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

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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 Undecane, 3,7-dimethyl- Hexadecanoic acid methyl ester, 11,14-Octadecadienoic acid methyl ester, 6-Octadecenoic acid methyl ester, 9-Octadecenoic acid methyl ester, Methyl stearate, Phenol 4,4'-(1-methylethylidene), Naphthalene-1-sulfonic acid 4-methoxy-,(2-adamantan-1-ylethyl)amide, Silane, [(10-iododecyl)oxy]trimethyl, and 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 ± 2% to 25% as concentrations are increased from at 100 µg/ml to 700 µg/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

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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). Plant phenolics are in great demand because they are safe and low toxicity. As a result, they function well in a variety of biological systems as metal chelators or free radical scavengers. The use of medicinal plants as remedy against various diseases treat a variety of diseases (Kupnik et al., 2022; Ko et al., 2021).

P. granatum has a rich phytochemical composition; polyphenols, which make up the majority of the phytochemicals in the fruit, are primarily responsible for their antioxidant properties (Eid et al., 2024). These antioxidant metabolites exhibit free radical scavenging activities, enabling them effective in neutralizing reactive oxygen and nitrogen species that can contribute to oxidative stress and inflammation (Conidi et al., 2020). The antioxidants in *P. granatum* are essential for reducing oxidative stressors, which that could be the cause of a number of chronic diseases, including cancer, heart disease, and neurological conditions. The antioxidant potential of pomegranate can be assessed using various in vitro methods, one of which is the 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a widely used technique for evaluating the free radical scavenging ability of natural compounds, as it allows for the quantification of the antioxidant activity (Heryanto et al., 2023).

Due to the wide range of phytochemicals found in plants that are known to have therapeutic potential, plant metabolites and their bioactive qualities have attracted a lot of attention (Polcaro et al., 2023). phytochemical composition in P. granatum can be better understood by using Gas Chromatography-Mass Spectrometry (GC-MS) to profile the plant's chemical components (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 the expand information about phytochemical profile in the extract of *P. granatum* as well as would shed 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 shady and dry area. Sample weighed 500 g is ground into a fine powder and added with 2000 ml of 75% ethanol and incubated at 25 ± 2°C for 48 h on continuous stirring. The ethanolic extract is evaporated using a rotatory 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°C to prepared sample for the gas chromatograph coupled with a mass-selective detector (GC-MS, Agilent, USA). A sample is injected and the amount of 1 µl and the helium (He) gas is used carrier and the flow rate is kept at 0.5 ml/min. the temperature 250°C is se as inlet temperature whereases the oven temperature is initially set at 110°C which reached 240°C after 4 h and persisted for 5 min at 280°C. The MS transfer line temperature is set at 200°C, whoever, the source temperatures are 180°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 µg/ml to 700 µg/ml are prepared for the analysis. Exactly 2 ml of DPPH solution is added into each concentration sample and incubated at 25C 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:
% Scavenging Activity = [(Ab - Aa) / Ab] × 100,
where Ab = the absorbance of the control (without antioxidant) and Aa = 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 (Fig. 1).

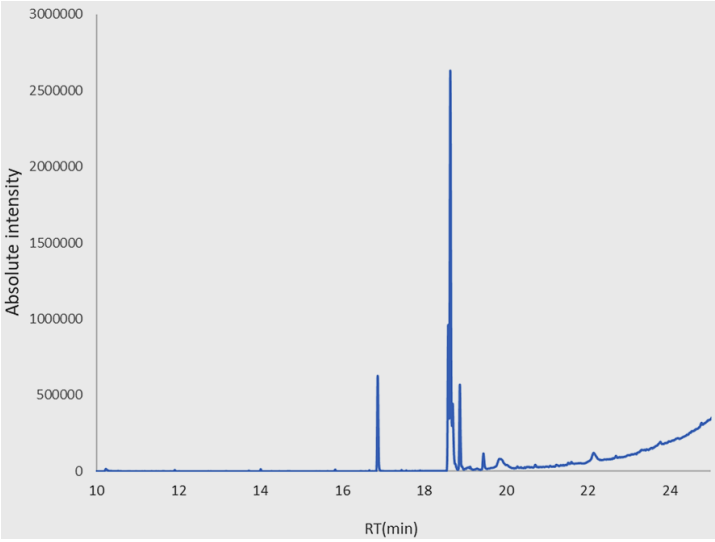

Figure 1: The GC chromatogram of the metabolites in the extract. The chromatogram shows the height of the peak and retention time (RT).

The identified metabolites include Undecane, 3,7-dimethyl-Hexadecanoic acid methyl ester, 11,14-Octadecadienoic acid methyl ester, 6-Octadecenoic acid methyl ester, 9-Octadecenoic acid methyl ester, Methyl stearate, Phenol 4,4'-(1-methylethylidene), Naphthalene-1-sulfonic acid 4-methoxy-,(2-adamantan-1-ylethyl)amide, Silane, [(10-

iododecyl)oxy]trimethyl, and Cyclodecasiloxane, eicosamethyl. These metabolites are determined based on their molecular weight, retention time, and concentration. The spectra of the identified metabolites are compared with standard compounds from the NIST libraries, and the metabolites are compiled in Table 1.

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	6.23	101082	27479	3.68	Undecane, 3,7-dimethyl-
2	16.853	496357	268445	1.85	Hexadecanoic acid, methyl ester
3	18.575	773241	410523	1.88	11,14-Octadecadienoic acid, methyl ester
4	18.626	2502182	1E+06	1.93	6-Octadecenoic acid, methyl ester, (Z)-
5	18.683	434350	199550	2.18	9-Octadecenoic acid, methyl ester, (E)-
6	18.86	469366	252574	1.86	Methyl stearate
7	19.435	95315	43196	2.21	Phenol, 4,4'-(1-methylethylidene)bis-
8	28.025	74794	6585	11.4	Naphthalene-1-sulfonic acid, 4-methoxy-, (2-adamantan-1-ylethyl)amide
9	28.467	121618	11207	10.9	Silane, [(10-iododecyl)oxy]trimethyl-
10	28.58	211545	21639	9.78	Cyclodecasiloxane, eicosamethyl-

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 ± 2%. When the concentration is increased up to 200 µg/ml and 300 µg/ml, the activity is recorded to be 18.4 ± 2% to 19.3 ± 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 (Fig. 2).

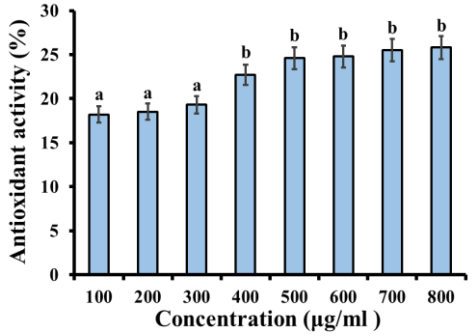

Fig.2: the antioxidant activity of the extract assessed by DPPH scavenging assays. Different letter represent the level of significance (p< 0.05).

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 Undecane, 3,7-dimethyl- Hexadecanoic acid methyl ester, 11,14-Octadecadienoic acid methyl ester, 6-Octadecenoic acid methyl ester, 9-Octadecenoic acid methyl ester, Methyl stearate, Phenol 4,4'-(1-methylethylidene), Naphthalene-1-sulfonic acid 4-methoxy-,(2-adamantan-1-ylethyl)amide, Silane, [(10-iododecyl)oxy]trimethyl, and 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 Undecane, 3,7-dimethyl- Hexadecanoic acid methyl ester, 11,14-Octadecadienoic acid methyl ester, 6-Octadecenoic acid methyl ester, 9-Octadecenoic acid methyl ester, Methyl stearate, Phenol 4,4'-(1-methylethylidene), Naphthalene-1-sulfonic acid 4-methoxy-,(2-adamantan-1-ylethyl)amide, Silane, [(10-iododecyl)oxy]trimethyl, and 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.

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