Phytochemical and antioxidant study of leaves extract of Punica granatum

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Abstract:

Objective: This research paper aimed to assess the hemolytic activity of solvent extracts derived from Punica granatum leaves using in vitro assays. Additionally, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted on the solvent extracts to identify their chemical composition. Furthermore, the thrombolytic potential of the Punica granatum leaf extracts was investigated through in vitro experiments. Lastly, the antibacterial and antifungal activities of the ingredients were determined against various pathogen genera. Through these methodologies, the study aimed to provide insights into the potential biomedical applications of Punica granatum leaf extracts.

Introduction:

Punica granatum, a native shrub in western Asia, is used in traditional medicine for extracting bioactive chemicals from fruit peels, and as a vermifuge or taenicide for intestinal worm treatment(Das et al., 1999).Punica granatum, a native shrub in western Asia, is used in traditional medicine for extracting bioactive chemicals from fruit peels, and as a vermifuge or taenicide for intestinal worm treatment (Satomi et al., 1993). Pomegranates are a popular shrub with various unique varieties, including Wonderful, Haku Botan, Nana, Red Silk, Eversweet, Angel Red, Purple Heart, Gulosha, Molar de Elche, and Parfianka. Pomegranate leaves extract, derived from the fruit's outer skin, contains bioactive substances beneficial for health (Yasoubi, Barzegar, SAHARI, & Azizi, 2007). It's used in traditional medical systems for treating diseases like microbial infections, diabetes, and cardiovascular problems. Pomegranate leaves are rich in antioxidants, which help the body remove free radicals and reduce oxidative stress(Jalikop, 2003). The intense red color of the peel is due to these polyphenols. Pomegranate leaves have been used as a dye for textiles since antiquity(Parmar, Kanwar, & Thakur, 2012).This study aims to extract antioxidants from pomegranate peel and investigate its potential for nutritional supplements(Ismail, Sestili, & Akhtar, 2012). Pomegranate leaves extract has been investigated for its anti-inflammatory effects, including reduced inflammation in joints, gastrointestinal health support, and skin relief. Clinical trials are needed to determine its efficacy and safety(Deshpande, Sengupta, & Raghuvanshi, 2014). Pomegranate leaves extract (PLE) has antibacterial and antifungal properties due to its high concentration of bioactive components, including tannins, polyphenols, and flavonoids(Casquete et al., 2015). Its broad-spectrum antimicrobial activity targets both positive

andnegative gram-positive bacteria, disrupting cell walls and inhibiting biofilm formation(Hashem et al., 2023). PLE has potential applications in developing new antibiotics, food preservatives, and combating antibiotic resistance. However, further research is needed to evaluate its safety and efficacy in real-world applications. Its antifungal properties could be used in antifungal creams, ointments, mouthwashes, and dietary supplements.Pomegranate leaves contain phytochemicals, antioxidants, and bioactive elements. However, there is limited research on their thermolytic potential. High temperatures may cause specific products from thermal breakdown. Variables like plant variety and extraction technique can affect bioactive.

Experimental Work:

Sample Collection:

Pomegranates, or *Punica grantum*, are grown in Pakistan. This plant grows gregariously on arid limestone soils inside the Salt Range in Baluchistan, North and South Waziristan, NWFP (now Khyber Pakhtunkhwa), Kurram, Dir, and Chitral. It can also be found in the Himalayan and Kashmir regions. Pomegranate peels were extracted from them. We received assistance in identifying and validating the plant samples that were gathered from the taxonomist at the Department of Chemistry, Riphah University Faisalabad, in Faisalabad, Pakistan. The pomegranate peels were sun-dried for fifteen days. After being finely ground, it was stored in fine jars and turned into a fine powder.

Extraction from plant materials:

A round-bottom flask was gently washed, dried, and cleaned in methanol to remove contaminants. The flask was then baked dry. A 0.1-gram pomegranate peel sample was weighed and transferred to a round-bottom flask filled with solvent. The flask was heated and bathed in water, then refluxed for two hours. The pipette, beaker, spatula, and magnetic beat were also rinsed with methanol. The process was repeated for pomegranate seeds sample.

Determination of antioxidant activity of plant extracts:

DPPH Radical Scavenging Activity:

The DPPH free radical scavenging test involves a mixture of DPPH and pomegranate extract, then diluted and measured using a UV spectrophotometer. The absorbance difference between the samples is used to calculate the percentage of radical scavenging activity, based on the difference in absorbance between the samples.

Radical scavenging (%) = [(A0 - A1 / A0) × 100]

where A1 represents the absorbance of the sample extracts and A0 represents the absorbance of the control.

Determination of Total Flavonoids

The colorimetric approach with a Each extract was mixed with aluminum chloride was utilized to determine the total flavonoid concentration. Every extract was combined with (0.08 L) of 2% $AlCl_3$. 5 ml of CH_3CO_2K (5%), afterward, was included. For forty minutes, the solution was left at room temperature. This reaction mixture's absorbance at 425 nm was determined applying a spectrophotometer. Several substance equivalent concentrations were utilized for the calibration curve plotting.

Total Phenolic Content

The folin ciocalteu reagent applied to determine the amount of phenol overall of pomegranates based on 8. Add 0.9 ml of folin ciocalteu (10%) to 0.5 ml of pomegranate extract sample. Samples were blended and 1 mL of Na_2CO_3 at 7.5% (w/v) added. After stirring the mixture, it was left to settle for six minutes. After allowing solutions to stand at room temperature for two hours, at 755 nm, A measurement of the absorbance was pmade. with a spectrophotometer. Expressed the results in Gallic acid equivalents in ml.

Determination of Total Carotenoids

Using the procedure outlined by Talcott and Howard (1999), the total amount of carotenoids was derived with a few minor changes. A 2-gm sample was extracted with a solvent mixture consisting of 25 milliliters of ethanol and acetone in a 1:1 volume ratio. A fortification of 200 mg/L of butylated hydroxytoluene was added to the extraction solvent. Every procedure performed in a room with a fluorescent bulb that produced a yellow light to prevent changes brought on by light (Thorn). After separation, the material was centrifuged for 15 minutes at 1500 g and 4 to 5°C. After finished product gathered, the remaining residue removed again using the same method until it was clear. Ultimately, the absorbance was measured at with a spectrophotometer 470 nm after the supernatants were combined with 100 ml of the solvent used for extraction. The number of total carotenoids, represented in milligrams per g of dry weight, was computed as follows:

Total carotenoids = Ab× V× 10^6 / A^1%× 100G

Where A^1% is the extinction coefficient for a 1% combination of carotenoids at 2500 nm, G is sample weight, V is volume of the total extract, and Ab is the absorbance at 470 nm.

Characterization:

Fourier Transform Infrared Spectrometer

All of prepared samples underwent characterization by FT-IR in order to analyze their functional groups

Result and Discussion:

Extraction of Yield

The antioxidant extract production from Qandhari and Golden pomegranate leaves showed significant heterogeneity, with yields varying from 7.32 to 16.21 g/100g of dry stuff. Water from Golden fruit yielded the least (21%), while distilled water from Golden pomegranate fruit yielded the highest (36%). The solvent extraction capabilities of aqueous ethanol, aqueous methanol, absolute ethanol, absolute methanol, ethyl acetate, n-hexane, and distilled water were arranged in order of their effectiveness. The study reveals that the antioxidant extract from golden and Qandhari pomegranate leaves is highest in 80% methanol, while in 100% ethanol, it yields 12.89 g/100g. In n-hexane, it yields 8.16g/100g. The highest yield of leaf extracts was found at an 80% ethanol content, indicating that ethanol is a more effective solvent for extracting antioxidant components. Distilled water from golden

pomegranate leaves yields the lowest.The study reveals that using 100% methanol yields 31.33 g/0.1kg from Qandhari and 30.46 g/0.1kg of solid content from Golden pomegranate seeds. When using 80% Methyl Alcohol, the protective agent extracts yield 34.19 g/0.1kg and 34.67 g/0.1kg of solid content. In 80% ethanol, the antioxidant extracts yield 32.13 g/0.1kg and 29.31 g/0.1kg of solid content.In distilled water, the antioxidant extracts yield 27.51 g/0.1kg and 27.21 g/0.1kg of solid content.

Sr	Extracts	Qandhari Leaves (g/100g)	Golden Leaves (g/100g)
1	80 % EtOH	16.23 ± 0.23	15.33 ± 0.23
2	Distilled Water	6.88 ± 0.23	8.23 ± 0.23

Assay for Scavenging DPPH Radicals

DPPH is a persistent organic free radical that has a vivid violet color. This causes absorption peaks between 515 and 528 nm. A hydrogen donor, usually phenolics, contributes a proton, causing the substance to become yellow. Leaf extracts are known to have a greater capacity to scavenge DPPH radicals and, as a result, act as antioxidants when the amount of components

Sr	Extracts	Qandhari Leaves(GAE)	Golden Leaves (GAE)
1	80% EtOH	14.33 ± 0.02	15..55 ± 0.02
2	Distilled Water	8.76 ± 0.02	8.33 ± 0.02

that are phenolic or the degree of hydroxylation increases. Extracts of Qandhari, golden pomegranate leaves, with IC_{50} values ranging from 0.052 to 0.309 mg/mL and 0.030-0.164 mg/mL, respectively, exhibited radical scavenging action. With an IC_{50} value as low as 0.070 mg/mL, the ethanol-based leaf extract demonstrated the highest free radical scavenging efficacy. With an IC_{50} value as low as 0.030 mg/mL, the ethanol-based leaf extract exhibited the highest free radical scavenging activity. This was most likely due to its reduced phenolic concentration when compared to the other extracts. The Qandhari and golden pomegranate leaves exhibit DPPH radical scavenging activity with IC50 values of 0.107 and 0.087 mg/mL, respectively. Extracts from these leaves in

Sr	Extracts	Qandhari Leaves(mg/mL)	Golden Leaves (mg/mL)
1	Distilled Water	0.309 ± 0.40	0.164 ± 0.40
2	80% MtOH	0.070 ± 0.40	0.054 ± 0.40

80% methanol yielded IC50 values of 0.070 and 0.054 mg/mL, respectively. The leaves also produced extracts with IC50 values of 0.079 mg/mL and 0.062 mg/mL, respectively. The Qandhari leaf extract in aqueous ethanol showed the highest DPPH radical scavenging activity.

Total Flavonoid Content

Research emphasizes the significance of total flavonoids in evaluating antioxidant potential in fruits and vegetables, with Qandhari and golden pomegranate extracts demonstrating high total phenolic content. When Qandhari and golden pomegranate leaves were extracted in various solvents, the total flavonoids that were found ranged from 8.76 to 14.33 GAE (milligram per 100 gram) and 8.33 to 14.55 CE (milligram per 100 gram). Under different solvent conditions, there were significant differences in the number of flavonoids extracted from the leaves of Qandhari and

Golden pomegranates ($p<0.05$). Varying extraction solvents' abilities to dissolve naturally occurring floral antioxidant chemicals could be the reason for variations in Total Flavonoid Content.

Total Flavonoid Content

The importance of total flavonoids in assessing the antioxidant potential of fruits and vegetables has been reinforced by a number of research. Consequently, it's imperative that you make sure for flavonoids in freshly harvested plant. The total phenolic content found in the Qandhari and golden pomegranate extracts has been demonstrated. When Qandhari and golden pomegranate leaves were extracted in various solvents, the total flavonoids that were found ranged from 7.7 to 13.20CE (milligram per 100 gram) and 8.10 to 14.55 CE (mg/100g).

Sr	Extracts	Qandhari Leaves(CE)	Golden Leaves (CE)
1	80% EtOH	13.20 ± 0.02	14.55 ± 0.02
2	Distilled Water	7.70 ± 0.02	8.10 ± 0.02

FT-IR of Pomegranate leaves

The FT-IR spectra of compound revealed the presence of C-H peak at 1483 cm-1, While C-C peak at 2369 cm-1. Further peak of C-OH appeared at 830 cm.

Conclusion:

The results indicated Pomegranate leaves possess significant antioxidant properties. The effect can be enhanced by modifications in the extraction methodology. The method should be efficient way for extracting frompomegranate leaves.

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