



POMEGRANATE PEEL : A MULTIFUNCTIONAL ACTIVE

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Abstract-

Pomegranate (*punicagranatum*) is an ancient fruit widely known for its medicinal and nutritional purposes. It is a potent source of bioactive compounds widely utilized for its multifunctional benefits including antimicrobial, anti – inflammatory and antioxidant properties. It consists of flavonoids mainly anthocyanins, flavonols and phenolic acid found in the peel and juice of the fruit whereas hydrolysable tannins (ellagitannins and gallotannins) found are responsible for maximum level of antioxidant activity. Food industries generate ample amount of biowaste from fruits and vegetables, out of which peels and seeds are often discarded. This research aims to study the multifunctional activity of pomegranate peel extract obtained from biowaste of food processing industries. As the world is leading towards eco-safety and eco-preservation, food quality and safety has been brought into consideration and therefore the utilization of natural constituents in food is high in demand. In this study pomegranate peels were extracted through soxhlation using hydro-alcoholic solvent (40 : 60) and subjected to spectrophotometric analysis over the range of 200 – 800 nm. The absorbance of the extract in the range of 230-290 nm confirms the presence of polyphenols and the absorbance at 460nm, confirms the presence of natural color in the same extract (yellowish – orange) amber. To study the antioxidant property the extract was subjected for HPLC analysis to find out the active responsible for antioxidant property. The HPLC analysis showed the presence of gallic acid with retention time 2.89 min in

accordance with the standards results of gallic acid. The stability of the natural color was studied at different pH and temperatures. Safety of natural color as a food additive issues a major public concern in terms of heavy metals therefore analysis of heavy metal was done by ICP-MS and it was found to be present in trace quantities within the permissible limits. To ensure the safety of the use of bio-waste as a food additive the sample was tested for total viable micro - organisms and were found to be in low concentration.

Keywords – gallic acid, antioxidant, polyphenols, absorbance, natural color

Introduction

Pomegranate (*Punica granatum*) is a deciduous tree of genus *punica* and family *Lythraceae*. It is a long lived and drought -tolerant plant cultivated in Iran, India Turkey, Egypt, Tunisia Spain and Morocco[1]. Pomegranate fruit owing to its good taste and nutrition is produced and consumed on a large scale. During food processing biowaste such as seeds and peels can lead to environmental pollution if not handled properly. Fruit industries generate ample amount of biowaste. The peel and the seeds amount to more than 50% of the fruit[2]. The peels of the fruit constitutes about (39 – 53%), juice varies between (38-50%) and the seeds represents (8-12%) of the total fruit weight[3,4]. The peel/ juice of the fruit consists of flavonoids which includes flavonols, catechin, epicatechin, anthocyanins and phenolic acids whereas hydrolyzable tannins (ellagitannins and gallotannins) are present in the mesocarp and pericarp of the fruit. Punicaligin present in the

peel of the fruit is responsible for maximum level of antioxidant activity. The rising interest in pomegranate peel is due to their sensorial attributes and remarkable amount of bioactive compounds. Consumers concern with relationship between health and diet has led to the search of multifunctional properties of biowaste beyond nutrition[5]. Pomegranate being rich in bioactive compounds shows properties such as anti-inflammatory, hypersensitivity and antioxidant. The need to find alternatives to substitute the synthetic additives has led to use renewable biomass as a natural source with multifunctional properties hence contributing to circular economy model. Color is an essential aspect of food products, it makes the food products to be more appetizing and appealing. In the present era of eco safety growing worldwide, food safety has been brought more into consideration. The use of synthetic colors has been proven to be hazardous to health. Synthetic additives such as tartrazine used in processed cheese, sauces has been identified to cause migraine, many allergic reactions[6]. These increasing health risks caused due to the use of synthetic colors has led to greater demand of natural colors, with the change in consumer lifestyle since last few years[7]. Natural colors are derived from mineral, plant and animal based origins. Some of the natural food colorants, include carotenoids (orange -yellow - red) color largely found in annatto seeds, saffron, anthocyanins (red - blue) color found in blue berries, grapes, apples. Betalains (red - purple) color found in beet root, cactus, amaranths and chlorophylls found in green plants[8]

2) Materials and Methods

2.1 Sample collection and processing

The waste peels of pomegranate were collected from (Santosh juice centre, Sitabuldi, Nagpur). Sodium bicarbonate was procured from (Vaibhav organics, Wadi, Nagpur). The freshly collected peels were soaked in a bowl of water consisting of sodium bicarbonate for 15 - 20 mins. Subsequently the peels were allowed to sun dry for 2 - 3 days. After being fully parched, the peels were blended into a fine powder and a mesh of size 120 was used in order to remove coarse granules.

2.2 Extraction of active

The weighed (coarse) powder of pomegranate peel (60g) was taken in a round bottom flask with hydro-alcoholic solvent, ethanol :water (60:40). The solution in the flask was heated at 70°C for 18 cycles. The solvent was allowed to dry at room temperature. The obtained extract was kept in a moisture free container and was further utilized for phytochemical analysis.

2.3 Phytochemical Analysis

The obtained extract was subjected to phytochemical screening in order to detect the presence of alkaloids, tannins, phenols and flavonoids in the peel[9,10]

2.4 HPLC Analysis

HPLC analysis was performed to determine the antioxidant activity shown by gallic acid in the peel by comparing the retention time of standard gallic acid with sample. Identification of Gallic Acid in sample extract was performed by using HPLC using Cosmoil C18 column (150mm by 4.6mm, 5 µm particle) and mobile phase of mixture (ethyl acetate : ethanol: water, 1;5;4,v/v/v) to obtain peaks of gallic acid[11]

2.5 Spectrophotometric analysis

The absorbance for the natural color was measured over the range of 400 - 700nm by using UV-Vis Singlebeam spectrophotometer (NSP - 369)12

2.6 Heavy metal analysis

Heavy metal analysis was done to check the edibility of food color by using inductively coupled plasma mass spectroscopy (ICP-MS)[13,14]

2.7 Estimation of total viable count

For the enumeration of total viable bacteria (TVB) and the total fungal load, 0.1 ml of each sample was introduced onto the nutrient agar (na) plate (Himedia Laboratories Pvt. Ltd Mumbai, India) and Sabouraud Dextrose Agar (SDA) plates (Himedia Laboratories Pvt. Ltd Mumbai, India) respectively by spread plate technique. Plates were incubated at 37°C for 24 hr and at 25° C for 48 hr for total viable bacteria and fungi, respectively[15]

3. Results and Discussions

Since ancient times pomegranate has been widely utilized for its various multifunctional benefits[16]. However, the peels of the fruit are often considered as a biowaste and are discarded and improper waste management of it can lead to extensive environmental pollution.

Therefore, in this study an attempt has been made to upcycle this biowaste to extract valuable bioactives. For this waste peels of pomegranate were collected from (Santosh Juice centre, Sitabuldi, Nagpur). The peels were processed and extracted using soxhlation.

To confirm the presence of bioactives present in the peel, the extract was subjected to phytochemical screening as shown in Table no.1

Compound detected	Inference
Alkaloids	++
Phenols	++
Tannins	++
Flavonoids	++
Carotenoids	--
Cholesterol	--

Table No.1 Phytochemical screening of extract

The presence of bioactives such as alkaloids, phenols, tannins and flavonoids were confirmed in the peel whereas the extract showed the absence of carotenoids and cholesterol.

The natural pigment of the obtained extract which was yellowish-orange in color was estimated by using UV – Vis Single beam spectrophotometer (NS 369) and the maximum absorbance was obtained at 280 nm and 460 nm shown in Fig no.1

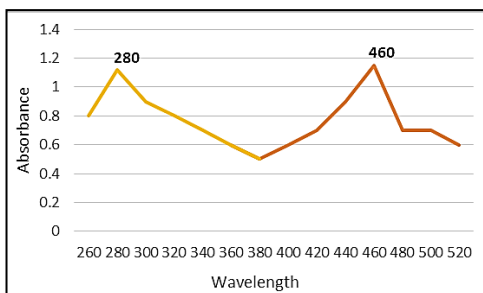


Fig no.1 Absorbance spectrum of pomegranate peel extract

Pomegranate peel is a rich source of bioactive compounds (table no.1) and a reservoir of antioxidants like polyphenols. Studies have demonstrated that polyphenols absorb at 230 – 290 nm [17]. In the present study absorption spectrum of pomegranate peel extract showed a λ - max at 280 nm which is in accordance with the cited literature. Lampakis et al. (2021)

showed that the main phytochemical component classes identified so far in pomegranate peels are phenolic acids (ellagic acid and gallic acid). [18] He also pointed out that the main phenolic compound of pomegranate fruit with high antioxidant activity is gallic acid.

The extract was subjected to HPLC analysis in order to detect the presence of gallic acid responsible for the antioxidant property. Peak of gallic acid was identified by comparison with retention time of standard gallic acid. The HPLC analysis of peel extract showed the presence of gallic acid with retention time 2.89 min as shown in (Fig no.3) in accordance with the standard results of gallic acid 2.96 min as shown in (Fig no.2) [19]

No	Heavy metals	Permissible limits (as per FSSAI)	Results (ppm)
1	Arsenic	3	0.327
2	Chromium	1	0.862
3	Lead	10	0.527
4	Mercury	0.5	0.127

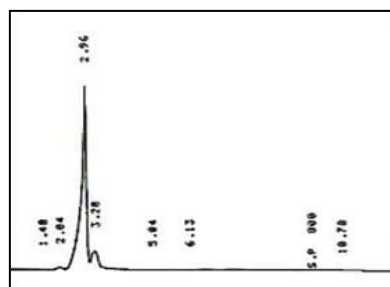
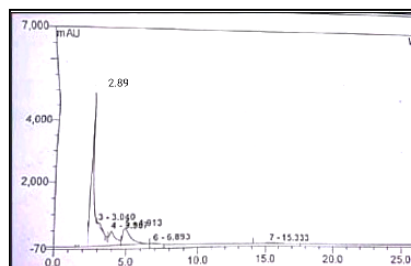


Fig no.2 HPLC spectra of standard gallic acid standard

Fig no.3 HPLC spectra of gallic acid sample

The UV – Vis spectrum of pomegranate peel extract showed two basic clusters of absorbance the first one at 280 nm which confirmed the presence of gallic acid and the other one at 460 nm, confirmed the presence of yellowish –



orange (amber) color dye which can be used as a colorant in food industry which is a futuristic and eco – friendly approach. An attempt was made to establish the obtained color as a natural food color and hence, the test for heavy metals was carried out.

The analysis of heavy metals was established by using Inductively Coupled Plasma Mass Spectroscopy(ICP – MS).

The heavy metals present in the sample were within the admissible limits according to FSSAI as shown in table no.2^[20]

Microbial load cfu /ml		
Extract	TVBC	TFC
Sample	9.2×10^2	0

Table no.2 Analysis of heavy metals in the pomegranate peel extract

Conclusion

Studies have demonstrated antioxidant properties of pomegranate peel owing to the presence of phytochemical gallic acid. We have studied and concluded that the pomegranate peel extract appeared to have a high potential as a food supplement rich in natural antioxidant and natural color. This color can be very safely exploited in pharmaceutical, personal care and textile domain and merits further for intensive study. Reusing pomegranate peel extract in food industry could be a sustainable way to reduce environmental impact and cost associated with waste disposal with significant advantages to product quality being natural thus improving human health

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