RESEARCH ON THE EXTRACTION, PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITY OF THE METHANOL EXTRACT FROM *MIRABILIS JALAPA* L. FLOWERS

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Abstract - The concentrated methanolic extract of *Mirabilis jalapa* L. flowers was prepared using ultrasound-assisted extraction at a ratio of flower power (g)/methanol (mL) of 1/25, for 40 minutes with 3 ultrasonic cycles. Phytochemical screening indicated the presence of saponins, alkaloids, coumarins, flavonoids, polyphenols, steroids, and carboxylic acids. GC-MS analysis of the volatile constituents of the methanol extract identified 16 compounds, with carboxylic acids and their derivatives constituting the major components. Simultaneously, the extract exhibited DPPH antioxidant activity (IC_{50} of 41.47±2.28 µg/mL), inhibited HepG2 cells with a percentage of 29.58±1.54% at a concentration of 100 µg/mL, and α-glucosidase enzyme with a percentage of 30.56±1.37% at a concentration of 500 µg/mL.

Key words - *Mirabilis jalapa*; methanolic extract; DPPH; HepG2; α-glucosidase.

1. Introduction

Mirabilis jalapa L., commonly known as the four o'clock flower, belongs to the family Nyctaginaceae. It originates from tropical America and is distributed in tropical and subtropical regions [1]. In folk medicine, aqueous extracts from the leaves, seeds, roots, and flowers of *Mirabilis jalapa* have been used to treat muscle pain, diarrhea, diabetes, hepatitis, and skin rashes [2]. Its extracts have been reported to possess antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, cytotoxic, protein synthesis-inhibitory, and antispasmodic activities [3-7].

Chemical studies on different parts of the plant have indicated the presence of several classes of compounds, including phenols, flavonoids, alkaloids, steroids, tannins, triterpenes, lignins, saponins, and glycosides, as well as compounds such as trigonelline, isoquinoline, the flavonoid isorhamnetin, rhamnoside glycosides, β-sitosterol, stigmasterol, ursolic acid, oleanolic acid, brassicasterol, and rotenoids. Among these plant parts, the flowers are rich in polyphenols and flavonoids [8-11].

However, reports on the phytochemical profile, DPPH antioxidant activity, HepG2 cell inhibitory activity, and α-glucosidase enzyme inhibitory activity of *Mirabilis jalapa* flowers collected in Vietnam remain limited. This study reports several findings on the extraction, chemical characteristics, DPPH radical scavenging activity, HepG2 cell inhibitory activity, and α-glucosidase enzyme inhibitory activity of the concentrated methanolic extract from *Mirabilis jalapa* flowers collected in Da Nang, Vietnam.

2. Materials and methods

2.1. Materials, chemicals, and equipment

2.1.1. Materials

Flowers of *Mirabilis jalapa* L. were collected in Da Nang in May 2024 and identified as *Mirabilis jalapa* L. (MJF002) by Dr. Tran Quang Dan, The University of Danang - University of Science and Education. The flowers were selected as raw material when they were free from pests, bruising, or damage, while also ensuring uniformity in quality.

The flower powder of *Mirabilis jalapa* was obtained after the flowers had been cleaned, dried, and finely ground. The flower powder was brown in color and stored under dry conditions for experiments to determine physicochemical parameters as well as for the extraction process.

2.1.2. Chemicals

CH_3OH, Mayer, Wagner, Dragendorff, Fehling A, Fehling B, Liebermann-Burchard, $NaNO_2$, $AlCl_3$, NaOH, HCl, $FeCl_3$, Na_2CO_3, SRB, DMSO, CCl_3COOH, CH_3COOH, $(CH_2OH)_3CNH_2$ (China).

Ellipticine (Sigma-Aldrich, 519-23-3), 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, 84077-81-6), L-ascorbic acid (Sigma-Aldrich, 50-81-7), acarbose (Sigma-Aldrich, 56180-94-0), *p*-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich, 2492-87-7) and α-glucosidase (Sigma-Aldrich, 9001-42-7).

2.1.3. Equipment

The equipment used in this study included an ultrasonic bath digital ultrasonic cleaner, a rotary vacuum evaporator, an ELISA reader Biotek, USA, and a GC-MS system Agilent 7890A/5975C.

2.2. Research methods

2.2.1. Physicochemical parameters

The moisture content was determined according to Appendix 9.6 of the Vietnamese Pharmacopoeia V. The flower powder of *Mirabilis jalapa* was placed in a porcelain crucible and dried to constant weight at 105°C.

The total ash content was determined according to Appendix 9.8 of the Vietnamese Pharmacopoeia V. After being dried to constant weight, the flower powder of *Mirabilis jalapa* was incinerated at 500°C for 6 hours to obtain white ash. After incineration, the porcelain crucible containing the ash was cooled in a desiccator to room temperature and then weighed.

The heavy metal content was determined using the AAS method. The obtained ash sample was dissolved in 10% HNO_3 solution and then made up to the specified volume with distilled water before analysis.

2.2.2. Extraction of the concentrated methanolic extract

Ultrasound-assisted extraction was performed at a frequency of 40 kHz and a temperature of 50°C. The flower powder of *Mirabilis jalapa* was extracted with methanol at different solid-to-solvent ratios, as investigated in section a, for different extraction times, as investigated in section b, with three ultrasonic cycles.

The extraction yield H (%) of the concentrated methanolic extract from *Mirabilis jalapa* flowers was calculated according to Formula 1:

$$H\,(\%) = \frac{m_c}{m_{ngl}} \times 100\% \quad (1)$$

Where:

m_c: mass of the concentrated methanolic extract (g);

m_{ngl}: mass of *Mirabilis jalapa* flower powder (g).

a. Solid-to-solvent ratio

Each sample, consisting of 1.0 g of *Mirabilis jalapa* flower powder, was extracted by ultrasound at a frequency of 40 kHz and a temperature of 50°C for 30 minutes, using methanol volumes of 10, 15, 20, 25, and 30 mL, respectively. After extraction, five corresponding extracts were obtained. The solvents of these extracts were removed using a rotary vacuum evaporator to obtain five crude extracts.

b. Extraction time

A 1.0 g sample of *Mirabilis jalapa* flower powder was sonicated at a frequency of 40 kHz and a temperature of 50°C using the flower powder/methanol ratio selected from section a, for extraction times of 25, 30, 35, 40, and 45 minutes, respectively, to obtain five corresponding extracts. The solvent was recovered using a rotary vacuum evaporator to obtain five crude extracts.

2.2.3. Determination of chemical classes and chemical composition

a. Chemical classes

- Saponins were determined by reaction with distilled water, HCl, and NaOH. The appearance of stable foam confirmed the presence of saponins.

- Alkaloids were detected using Mayer's, Wagner's, and Dragendorff's reagents. The formation of a yellow or white precipitate with Mayer's reagent, a brown precipitate with Wagner's reagent, or an orange precipitate with Dragendorff's reagent indicated the presence of alkaloids.

- Flavonoids were identified through reaction with a mixture of $NaNO_2$, $AlCl_3$ and NaOH. The appearance of an orange-yellow color indicated the presence of flavonoids.

- Coumarins were determined using 10% NaOH solution. The occurrence of turbidity indicated the presence of coumarins.

- Polyphenols were detected using $FeCl_3$ reagent. The appearance of a dark blue precipitate indicated the presence of polyphenols.

- Reducing sugars were identified using Fehling A and Fehling B reagents. A brick-red precipitate was the characteristic sign of a positive reaction, indicating the presence of reducing sugars.

- Steroids were determined using Liebermann-Burchard reagent. The formation of a green color indicated the presence of steroids.

- Carboxylic acids were identified by reaction with Na_2CO_3. The release of gas bubbles indicated the presence of carboxylic acids.

b. Chemical composition

The volatile compounds present in the concentrated methanolic extract of *Mirabilis jalapa* flowers were analyzed by GC-MS using an Agilent 7890A/5975C system.

2.2.4. Cytotoxic activity against liver cancer cells

The HepG2 liver cancer cell line was provided by Prof. J. M. Pezzuto, Long Island University, USA [12]. The *in vitro* assay was conducted according to the method described by Monks [13].

The test sample was prepared as a stock solution in 100% dimethyl sulfoxide DMSO in the first sample tube at an initial concentration of 20 mg/mL. The sample was then diluted to 2 mg/mL, or 2000 µg/mL, by transferring 100 µL of the stock solution into a second sample tube containing 900 µL of FBS-free medium.

Finally, a sample dilution plate was prepared by transferring 50 µL of the sample from the second tube into well A1 of a 96-well plate to perform the second dilution step. Then, 10 µL of the sample from well A1 was transferred into well B1, which already contained 90 µL of cell culture medium without FBS, and the dilution was continued in the same manner to generate a series of four concentrations from high to low: A1, B1, C1, and D1. Thus, at this step, the sample was diluted to obtain a concentration series of 2000, 400, 80, and 16 µg/mL.

The cells were treated with trypsin to detach them from the culture surface and then counted using a cell counting chamber to obtain a density of 3×10^4 cells/mL. Subsequently, 190 µL of the cell suspension in culture medium supplemented with 5% FBS was distributed into each well of a 96-well plate.

From the sample dilution plate, 10 µL of the prepared sample was transferred into the experimental plate containing the test cells, which had 190 µL of cell suspension. The day-0 control well contained 190 µL of cancer cell suspension and 10 µL of 1% DMSO, without the test sample.

Ellipticine was used as the positive control, while 1% DMSO was used as the negative control, with a final concentration of 0.05% in the test well. The IC_{50} value was determined using TableCurve 2Dv4 software.

2.2.5. DPPH antioxidant activity

The *in vitro* DPPH free radical scavenging assay was conducted with modifications according to the reference method [14]. A 0.25 µM DPPH solution was prepared in methanol. The test sample was dissolved in methanol to obtain a stock solution. Deionized water was used for dilution to obtain the investigated concentrations.

L-ascorbic acid was used as the positive control and was prepared at a test concentration range similar to that of the test sample. In each well of a 96-well plate, 100 µL of sample solution at different concentrations was mixed with 100 µL of DPPH solution at a volume ratio of 1:1.

The control well contained 100 µL of distilled water and 100 µL of DPPH solution, without the test sample. The blank sample was prepared in the same manner as the test sample; however, the DPPH solution was replaced with 100 µL of methanol.

2.2.6. α-glucosidase enzyme inhibitory activity

The assay was performed with modifications according to B. Elya *et al*. [15]. The sample was dissolved in 100% DMSO to obtain a stock solution of 20 mg/mL and then diluted with 10 mM phosphate buffer pH 6.8 to obtain the investigated concentrations.

In each well of a 96-well plate, 50 µL of the diluted sample solution was mixed with 20 µL of *α*-glucosidase 0.5 U/mL and 130 µL of 100 mM phosphate buffer pH 6.8. The mixture was mixed thoroughly and incubated at 37°C for 15 minutes. The final concentrations of the test sample in the wells were 500, 100, 20, and 4 µg/mL, respectively.

Then, 50 µL of 5 mM *p*-nitrophenyl-*α*-D-glucopyranoside pNPG substrate solution was added to each well, followed by further incubation at 37°C for 60 minutes. The reaction was terminated by adding 80 µL of 0.2 M Na_2CO_3 solution to each well.

Wells containing the test sample, phosphate buffer, and pNPG but without enzyme were used as blanks. The control wells contained 10% DMSO, phosphate buffer, *α*-glucosidase enzyme, and pNPG, without the test sample. Acarbose was used as the positive control. The optical density was recorded at 405 nm using an ELISA reader.

3. Results and discussion

3.1. Physicochemical parameters

- Moisture content: 10.75%
- Total ash content: 9.47%
- The heavy metal content is presented in Table 1.

Table 1. *Contents of selected heavy metals*

Metal	mg metal/kg dry weight	Permitted mg metal/kg dry weight
Pb	$20.040.10^{-4}$	≤ 0.05
Cu	$0.154.10^{-4}$	≤ 30
Zn	$1.516.10^{-4}$	≤ 40
As	$3.943.10^{-4}$	≤ 0.1

The results in Table 1 show that the contents of heavy metals in *Mirabilis jalapa* flower powder were all considerably lower than the maximum permitted limits [16].

3.2. Extraction of the concentrated methanolic extract

a. Solid-to-solvent ratio

The results in Table 2 and Figure 1 show that the highest extraction yield reached 31.5% when 1.0 g of raw material was extracted with 25 mL of methanol. As the methanol volume increased from 10 mL to 25 mL, the mass of the obtained extract increased accordingly. However, further increasing the solvent volume to 30 mL did not increase the amount of recovered extract. This indicates that most of the extractable compounds had been separated from the raw material at the ratio of 1.0 g/25 mL methanol. Therefore, 25 mL of methanol was selected for the subsequent experiment.

Table 2. *Extraction yield results at different methanol volumes*

No.	Volume of methanol (mL)	Mass of methanol extract (g)	Extraction yield (H %)
1	10	0.132	13.2
2	15	0.215	21.5
3	20	0.284	28.4
4	25	0.315	31.5
5	30	0.315	31.5

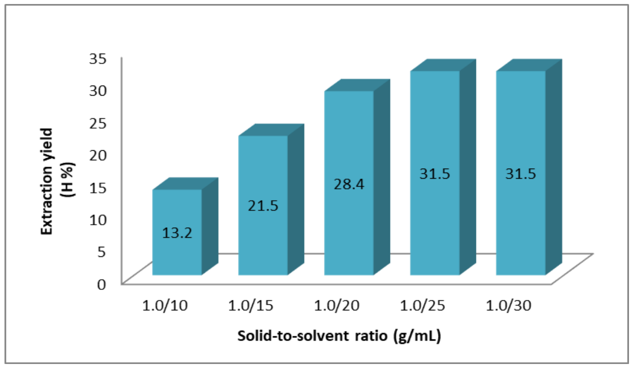

Figure 1. *Effect of solid-to-solvent ratio on methanol extraction yield*

b. Extraction time

Table 3 and Figure 2 show that the extraction yield reached a maximum of 35.1% at an extraction time of 40 minutes. Further extending the extraction time did not increase the amount of extract obtained, indicating that the compounds in the raw material had been almost completely extracted.

Table 3. *Extraction yield results at different extraction times*

No.	Extraction time (min)	Mass of methanol extract (g)	Extraction yield (H %)
1	25	0.276	27.6
2	30	0.315	31.5
3	35	0.338	33.8
4	40	0.351	35.1
5	45	0.351	35.1

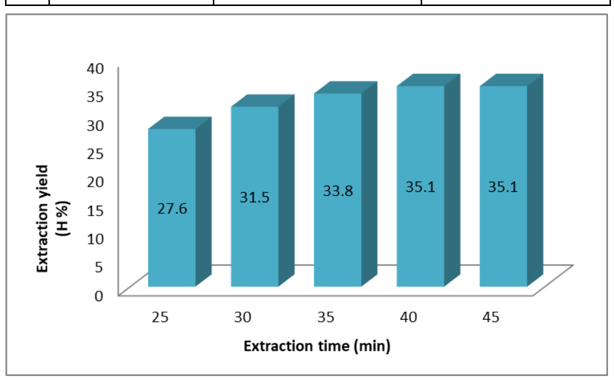

Figure 2. *Effect of extraction time on methanol extraction yield*

Thus, ultrasound-assisted extraction of the concentrated methanolic extract from *Mirabilis jalapa* flowers under the conditions of a solid-to-solvent ratio of 1.0 g of *Mirabilis jalapa* flower powder/25 mL methanol and an extraction time of 40 minutes resulted in an extract yield of 35.1%.

3.3. Determination of chemical classes and chemical composition

a. Chemical classes

The qualitative screening results of chemical classes in the concentrated methanolic extract of *Mirabilis jalapa* flowers are presented in Table 4.

The qualitative phytochemical results in Table 4 show the presence of saponins, alkaloids, coumarins, flavonoids, polyphenols, steroids, and carboxylic acids in the concentrated methanolic extract from *Mirabilis jalapa* flowers. These results are consistent with the report by Md. H. O. Rashid *et al*. [7] on the presence of flavonoids, polyphenols, steroids, and reducing sugars in the methanolic extract of *Mirabilis jalapa* flowers collected in Pabna, Bangladesh. However, the findings regarding the presence of saponins and alkaloids differ from those reported for the concentrated methanolic extract of *Mirabilis jalapa* flowers collected in Da Nang, Vietnam.

Table 4. *Phytochemical constituents*

Phytochemical constituents	Reagent	Observation	Conclusion
Saponins	Distilled water	Sustainable foam	Present
	HCl	Sustainable foam	Present
	NaOH	Unsustainable foam	Present
Alkaloids	Mayer	Pale yellow precipitate	Present
	Wagner	Brown precipitate	Present
	Dragendroff	Organe precipitate	Present
Coumarins	NaOH	Turbidity observed	Present
Flavonoids	$NaNO_2$ + $AlCl_3$ + NaOH	Organe yellow coloration	Present
Polyphenols	$FeCl_3$	Dark green precipitate	Present
Reducing sugars	Fehling A + Fehling B	No observable change	Absent
Steroids	Liebermann-Burchard	Green coloration	Present
Carboxylic acids	Na_2CO_3	Effervescence observed	Present

b. Chemical composition

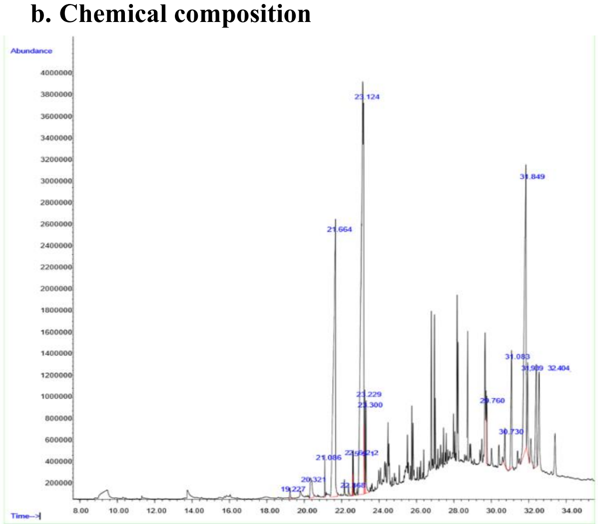

Figure 3. *GC-MS chromatogram*

The GC-MS results of the concentrated methanolic extract from *Mirabilis jalapa* flowers, shown in Figure 3 and Table 5, indicate that among the 16 identified compounds, compounds belonging to the class of carboxylic acids and their derivatives accounted for 49.51%, steroids accounted for 21.26%, and other organic compounds accounted for 0.31%.

The results of this study showed several volatile chemical constituents that differed from the report by F. I. Liya *et al*. [2], which identified 20 chemical compounds in the methanolic extract of the whole *Mirabilis jalapa* plant using GC-MS, and from the report by V. N. Pangi [17], which identified 29 compounds in the methanolic extract of *Mirabilis jalapa* leaves using GC-MS.

Table 5. *Chemical composition*

No.	Retention time (min)	Compound	Peak area (%)
1	19.227	Tetradecanoic acid	0.20
2	20.321	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)	1.44
3	21.086	Hexadecanoic acid, methyl ester	0.44
4	21.664	*n*-Hexadecanoic acid	15.58
5	22.368	Heptadecanoic acid	0.20
6	22.591	9,12-Octadecadienoic acid (*Z*, *Z*)-, methylester	0.45
7	22.622	9,12,15-Octadecatrienoic acid, methyl ester, (*Z*, *Z*, *Z*)-	0.42
8	23.124	9,12,15-Octadecatrienoic acid, (*Z*, *Z*, *Z*)-	28.49
9	23.229	Oleic acid	1.07
10	23.300	Octadecanoic acid	1.22
11	29.760	Vitamin E	0.31
12	30.730	Campesterol	0.80
13	31.083	Stigmasterol	2.77
14	31.849	*β*-Sitosterol	12.75
15	31.939	Isofucosterol	1.94
16	32.404	Dihydrochondrillasterol	3.00

3.4. Cytotoxic activity against liver cancer cells

The concentrated methanolic extract from *Mirabilis jalapa* flowers showed inhibitory activity against HepG2 cells, with an inhibition percentage of 29.58±1.54% at a concentration of 100 µg/mL (Table 6). Y. H. Suselo *et al*. [18] reported that the ethanolic extract from *Mirabilis jalapa* flowers collected in Yogyakarta, Indonesia, exhibited cytotoxic activity against HepG2 cells with an IC_{50} value of 187.79±4.13 ppm.

Table 6. *Cytotoxic activity against HepG2 cancer cells*

Percentage inhibition of HepG2 cells				
Concentration (µg/mL)	Methanol extract		Ellipticine	
	Percentage of inhibition	SD	Percentage of inhibition	SD
100	29.58	1.54	93.01	1.71
20	15.57	2.34	79.38	1.02
4	6.28	0.31	52.69	1.31
0.8	1.70	0.15	23.10	0.95
IC_{50}	> 100		0.33±0.02	

Ellipticine was tested at a concentration range of 10, 2, 0.4, and 0.08 µg/mL

3.5. Antioxidant activity

***Table 7.** DPPH radical scavenging activity of the methanol extract*

Concentration (μg/mL)	Percentage of free radical scavenging			
	Methanol extract		L-Ascorbic acid	
	Mean	SD	Mean	SD
100	84.69	2.72	92.55	1.35
20	29.85	1.13	72.51	1.67
4	17.46	0.65	32.72	1.03
0.8	2.35	0.14	13.41	0.75
IC_{50}	41.47±2.28		7.84±0.35	

The concentrated methanolic extract from *Mirabilis jalapa* flowers exhibited antioxidant activity through the DPPH free radical scavenging mechanism, with an IC_{50} value of 41.47±2.28 μg/mL (Table 7). Md. H. O. Rashid [7] reported this activity for the methanolic extract from *Mirabilis jalapa* flowers collected in Pabna, Bangladesh, with an IC_{50} value of 13.70±0.32 μg/mL.

3.6. α-glucosidase enzyme inhibitory activity

The concentrated methanolic extract from *Mirabilis jalapa* flowers inhibited *α*-glucosidase, with an inhibition percentage of 30.56±1.37% at a concentration of 500 μg/mL (Table 8). D. D. Kodical [19] reported this activity for the ethanolic and aqueous extracts from *Mirabilis jalapa* flowers collected in Dakshina Kannada, India, with IC_{50} values of 221.45 and 299.84 μg/mL, respectively.

***Table 8.** α-Glucosidase inhibitory activity of the methanol extract*

Concentration (μg/mL)	Percentage of *α*-glucosidase inhibition			
	Methanol extract		Acarbose	
	Percentage of inhibition	SD	Percentage of inhibition	SD
500	30.56	1.37	78.88	1.54
100	10.06	1.04	52.69	1.72
20	8.63	0.69	17.84	1.47
4	6.10	0.59	8.46	0.49
IC_{50}	> 500		113.41±11.17	

4. Conclusion

The concentrated methanolic extract from *Mirabilis jalapa* flowers was obtained by ultrasound-assisted extraction at a flower powder (g)/methanol (mL) ratio of 1/25, with an extraction time of 40 minutes over three ultrasonic cycles. Saponins, alkaloids, coumarins, flavonoids, polyphenols, steroids, and carboxylic acids were detected in the concentrated methanolic extract through chemical reactions with specific reagents.

In addition, GC-MS analysis preliminarily screened the presence of 16 volatile compounds in the concentrated methanolic extract, among which compounds belonging to the class of carboxylic acids and their derivatives accounted for the majority. Furthermore, the concentrated methanolic extract exhibited DPPH antioxidant activity with an IC_{50} value of 41.47 ± 2.28 μg/mL.

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