactivity assays such as antioxidant assays are valuable for identifying the constituents of plant extracts, including the predominant compounds, and for discovering bioactive agents that could be used in developing pharmaceuticals. Among these, phenolic compounds are particularly notable for their wide-ranging health benefits due to the presence of hydroxyl groups that neutralize singlet and triplet oxygen, break down peroxides, and repair oxidative damage in debilitating diseases such as Parkinson's, Alzheimer's, and heart disease [13–15]. Therefore, medicinal plants with antioxidant potential are critical as the demand for it is high, minimizing the risk of serious diseases [12,16]. Rising global demand for natural products and safer alternatives to conventional medicine has driven the exploration of medicinal plants such as *Mirabilis jalapa*. Therefore, in this study, the aqueous, methanol, dichloromethane (DCM), and hexane extracts of *Mirabilis jalapa* tubers extracted using maceration, sonication and Soxhlet extraction were investigated for their bioactive compounds through thin layer chromatography coupled with spray reagents, then subjected to total phenolic content assay and antioxidant assays.

Although the phytochemical composition and antioxidant activity of *M. jalapa* have previously been investigated, limited information is available comparing different extraction methods and solvents for the tuberous roots under the same experimental conditions. Therefore, this study comparatively evaluated maceration, sonication and Soxhlet extraction using solvents of different polarities, followed by preliminary phytochemical screening and assessment of phenolic content and antioxidant activity in selected extracts.

2. Materials and Methods

2.1. Sampling, Identification and Authentication

About 1 kg of tubers was collected from well-grown *Mirabilis jalapa*; see Figure 1. The plant materials were obtained from Kandy District, Central Province, Sri Lanka (7°19'09.4" N 80°37'56.5" E) during the northeast monsoon season in Sri Lanka. Before the study, the plant material was identified as *Mirabilis jalapa* L. (Nyctaginaceae) (locally known as hendirikka in Sri Lanka in the Sinhala language, and also referred to as the four o'clock flower and marvel of Peru in English) based on morphological identification by a qualified scientific officer (pharmacognosist) in the pharmaceutical botany division, Bandaranaike Memorial Ayurveda Research Institute (BMARI), Nawinna, Maharagama, Sri Lanka. A voucher specimen was deposited at BMARI under the accession number 3136 on 27 March 2024.



Figure 1. Well-grown *Mirabilis jalapa* L. and collected tubers of *Mirabilis jalapa* L.

2.2. Preparation of Extracts

The tuberous roots were cleaned, air-dried at room temperature (25 °C) in the shade until constant weight was achieved, then ground into a coarse powder (refer to Figure 2) [2,13]. Using a 1:5 sample-to-solvent ratio, we used three extraction methods: maceration, sonication, and Soxhlet. For maceration, 50 g of root powder and 250 mL of solvent were macerated at room temperature for 3 days with intermittent shaking, then filtered, centrifuged (5000 rpm, 20 min), and concentrated by rotary evaporation. For sonication, 25 g of root sample was sonicated for 15–30 min at 29–35 °C, then filtered and evaporated using a rotary evaporator. Soxhlet extraction involved processing 2.5 g of sample for 3 h at 20–30 °C for hexane, DCM, and methanol, and at 40–45 °C for aqueous, followed by evaporation using a rotary evaporator [13].



Figure 2. Cut tubers and coarse powder of *Mirabilis jalapa*.

2.3. Thin Layer Chromatography and Preliminary Phytochemical Screening

TLC operates on the principle of differential adsorption on a thin adsorbent layer, with the mobile phase driving the solutes via capillary action (refer to Figure 3) [17]. Different solvent systems were developed for each extract hexane, DCM, methanol and aqueous of *Mirabilis jalapa* Linn. tuberous roots, considering their different polarities; Toluene: Ethyl acetate (4:1), Toluene: Ethyl Acetate: Formic acid (4:1:0.2), Toluene: Ethyl Acetate: Formic Acid (4:1:0.4), Chloroform: Methanol (3.5:1.5). Before analysis, the glass chamber was saturated with the following chosen solvent system allowed to stand for 20 min, ensuring optimal development conditions before placing the TLC plates. The TLC plates (silica gel 60) were cut into the required sizes, estimated 6.5 cm (height) × 4 cm (width), labelled, and heated for activation in an oven at 105 °C for 30 min. Dried extracts were properly reconstituted with respective fresh solvents and spotted three times at marked positions on the TLC plate. The plate was placed in the chamber containing the chosen solvent system with only its lower edge touching the solvent. Once the solvent spotted reached the designated top line, the plate was removed, and bands were observed using UV light at 254 nm and 365 nm in a UV light cabinet. To enhance visualization, the spray reagents were used (refer to Table 1) [18–21]. The distinct bands were then compared and documented for further analysis.

Table 1. Spray reagents used for detection.

Spray Reagent	Preparation and Usage	Phytochemical/s
Dragendorff reagent [19]	Solution A: 0.85 g of bismuth nitrate was dissolved in 10 mL glacial acetic acid, followed by 40 mL of deionized water while heating. Solution B: 8 g of potassium iodide was dissolved in 30 mL of deionized water For the stock solution, solution A and B were mixed in 1:1 ratio. About 2 mL of stock was added along with 4 mL glacial acetic acid and 20 mL of deionized water for a working spray reagent. The Spray plate was checked.	Alkaloids
1% Ferric chloride [20]	1 g of ferric chloride was dissolved in 50 mL methanol, followed by 50 mL of deionised water. The Spray plate was checked for visible compounds.	Phenolics, tannins
5% alcoholic Potassium hydroxide [19]	5% ethanolic potassium hydroxide was prepared using 5 g of potassium hydroxide in 100 mL ethanol. Sprayed plate was dried and observed with and without UV-365 nm, with and without warming.	Anthraquinones, anthrones, coumarins
Anisaldehyde-sulphuric acid reagent [19]	0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol and 5 mL of concentrated sulphuric acid was added in the same order to prepare the spray reagent. Once sprayed the plate was heated to 100 °C for ten minutes to see the developed plate.	Terpenoids, saponins, bitter principles
10% ethanolic sulphuric acid [19]	10% ethanolic sulphuric acid was prepared by slowly adding 10 mL of concentrated sulphuric acid (98% w/w; approximately 18.4 M) to 90 mL of ethanol while cooling. The prepared reagent was sprayed onto the TLC plate, dried, and observed with and without UV light at 365 nm, before and after heating.	Cardiac glycosides, lignans
Iodine [19]	A few iodine crystals were added to an empty glass jar and allowed to vaporize before adding the developed TLC plate. About 15 min later, the plates were examined.	Hydrocarbons
0.02% methanolic 2,2-Diphenyl-1-picrylhydrazyl (DPPH)	10 mg of DPPH powder was added to 50 mL of methanol, and the solution was sprayed onto the developed TLC plate. The Spray plate was checked for visible compounds.	Antioxidants

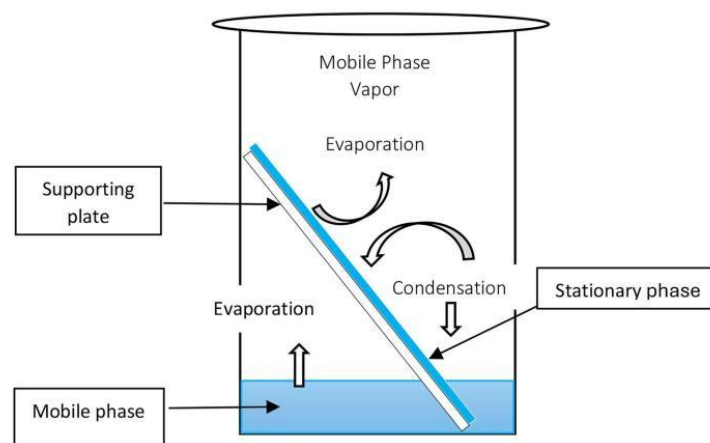


Figure 3. Schematic diagram of the process of thin layer chromatography principle (Side view).

2.4. Total Phenolic Content Assay (TPC)

2.4.1. Preparation of Materials for TPC

The working reagents, Folin reagent and sodium carbonate solution of 10% and 7.5%, respectively, were prepared using distilled water as the diluent. The positive control was prepared with a gallic acid stock solution at 20 mg/mL and reagents. From the stock, six different dilutions were made serially (2, 1, 0.5, 0.25, 0.125, 0.0625 mg/mL). This series was used to plot the calibration curve. The negative control only contained reagents. The test samples were prepared by dissolving the extract in methanol to reach a starting concentration of 1 mg/mL. No dilution series was performed.

2.4.2. Total Phenolic Content Assay

Appropriately labelled 1.5 mL microcentrifuge tubes (samples and controls) were prepared with a set concentration of 1 mg/mL, and 100 μ L from each were added into another, respectively labelled tube. Extracts to be tested were chosen from the TLC results. After that, 400 μ L of 7.5% Na_2CO_3 and 500 μ L of 10% Folin reagent were added to the tubes containing the control or sample to be tested. Then, all tubes were incubated in the dark at room temperature for one hour. After incubation, 200 μ L of each tested sample/control was transferred into a 96-well plate, and the absorbance was read at 765 nm using an ELISA plate reader. The assay was done in triplicate to ensure accurate measurement.

2.4.3. Data Analysis

For calculating the TPC of the samples, a scatter graph of gallic acid concentrations (x-axis) versus absorbance (y-axis) was plotted using six different concentrations with three replicates each. A plot of the data was fitted to the equation of the line,

$$Y = mx + c$$

where x is the concentration, and Y is the absorbance. The gallic acid equivalent for each sample was calculated and expressed as milligrams (mg) of gallic acid equivalent per gram of extract. The results are mentioned as mean $\pm$ standard deviation.

2.5. Evaluation of In Vitro Antioxidant Activity

2.5.1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

Methodology

The DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) assay measures antioxidant activity through an electron-transfer process: the purple-colored solution of stable DPPH radical in methanol is reduced (yellow) after a reaction with antioxidant compounds [22]. The DPPH solution was prepared by dissolving 20 mg of DPPH powder in 100 mL of methanol to a concentration of 0.2 mg/mL. For the assay, a control was prepared with only methanol (160 μ L) and DPPH (40 μ L) in 96-well plates. Butylated hydroxytoluene (BHT) acted as the standard, prepared at 1.25 mg/mL and diluted to six concentrations. This resulted in in-well concentrations of 1–0.03125 mg/mL. Test samples were also dissolved in methanol at 1.25 mg/mL and diluted to match the same in-well concentrations.

Each well received 160 µL of sample or standard along with 40 µL DPPH solution. The mixture was gently mixed, covered to prevent evaporation, and incubated in the dark at room temperature for 10 min. Absorbance was then measured at 517 nm using an ELISA plate reader [16].

Data Analysis

The percentage radical scavenging activity was calculated using the following equation:

$$\% \text{ Radical Scavenging} = \{B - A\} / \{B\} * 100$$

where B was the absorbance of the control, and A was the absorbance of the sample. For IC_{50} determination, the Quest Graph™ IC_{50} Calculator and GraphPad Prism software were used, along with Microsoft Excel regression analysis for R^2 (goodness of fit) and p -value. The data were plotted as a scatter graph of concentration against % scavenging activity. The results are mentioned as mean $\pm$ standard deviation. A sigmoid regression equation was used to determine the IC_{50} value:

$$Y = Min + \frac{Max - Min}{1 + \left(\frac{X}{IC_{50}}\right)^{Hill \text{ coefficient}}}$$

where Y is the percentage radical scavenging activity, X is the sample concentration, Min and Max represents the lower and upper plateaus, respectively. IC_{50} is the concentration corresponding to 50% radical scavenging activity under the specified model. For the constrained model Min and Max were fixed at 0% and 100%, respectively.

2.5.2. ABTS (2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Radical Scavenging Assay

Methodology

The generation of the ABTS radical cation happens through a reaction with potassium persulfate, yielding a characteristic green-blue color. Antioxidants interact with the ABTS cation by reducing it; the decrease in absorbance, which quantitatively accounts for antioxidant capacity, is measured. The reaction pathways proposed include both the direct oxidation of antioxidants and the coupling adduct formation between ABTS and some phenolic antioxidants [23]. The stock ABTS solution was prepared by dissolving 0.0196 g of ABTS and 0.0033 g of potassium persulfate in 10 mL of distilled water. After 24 h for radical generation, the reagent was diluted as needed which achieved 0.75 absorbance to be used as a working solution. For the assay, controls were made by adding 20 µL methanol with 180 µL of ABTS reagent in a well.

BHT was the standard, prepared at 1 mg/mL and serially diluted. 20 µL of each concentration was combined with 180 µL of ABTS in separate wells. Similarly, test samples were dissolved at 1 mg/mL, serially diluted to five concentrations, and 20 µL was added to 180 µL of ABTS. All plates were gently mixed, covered to prevent evaporation, incubated in the dark at room temperature for 10 min, and absorbance was measured at 734 nm using an ELISA plate reader.

Data Analysis

The percentage radical scavenging activity was calculated using the following equation:

$$\% \text{ Radical Scavenging} = \{B - A\} / \{B\} * 100$$

where B was the absorbance of the control and A was the absorbance of the sample. IC_{50} was not determined as the dataset did not have radical scavenging of more than 50%. The results are mentioned as mean $\pm$ standard deviation.

3. Results

3.1. Thin Layer Chromatography (TLC) and Preliminary Phytochemical Examination

The qualitative TLC analysis was performed to obtain a preliminary chromatographic profile of the different extracts. The profiles obtained under different conditions are described below, and the corresponding materials are provided in the supplementary materials (Supplementary Figures S1–S5). Several distinct spots were observed on the TLC plate, with varying numbers, colors, intensities, and migration patterns. These spots indicated different phytochemical constituents in the plant extracts.

Under a toluene: ethyl acetate (4:1) solvent system, the hexane fraction depicted two similar compounds in all three methods, marked as (F1) and (F2), comparing all three extraction methods: maceration (M), sonication (S), and Soxhlet (X). Moreover, two different compounds were separated in the Soxhlet and sonication methods, with spots indicated as (F3) and (F4). Under UV 254 nm (A), F1, F2, F3, and F4 appeared as weak bands; however,

F1 appeared as a fluorescent band under 365 nm (B). After treatment with 10% ethanolic H₂SO₄, more visible bands were observed under 365 nm UV light, as blue and purple bands (C) (Supplementary Figure S1).

Under a toluene: ethyl acetate: formic acid (4:1:0.2) solvent system, in the DCM fraction, most compounds were separated by the maceration and Soxhlet extraction methods. Without chemical treatment, quenched spots appeared at UV 254 nm (A), and no fluorescence bands were observed at UV 365 nm (B), but different colors of spots appeared after being treated with 10% ethanolic H₂SO₄ and visualized under 365 nm UV light (C), where a specific orange-colored band separated in the Soxhlet extraction method but was not significantly observed in other techniques (Supplementary Figure S2).

Under a Toluene: Ethyl Acetate: Formic Acid (4:1:0.4) solvent system, in the methanol fraction, similar clear bands (G1) were observed in all three extraction methods. A different band (G2) was observed only in the extract obtained from the Soxhlet extraction method and showed weak quenching and a blue band under UV 254 nm (A) and UV 365 nm (B), respectively (Supplementary Figure S3). When the TLC plate was sprayed with 10% ethanolic H₂SO₄ and heated for a long time, some compounds disappeared in the extract derived from maceration extraction. Further, some more bands appeared clearly, denoted as more compounds, in the extracts derived from sonication and Soxhlet extraction methods (Supplementary Figure S4).

Under the chloroform: methanol (3.5:1.5) solvent system, in the aqueous fraction, most of the compounds were extracted by the maceration and Soxhlet methods, as a number of bands were observed. A faded band was observed at UV 365 nm (B) for sonication; however, a significant band was not observed with 10% ethanolic H₂SO₄ (C) or iodine vapour (D) (Supplementary Figure S5).

Qualitative Preliminary Phytochemical Screening

As indicated in Table 2, the results are demonstrated by (-) negative, (+) trace positive, (++) moderately positive, (+++) highly positive based on the observation of the band appeared in the TLC plate after treated with spray reagent. According to the results the tannins and phenolic compounds are present in the methanol extract of maceration and the Soxhlet extraction method. Terpenoids and saponins were present in all four extracts except in the aqueous extract of sonication. Alkaloids were less significantly observed in all four extracts in the qualitative preliminary phytochemical screening using dragendorff reagent as the spray reagent that was used to screen for alkaloids. In the screening of antioxidant activity, colorless spots appeared in all four extracts except the DCM and methanol extracts of sonication.

Table 2. Qualitative preliminary phytochemical Screening of hexane, DCM, methanol, and aqueous extract from the maceration (M), sonication (S), and Soxhlet (X) extraction methods of tuberous roots of *Mirabilis jalapa* Linn.

Phytoconstituents	Hexane			DCM			Methanol			Aqueous		
	M	S	X	M	S	X	M	S	X	M	S	X
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Phenolics	-	-	-	-	-	-	+	-	+++	+	-	++
Tannins	-	-	-	-	-	-	+	-	+++	+	-	++
Terpenoids	+++	+++	+++	+++	+++	+++	++	+	++	+	-	+
Saponins	+++	+++	+++	+++	+++	+++	++	+	++	+	-	+
Cardiac glycosides	+++	+++	+++	+++	++	+++	++	++	++	-	-	-
Lignans	+++	+++	+++	+	-	+	++	++	+++	++	-	+++
Anthraquinones	-	-	-	-	-	-	+	-	+++	+	-	+++
Anthrones	-	-	-	++	-	++	-	-	-	-	-	-
Coumarins	-	+++	+	+	-	+++	++	-	++	-	-	++
Antioxidant	+	+	+++	+	-	+	++	-	++	++	+	+++

- Negative; + Trace positive; ++ Moderately positive; +++ Highly positive.

3.2. Evaluation of Antioxidant Activity

3.2.1. Total Phenolic Content

The phenolic content was calculated from the regression equation of the calibration curve ($R^2=0.9271$, $y=0.6417x-1.0173$), expressed as mg GAE/g of extract. Only well-performing extracts in TLC were included in the analysis. The total phenolic content was carried out for hexane, DCM, methanol and aqueous extracts from the maceration (M) technique and for hexane, aqueous from Soxhlet extraction (X) has shown larger variations. The TPC value follows the descending order as methanol (M) > aqueous (M) > hexane (X) > DCM (M) > hexane (M) > aqueous (X).

As shown in Table 3, the highest phenolic content was reported in the methanol macerate (320.12 ± 42.06 mg GAE/g), while the lowest was in the aqueous extract prepared using Soxhlet extraction (167.30 ± 4.28 mg GAE/g).

Table 3. Total phenolic content of hexane, DCM, methanol, and aqueous extracts from the maceration (M) technique, and hexane and aqueous extracts from the Soxhlet (X) extraction method.

Extracts	Total Phenolic Content (mg GAE/g)
Hexane ^M	168.11 ± 5.47
DCM ^M	170.03 ± 6.22
Methanol ^M	320.12 ± 42.06
Aqueous ^M	232.31 ± 5.60
Hexane ^X	179.12 ± 6.51
Aqueous ^X	167.30 ± 4.28

M—Maceration; X—Soxhlet.

3.2.2. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Only well-performing extracts in TLC were included in the analysis. In Table 4, the DPPH radical scavenging percentage for hexane, DCM, methanol, and aqueous extracts from the maceration (M) technique, and hexane and aqueous extracts of *M. jalapa* tuberous roots extracted by the Soxhlet (X) technique shows that the highest DPPH radical scavenging percentage is observed for methanol and aqueous extracts from the maceration technique compared to other extracts. The hexane extract from the Soxhlet method showed a higher RSA percentage compared to the hexane extract from the maceration. The comparison of the DPPH radical scavenging activity of methanol and aqueous extracts from the maceration technique to the standard BHT is shown in Table 5. The dose-response curves for hexane, DCM, methanol, and aqueous extracts obtained by maceration (M), as well as the hexane and aqueous extracts obtained by Soxhlet (X) technique, are presented in Figure 4. The estimated IC₅₀ values for these extracts, representing the concentrations required to scavenge 50% of DPPH radicals, are summarized in Table 6.

Table 4. DPPH radical scavenging activity for hexane, DCM, methanol, and aqueous extracts from maceration (M) technique, and for hexane and aqueous extracts from Soxhlet (X) technique of *M. jalapa* tuberous roots.

Concentration mg/mL	Hexane ^M	DCM ^M	Methanol ^M	Aqueous ^M	Hexane ^X	Aqueous ^X
1	42.53 ± 4.30	65.35 ± 3.83	89.33 ± 0.28	84.56 ± 0.57	58.62 ± 2.74	77.83 ± 3.94
0.5	37.93 ± 2.15	51.89 ± 2.97	88.34 ± 0.28	82.43 ± 1.99	48.93 ± 4.86	60.43 ± 0.57
0.25	37.27 ± 2.48	44.83 ± 2.61	86.86 ± 0.75	82.10 ± 1.14	48.11 ± 3.63	51.72 ± 1.78
0.125	36.12 ± 2.43	36.29 ± 2.05	86.67 ± 1.71	74.06 ± 7.86	43.51 ± 2.22	46.14 ± 3.73
0.0625	35.96 ± 4.29	25.94 ± 5.84	86.53 ± 2.80	69.62 ± 0.57	39.57 ± 1.50	40.39 ± 1.48
0.03125	28.08 ± 2.71	24.45 ± 7.64	76.68 ± 17.22	56.65 ± 2.56	39.08 ± 4.58	40.23 ± 3.63

M—Maceration; X—Soxhlet.

Table 5. Comparison of DPPH radical scavenging activity of methanol and aqueous extracts of maceration technique (M) of tuberous roots of *Mirabilis jalapa* to the standard BHT.

Concentration mg/mL	Standard (BHT)	Methanol ^M	Aqueous ^M
1	94.09 ± 1.39	89.33 ± 0.28	84.56 ± 0.57
0.5	92.61 ± 2.43	88.34 ± 0.28	82.43 ± 1.99
0.25	91.63 ± 1.46	86.86 ± 0.75	82.10 ± 1.14
0.125	84.24 ± 0.92	86.67 ± 1.71	74.06 ± 7.86
0.0625	61.58 ± 1.86	86.53 ± 2.80	69.62 ± 0.57
0.03125	49.26 ± 1.22	76.68 ± 17.22	56.65 ± 2.56

M—Maceration.

Table 6. Estimated IC₅₀ results in DPPH radical scavenging assay.

Extract	IC ₅₀ (mg/mL)	R ²	p-Value
Standard (BHT)	0.034	0.968	0.0010
Hexane ^M	Not determined > 1 mg/mL	0.810	0.0078
DCM ^M	0.366	0.980	0.0004
Methanol ^M	Not determined < 0.03125 mg/mL	0.706	0.0863
Aqueous ^M	Not determined < 0.03125 mg/mL	0.934	0.0043
Hexane ^X	0.353	0.912	0.0078
Aqueous ^X	0.137	0.873	0.0161

M—Maceration; X—Soxhlet.

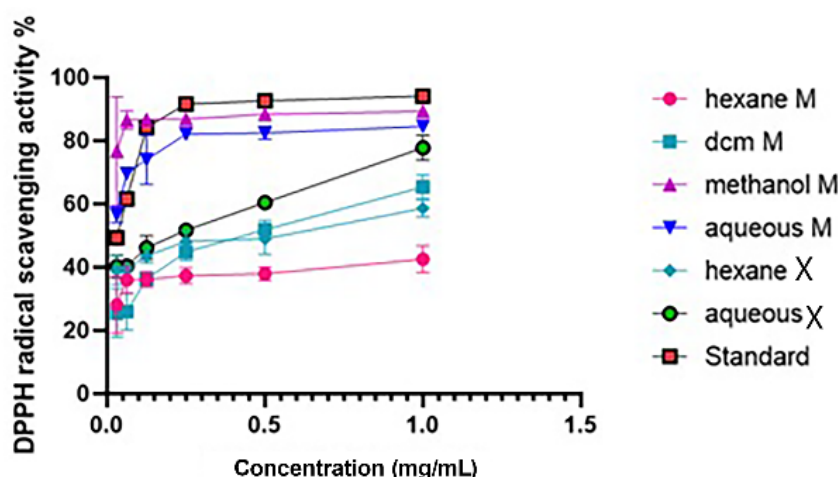


Figure 4. Dose-response curve of DPPH radical-scavenging activity vs. concentration for hexane, DCM, methanol, and aqueous extracts from maceration (M) technique, and for hexane and aqueous extracts from Soxhlet (X) technique of *M. jalapa* tuberous roots.

3.2.3. ABTS (2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Assay

Only well-performing extracts in TLC were included in the analysis. In Table 7, the ABTS radical scavenging percentage for hexane, DCM, methanol, and aqueous macerates (M), and hexane and aqueous extracts from Soxhlet extraction (X) of *M. jalapa* tuberous roots shows that the highest ABTS radical scavenging activity was observed for methanol and aqueous extracts from the maceration technique compared to other extracts. The hexane extract from the Soxhlet method showed a higher ABTS scavenging activity compared to the hexane extract from the maceration method. The comparison of the ABTS radical scavenging activity of methanol and aqueous extracts from the maceration technique to the standard BHT is shown in Table 8. The dose-response curves for hexane, DCM, methanol, and aqueous extracts obtained by maceration (M), as well as the hexane and aqueous extracts obtained by Soxhlet (X) technique, are presented in Figure 5. Estimated IC₅₀ values of the test extracts were not calculated, as the peak performance of the above extracts did not exceed 50% activity.

Table 7. ABTS radical scavenging percentage for hexane, DCM, methanol, and aqueous extracts from maceration (M) technique, and for hexane and aqueous extracts of tuberous roots of *M. jalapa* using Soxhlet (X) technique.

Concentration mg/mL	Hexane ^M	DCM ^M	Methanol ^M	Aqueous ^M	Hexane ^X	Aqueous ^X
1	17.89 ± 4.52	22.29 ± 1.29	43.22 ± 4.74	43.30 ± 5.17	16.79 ± 2.77	14.30 ± 0.94
0.5	15.18 ± 3.66	20.05 ± 0.95	31.35 ± 4.69	38.50 ± 6.86	16.52 ± 0.97	13.51 ± 1.43
0.25	14.66 ± 5.64	15.97 ± 4.50	24.08 ± 1.09	27.81 ± 3.77	16.34 ± 0.34	13.13 ± 0.35
0.125	12.69 ± 1.54	15.38 ± 2.43	20.40 ± 2.25	19.46 ± 6.60	15.80 ± 0.48	12.89 ± 0.73
0.0625	11.08 ± 0.55	14.69 ± 0.25	19.11 ± 1.01	17.26 ± 2.93	15.37 ± 3.78	12.62 ± 1.44
0.03125	10.09 ± 3.62	12.88 ± 2.29	15.84 ± 1.67	15.24 ± 6.24	13.36 ± 4.91	11.90 ± 0.68

M—Maceration; X—Soxhlet.

Table 8. Percentage of ABTS radical scavenging activity comparing methanol and aqueous extracts of *Mirabilis jalapa* tuberous roots using the maceration technique to the BHT standard.

Concentration mg/mL	Standard (BHT)	Methanol ^M	Aqueous ^M
1	91.57 ± 4.33	43.22 ± 4.74	43.30 ± 5.17
0.5	91.16 ± 4.41	31.35 ± 4.69	38.50 ± 6.86
0.25	89.56 ± 4.64	24.08 ± 1.09	27.81 ± 3.77
0.125	85.54 ± 4.97	20.40 ± 2.25	19.46 ± 6.60
0.0625	74.70 ± 5.19	19.11 ± 1.01	17.26 ± 2.93
0.03125	64.66 ± 5.65	15.84 ± 1.67	15.24 ± 6.24

M—Maceration.

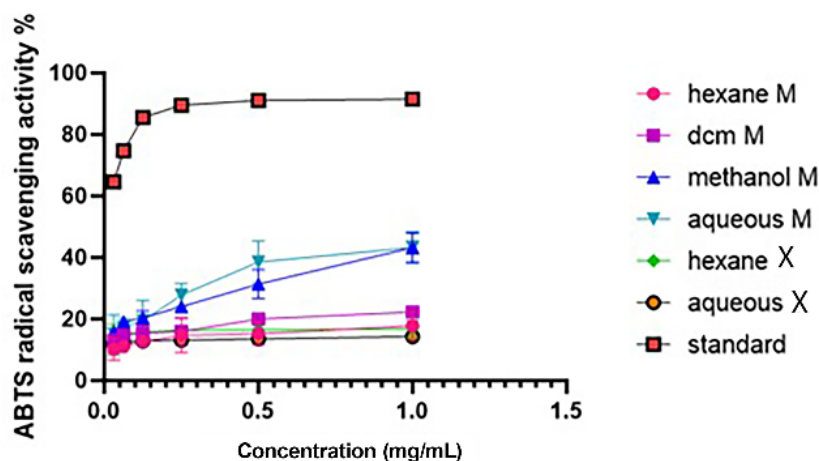


Figure 5. Dose- response curve of ABTS radical scavenging activity vs. concentration for hexane, DCM, methanol, and aqueous extracts from the maceration (M) technique, and for hexane and aqueous extracts from the Soxhlet (X) technique of *M. jalapa* tuberous roots.

4. Discussion

TLC and preliminary phytochemical screening revealed an array of bioactive compounds occurring in the tuberous roots of *Mirabilis jalapa* for various solvent extracts: hexane, DCM, methanol, and aqueous. This implies that there are phytochemicals that only dissolve in some solvents, indicating a complex chemical profile with therapeutic uses. The extraction methods used: maceration, sonication, and the Soxhlet technique, played significant roles in recovering phytochemicals.

Terpenoids, saponins, cardiac glycosides, and lignans were present in high amounts in all the tests with hexane extracts. This indicates that hexane, a nonpolar solvent, is effective in extracting these compounds regardless of the method used. Soxhlet extraction, however, can yield a larger amount because of the continuous circulation of hot solvent, which enhances solubility [24].

Similarly, DCM extracts remained highly active for the terpenoids, saponins, and cardiac glycosides, as did hexane across all approaches used, and coumarins and anthrones in maceration and Soxhlet methods. Both maceration and Soxhlet extractions are proven to be useful.

Methanol extracts showed a significant occurrence of phenolics, tannins, terpenoids, saponins, cardiac glycosides, and lignans, confirming methanol's effectiveness in the extraction of polar compounds. Maceration can be a gentler method for extracting active phenolics and antioxidants without losing their activity, while Soxhlet extraction might be more appropriate for metabolites that need more thorough conditions [16,25].

Aqueous extracts contained medium activities for antioxidants, phenolics, and tannins, with a strong emphasis on water as a good medium for the extraction of polar substances. In addition, saponins and lignans have not been extracted as much in this regard; maceration can be particularly helpful as relatively low temperatures favor heat-sensitive polar substances. Soxhlet extraction is also favorable, bearing in mind the consideration that it will extract the same or significant number of phytochemicals [26,27].

Alkaloids and anthrones were not present or were weakly present in all extracts, while anthraquinones were significantly found in methanol and aqueous extracts by maceration and the Soxhlet method, and coumarins were present with trace to moderate activity, mainly in hexane and methanol extracts. This might suggest that the compounds are poor in roots or require other solvents for effective extraction. Therefore, the extraction procedure should be suitable for the targeted phytochemicals. Terpenoids, saponins, and lignans are extracted optimally by Soxhlet because it is ideal for non-polar compounds. Maceration and Soxhlet extraction with methanol or water are, however, more ideal for maintaining antioxidant and phenolic bioactivities [28].

The TPC results indicate that the methanol extract (320.12 mg GAE/g) obtained by maceration yielded the highest TPC. Methanol is a widely utilized solvent for phenolic compound extraction due to its polarity and ability to disrupt plant cell walls, enhancing the solubility of phenolics [29,30]. The aqueous extract also exhibited a relatively high TPC, which conforms to previous findings that water, although a less efficient solvent than methanol, can extract sizable quantities of polar phenolics.

Among the non-polar solvents, hexane (168.11 mg GAE/g) presented the lowest TPC values because phenolic compounds are generally polar in nature and are not well extracted by non-polar solvents like hexane [31]. DCM

(170.03 mg GAE/g) presented a relatively higher TPC compared to hexane, perhaps due to the moderate polarity, which allowed partial extraction of some phenolic compounds.

In comparison with Soxhlet extraction with maceration, the findings indicate that Soxhlet extraction did not considerably increase the yield of phenolic compounds. In certain instances, the values were marginally greater or approximately like maceration. Although Soxhlet extraction provides continuous refluxing of the solvent, which enhances solubility and diffusion of phenolics, heat exposure over a longer period can cause thermal decomposition of heat-sensitive phenolic compounds, which could be the reason for the similar values [32]. Future studies should involve optimizing extraction conditions such as solvent concentration, time, and temperature for the realization of maximum obtainable phenolic yield with minimum degradation.

Antioxidant activity of tuberous roots of *Mirabilis jalapa* was evaluated using ABTS and DPPH free radical scavenging assays. The standard antioxidant BHT exhibited maximum radical scavenging activity in both assays ($IC_{50} = 0.034$ mg/mL), confirming its strong antioxidant activity as the standard. Among the tested extracts, methanol and aqueous macerates showed the highest DPPH radical-scavenging activity. Model-derived IC_{50} estimates were below the lowest experimentally tested concentration and should therefore be interpreted cautiously ($IC_{50} = \text{not determined} < 0.03125$ mg/mL). This result agrees with previous studies indicating that the polarity of the solvent considerably influences the antioxidant compound extraction efficiency, where polar solvents, methanol and water, are more effective in extracting flavonoid and phenolic compounds responsible for antioxidant activity [2]. The macerated DCM and hexane extracts were less active in both tests, indicating minimal antioxidant potential.

The Soxhlet extracts were generally as active or slightly more active than their macerated counterparts, with aqueous and hexane Soxhlet extracts being moderately scavenging. Surprisingly, in the ABTS assay, most of the extracts were comparatively less active than in DPPH, except for methanol macerate, which showed an increasing trend. These results suggest that the polarity of the solvent significantly influences antioxidant activity, with aqueous extracts and methanol exhibiting the greatest ability to scavenge free radicals. This agrees with evidence that solvent polarity affects the efficiency of yield of antioxidant compounds, where polar solvents enhance the yield of phenolic and flavonoid compounds [2,29,33].

In general, the tuberous roots of *Mirabilis jalapa* in the present study has shown significant antioxidant potential, although similar to other findings but differing in intensity possibly because of various reasons such as the geographical location of collected plant material, the time collected, the stage of growth, etc. [34]. Employment of proper extraction methods, usage of combination solvents, proper storage conditions, and usage of bioassays other than spectrophotometry to confirm activity would validate current findings [35,36].

5. Limitations of the Study

This study has several limitations that should be considered when interpreting the findings. Extraction yields were not recorded for all solvent and extraction method combinations, which limited our ability to directly compare extraction efficiency. The phytochemical screening was preliminary and qualitative, and the compounds detected by TLC were not further confirmed using reference standards or instrumental techniques. In addition, quantitative TPC and antioxidant assays were carried out only for selected extracts based on the initial TLC findings, and therefore a complete quantitative comparison of all three extraction methods was not possible.

The TPC calibration curve also showed some variation ($R^2 = 0.9271$), which may have affected the accuracy of the estimated phenolic contents. For the DPPH assay, the estimated IC_{50} values of the methanol and aqueous macerates were below the lowest concentration tested and should therefore be interpreted with caution. The relatively low model fit obtained for the methanol extract further limits the reliability of its estimated IC_{50} value. Moreover, the measurements were mainly technical replicates rather than independent biological replicates, limiting further statistical comparisons and correlation analysis. Future studies with a wider concentration range, independent biological replicates, extraction-yield measurements, and further chemical characterization would help confirm and extend the present findings.

6. Conclusions

The findings confirm that tuberous roots of *Mirabilis jalapa* have significant antioxidant activity, particularly in methanol and aqueous extracts. TLC and preliminary phytochemical screening confirmed the presence of various bioactive compounds in *Mirabilis jalapa* tuberous roots under different solvent polarities. Hexane and DCM extracts effectively partitioned terpenoids, saponins, cardiac glycosides, and lignans. Methanol and aqueous extracts obtained by maceration and the Soxhlet technique yielded phenolics and tannins.

Overall, *M. jalapa* tuberous roots exhibited significantly higher antioxidant activity under the tested conditions. These findings provide preliminary evidence of their antioxidant potential and require further studies to confirm antioxidant activity, other biological activities, and the phytochemicals in the tuberous root of *Mirabilis jalapa* Linn.

Supplementary Materials: The following supporting information can be downloaded at: URL.

Author Contributions: Concept and design, Data analysis/interpretation done by L.N., L.M., N.S. and C.B.R.; Statistical analysis and Data acquisition by L.N. and L.M.; Drafting manuscript and Critical revision of manuscript by all authors; admin, technical or material support by C.B.R., A.M. and T.N.; Supervision by C.B.R., A.M. and A.I.K. All authors have read and agreed to the published version of the manuscript.

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