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Extraction Of Biological Active Compounds From Pomegranate Peels

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Abstract: *In India, pomegranate peel (PP) is a notable agri-food by-product due to its rich content of bioactive compounds. Recently, interest in the extraction and application of these bioactive compounds has grown significantly, especially for various disease management applications. In response to an increasing focus on extraction techniques, this study investigates the yield of bioactive compounds from PP. Ripened fruit samples of two pomegranate varieties namely Mridula and Ganesh were sourced from Azadpur fruit market, New Delhi (28°42′47″N 77°10′36″E). Using cold percolation and soxhlet extraction methods, the study obtained pomegranate peel extracts and measured their yields. The results demonstrated that ethanol as a solvent produced the highest extraction yields for both Ganesh and Mridula varieties, at 36.78% and 39.64%, respectively using cold percolation as the extraction method with a dipping duration of 48 hours.*
Keywords: *Extraction, Utilization, Pomegranate peel, Bioactive compounds, Phenolic compounds, Antioxidant activity*

INTRODUCTION

Pomegranate (Punica granatum L.) is one of the most important indigenous fruits cultivated in many countries. In pomegranates, a high percentage of the fruit is converted to waste that varies from some percent depending on the varieties [1,2]. The industrial processing of the fruit generates huge amounts of by-products, mainly peels and seeds, which are discarded as waste. Among the residues, pomegranate peel is an excellent source of phenolic compounds like flavonoids, phenolic acids, and tannins[3,4]. Pomegranate peel has bioactive compounds that could be exploited as functional ingredients and offer additional value to the industry. Bioactive compounds have actions that promote good health[5,6]. The utilization of by-products from pomegranate processing, which are abundant in beneficial bioactive compounds, has the potential to contribute to the development of a wide range of products. Extraction is a key step for obtaining antioxidants with an acceptable yield. Several extraction techniques have been reported for the extraction of phenolic compounds from different matrices using solvents with different polarities, such as methanol, ethanol, acetone and distilled water. Figure 1 pictures the extraction from pomegranate peels.

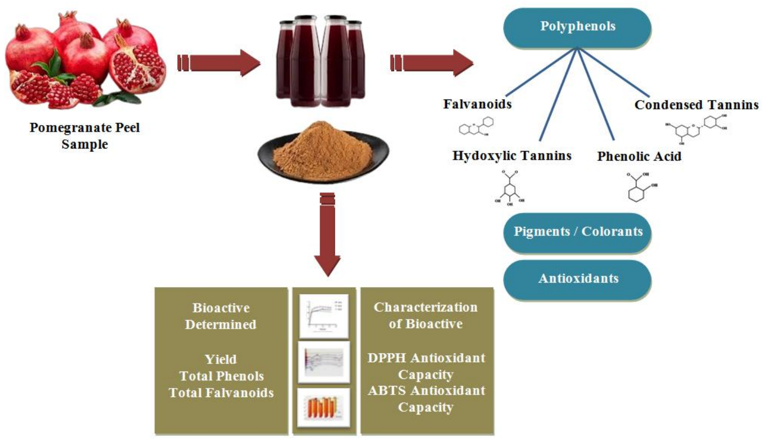

Figure 1: Graphical Abstract of bioactive compounds of pomegranate peels

The bioactive compounds include functional ingredients that improve the quality of food and additives employed in the food industry to extend the shelf life of products [7, 8 and 9]. PP is one of the major by-products of the pomegranate juice industry [10]. Existing studies focused on various methods of extraction and utilization of pomegranate peels. Many studies expressed these views, particularly on the extraction of bioactive compounds. However, only a few studies have explained the evaluation of anti-oxidant activity and tannin estimation. Therefore, the study analyzed the effectiveness of the extraction and utilization of pomegranate peels using cold percolation and soxhlet methods. The objective of the research is to illustrate the yield obtained from different parts of pomegranate, namely arils, juice, seeds and peels, by cold percolation and the soxhlet method, using four different solvents (distilled water, methanol, ethanol and acetone) and three different time frames.

LITERATURE REVIEW

Mariana P. Santos *et.al* 2019 illustrated the extraction of biologically active compounds from PP. The collected sample was analyzed using statistical analysis. The results of the study revealed that the combination of PLE was used to improve the extraction of phenolic compounds from pomegranate peels. Moreover, the study results were produced with an ultrasound power between 400 and 600 W combined with expansion gas pressure between 5 and 10 bar.Paula Garcia *et.al, 2021* investigated the recovery of bioactive compounds from the peel of pomegranate using pressurized liquid extraction. Pomegranate fruits were collected at the ripening stage. Pomegranate peel extract and pressurized liquid extraction (PPE-PLE) concentration was analyzed by ANOVA. Findings in the study showed that the combination of pressurized water and ethanol resulted in a PPE-PLE with punicalagin content obtained under optimal conditions.Mohamed Taha Yassin *et.al*, 2021 evaluated the in vitro evaluation of biological activities and phytochemical analysis of pomegranate peels. The sample was extracted and analyzed using statistical methods. Findings illustrated that the methanolic extract of pomegranate peels showed the highest antibacterial efficiency, while the acetonic extract recorded the highest antioxidant activity.

Pavan Kumar and Shalini 2021 examined the extraction of biologically active compounds from pomegranate peel. The pomegranate fruits were collected as the study sample. The extracts from the peels of pomegranate were analyzed using statistical analysis. The study results figured that the application could be effective for the extraction of bioactive from any food and plant systems with minimal process time and power consumption with the green label. Moreover, significant correlation analysis was established with phenols and flavonoids with the highest coefficient. Nishant Kumar *et.al*, 2022 explored the solvent extraction of bioactive compounds in pomegranate peel. The samples of the study were pomegranate fruits. Completely randomized design (CRD) was performed in the study. Results of the study revealed that freeze-drying was the most desirable method to retain the higher amount of bioactive compounds of pomegranate peel. Merve Balaban *et.al* 2022 explained the antimicrobial activities of the bioactive compound in pomegranate peel extracts. The extracted compounds were analyzed by qualitative analysis. The highest levels of punicalagin and ellagic acid were detected in the EA extract at 13.86% and 17.19%, respectively, whereas the lowest levels in HPLC analyses were obtained as the findings. Moreover, the antimicrobial activities were assessed by the presence or absence of inhibition zones.

Yanlin Feng *et.al*, 2022 intended to analyze the biological activities and compositions of pomegranate peel. The results of the study from the principal component analysis indicated that flavor was responsible for both the Ferric-reducing antioxidant power (FRAP) and resistance against lipid peroxidation. Also, total tannins (TT)had a weak positive correlation with the inhibition of IL-6. Furthermore, the PPE with higher contents of punicalagin had poor strength in preventing their lease of nitrogen oxide.

MATERIALS AND METHODS

Sample Collection and Extract Preparation

In this study, pomegranate fruits of Mridula and Bhagwa varieties were sourced from Azadpur fruit market, New Delhi. Extract preparation involved manual separation of peels and arils, followed by juice and seed extraction. After air-drying in shade for a week, peels and seeds were tray-dried at 50 °C for 6 hours, ground into fine powder, and stored in airtight packets under refrigeration. A constant amount of sample was utilized for antioxidant extraction (in triplicate) using solvents like 80% ethanol, 80%

methanol, 80% acetone, and distilled water for varying durations with Soxhlet apparatus and cold percolation methods. Filtered extracts underwent solvent evaporation at room temperature and were preserved in amber vials under refrigeration for further analysis.

Chemicals and reagents

Ethanol, Methanol, Acetone, Distilled water, Beakers, whatmann filter paper. Purchased from Star Scientific House in Badarpur, Delhi. All chemicals used are of analytical grade.

Determination of Extraction Efficiency (EE)

The dried extracted sample is weighed to calculate the yield by the following equation,

$$Yield\,(\%, wet\ weight\ basis) = \frac{W_1 X 100}{W_2}$$

Where, W_1 = Weight of dried residue, and W_2 = Weight of dried sample concentrated.

RESULTS AND DISCUSSIONS

The extraction yield from various parts of the pomegranate was determined using two distinct methods: cold percolation and Soxhlet extraction. The percentage yield was calculated for each part of the pomegranate, utilizing three different time intervals and four types of solvents. These methodological variations allowed for a comprehensive assessment of the efficiency of the extraction processes and their impact on yield.

4.1 Extraction yield from various parts of the pomegranate, obtained using the cold percolation method

Extracts from various parts of the pomegranate using two different varieties namely Ganesh and Mridula were obtained using a variety of solvents, thus resulting in significant differences in extraction efficiency for each solvent. The yields of extract from different parts of the fruit were obtained in the order of peels > juice > arils > seeds.

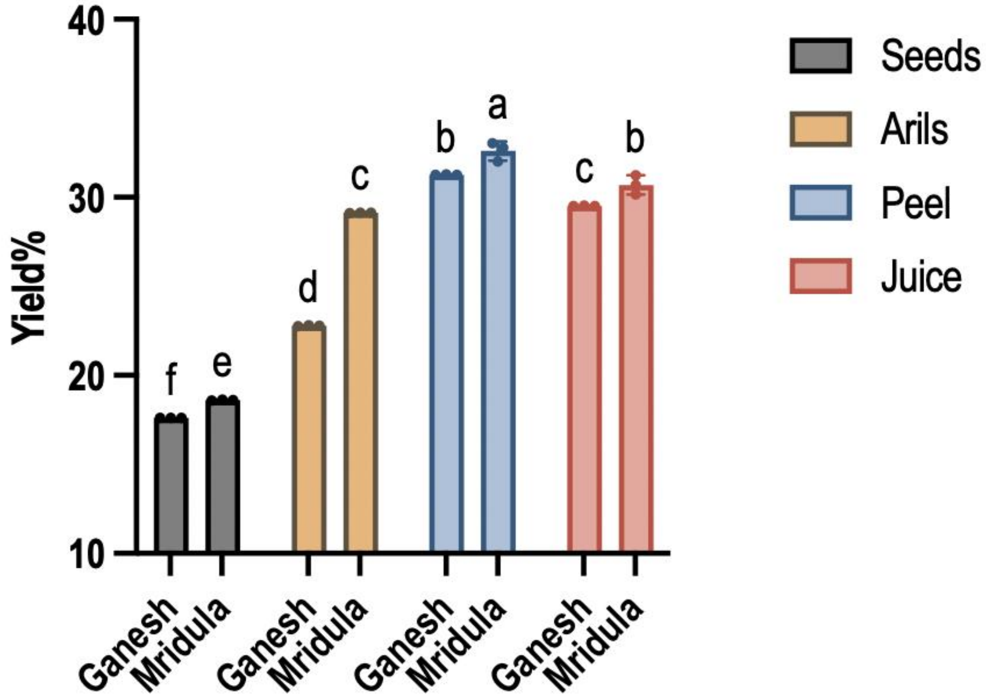

Fig. 2: Two-way ANOVA was performed to evaluate the effects of Ganesh and Mridula- seeds, arils, peel and juice on yield% using Cold percolation extraction. Significant interactions and main effects were further analyzed using Šídák's multiple comparisons test for multiple comparisons. Results are displayed using a compact letter display (CLD), where groups that do not share a letter are significantly different (p

< 0.05). Data are presented as mean ± SEM, with n = 3 per group. Software used is Graphpad prism India version 10.

4.2 Extraction yield from various parts of pomegranate, obtained using the soxhlet extraction method

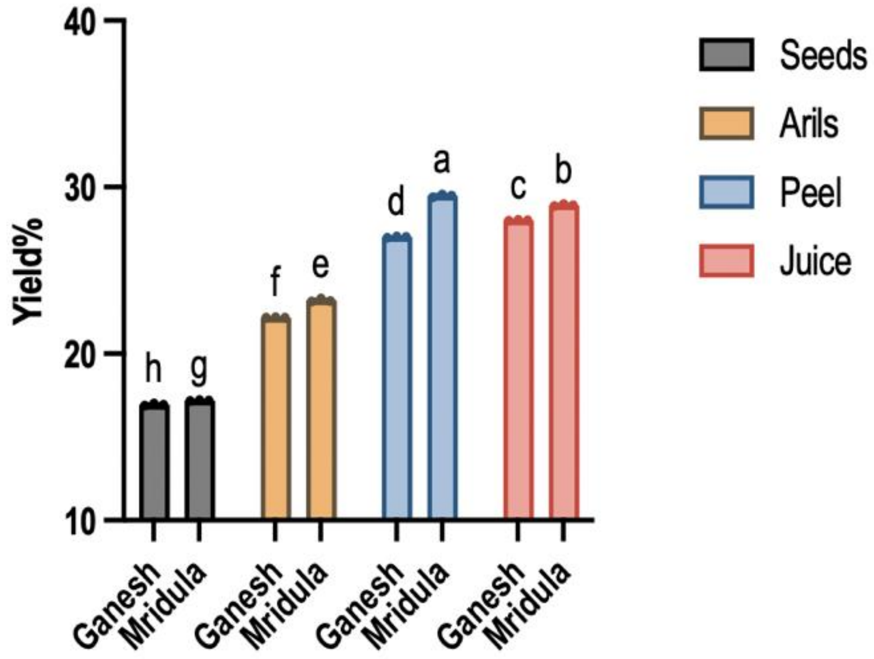

Fig .3 : Two-way ANOVA was performed to evaluate the effects of Ganesh and Mridula- seeds, arils, peel and juice on Yield% using Soxhlet extraction. Significant interactions and main effects were further analyzed using Šídák's multiple comparisons test for multiple comparisons. Results are displayed using a compact letter display (CLD), where groups that do not share a letter are significantly different ($p < 0.05$). Data are presented as mean ± SEM, with n = 3 per group.

Extracts from the pomegranate were obtained utilizing a diverse array of solvents, thereby resulting in notable disparities in extraction efficacy for each solvent. The yield obtained in different parts using soxhlet extraction and cold percolation is illustrated in Table 1&2 respectively.

Peel Variety	Solvent	Yield (%)		
		2 hrs	4hrs	6hrs
Mridula	Distilled Water	29.4±0.23	30.01±0.31	30.67±0.28
	Acetone (80%v/v)	34.08±0.16	34.29±0.19	34.51±0.17
	Ethanol (80%v/v)	37.06±0.36	37.14±0.39	37.46±0.41
	Methanol (80%v/v)	36.47±0.43	36.81±0.47	37.51±0.4
Ganesh	Distilled Water	27±0.11	29.42±0.19	30.76±0.1
	Acetone (80%v/v)	34.12±0.71	34.76±0.7	35.19±0.78
	Ethanol (80%v/v)	36.46±0.75	37.02±0.7	37.38±0.81
	Methanol (80%v/v)	36.14±0.09	36.26±0.11	37.12±0.13

Table 1: Extraction yield of pomegranate peel obtained through Soxhlet extraction.

Peel Variety	Solvent	Yield (%)		
		24 hrs	48 hrs	72 hrs
Mridula	Distilled Water	32±0.03	32.13±0.07	32.84±0.04
	Acetone (80%v/v)	36.54±0.88	36.89±0.83	37.24±0.81
	Ethanol (80%v/v)	39.64±0.71	42.55±0.7	41.29±0.77
	Methanol (80%v/v)	39.41±0.35	40.16±0.39	42.61±0.3
Ganesh	Distilled Water	31.27±0.75	31.84±0.7	32.54±0.73
	Methanol (80%v/v)	36.52±0.11	38.41±0.17	40.76±0.1
	Ethanol (80%v/v)	36.78±0.85	39.5±0.88	39.67±0.81
	Acetone (80%v/v)	35.06±0.88	35.42±0.83	36.13±0.81

Table 2: Extraction yield of pomegranate peel obtained through cold percolation.

Pomegranate peels demonstrated the highest yield of bioactive compounds compared to seeds, juice, and arils, as evidenced by quantitative extraction studies. This superior yield can be attributed to the peel's rich phytochemical composition, including phenolic compounds, flavonoids, and tannins, which are present in significantly higher concentrations than in other parts of the fruit. These bioactive constituents contribute to the peel's potent antioxidant properties. The findings underscore the potential of pomegranate peels as a valuable source for bioactive extraction, promoting their utilization in nutraceutical and pharmaceutical applications. Fig 4. demonstrates that the highest yields were obtained from the peels of pomegranates in both varieties of pomegranate.
Table 2 depicts that the extraction yield was highest in the peels of the Mridula variety (i.e., 41.29%) with ethanol as the solvent. Also, the yield was extracted using the cold percolation method for a dipping time of 48 hours. This combination produced the most efficient extraction of bioactive compounds, maximizing anti-oxidant activity. In comparison, methanol produced a lower yield in the peels of the Ganesh variety, while other solvents yielded relatively lower values across both varieties. Additionally, distilled water in the seeds of the Mridula variety showed a particularly low extraction yield (19.12%), highlighting the effectiveness of ethanol for maximizing extraction in pomegranate peels.

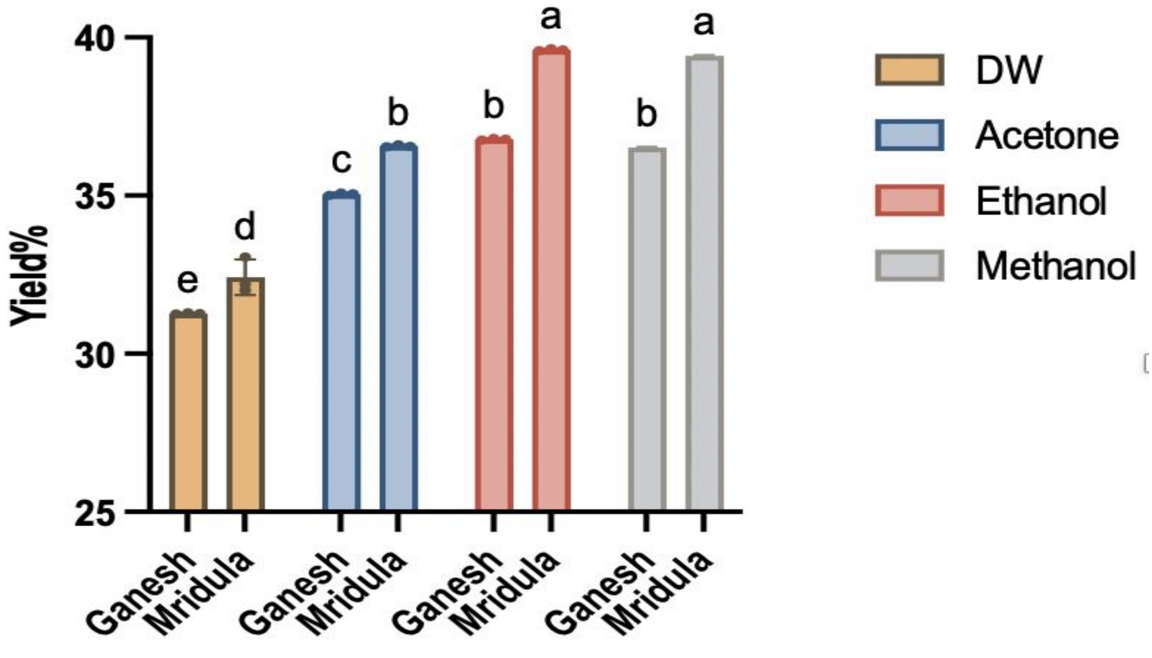

Fig : 4 Comparison of extraction yields (%) from two distinct peel varieties using cold percolation with four different solvents.

Two-way ANOVA was performed to evaluate the effects of Ganesh and Mridula peel extract on yield% using four different solvents. Also, the extract using acetone as solvent in the variety of Ganesh yielded a low value (i.e., 16.37%) in the arils of pomegranate. Significant interactions and main effects were further analyzed using Šídák's multiple comparisons test for multiple comparisons. Results are displayed using a compact letter display (CLD), where groups that do not share a letter are significantly different ($p < 0.05$). Data are presented as mean ± SEM, with n = 3 per group.

The yields of pomegranate extract from different solvents were in the order Ethanol (80% v/v) in water> Methanol (80% (v/v) >distilled water>Acetone (80% (v/v). Broadly, the yield obtained in ethanolic extract for the Mridula variety of pomegranate had achieved a maximum of 39.64% from peels.

Cold Percolation Yield of Pomegranate Peel Extract at Varying Time Frames

Pomegranate peels demonstrated the highest yield of bioactive compounds when extracted using the cold percolation method at three time intervals: 24, 48, and 72 hours. The extraction efficiency was optimized at 48 hours, yielding a superior concentration of bioactive constituents, including phenolic compounds, flavonoids, and tannins. The results indicate that extending the percolation time beyond 48 hours did not significantly enhance yield, suggesting 48 hours as the optimal extraction duration

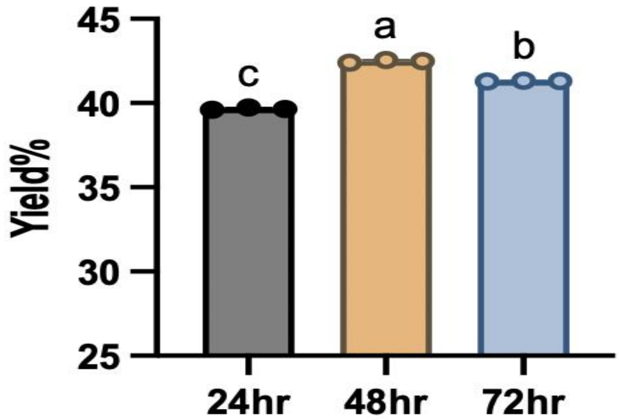

Fig. 5: Extract yield (%) from peels of Mridula variety at three time points using cold percolation.

One-way ANOVA was performed to evaluate the effects of different time points on extract yield from Mridula peels using ethanol (80% v/v) as solvent and cold percolation as extraction method. Significant I nteractions and main effects were further analyzed using Tukey's HSD post hoc test for multiple comparisons. Results are displayed using a compact letter display (CLD), where groups that do not share a letter are significantly different ($p < 0.05$). Data are presented as mean ± SEM, with n = 3 per group.

CONCLUSION

This study investigated the extraction of bioactive compounds from pomegranate peels. Extracts from various pomegranate parts were obtained using solvents such as distilled water, acetone, methanol, and ethanol. Using both cold percolation and Soxhlet extraction methods, ethanol produced the highest yield of 41.9% for the Mridula variety of pomegranate peels. Unlike the findings in this study, Abderrezak Kennas and Hayat Amellal Chibane [18] reported similar yields for methanol and ethanol (27.21 ± 0.47 and 26.20 ± 0.45%, respectively) without significant differences. Another study by Rayeheh Ghasemi et al. [19] reported a pomegranate peel yield of 18.90%, which is considerably lower than the yield in the present study. Results indicated that, compared to the Ganesh variety, the Mridula variety extracted with ethanol demonstrated the highest values across all tests after 48 hours. Limitations of this study included the lengthy extraction times and the need for pure, costly solvents. Future research will aim to conduct a comprehensive analysis across various fruits, focusing on solvents for extracting bioactive and phenolic compounds.

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