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Evaluation of Antioxidant Activities of Methanolic Extract Leaves of Punica Granatum Linn

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A B S T R A C T

Pomegranate (Punica granatum L.), an edible fruit native to Persia that is cultivated and consumed worldwide, including in Iran, has been esteemed throughout history for its medicinal properties. Pomegranate leaves serve as a valuable source of potentially beneficial bioactive compounds. This study aimed to explore the in vitro antioxidant properties of the methanolic extract obtained from Pomegranate leaves (Punica granatum L.) using the DPPH radical scavenging assay. The assessment of antioxidant activity in Punica granatum L. leaves demonstrated that the methanolic extract exhibited a promising IC50 value of 120.345 µg/ml, in comparison to the reference standard ASA, which had an IC50 of 90.545 µg/ml in the DPPH assay. These significant biological activities suggest that P. granatum leaves could potentially serve as a source of active compounds with applications in the pharmaceutical industry, contingent upon further in vivo experiments.

Keywords: *Punica granatum. Antioxidant, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Plant extracts, Plant leaves*

1. Introduction

Pomegranate (Punica granatum L.) is a prominent commercial fruit crop extensively cultivated in various regions, including parts of Asia, North Africa, the Mediterranean, and the Middle East (1). Pomegranate fruits find wide consumption, whether eaten fresh or processed into juice, jams, syrup, and sauce. The edible part, known as the aril, constitutes approximately 55-60% of the total fruit weight, comprising roughly 75-85% juice and 15-25% seeds (2). It's worth noting that commercial pomegranate juice (PJ) boasts one of the highest antioxidant activities when compared to other fruit juices, red wine, and green tea (3). This can be attributed to its high polyphenol content, primarily ellagitannins, with the major components being punicalagins (3, 4). (Note that punicalagins naturally exist in two reversible anomers, R and β, hence the plural form) Additionally, pomegranates contain various polyphenols like condensed tannins, anthocyanins, and minor flavonoids (5). Recent research has highlighted the remarkable antioxidant activity of extracts from different parts of the pomegranate fruit, including the peel, juice, and seeds (3, 6, 7). Pomegranate juice stands out with its superior antioxidant capacity compared to other fruit juices

and beverages (8). This heightened antioxidant potential is attributed to its substantial phenolic compound content (3). Pomegranates are known to contain a variety of phenolic compounds, such as anthocyanins (3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin), ellagic acid, punicalin, punicalagin, pedunculagin, and various flavanols (9). Pomegranate has gained recent attention due to its nutritional and antioxidant properties. Al-Maiman and Ahmad (2) conducted an analysis of the physical and chemical changes during pomegranate fruit maturation (10). Others have explored the chemical and antioxidant properties of pomegranate cultivars cultivated in the Mediterranean region of Turkey (11-14). It's important to note that the composition of pomegranate fruit is significantly influenced by factors such as the cultivar type, growing region, climate, maturity, and agricultural practices (11-14). Furthermore, numerous studies have reported noteworthy variations in organic acids, phenolic compounds, sugars, and water-soluble vitamin composition of pomegranates across different years (2, 10, 13, 15-17). These parameters are of particular importance for consumers seeking to recognize and select more nutritious fruits.

2. Methods and Materials

The methodology employed in this research involves a series of structured steps to assess the antioxidant activities of methanolic extracts from Punica granatum L. (pomegranate) leaves. It begins with the collection and preparation of the plant material, followed by the extraction of bioactive compounds using methanol.

2.1. Plant Material

The Leaves of Punica granatum L. were collected from the Jessore region in Bangladesh. The authenticity of the plant leaves was verified at the Bangladesh National Herbarium, Mirpur, Dhaka. The leaves were sun-dried for several days and then oven-dried for 24 hours at a controlled low temperature (not exceeding 40°C) to facilitate grinding. The dried leaves were subsequently ground into a coarse powder using a high-capacity grinding machine.

2.2. Extraction

A quantity of 300 grams of the powdered material was placed in a clean amber-colored reagent bottle (2.5 liters) and soaked in 2.0 liters of methanol. The container, along with its contents, was sealed with a bottle cap and left undisturbed for 15 days, with occasional shaking and stirring. The entire mixture was then filtered through a fresh cotton plug and, finally, through a Whatman No.1 filter paper. The filtrate was allowed to evaporate at ambient temperature until approximately 70% of the solvent had evaporated.

2.3. Solvent-Solvent Partitioning

Solvent-solvent partitioning was conducted following the method initially designed by Kupchan and later modified by Wagnen ((18)). The crude extract (5 grams) was dissolved in 10% aqueous methanol.

2.4. Antioxidant Test

DPPH Assay. The free radical scavenging activity was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (20,21=14,15). Ascorbic acid and tert-butyl-1-hydroxytoluene were used as positive controls. A total of 20 milligrams of DPPH powder was weighed and dissolved in methanol to prepare a DPPH solution with a concentration of 20 µg/mL. The solution was placed in an amber reagent bottle and stored in a lightproof container. Approximately 2.0 mL of a methanol solution of the sample (extract/control) at various concentrations (ranging from 500 to 0.977 µg/mL) was mixed with 3.0 mL of a DPPH methanol solution (20 µg/mL). After a 30-minute incubation at room temperature in a dark place, the absorbance was measured at 517 nm using a UV spectrophotometer against methanol as a blank. The inhibition of the DPPH free radical in percentage (I%) was calculated as follows:

I%=(1-Asample/Ablank) ×100 where, Ablank is the absorbance of the control reaction (containing all reagents except the test material). Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract concentration.

3. Findings

The antioxidant activity of the aqueous crude extracts of Punica granatum leaves was evaluated using the DPPH method. The IC50 values, a measure of the antioxidant potency, were determined for the methanol extract of

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pomegranate leaves and compared to Ascorbic Acid (ASA) as a reference standard. The results indicated that the methanol extract exhibited mild free radical scavenging activity with an IC50 value of 120.345µg/ml. In contrast, the reference standard Ascorbic Acid demonstrated a more potent antioxidant activity with an IC50 value of 90.545µg/ml. These findings are presented in Figure 1 and Figure 2.

Figure 1

DPPH free Radical scavenging activity curve for ascorbic acid (Standard)

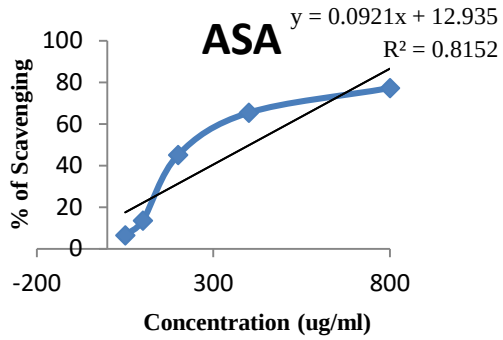
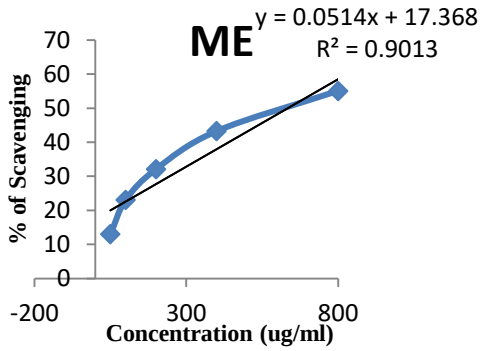


Figure 2

DPPH free Radical scavenging activity curve of methanol extract of Punica granatum leaves



4. Discussion

The results of this study have unveiled significant biological activities associated with the methanolic extract of Punica granatum leaves, offering promising prospects for potential applications in the pharmaceutical industry. While the antioxidant activity of these extracts has been well-demonstrated, it is essential to acknowledge that their utilization in a pharmaceutical context hinges on further in vivo experiments. Oxidative stress, characterized by the generation of substantial quantities of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS), is an early response to stress and has implications for disease and aging in animals (19). To counteract ROS-induced oxidative damage, organisms rely on their antioxidative defense systems. However, the use of synthetic antioxidants is limited due to concerns about potential carcinogenicity (7), leading to the increasing importance of natural antioxidants.

This study focused on investigating the antioxidant activity using the DPPH assay in methanol extract (ME) prepared from Punica granatum leaves. The principle of the DPPH method is based on the interaction between antioxidants and a stable free radical, 2,2-diphenyl-1-picrylhydrazyl, causing a change in color as the radical is converted to 2,2-diphenyl-1-picrylhydrazine. The extent of discoloration serves as an indicator of the sample's scavenging potential (20). When the IC50 value is lower, it indicates higher scavenging ability. The results displayed in Figure 1 and Figure 2 show the IC50 determinations for the extracts. Previous studies have suggested that the antioxidant activity of pomegranate leaves is positively correlated with the content of total phenolics and flavonoids (21). Phenolic acids and flavonoids, common natural antioxidants in fruits and vegetables, are also present in pomegranate leaves. Reports have shown that aqueous extracts of pomegranate leaves exhibit potent radical scavenging capacity against reactive oxygen species (18). Additionally, a radical scavenging antioxidant, punicalagin, has been isolated from pomegranate fruit, suggesting an electron-donor mechanism for its antioxidant action (22). It is plausible that the antioxidant activity of pomegranate leaves results from a combination of phytochemicals, including phenolic acids and flavonoids, which are recognized natural antioxidants. Previous research has highlighted the dependence of pomegranate leaf antioxidant activity on total phenolics, flavonoids, and harvest time (18). It is worth noting that the antioxidant activity of the extracts in this study exceeded that reported by Kaneria et al. (23).

5. Conclusion

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The results of this study demonstrate that the methanol extract of pomegranate leaves exhibits mild antioxidant activity. This suggests that the methanolic extract, pending further investigations into its potential health benefits for experimental animals in in vivo studies, could serve as a natural source of potent antioxidant compounds. Such compounds might find application as natural additives in the food and pharmaceutical industries. Moreover, it holds promise for further exploration and development as valuable nutraceuticals. However, additional research is necessary to evaluate in vivo biological activities and identify the specific phytochemicals responsible for the observed antioxidant properties.

6. Limitations and Suggestions

While this study provides valuable insights into the antioxidant potential of Punica granatum leaf extracts, several limitations need to be considered. The study primarily focused on in vitro antioxidant assays. To establish the practical applicability of these findings, it is crucial to conduct in vivo experiments on experimental animals to determine the health benefits and potential side effects. Although the overall antioxidant activity is demonstrated, further research is needed to identify and quantify the specific phytochemical compounds responsible for these properties. To ensure safety and efficacy, it is important to determine the optimal dosages for potential use as nutraceuticals or pharmaceutical additives. The study acknowledges variations in antioxidant activity based on factors such as total phenolics, flavonoids, and harvest time. Further investigations can delve into these variables to optimize the extraction process. Addressing these limitations and conducting additional research will provide a more comprehensive understanding of the antioxidant properties of Punica granatum leaf extracts and their potential applications.

Authors' Contributions

I.A: wrote the manuscript and performed the calculations. U.A: supervised the project and verified the results. A.H.B: did the literature review and revised the manuscript

Transparency Statement

The authors are willing to share their data, analytics methods, and study materials with other researchers. The material will be available upon reasonable request.

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Declaration of Interest

All authors declare that they have no conflict of interest and therefore have nothing to declare.

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References

1. Sarkhosh A, Zamani Z, Fatahi R, Ebadi A. RAPD markers reveal polymorphism among some Iranian pomegranate (Punica granatum L.) genotypes. Scientia Horticulturae. 2006;111(1):24-9. https://doi.org/10.1016/j.scienta.2006.07.033
2. Al-Maiman SA, Ahmad D. Changes in physical and chemical properties during pomegranate (Punica granatum L.) fruit maturation. Food Chemistry. 2002;76(4):437-41. https://doi.org/10.1016/S0308-8146(01)00301-6
3. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food chemistry. 2000;48(10):4581-9. [PMID: 11052704] . https://doi.org/10.1021/jf000404a
4. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem. 2005;16(6):360-7. [PMID: 15936648] . https://doi.org/10.1016/j.jnutbio.2005.01.006
5. Aviram M. Pomegranate juice as a major source for polyphenolic flavonoids and it is most potent antioxidant against LDL oxidation and atherosclerosis. Free Radical Research. 2002;36:71-.
6. Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, et al. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr. 2000;71(5):1062-76. [PMID: 10799367] . https://doi.org/10.1093/ajcn/71.5.1062
7. Singh RP, Chidambara Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models. J Agric Food Chem. 2002;50(1):81-6. [PMID: 11754547] . https://doi.org/10.1021/jf010865b

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E-ISSN: 2981-2569

8. Seeram NP, Aviram M, Zhang Y, Henning SM, Feng L, Dreher M, Heber D. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J Agric Food Chem. 2008;56(4):1415-22. [PMID: 18220345] . https://doi.org/10.1021/jf073035s
9. González-Molina E, Moreno DA, García-Viguera C. A new drink rich in healthy bioactives combining lemon and pomegranate juices. Food Chemistry. 2009;115(4):1364-72. https://doi.org/10.1016/j.foodchem.2009.01.056
10. Ozgen M, Durgaç C, Serçe S, Kaya C. Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food chemistry. 2008;111(3):703-6. https://doi.org/10.1016/j.foodchem.2008.04.043
11. Heshi A, Garande V, Wagh A, Katore H. Effect of pre-harvest sprays of chemicals on the quality of pomegranate fruit (Punica granatum L.). Cv. G-137. Agricultural Science Digest. 2001;21(1):25-7.
12. Holcroft DM, Gil MI, Kader AA. Effect of carbon dioxide on anthocyanins, phenylalanine ammonia lyase and glucosyltransferase in the arils of stored pomegranates. Journal of the American Society for Horticultural Science. 1998;123(1):136-40. https://doi.org/10.21273/JASHS.123.1.136
13. Melgarejo P, Salazar DM, Artes F. Organic acids and sugars composition of harvested pomegranate fruits. European Food Research and Technology. 2000;211:185-90.
14. Özkan M. Degradation of anthocyanins in sour cherry and pomegranate juices by hydrogen peroxide in the presence of added ascorbic acid. Food Chemistry. 2002;78(4):499-504. https://doi.org/10.1016/S0308-8146(02)00165-6
15. Kulkarni AP, Aradhya SM. Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food chemistry. 2005;93(2):319-24. https://doi.org/10.1016/j.foodchem.2004.09.029
16. Poyrazoğlu E, Gökmen V, Artık N. Organic acids and phenolic compounds in pomegranates (Punica granatum L.) grown in Turkey. Journal of food composition and analysis. 2002;15(5):567-75. https://doi.org/10.1006/jfca.2002.1071
17. Tezcan F, Gültekin-Özgüven M, Diken T, Özçelik B, Erim FB. Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food chemistry. 2009;115(3):873-7. https://doi.org/10.1016/j.foodchem.2008.12.103
18. Zhang L, Gao Y, Zhang Y, Liu J, Yu J. Changes in bioactive compounds and antioxidant activities in pomegranate leaves. Scientia Horticulturae. 2010;123(4):543-6. https://doi.org/10.1016/j.scienta.2009.11.008
19. Melov S. Animal models of oxidative stress, aging, and therapeutic antioxidant interventions. Int J Biochem Cell Biol. 2002;34(11):1395-400. [PMID: 12200034]. https://doi.org/10.1016/S1357-2725(02)00086-9
20. Soria EA, Goleniowski ME, Cantero JJ, Bongiovanni GA. Antioxidant activity of different extracts of Argentinian medicinal plants against arsenic-induced toxicity in renal cells. Hum Exp Toxicol. 2008;27(4):341-6. [PMID: 18684805] . https://doi.org/10.1177/0960327108092192
21. Adhami VM, Mukhtar H. Polyphenols from green tea and pomegranate for prevention of prostate cancer. Free Radic Res. 2006;40(10):1095-104. [PMID: 17015254] . https://doi.org/10.1080/10715760600796498
22. Kulkarni AP, Aradhya SM, Divakar S. Isolation and identification of a radical scavenging antioxidant–punicalagin from pith and carpellary membrane of pomegranate fruit. Food chemistry. 2004;87(4):551-7. . https://doi.org/10.1016/j.foodchem.2004.01.006
23. Kaneria MJ, Bapodara MB, Chanda SV. Effect of extraction techniques and solvents on antioxidant activity of pomegranate (Punica granatum L.) leaf and stem. Food Analytical Methods. 2012;5:396-404. https://doi.org/10.1007/s12161-011-9257-6