ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 10, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS AND CYTOTOXICITY ASSESSMENT OF PETROLEUM ETHER EXTRACT OF *TROPAEOLUM MAJUS* LEAVES ON MDA-MB-231 HUMAN BREAST CANCER CELL LINE

MAZIN SALEEM SHAKIR[1], AHMED ISMAIL HASSAN MOAD[2], ABDULLAH H MAAD[3,4]*, ALI MOHAMED SAED ALMASTAFA[5]

[1]Department of Pharmacognosy, College of Pharmacy, University of Al-Ameed, Karbala, Iraq. [2]Department of Medical Laboratories, Faculty of Medicine and Health Sciences, Hodeidah University, Al-Hodeidah, Yemen. [3]Department of Pharmaceutics, College of Pharmacy, University of Al-Ameed, Karbala, Iraq. [4]Department of Pharmacy Practice, College of Clinical Pharmacy, Hodeidah University, Al-Hodeidah, Yemen. [5]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Al-Ameed, Karbala, Iraq.
Corresponding Author: Abdullah H Maad; Email: dr.ph.abdullah.maad@gmail.com

Received: 26 June 2025, Revised and Accepted: 08 August 2025

ABSTRACT

Objectives: The aim of this study was to analyze the chemical components present in the petroleum ether extract of *Tropaeolum majus* leaves using gas chromatography–mass spectrometry (GC-MS), and to assess its cytotoxic effects on the MDA-MB-231 human breast cancer cell line.

Methods: Petroleum ether extract was obtained from shade-dried leaves of *T. majus* through Soxhlet extraction and analyzed by GC-MS to identify its lipophilic constituents. The cytotoxic potential of the extract was evaluated on MDA-MB-231 cells using the WST-1 assay across a concentration range of 0.01–100 µg/mL.

Results: The GC-MS analysis identified various chemical constituents, including fatty acids, fatty alcohols, and diterpenoids, with hexadecanoic acid and isophytol being predominant. According to the WST-1 assay, the extract exhibited minimal cytotoxicity toward MDA-MB-231 cells, as cell viability remained above 86% even at the maximum tested concentration of 100 µg/mL.

Conclusion: The petroleum ether extract of *T. majus* leaves was found to contain several bioactive lipophilic constituents; however, it did not demonstrate notable cytotoxic effects against MDA-MB-231 breast cancer cells at the concentrations tested. Additional research involving higher doses or different extract fractions is suggested to better assess its potential anticancer properties.

Keywords: Gas chromatography–mass spectrometry, *Tropaeolum majus*, MDA-MB-231, Petroleum ether extract, WST-1 assay.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i10.55775. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Tropaeolum majus L., commonly referred to as garden nasturtium, is an annual herbaceous plant valued for its ornamental appeal and medicinal uses. In traditional medicine, different parts of the plant, especially the leaves and flowers, have been linked to a range of therapeutic effects, such as antioxidant, antimicrobial, diuretic, and anti-inflammatory activities [1]. These pharmacological effects are largely attributed to the presence of diverse phytochemicals, such as flavonoids, glucosinolates, essential oils, and fatty acids [2,3]. In recent years, scientific interest has grown in identifying and characterizing the chemical components responsible for the therapeutic effects of *T. majus*. Analytical tools, such as Gas Chromatography-Mass Spectrometry (GC-MS), have become essential for profiling volatile and semi-volatile phytochemicals in plant extracts. GC-MS enables the identification of constituents based on their mass-to-charge ratios, retention times, and spectral library matches [4,5]. The solvent used for extraction significantly influences the yield and types of compounds extracted. Petroleum ether, being non-polar, is suitable for isolating lipophilic compounds, including fatty acids, fatty alcohols, terpenoids, and hydrocarbons [6,7]. Breast cancer is one of the most prevalent and challenging malignancies worldwide [8]. The MDA-MB-231 cell line, a triple-negative breast cancer (TNBC) model, is often used in cytotoxicity and drug-resistance studies due to its aggressive behavior and lack of hormonal receptors, making it a valuable *in vitro* model for evaluating potential chemotherapeutic agents [9]. This study investigates the chemical composition of *T. majus* leaves cultivated in Iraq through GC-MS analysis of their petroleum ether extract. It also evaluates the extract's cytotoxic effect on the MDA-MB-231 human breast cancer cell line using the WST-1 cell viability assay to assess its possible anticancer activity.

METHODS

Plant material and extractions

Fresh *T. majus* leaves were harvested from the garden of the College of Pharmacy at the University of Al-Ameed and authenticated by Dr. Neepal Imtair Al-Garaawi, a plant taxonomy professor at the University of Karbala. The leaves were dried in the shade at ambient temperature and subsequently pulverized into a fine powder using a mechanical grinder. Approximately 150 g of powdered leaves were extracted using a Soxhlet apparatus with 500 mL of petroleum ether (boiling range 40–60°C) for 10 h. The resulting extract was filtered, evaporated, and concentrated under reduced pressure using a rotary evaporator (Heidolph, Germany) to obtain the petroleum ether extract. The obtained extract was used in its crude form without further purification or fractionation for both GC-MS analysis and cytotoxicity assays.

Chemicals and reagents

Petroleum ether was of analytical grade and purchased from Sigma-Aldrich (Germany). Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Penicillin, and Streptomycin were obtained from Gibco® (Thermo Fisher Scientific, USA). The WST-1 Cell Proliferation Reagent (ab155902) was procured from Abcam (UK). All other chemicals and solvents used in the extraction and analytical procedures

were of analytical grade and were used without further purification. Helium gas used as the carrier gas in GC-MS analysis was supplied by the Iraqi Gas Company.

Cell culture

MDA-MB-231, a human breast cancer cells were provided by Nawah- Scientific Centre (Cairo, Egypt). Cells were cultured under identical conditions using DMEM supplemented with 100 µg/mL streptomycin, 100 U/mL penicillin, and 10% heat-inactivated FBS at 37°C in a 5% carbon dioxide incubator.

Experimental design

This experimental study aimed to analyze the chemical constituents and assess the cytotoxic activity of the petroleum ether extract from *T. majus* leaves cultivated in Iraq. The chemical profiling of non-polar constituents was performed using gas chromatography-mass spectrometry (GC-MS). To evaluate the cytotoxic potential, the extract was tested on MDA-MB-231 human breast cancer cells using the WST-1 colorimetric assay, measuring cell viability after 48 h of exposure to various concentrations.

GC-MS analysis

GC-MS analysis was conducted using a Shimadzu GCMS-QP2020 system to identify the chemical constituents present in the petroleum ether extract of *T. majus* leaves. A 1 µL volume of the petroleum ether extract was injected in split mode. Helium served as the carrier gas at a flow rate of 1.41 mL/min under a pressure of 80.0 kPa. The oven temperature was programmed to start at 45°C (held for 2 min), then ramped at 5°C per min–300°C, where it was held for an additional 5 min. The injector temperature was maintained at 250°C. Mass spectra were recorded in electron ionization mode, and compound identification was performed by matching the obtained spectra with those in the NIST and Wiley mass spectral libraries [10,11].

Cytotoxicity assessment of *T. majus* extract (WST-1 assay)

The cytotoxic potential of the petroleum ether extract was evaluated using the WST-1 assay. A total of 50 µL of cell suspension (3×10^3 cells/well) was seeded in 96-well plates and allowed to attach for 24 h in complete growth medium. Subsequently, 50 µL of medium containing various concentrations (0.01, 0.1, 1, 10, and 100 µg/mL) of petroleum ether extract of *T. majus* was added. After 48 h of treatment, 10 µL of WST-1 reagent was added to each well. Absorbance was measured at 450 nm after 1 h of incubation using a FLUOstar Omega microplate reader (BMG LABTECH®, Ortenberg, Germany) [12].

Statistical analysis

All experiments were carried out in triplicate, and the results are expressed as mean±standard deviation. Statistical differences between control and treated groups were evaluated using one-way analysis of variance, followed by Tukey's *post hoc* test to determine significance. A p-value below 0.05 was considered statistically significant. Data analysis and graphical representations were performed using GraphPad Prism software (Version 6.0, GraphPad Software, La Jolla, USA).

RESULTS

Extraction yield

The petroleum ether extraction of *T. majus* leaves yielded 26.25 g of dried extract from 150 g of powdered material, giving a yield of 17.5% w/w.

GC-MS analysis

The GC-MS chromatogram of the petroleum ether extract of *T. majus* leaves revealed multiple peaks, each corresponding to distinct chemical constituents. A total of nine compounds were identified based on retention time, peak area, molecular weight, and molecular formula. These included levulinic acid, hexadecanoic acid methyl ester, hexadecanoic acid, 3-decen-1-ol, methyl isoheptadecanoate, docosanoic acid ethyl ester, n-decanoic acid, isophytol, and cyclopentane undecanoic acid. A graphical representation of the chromatogram is shown in Fig. 1 and the identified compounds are listed in Table 1.

Cytotoxicity assessment of *T. majus* extract (WST-1 assay)

The petroleum ether extract of *T. majus* exhibited no significant cytotoxicity against MDA-MB-231 human breast cancer cells at concentrations up to 100 µg/mL. Specifically, the percent viability of MDA-MB-231 cells treated with the extract was 98.6±0.6% at 0.01 µg/mL, 97.5±0.6% at 0.1 µg/mL, 90.2±3.0% at 1 µg/mL, 86.9±0.07% at 10 µg/mL, and 86.6±0.7% at 100 µg/mL (Fig. 2). Although a slight dose-dependent decrease in viability was observed, the changes were not statistically significant compared to the untreated

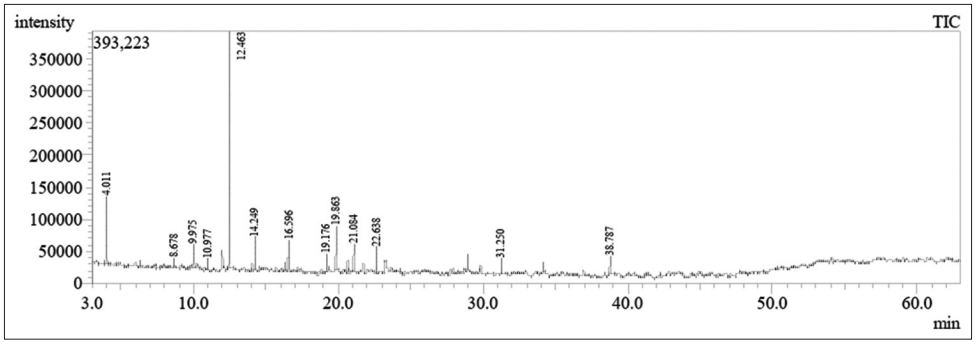

Fig. 1: Gas chromatography-mass spectrometry chromatogram of petroleum ether extract of *Tropaeolum majus*

Table 1: GC-MS identification of chemical constituents in the petroleum ether extract of *Tropaeolum majus*

Compound	Retention time (min)	Peak area (%)	Classification	Molecular formula
Levulinic acid	4.010	0.23	Fatty acid	$C_5H_8O_3$
Hexadecanoic acid, methyl ester	19.639	0.31	Fatty acid	$C_{18}H_{36}O_2$
Hexadecanoic acid	27.992	2.47	Fatty acid	$C_{16}H_{32}O_2$
3-Decen-1-ol	16.178	0.47	Fatty alcohol	$C_{10}H_{20}O$
Dianhydrogalactitol	24.510	0.58	Hexitolepoxide	$C_6H_{10}O_4$
Docosanoic acid, ethyl ester	20.012	0.29	Fatty acid	$C_{24}H_{48}O_2$
n-Decanoic acid	22.439	0.82	Fatty acid	$C_{10}H_{20}O_2$
Isophytol	23.615	5.30	Diterpenoid alcohol	$C_{20}H_{40}O$
Cyclopentaneundecanoic acid	28.204	6.67	Fatty acid	$C_{16}H_{30}O_2$

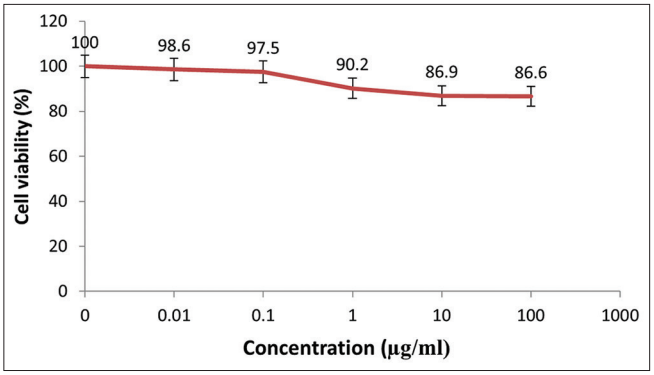

Fig. 2: Cytotoxicity assay of petroleum ether extract of *T. majus* on MDA-MB-231 cells

control group (p=0.76), suggesting a lack of cytotoxic activity at the tested concentrations.

DISCUSSION

GC-MS analysis is a powerful technique used to identify and quantify chemical compounds in complex mixtures. The results revealed that the petroleum ether extract of *T. majus* leaves contains a diverse range of phytochemicals, with fatty acids being the predominant class. The major constituents identified included hexadecanoic acid (palmitic acid), levulinic acid, docosanoic acid ethyl ester, and isophytol. Fatty acids, such as palmitic acid (hexadecanoic acid) and decanoic acid recognized for their biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties. Palmitic acid was also reported as a dominant compound in *T. majus* extracts by Musolino *et al*. [13], suggesting consistency in the lipophilic profile across geographic locations. Isophytol, a diterpenoid alcohol detected in this study, has been documented to possess anti-inflammatory and antioxidant activities. It has been reported in essential oils of other medicinal plants [14], further supporting its role as a biologically relevant component in medicinal flora. Levulinic acid, a keto acid detected here, is a known platform chemical derived from biomass and is utilized in pharmaceutical formulations [15]. Its presence in *T. majus* adds value to the extract's industrial applicability [16]. The composition reported in this study is largely consistent with the findings of Ivanov *et al*., who performed GC-MS on non-polar extracts of *Ficus carica* leaves and found similar classes of compounds, such as fatty acids and alcohols [17]. However, in contrast to studies focusing on methanolic or hydroalcoholic extracts of *T. majus,* where flavonoids and phenolic acids were prominent [18,19], our petroleum ether extract selectively highlighted lipophilic constituents, underscoring the importance of solvent choice in phytochemical profiling. Some unidentified peaks in the chromatogram may correspond to novel or less-common compounds not covered in present spectral libraries, indicating the potential for future in-depth structural elucidation studies using NMR or LC-MS/MS. Regarding cytotoxicity, the petroleum ether extract of *T. majus* leaves exhibited no significant cytotoxic effect on MDA-MB-231 human breast cancer cells at concentrations up to 100 µg/mL. Cell viability remained high across all tested concentrations, with values exceeding 86%, indicating that the extract, in its present form and dose range, does not exert strong antiproliferative effects on this aggressive TNBC cell line. These findings are in agreement with previous studies that reported limited or no cytotoxic activity for non-polar plant extracts at low-to-moderate concentrations. For example, Aly *et al*. observed weak cytotoxicity in petroleum ether extracts of *Sophora tomentosa* and *Sophora secundiflora*, and suggested that the anticancer activity of these species may be more prominent in polar or semi-polar fractions rich in alkaloids or flavonoids [20]. Similarly, Ivanov *et al*. noted that the unpolar fraction of *Ficus carica* leaves showed moderate cytotoxicity only at higher concentrations, emphasizing the need to test broader dose ranges [17]. Interestingly, although a dose-dependent decline in cell viability was noted (from 98.6% at 0.01 µg/mL to 86.6% at 100 µg/mL), the reduction was not statistically significant (p=0.76), suggesting that higher concentrations may be necessary to reach the half-maximal inhibitory concentration threshold. Future investigations should explore the cytotoxic potential of other solvent fractions (e.g., methanolic or ethyl acetate extracts), higher doses, and potential mechanisms of action, such as apoptosis or cell cycle modulation.

CONCLUSION

This study evaluated the phytochemical profile and cytotoxic activity of the petroleum ether extract of *T. majus* leaves grown in Iraq. GC-MS analysis identified multiple bioactive lipophilic compounds, including fatty acids, fatty alcohols, and diterpenoid alcohols, such as hexadecanoic acid and isophytol. However, despite the presence of these constituents, the extract showed no significant cytotoxicity against MDA-MB-231 human breast cancer cells at concentrations up to 100 µg/mL, as measured by the WST-1 assay. These results indicate that although the extract contains potentially active compounds, its anticancer effect within the tested dose range is minimal. Further research involving higher concentrations, alternative solvent extracts, or isolated compounds is warranted to better assess the plant's therapeutic potential, especially regarding anticancer properties.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the College of Pharmacy, University of Al-Ameed, for providing laboratory facilities and technical support throughout the study. The authors also acknowledge the staff of the analytical laboratory for their support during the GC-MS analysis.

AUTHORS CONTRIBUTION

Mazin Saleem Shakir was responsible for plant collection, extraction procedures, experimental work, and initial drafting of the manuscript. Ahmed Ismail Hassan Moad and Ali Mohamed Saed Almastafa assisted with cytotoxicity assay analysis, data validation, and manuscript editing. Abdullah H. Maad contributed to study design, GC-MS data interpretation, and critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

FUNDING

The study was self-funded by the authors.

REFERENCES

1. Saini I, Chauhan J, Kaushik P. Medicinal value of domiciliary ornamental plants of the Asteraceae family. J Young Pharm. 2020;12(1):3-10. doi: 10.5530/jyp.2020.12.2
2. Al-Jassani MJ. *Tropaeolum majus* leaves extract as an antifungal, antiaflatoxigenic and antiaflatoxin agent. J Glob Pharm Technol. 2017;12(9):328-33.
3. Hamed MM, Abdalla AM, Ghareeb MA, Saleh SA. Chemical constituents, *in vitro* antioxidant activity, oral acute toxicity and LD50 determination of *Moringa oleifera* leaves. Int J Pharm Pharm Sci. 2017;9(5):240-7. doi: 10.22159/ijpps.2017v9i5.17834
4. Ranjan S, Chaitali R, Sinha SK. Gas chromatography-mass spectrometry (GC-MS): A comprehensive review of synergistic combinations and their applications in the past two decades. J Anal Sci Appl Biotechnol. 2023;5(2):72-85.
5. Ma AK, Mohajab AA, Al-Masrafi I. Impact monitoring of low and high dexamethasone doses on COVID-19 outcomes in Al Hudaydah, Yemen: A pharmacoepidemiological study. Asian J Pharm Clin Res. 2025;18(1):52-5.
6. Zarrinmehr MJ, Daneshvar E, Nigam S, Gopinath KP, Biswas JK, Kwon EE, *et al*. The effect of solvents polarity and extraction conditions on the microalgal lipids yield, fatty acids profile, and biodiesel properties. Bioresour Technol. 2022;344(B):126303. doi: 10.1016/j.biortech.2021.126303, PMID 34752885

7. Ahmed Y, Maad AH, Hassan HA, Abdallah DB, Yousef M, H. Kadhum AA, *et al*. Evaluation of carboxymethyl millet starch and pregelatinized millet starch as pharmaceutical excipients using factorial experimental designs. Int J Appl Pharm. 2025;17(2):321-8. doi: 10.22159/ijap.2025v17i2.52161
8. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. Br J Radiol. 2022;95(1130):20211033. doi: 10.1259/bjr.20211033, PMID 34905391
9. Sharma MP, Shukla S, Misra G. Recent advances in breast cancer cell line research. Int J Cancer. 2024;154(10):1683-93. doi: 10.1002/ijc.34849, PMID 38230499
10. Aziz SS, Al Saiqali MA, Begum N, Najmuddin M. GC-MS characterization and therapeutic potential of phytochemical constituents of the Unani medicine Uterotibb's aqueous alkaline extract. Haya Saudi J Life Sci. 2025;10(5):146-56. doi: 10.36348/sjls.2025.v10i05.001
11. Firdouse S, Ahmed S, Munaim MA. Qualitative and quantitative analysis of phytoconstituents in the Unani formulation Habb-E-Bukhar. Int J Curr Pharm Res. 2024;16(3):36-41. doi: 10.22159/ijcpr.2024v16i3.4060
12. Abdou SA, Mohamed AI, Aly Y. Cytotoxicity evaluation of three different types of intracanal medications. J Int Oral Health. 2023;15(4):384-90. doi: 10.4103/jioh.jioh_50_23
13. Musolino V, Marrelli M, Perri MR, Palermo M, Gliozzi M, Mollace V, *et al*. *Centranthus ruber* (L.) DC. and *Tropaeolum majus* L.: Phytochemical profile, *in vitro* anti-denaturation effects and lipase inhibitory activity of two ornamental plants traditionally used as herbal remedies. Molecules. 2022;28(1):32. doi: 10.3390/molecules28010032, PMID 36615228
14. Al-Snafi AE. Oils and fats contents of medicinal plants, as natural ingredients for many therapeutic purposes-A review. IOSR J Pharm. 2020;10(7):1-41.
15. Zhang M, Wang N, Liu J, Wang C, Xu Y, Ma L. A review on biomass-derived levulinic acid for application in drug synthesis. Crit Rev Biotechnol. 2022;42(2):220-53. doi: 10.1080/07388551.2021.1939261, PMID 35105271
16. Mudhafar M, Alsailawi H, Abdulrasool MM, Jawad RK, AD. Mini-review of phytochemicals of ten *Ficus* species. Int J Chem Res. 2021;5:7-18. doi: 10.22159/ijcr.2021v5i4.185
17. Ivanov I, Dincheva I, Badjakov I, Petkova N, Denev P, Pavlov A. GC-MS analysis of unpolar fraction from *Ficus carica* L.(fig) leaves. Int Food Res J. 2018;25(1):282-6.
18. Vrca I, Jug B, Fredotović Ž, Vuko E, Brkan V, Šestić L, *et al*. Significant benefits of environmentally friendly hydrosols from *Tropaeolum majus* L. Seeds with multiple biological activities. Plants (Basel). 2023;12(22):3897. doi: 10.3390/plants12223897, PMID 38005794
19. Maad AH, AL-Gamli AH, Shamarekh KS, Refat M, Shayoub ME. Antiproliferative and apoptotic effects of *Solenostemma argel* leaf extracts on colon cancer cell line HCT-116. Biomed Pharmacol J. 2024;17(3):1987-96. doi: 10.13005/bpj/3001
20. Aly SH, Elissawy AM, Eldahshan OA, Elshanawany MA, Singab AN. Variability of the chemical composition of the essential oils of flowers and the alkaloid contents of leaves of *Sophora secundiflora* and *Sophora tomentosa*. J Essent Oil Bear Plants. 2020;23(3):442-52. doi: 10.1080/0972060X.2020.1750489