

## Metabolite Profiling by GC-MS and Antioxidant Properties of *Punica granatum*

Bander Almuntashiri\*, Ali Zari†, Ihsan Ullah‡

### Abstract

*Punica granatum* has a great medicinal use due to a wide range of secondary metabolites which have very important roles in cell signaling immunity, and reproduction. In the present study, metabolites from *P. granatum* are extracted using 75% ethanol and dehydrated using a rotatory. The metabolites are identified through Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The resultant metabolites in the ethanolic extract are including Palmitic acid, methyl ester; 11,14-Octadecadienoic acid, methyl ester; 2-Bis(hydroxyphenyl)propane, Methyl octadecenoate, Naphthalene-1-sulfonic acid, Silane, [(10-iododecyl)oxy]trimethyl, Cyclodecasiloxane, eicosamethyl. The metabolites are assessed based on peak area, retention time and height of the peak and are compared standard reference compounds from NIST libraries. Moreover, antioxidant properties of the extract are evaluated via 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays. The DPPH results revealed a dose-dependent increase in activity from  $18 \pm 2\%$  to 25% as concentrations are increased from at 100  $\mu\text{g/ml}$  to 700  $\mu\text{g/ml}$ . these results suggested the potential health benefits and antioxidant properties of *P. granatum* metabolites. However, further studies are recommended to explore the in-depth medicinal potential of the *P. granatum*.

**Keywords:** 2,2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging; Antioxidant; Medicine; Secondary Metabolites.

### Introduction

Biologically active metabolites (secondary metabolites) in plants regulate and maintain plant health as well as protect them from the bio-stressors and abio-stressors. The plant secondary metabolites have been reported significant activities against many diseases including infectious diseases as well as different types of cancers (Calderón-Oliver, & Ponce-Alquicira, 2021). Phenolic compounds obtained from plants are well known for their antioxidant potential and known as food additives. These antioxidants are of great importance even with low concentration which reduce the harmful effects induced by reactive oxygen species ROS and free radicals (Hamady et al., 2015; Hayder et al., 2020). High levels of free

---

\*Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia, [A7-com@hotmail.com](mailto:A7-com@hotmail.com)

†Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia, [azari@kau.edu.sa](mailto:azari@kau.edu.sa)

‡Corresponding Author: Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia, [iullah@kau.edu.sa](mailto:iullah@kau.edu.sa)

radicals and ROS in an organism promote the development of a variety of chronic diseases (Abdelmonsef et al., 2024).

Foods derived from plants are abundant in a variety of phytochemicals and antioxidants, which have been associated with many health benefits (Hamid et al., 2022). The pomegranate (*Punica granatum* L.) is one of these that has drawn the most attention because of its high concentration of polyphenolic compounds, especially anthocyanins and ellagitannins, which have strong antibacterial and antioxidant qualities (Conidi et al., 2020). Phenolic compounds of plant origin are safe and cause no toxicity and play a good role in different biological systems, therefore, they are preferably used as metal chelators or free radical scavengers (Kupnik et al., 2022; Ko et al., 2021).

*P. granatum* has a number of secondary metabolites, including flavonoids, polyphenols, alkaloids, and many others. These metabolites are present in the fruit, peel, and other parts, responsible for their antioxidant activities (Eid et al., 2024). The antioxidant activities of the metabolites are due to free radical scavenging capability, which enables metabolites to neutralize reactive oxygen and nitrogen species effectively, causing oxidative stress leading to inflammation (Conidi et al., 2020). The antioxidant properties of metabolites are important to decrease oxidative stress, which cause cancer, heart disease, and many other diseases. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is widely used to evaluate the free radical scavenging ability of the metabolites (Heryanto et al., 2023).

*P. granatum* and its extract are used because the higher concentration of antioxidant metabolites is present in its fruit and peel. (Polcaro et al., 2023). The secondary metabolite profile of *P. granatum* through Gas Chromatography-Mass Spectrometry (GC-MS) enable us to identify the bioactive antioxidant metabolites in its extract (Bozkurt & Ergun, 2021). According to Bozkurt and Ergun (2021) this is the reason why the analysis of the plant-associated chemical components by GC-MS can yield the most general expressive profile of *Punica granatum* through its phytochemical composition.

The aim of the present study is to examine the chemical composition of *P. granatum* and assess its anti- antioxidant properties. The metabolites profiling is carried out through GC-MS to identify specific compounds in the extract of *P. granatum*. Moreover, the antioxidant properties of the extract are assessed using the DPPH assay. The analysis would be contributing to expanding information about phytochemical profile in the extract of *P. granatum*, as well as shedding light on its medicinal applications by counting the oxidative stress-related disorders (Hayder et al., 2020; Hussain et al., 2016; Kaderides et al., 2019).

## **Materials and Methods**

### ***Sample Collection***

The *P. granatum* samples are collected from a nearby market situated in Jeddah, Saudi Arabia, and the samples are dried in a shady and dry area. A sample weighing 500 g is ground into a fine powder and added with 2000 ml of 75% ethanol and incubated at  $25 \pm 2^{\circ}\text{C}$  for 48 h on continuous stirring. The ethanolic extract is evaporated using a rotary evaporator. The dehydrated residues are quantified and stored for future investigations.

### ***Gas Chromatography-Mass Spectrometry Analysis***

The dehydrated residues are filtered using and are added with ethanol (100%). The extracts are further dehydrated in a rotary evaporator under vacuum at a temperature of  $40^{\circ}\text{C}$  to prepare sample for the gas chromatograph coupled with a mass-selective detector (GC-MS, Agilent, USA). A sample is injected and the amount of 1  $\mu\text{l}$  and the helium (He) gas is used carrier and the flow rate is kept at 0.5 ml/min. the temperature  $250^{\circ}\text{C}$  is set as inlet temperature whereas the oven temperature is initially set at  $110^{\circ}\text{C}$  which reached  $240^{\circ}\text{C}$  after 4 h and persisted for 5 min at  $280^{\circ}\text{C}$ . The MS transfer line temperature is set at  $200^{\circ}\text{C}$ , whereas, the source temperatures are  $180^{\circ}\text{C}$ . Electron impact ionization at 70 eV in the GC-MS analysis with Total Ion Count (TIC) for identifying and quantifying compounds. Data are compared to the GC-MS database, to identify the names, molecular weights, and structures of test substances.

### ***Antioxidant Assay (Free radical scavenging)***

The antioxidant assay through DPPH known as stable free radicals to assess the ability of an antioxidant to neutralize reactive oxygen species. A series of sample solutions at concentrations from 100  $\mu\text{g}/\text{ml}$  to 700  $\mu\text{g}/\text{ml}$  are prepared for the analysis. Exactly 2 ml of DPPH solution is added into each concentration sample and incubated at  $25^{\circ}\text{C}$  in the dark for 1 h. Vitamin C (100 mg/ml) is used as an extra positive control in the same way and a blank solution is prepared by adding 1 ml of ethanol only in 2 ml of DPPH solution. After incubation, the absorbance of the solution is recorded at 517 nm using a spectrophotometer.

The free radical scavenging activity in percent (%) is calculated through the following formula:

$$\% \text{ Scavenging Activity} = [(A_b - A_a) / A_b] \times 100,$$

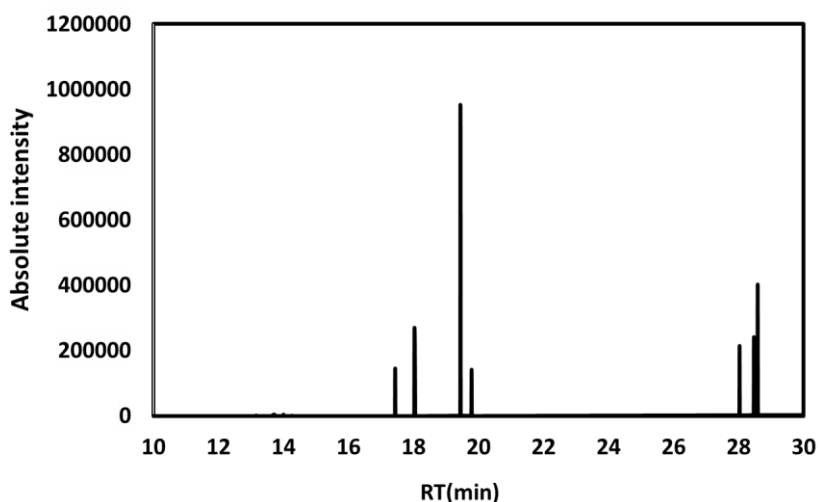
where  $A_b$  = the absorbance of the control (without antioxidant) and  $A_a$  = absorbance of the antioxidant sample.

### Statistical Analysis

Microsoft excel is used to analyze the data. The values are presented as of mean + SD and the analysis is carried out using one way and ANOVA.

### Results

The *P. granatum* is known for its significant medicinal value due to its diverse secondary metabolite profile. These bioactive metabolites play important roles in numerous physiological functions including immunity, signaling, defense and mechanisms. The GC-MS analysis of the ethanolic extract of *P. granatum* exhibited distinct peaks, indicating the presence of these metabolites (Figure 1).



**Figure 1:** The GC chromatogram of the metabolites in the extract. The chromatogram shows the height of the peak and retention time (RT).

The identified metabolites including Palmitic acid, methyl ester, 11,14-Octadecadienoic acid, methyl ester, 2-Bis(hydroxyphenyl)propane, Methyl octadecenoate, Naphthalene-1-sulfonic acid, Silane, [(10-iododecyl)oxy]trimethyl, Cyclodecasiloxane, eicosamethyl. The spectra of the identified metabolites are compared with standard compounds from the NIST libraries, and the metabolites are compiled in Table 1.

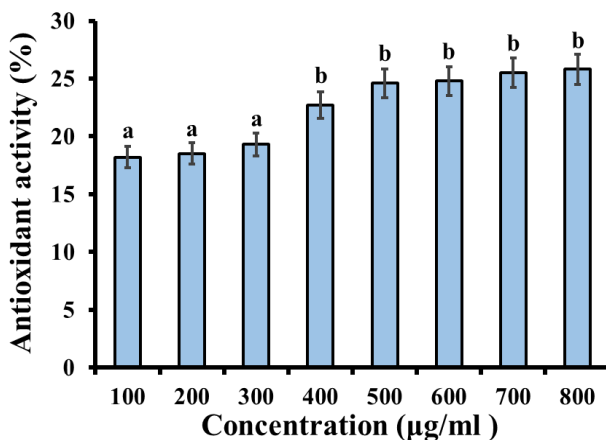
### Antioxidant Activities of the Extracts of Pomegranate Peels

The antioxidant potential of the extracts is assessed using DPPH scavenging assays. The ethanolic extract at a concentration of 100 µg/ml exhibited a scavenging efficiency of  $18.2 \pm 2\%$ . When the concentration

is increased up to 200 µg/ml and 300 µg/ml, the activity is recorded to be  $18.4 \pm 2\%$  to  $19.3 \pm 2.5\%$  which showed insignificant changes in antioxidant activities as compared to the activity at 100 µg/ml. However, at the concentration of 400 µg/ml, the antioxidant activity is 22.7%, which is significantly higher ( $p < 0.05$ ) as compared to the activity control and 100 µg/ml. Furthermore, the activity reached a 25% maximum at higher concentrations ranging from 500 µg/ml to 700 µg/ml (Figure 2).

**Table 1: List of the metabolites detected by GC-MS in the extract. The list shows the peak, retention time, peak area, height.**

Peak #	Ret. Time	Area	Height	A/H	Name
1	17.433	496357	268445	1.85	Palmitic acid, methyl ester
2	19.14	773241	410523	1.88	11,14-Octadecadienoic acid, methyl ester
3	19.78	95315	43196	2.21	2-Bis(hydroxyphenyl)propane;
4	20.712	469366	252574	1.86	Methyl octadecanoate
5	28.025	74794	6585	11.36	Naphthalene-1-sulfonic acid,
6	28.467	121618	11207	10.85	Silane, [(10-iododecyl)oxy]trimethyl-
7	28.581	211545	21639	9.78	Cyclodecasiloxane, eicosamethyl-



**Figure 2: the antioxidant activity of the extract assessed by DPPH scavenging assays. Different letter represent the level of significance ( $p < 0.05$ ).**

## Discussion

*P. granatum* has very high medicinal values and is rich with valuable nutrients and secondary metabolites (Ko et al., 2021). Previous studies have reported the extract of *P. granatum* is containing 17.9% fiber, 3.3% protein, 1.2% fat, essential vitamins, and minerals (Latiff et al., 2021). The bioassay of the secondary metabolite exhibited antimicrobial and antioxidant properties reported by Kupnik et al. (2022). The extraction efficacy of the metabolites is dependent on several factors e.g., solvent

type, extraction method, and temperature conditions. Sharmin et al. (2016) reported that high temperatures can enhance extraction efficiency by increasing cell wall permeability and decreasing solvent viscosity. Further they also reported that when temperatures reach more than 40°C, some metabolites such as flavonoids and polyphenols start degrading. Furthermore, studies showed that ethanol extraction resulted higher yields compared to other polar solvents used for the extraction (Ko et al., 2021; Latiff et al., 2021). Efficacy is attributed to the polar nature of flavonoids and phenolics metabolites which are better dissolved in the solvents like ethanol (Beghè et al., 2024).

GC-MS analysis of the present study revealed the list of metabolites including Palmitic acid, methyl ester, 11,14-Octadecadienoic acid, methyl ester, 2-Bis(hydroxyphenyl)propane, Methyl octadecenoate, Naphthalene-1-sulfonic acid, Silane, [(10-iododecyl)oxy]trimethyl, Cyclodecasiloxane, eicosamethyl. Previous studies reported the anticancer properties of extracts, building significant interest within the researchers to investigate natural sources for the development of innovative cancer treatments.

The growing interest in innovative cancer treatments is largely driven by the vast biological and chemical diversity found in natural resources. This diversity opens up promising avenues for the development of novel therapies (Kupnik et al., 2022; Ko et al., 2021; Latiff et al., 2021). Plant bioactive compounds, recognized for their antioxidant properties, are particularly noteworthy. These compounds work by inhibiting lipid peroxidation, scavenging oxygen radicals, modulating signaling pathways, and regulating gene expression (Abdelmonsef et al., 2024; Hussain et al., 2016).

In vitro studies by Hamady et al. (2015) demonstrated that pomegranate extract exhibited antioxidant activity comparable to BHT, and even surpassed BHA at concentrations of 25 ppm and higher (Abdelmonsef et al., 2024). Interestingly, pomegranate peel extract, rich in phenolic compounds, has been shown to outperform non-polar solvents like petroleum ether and chloroform in DPPH radical scavenging efficiency when compared with polar solvents such as ethanol, acetone, and ethyl acetate, as reported by Han et al. (2007). The DPPH assay results demonstrate that the antioxidant activity of pomegranate peeling extract is higher about 6 times than that of the seed extract, as reported by Sabraoui et al. (2022). The antioxidant capacity of phenolic compounds is considered to be due to the donation of hydrogen atoms to neutralize free radicals, as explained by Hamady et al. (2015).

Bioactive compounds in plants, particularly those rich in phenolics, show variety of antioxidant roles in plants, including inhibiting

lipid peroxidation, scavenging reactive oxygen species (ROS), and influencing gene expression (Kiran et al., 2024).

### Conclusion

This study determined the significant medicinal importance of *P. granatum*, showing diverse bioactive metabolites identified through GC-MS. The analysis resulted list of metabolites, including Palmitic acid, methyl ester, 11,14-Octadecadienoic acid, methyl ester, 2-Bis(hydroxyphenyl)propane, Methyl octadecenoate, Naphthalene-1-sulfonic acid, Silane, [(10-iododecyl) oxy]trimethyl, Cyclodecasiloxane, eicosamethyl. In addition, DPPH scavenging assays provided evidences of a concentration-dependent increase in the antioxidant activity of extracts. The results supported the use of *P. granatum* in pharmaceutical and nutraceutical values and its antioxidant properties.

### References

- Abdelmonsef, M., Shawky, E., Ghareeb, D.A., El Naggar, E.M.B., & El Newehy, N.M. (2024). Comprehensive metabolomics and chemometrics unravel potential anti-diabetic metabolites of pumpkin (*Cucurbita pepo* L.) fruits through UPLC-QqQ-MS and GC-MS analyses. *Food Research International*, 192: 114771.
- Abdel-salam, F.F., Moharram Y.G.E., & El-Zalaki, E.M. (2018). Characterization of wastes from pomegranate (*Punica granatum* L.) juice and its use as a functional drink. *Egypt Journal of Food Science*, 46: 91-100.
- Beghè, D., Cirilini, M., Beneventi, E., Dall'Asta, C., Marchioni, I., & Petruccielli, R. (2024). Exploring Italian autochthonous *Punica granatum* L. accessions: Pomological, physicochemical, and aromatic investigations. *Plants*, 13: 2558.
- Bozkurt, T., & Ergun, Z. (2021). Fatty acid composition and antioxidant capacity of pomegranate seed oil. *GSC Biological and Pharmaceutical Sciences*, 15(02): 103–110.
- Büyüktuncel, E. (2012). Gelişmiş ekstraksiyon teknikleri I. *Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi*, (32): 209-242.
- Calderón-Oliver, M., & Ponce-Alquicira, E. (2021). Environmentally friendly techniques and their comparison in the extraction of natural antioxidants from green tea, rosemary, clove, and oregano. *Molecules*, 26(7): 1869.
- Eid, A.M., Issa, L., Arar, K., et al. (2024). Phytochemical screening, antioxidant, anti-diabetic, and anti-obesity activities, formulation, and characterization of a self-nanoemulsion system loaded with

- pomegranate (*Punica granatum*) seed oil. *Scientific Reports*, 14: 18841.
- Hamady, A.A., Abdel-Moneim, M.A., El-Chaghaby, G.A., Abd-El-Ghany, Z.M., & Hassanin, M.S. (2015). Effect of Pomegranate peel extract as a natural growth promoter on the productive performance and intestinal microbiota of broiler chickens. *African Journal of Agricultural Science and Technology*, 3(12): 514-519.
- Hayder, Z., Walid, E., Ben, O.K., Ali, B.M., & Hannachi, H. (2020). Modeling of polyphenols extraction from pomegranate by-product using rotatable central composite design of experiments. *Acta Ecologica Sinica*.
- Heryanto, R., Putra, C.A., Khalil, M., Rafi, M., Putri, S.P., Karomah, A.H., & Batubara, I. (2023). Antioxidant activity and metabolite profiling of *Xylocarpus granatum* extracts using gas chromatography-mass spectrometry. *Metabolites*, 13(2): 156.
- Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M.C., & Rahu, N. (2016). Oxidative stress and inflammation: what polyphenols can do for us? *Oxidative Medicine and Cellular Longevity*.
- Kaderides, K., Papaoikonomou, L., Serafim, M., & Goula, A.M. (2019). Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction. *Chemical Engineering and Processing - Process Intensification*.
- Kiran, S., Aslam, A., Parveen, A., Dilshad, M., & Hussain, S. (2024). Phytochemistry of *Punica granatum* fruit: Its nutritional and biological potential. *Bioactivities*, 2(1): 57–73.
- Ko, K., Dadmohammadi, Y., & Abbaspourrad, A. (2021). Nutritional and bioactive components of pomegranate waste used in food and cosmetic applications: A Review. *Foods*, 10, 657.
- Kupnik, K., Leitgeb, M., Primožič, M., Postružnik, V., Kotnik, P., Kučuk, N., Knez, Z., & Marevci, M.K. (2022). Supercritical fluid and conventional extractions of high value-added compounds from pomegranate peels waste: production, quantification and antimicrobial activity of bioactive constituents. *Plants*, 11, 928.
- Latiff, N.A., Ong, P.Y., Abd Rashid, S.N.A., Abdullah, L.C., Amin, N.A.M., & Fauzi, N.A.M. (2021). Enhancing recovery of bioactive compounds from *Cosmos caudatus* leaves via ultrasonic extraction. *Scientific Reports*, 11:17297.
- Polcaro, L.M., Samani, M.R., Piacente, S., & Masullo, M. (2024). Development of a new extraction method for pomegranate and metabolite profiling by an LC-MS and <sup>1</sup>H NMR combined approach. *Foods*, 13: 1429.



- Sharmin, T., Ahmed, N., Hossain, A., Hosain, M.M., Mondal, S.C., Haque, M.R., Almas, M., & Siddik, M.A.B. (2016). Extraction of bioactive compounds from some fruits and vegetables (Pomegranate Peel, Carrot, and Tomato). *American Journal of Food and Nutrition*, 4(1): 8-19.
- Shaygannia, E., Bahmani, M., Zamanzad, B., & Rafieian-Kopaei, M. (2016). A review study on *Punica granatum* L. *Journal of Evidence-Based Complementary & Alternative Medicine*, 21(3): 221-227.