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PHYTOCHEMICAL INVESTIGATION OF PUNICA GRANATUM L. AND THEIR ANTIMICROBIAL ACTIVITIES AGAINST HUMAN PATHOGENS

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ABSTRACT

Pomegranate fruit (*Punica granatum L.*) is gaining popularity because of its many health benefits and nutraceutical properties. The peels, seeds and mesocarp that are typically produced by pomegranate fruit are rich in components that are biologically active and have a major favorable impact on health. The purpose of this study was to compare the phytochemical potential of pomegranates grown wild and bhagwa variety using their peels, mesocarp and seeds. The byproduct samples of both varieties contained a number of valuable compounds, as shown by qualitative analysis. The results indicate that phytochemicals with therapeutic effect can be used to combat bacteria, heal a range of diseases, and aid in the discovery of new and novel compounds.

KEYWORDS: *Punica granatum L.*, Phytochemicals and Anti-microbial.

INTRODUCTION

Pomegranate (Punica granatum L.), a member of Lythraceae family, is a fruit-bearing deciduous shrub with medicinal properties. Pomegranate has been cultivated since ancient times. The origin of this species is Iran, Afghanistan, Georgia, and other Central Asian regions, and it spread eastward to China and India.[1,2] In recent decades pomegranate (Punica granatum L.) is one of the most popular and widely used fruit for research studies.[3] Typical morphological features of pomegranate fruit include the fusion of the ovary with the receptacle. Pomegranate is an example of balausta, that is, the characteristic structure of the fruit. Botanically, the balausta develops from an inferior syncarpous ovary, which is an

indehiscent, multilocular, multispeed fruit and its pericarp, which is derived from the wall of the ripened ovary, encompassing the reddish-colored exocarp (rind), and the outermost part of the mesocarp (white albedo) is hard and leathery in appearance. The inner spongy structure i.e. mesocarp also forms creamy, papery, nearly tasteless, plate-like infoldings i.e. locular septa covering the independent clusters of closely packed seeds, inadequately known as arils. The innermost part is the endocarp, which is in direct contact with seeds and usually consists of two rows of multi-ovule chambers, that is asymmetrically arranged locules, usually 2–3 in the upper row and 6–9 in the bottom. The calyx is tenacious and its shape resembles a crown. Kernels are the inner parts of the arils and an essential part of a seed, entail the embryo, and their structure is protected by the tegmen and the sclerotic mesotesta. Kernels are irregularly arranged on the placenta and inbounded in a pulpy and juicy coat.[4,5] Generally, a typical pomegranate fruit consists of various parts, such as peels, which constitute 30–40% of the fresh fruit weight, while the remaining edible part, which constitutes nearly 52% of the total fresh fruit weight, consists of 78% juice and approximately 22% kernels, with specific variability among the cultivated varieties. Variable by-products and parts of pomegranate fruits, such as bark, leaves, immature fruits, and fruit rind, have valuable therapeutic potential against many diseases.[6,7]

Punica granatum L. has been broadly used in traditional medicine by America, Asia, Africa and Europe for the treatment of different types of diseases. The *Punicagranatum L.* was esteemed as a symbol of aspiration and prosperity in the ancient Egyptian culture, leading to the customary adornment of sarcophagi with images of the plant. As stated by Eber's Paprus (one of the ancient medical writings, dated Circa 1,500 BC), the plant was utilized by Egyptians for the treatment of tapeworm and other parasitic infections, although several studies have focused on the prevention and treatment of diseases such as cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and skin allergy. Investigations have been performed to determine the antitumorous, antioxidant, antibacterial, antifungal, anticarcinogenic, antiviral, and anti-inflammatory properties of pomegranate by-products.[8,9] Recently, some studies have shown that commercial pomegranate seed extract has been introduced as a source of antioxidants to design an innovative synbiotic formulation to diminish dysbiosis and uremic toxins in patients with chronic kidney disorders. A tannin-rich dried extract obtained from pomegranate peels was recently shown to be a novel oenotannin that can be used as a coadjuvant in the winemaking process.

The anti-cancer effects of pomegranates have been illustrated to various metabolic constituents including polyphenols, carbohydrates, amino acids, proteins, polysaccharides etc. Biological mechanisms underlying tumor inhibition are also diverse, spanning from suppression of cancer growth, angiogenesis and metastasis to excitation of cell cycle arrest and apoptosis. Hydro alcoholic extracts of pomegranate by-products have been shown to be effective against the herpes virus, whereas whole fruits have exhibited high activity against the influenza virus.[10] The antimicrobial activity of some general pomegranate cultivars has also been studied, and it was found that pomegranate extracts inhibit and delay Staphylococcus aureus growth and consequent production of enterotoxin at 0.01, 0.05, and 1% v/v concentrations. Melendez and Capriles also reported that extracts from *Punica granatum L.* fruits showed strong in vitro antibacterial activity against the tested bacterial strains.[11]

MATERIALS AND METHODS

1. Collection of plant materials

The plant material was collected from P.V.P. College, Loni (Located 19.5813^0N, 74.4745^0E) Ahmednagar district, Maharashtra in March 2023. A voucher specimen of plant has been deposited in the herbarium of Department of Botany P.V.P. College Pravaranagar. The plant was identified with the help of assemble literature.

2. Plant extract preparation

The plant part was washed thoroughly with running tap water, then air dried under shade, and the plant material was grinded in mixer. The powder was kept in plastic bags with labeling. The plant extract was prepared by soxhlet extraction method. About 5 gm of powdered plant material was uniformly packed into a thimble and extracted with 250 ml of different solvent separately. Solvents used for extraction ethanol. After that, extract was taken in a beaker and kept on hot air oven till all the solvent got evaporated. Dried extract was kept in small bottle for their future use in phytochemical analysis.

3. Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using the following standard methods.[12-15]

Test for alkaloids

Wagner's test

The crude extract was mixed with dilute HCl and placed in the water bath for five minutes and then filter 1-2 ml filtrate, 2 ml of Wagner'sreagent were added. The reddish-brown color precipitate indicated the presence of alkaloids.

Dragendorff's test

The filtrate was mixed with Dragendorff'sreagent and the formation of an orange color precipitate indicates the presence of alkaloid.

Test for phenolic

Ferric chloride test

The extract was dissolved in distilled water, and then 2 ml of 5% $FeCl_3$ solution was added. The formation of violet color or blue-green color indicated the presence of phenolic compounds.

Lead acetate test

The extract was dissolved in DW and then 1 to 2 drops of lead acetate solution were added. The appearance of white ppt. indicated the presence of phenolic compound.

Test for glycosides

Liebermann's test

Crude extract mixed with 2 ml of $CHCl_3$ and 2 ml of CH_3COOH. The mixture was cooled in the ice bath and then carefully concentrated sulphuric acid was added. A color change from violet to blue to green indicated the presence of glycosides.

Killer- Kiliani test

The extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of ferric chloride. The mixture was then poured into another test tube containing 2 ml of concentrated sulphuric acid. A brown ring at the interphase indicated the presence of glycosides.

Test for saponins

A test tube containing the extract and five to ten milliliters of distilled water was shaken briskly. The development of a steady foam suggested the presence of saponins.

Test for terpenoids

2 ml of the extract was treated with 2 ml of chloroform and conc. sulphuric acid. The presence of terpenoids is indicated by the formation of a reddish brown tint at the interface.

Test for tannin

The extract was mixed with 2 to 3 ml of 2% solution of $FeCl_3$.A blue-green or black color indicated the presence of tannins.

Test for flavonoids

Shinoda test

The crude extract was mixed with fragments of magnesium ribbon and concentrated hydrochloric acid; the pink color appeared after a few minutes which indicated the presence of flavonoids.

Lead acetate test

The extract treated with 2ml of lead acetate solution, yellow color precipitate or crimson color indicated the presence of flavonoids.

Test for steroids

The plant extract was mixed with 2 ml of $CHCl_3$ and concentrated H_2SO_4 was added. A red color in the lower chloroform layer indicated the presence of steroids or mixing of an extract with 2 ml of chloroform than 2 ml of each of concentrated H_2SO_4 and CH_3COOH where poured into the mixture, dark green color indicated the presence of steroids.

Test for anthraquinones

2 ml of extract few drops of 10% ammonia solution was added, appearance of pink color precipitate indicates the presence of anthraquinones.

Taste for phlobatanin

1 two 2 ml 2% HCL was added to 1 ml of the extract. Phlobatannins are present when a red-colored precipitate appears.

Test for Micro-organisms

The bacterial isolates were obtained from the laboratory unit of the Department of Microbiology, Pravara Medical Trust Loni, and authenticated using standard biochemical

tests as described by Cheesbrough.[16] The isolates were maintained on a freshly prepared nutrient agar and potato dextrose agar slant.

4. Antimicrobial assay

The freshly prepared nutrient agar and potato dextrose agar plates were dried in a drier for about 15 minutes to remove surface moisture. The plates were aseptically inoculated uniformly with the test organism by spread plate techniques. Positive control discs containing standard antibiotic gentamicin for bacteria and negative control discs containing DMSO extract were used. The petri plates were incubated at 37^0C for 24 to 36 hours in the incubator. The result was observed by measuring of the zone of inhibition in diameter and recorded in millimeters.[17-20]

RESULTS AND DISCUSSION

The results of the phytochemical screens of different parts of the wild and bhagwa varieties of *Punica granatum L*. were compiled into tables 1 and 2. According to reports, the majority of bioactive chemicals found in wild pomegranate mesocarp extract. All six extracts contain flavonoids and saponins. The *Punica granatum L*. wild and bhagwa varieties have not been found to contain phalobatanins. Table 3 and 4 shows the effects of plant extracts on antibacterial activity. By applying the disc diffusion method, the ethanol extract of all six samples was examined for its ability to inhibit both gram-positive and gram-negative bacteria. Antimicrobial action is shown by *Punica granatum L*. Fruit parts demonstrated a significant antibacterial effect against microorganisms. Based on the current research; it appears that the sample extracts have antibacterial properties that could be utilized as antimicrobial agents in novel therapeutic medications. Important pharmacological research that results in the creation of more potent medications is the antibacterial property of biochemical substances. The greatest activity was demonstrated by both ethanol extracts. Pharmacological analysis and therapeutic antibacterial isolation are used to these medicinally useful plants.

Table 1: Phytochemical screening of *Punica granatum L.* (wild) peels, Seed and Mesocarp.

Obs. No.	Test	Seed extract	Peels extract	Mesocarp extract
1	Alkaloids	+	-	+
2	Phenols	-	+	+
3	Glycosides	+	-	+
4	Saponins	+	+	+

5	Terpenoids	+	-	+
6	Tannin	+	+	+
7	Flavonoids	+	+	+
8	Steroids	-	+	-
9	Anthraquinos	+	+	+
10	Phlobatanins	-	-	-

+ indicates presence and – indicates absence of activity.

Table 2: Phytochemical screening of Punica granatum L.(Hybrid) peels, seed and mesocarp.

Obs. No.	Test	Seed extract	Peels extract	Mesocarp extract
1	Alkaloids	+	-	+
2	Phenols	+	+	+
3	Glycosides	-	-	-
4	Saponins	+	+	+
5	Terpenoids	+	+	+
6	Tannin	+	-	-
7	Flavonoids	+	+	+
8	Steroids	-	-	+
9	Anthraquinos	-	+	+
10	Phlobatanins	-	-	-

+ indicates presence and – indicates absence of activity.

Table 3: Anti-microbial activities of plant extract (Wild).

Sr. No.	Bacterial isolates	Zone of Inhibition (in mm diameter) Gentamicin (Gram negative and positive)		
		Seed extract	Peels extract	Mesocarp extract
1.	*Escherichia coli*	9	11	12
2.	*Pseudomonas aeroginosa*	11	10	11
3.	*Bacillus subtilis*	11	9	12
4.	*phylococcus aureus*	10	10	11

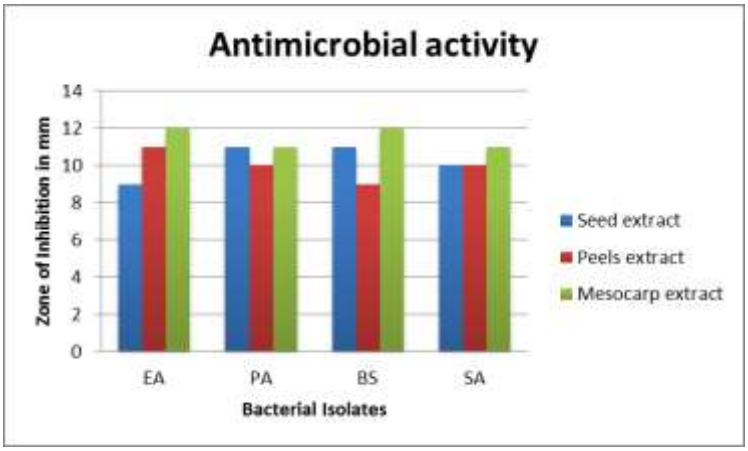

Fig. 1: Anti-microbial activities of plant extract (Wild).

Table 4: Anti-microbial activities of plant extract (Hybrid).

Sr. No.	Bacterial isolates	Zone of Inhibition (in mm diameter)		
		Gentamicin (Gram negative and positive)		
		Seed extract	Peels extract	Mesocarp extract
1.	*Escherichia coli*	9	9	10
2.	*Pseudomonas aeroginosa*	11	10	8
3.	*Bacillus subtilis*	10	10	11
4.	*phylococcus aureus*	10	8	10

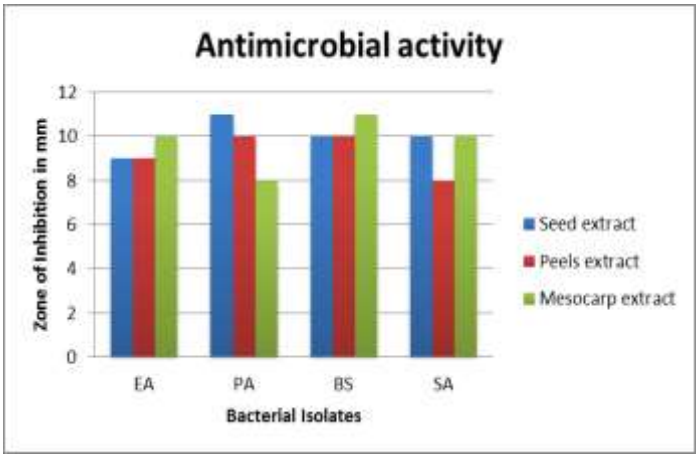

Fig. 2: Anti-microbial activities of plant extract (Hybrid).

CONCLUSION

Currently, a lot of research uses herbs because of their many chemical entities, which make them suitable for many kinds of investigations. Plant-based compounds have minimal adverse effects and a superior dosage response as compared to manufactured medications. The outcomes of anti-bacterial activity and phytochemical screening suggest that *Punica granatum L*.is a useful medicinal plant with a variety of therapeutic use. The fruit seeds, peels and mesocarp of *Punica granatum L.* beneficial for future research due to their increased ingredient content in the ethanol extracts. Better therapeutic and commercial application requires the completion of research and development activities.

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