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Bioactive Compounds from *Punica granatum* L. peel and leaf and their Antibacterial activity

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Abstract

Bioactive compounds such as polyphenols and flavonoids have received great attention due to their varied biological activities. These compounds are capable of balancing metabolic processes such as anti-oxidant, antimicrobial and anti-inflammatory effects. Humans have been using plant parts for its medicinal properties since thousands of years. Among these Pomegranate (*Punica granatum* L.), a deciduous shrub, widely considered to be a functional product of great benefit in the human diet due to presence of bioactive compounds in it. The fruit peel comprises of about 40-50% of the total fruit weight and a rich source of several bioactive compounds. Pomegranate leaf is rich in phytochemicals and traditionally was used to treat diarrhoea, skin ulcers and traumatic injuries.

Natural or synthetic compounds which check the growth of disease-causing microorganisms or kills it, known as antimicrobial compound. To combat antibiotic resistance, antimicrobial properties of traditional medicinal plants is being revisited. Pomegranate peel and leaf shows a wide spectrum of antimicrobial effects, which has an apparent inhibitory effect against bacteria, fungi and mould. The objective of this study was to investigate the total phenolic and flavonoid content of pomegranate peel and leaves and their antibacterial activity.

Key words : *Punica granatum* L., peel, leaf, bioactive compounds, antimicrobial activity.

Antimicrobials are naturally occurring or synthetic compounds used for checking the growth of several disease-causing microorganisms. These compounds generally act as safeguards from pathogenic microorganisms[15]. Life-style related issues frequently lead

towards the rapid increase in the rate of infections, antibiotic resistance inmicroorganisms. Synthetic antibiotics often are accompanied by side-effects, whichled to the rekindling interest in rediscovering natural products.The use of plants still plays an important role to cure number of human diseases[1]. In fact, plants serve as a gift from nature to counter the emergence of resistant microorganisms. Pathogenic microbes cause infectious diseases which is aproblem of developing countries and also remain one of the major factors behind high morbidity and mortality across the world[5].

Pomegranate (*Punica granatum* L.) is one of the oldest known fruits and naturally constitutes a rich source of phenolic compounds[10]. The plant is a shrub or small tree with several upright, thornystems, the leaves are elliptic, roughly 2 inches, the flowers are either white or red and belongs to the family Punicaceae[17]. The fruit has a leathery carp and is a rich source of organic acids, polyphenols, vitamins, polysaccharide and important mineral compound[8]. Its peel constitutes about half of the fruit weight, and is an important source of several bioactive compounds such as phenolics, flavonoids, ellagitannins, and proanthocyanidin compounds[18].

During the processing of pomegranate, peels are generated as a biowaste which are normally used for production of animal feed and fertilizer[19]. It represents an important raw material due to their phytochemicals and biological activities[7]. It has significant free radical scavenging, anti- microbial, anti-atherogenic and anti-mutagenic properties and are reported to produce ameliorating effects against many critical diseases[3]. It contains many such bioactive phytochemicals which control microbial activities[13,21].

The plant has elliptic to lance-shaped, bright green leaves of about 3 inches long[4]. Its leaves show potential antioxidant, antimicrobial, anti-inflammatory due to the presence of secondary metabolites, mainly phenolic compounds. Besides this, the lipophilic compounds present in leaves proves to be vital to some activities or contribute as support in the activity related to polyphenols[11].

Chemically, phenolic acids can be defined as substances possessing aromatic ring bound to one or more hydrogenated substituents, including their functional derivatives. Using the HPLC/ ultraviolet method, scientists quantified five phenolic compounds like vanillic, gallic and ellagic acids. Pomegranate peel extracts (PPEs) is a potential source of flavonoids such as catechin, epicatechin, quercetin, anthocyanin and procyanidins. Flavonoids are low molecular weight compounds consisting of 15 carbon atoms, arranged in a C6-C3-C6 configuration. Essentially, the structure consists of 2 aromatic rings joined by a 3-carbon bridge, usually in the form of heterocyclic ring[16].

On the basis of these facts the present study was designed to study the quantity of flavonoid and phenolic compounds in the peel extract of pomegranate and its potential antimicrobial activity.

Extracts preparation :

Pomegranate fruits were obtained from local market of Patna, Bihar. The peel

was separated from fruit to cut into small pieces. The leaves were collected from home garden. Washed peels and leaves were then dried by hot air oven at 50°C. The dried samples were milled as powder and stored at room temperature for further use.

The pulverized samples were packed in Soxhlet apparatus subjected to continuous hot percolation using 250 ml of solvent *i.e.,* acetone and methanol respectively. The extracts were further used for both qualitative and quantitative evaluation.

Qualitative Analysis

Test for Phenols :

A portion of the extract was treated with aqueous 5% ferric chloride and formation of deep blue or black colour indicates the presence of phenols.

Test for Flavonoids :

Two ml of extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Quantitative analysis :

Determination of total Phenolic content (TPC) :

The total phenolic content was determined using Folin-Ciocalteu assay. 1ml of sample was added to 25ml volumetric flask, containing 9ml of distilled water. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% sodium carbonate solution was added to the mixture. The solution was diluted to volume (25ml) with distilled water and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 765 nm. A set of standard solutions of gallic acid (10, 20, 40, 60, 80 and 100µg/ml) were prepared in the same manner as described for the extracts.Test samples were analysed in triplicate and the concentration of the sample value is reported in gallic acid equivalents (GAE) using units of µg/ml of extract.

Determination of total Flavonoids content (TFC) :

One ml of sample was added to 10ml volumetric flask containing 3ml of 95% methanol (v/v). To the flask was added 0.2 ml 10% aluminium chloride. After 5 min 0.2ml of 1M potassium acetate was added and total volume was made up to10ml with distilled water. The solution was mixed well and incubated at room temperature for 30 min. The absorbance of reaction mixture was measured at 415nm. A volume of 10% aluminium chloride was substituted by same volume of distilled water in blank. Test samples were analysed in triplicate and the concentration of the sample was determined using the calibration curve. The mean value is reported quercetin equivalent using units of µg/ml of extract.

Antimicrobial Analysis

Determination of inhibition zone :

An Inhibition Zone Method (also known as an Agar-Well Diffusion Method) was

employed[14] to determine the antimicrobial activity. Mueller Hinton agar was prepared by dissolving 38g in one-litre solution. The agar plate was inoculated by spreading a volume of the microbial inoculum over the entire agar. Then, a hole with a diameter of 6 mm had been punched aseptically with a sterile cork borer, and a volume (50µl) of the antimicrobial agent or extract solution at desired concentration was introduced into the well. Ciprofloxacin (antibiotic) was used as a positive control.

Determination of Activity Index :

The activity index (Table1) of the crude plant extract was calculated as Moorthy *et al.,*[12]:

$$\text{Activity Index (AI)} = \frac{\textit{Zone of inhibition of the extract}}{\textit{Zone of inhibition obtained for standard antibiotic drug}}$$

Table-1. Total phenolic, flavonoid contents of pomegranate peel and leaf extracts

Part of Plant	Solvent	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)
Peel	Acetone	133. 45 ± 0.05	68.35 ± 0.03
	Methanol	102.006 ± 0.004	23.72 ± 0.005
Leaf	Acetone	62.4±1.2	2.7±0.10
	Methanol	80.5±1.8	4.9±0.12

Note: Values are expressed as Mean ± S.D (n=5).

In current study, the phenolic content, flavonoid content and antimicrobial activity of the acetonic and methanolic extracts sample were evaluated.

Determination of total Phenolic content (TPC) and total Flavonoid content (TFC):

To perform the calculation of total phenolic content and total flavonoid content in the extracts, a standard calibration curve was obtained from a series of different Gallic acid (GA) and Quercetin (Q) concentrations respectively.

The mean values of total phenolics and flavonoids for acetonic peel extract were 133. 45±0.05 and 68.35±0.03 and for methanolic peel extract were 102.006±0.004 and 23.72±0.005 respectively. The mean values of total phenolics and flavonoids for acetonic leaf extract were 62.4±1.2 and 2.7±0.10and for methanolic leaf extract were 80.5±1.8 and 4.9±0.12 respectively. TPC is expressed as mg GAE/g DW and TFC is expressed as mg Q/g DW.

Antimicrobial assay :

The antimicrobial activity of the above-mentioned extracts was separately determined using a modified agar-well diffusion method (Table-1). The most known mechanism of antimicrobial activity by extracts containing phenolic compounds has been hypothesize to be due to the disruption of cell membrane[9].

Table-2. Antimicrobial activity of pomegranate peel and leaf extracts against *E. coli,* tested by agar well diffusion (Inhibition zone) method

Part of Plant	Solvent	Zone of Inhibition	Activity Index
Peel	Acetone	30	0.75
	Methanol	18	0.45
Leaf	Acetone	28	0.70
	Methanol	Not Sensitive	––
Ciprofloxacin (Control)	–––	40	––

Besides their known antioxidant activity many phenolic compounds are known to show significant antibacterial activity[6]. Among the phenolic compounds gallic acid was identified as the most active compound for inhibition of bacteria[2]. It has also been reported that chlorogenic acid can interact with the bacterial outer membrane, disrupt it, deplete the intercellular content and releases macro-molecules from cytoplasm which leads to the bacterial death[21].

The study demonstrated that the acetonic peel extract is a better source of phenolic compounds and flavonoids than the methanolic peel extractwhile for the leaf extract, methanol was proved to be a better solvent. The acetonic peel extract also showed a promising potential of antimicrobial activity against *E. coli* having maximum inhibition zoneof 30 mm among the following extracts. The control ciprofloxacin exhibited 40 mm zone of inhibition. The activity index for acetonic peel extract was found to be 0.75.

This antimicrobial activity could be due to the damage induced by the pomegranate peel extract and pomegranate leaf extract at the cell membrane of bacteria. Further the flavonoids also could have played active part in inhibiting through energy metabolism and DNA synthesis.

Thus, we can conclude that, the acetonic extract of pomegranate peel has the highest content of phenolics and flavonoids and the acetonic extract pomegranate leafhave the lowest content of these compounds. The data obtained for assessment of the preventive role of *P. granatum* L. against *E. coli* revealed that acetonic extract of peel showed the most promising potential of antibacterial activities having a maximum zone of inhibition of 30 mm and activity index of 0.75. This may be attributed to the individual or combined effect of phenolics and flavonoids which is highest in pomegranate acetonic peel extract.

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