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Phytochemical Screening, Antimicrobial and Antioxidant Activities of *Punica Grantum* Peel

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Abstract

Punica peel has been used for thousands of years to cure a wide range of diseases across different cultures and civilizations. It has great nutritional values and numerous health benefits. This study was carried out to investigate phytochemical classes, and to evaluate antimicrobial and antioxidant potential of *punica grantum* peel. The phytochemical screening showed presence of alkaloid, flavonoids, tannins, steroid, triterpenes, saponin and sterol. The antibacterial activity was high for *punica* ethanol extract with inhibition zone (30mm) against *Staphylococcus aureus*. The antifungal activity was high for *punica* methanolic extract against *Candida albicans* with inhibition zone (22mm). The three *punica grantum* peel extracts showed good antioxidant activity using DPPH radical a scavenging activity method.

Introduction

Medicinal plants have been used in virtually all cultures as a source of medicine. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. The wide spread use of herbal remedies and health care preparation is described in the Vedas and the Bible. Medicinal plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases across different cultures and civilization. It has great nutritional values and numerous health benefits. *Punica* has been used in holistic medicine to treat sore throat infection, cough, urinary infection, digestive disorders, skin disorders, arthritis and to expel tape worm, however modern research suggests that pomegranates might be useful in treating such serious condition as prostate cancer, skin cancer, Osteoarthritis and diabetes. Studies also show that pomegranate seeds might help ride the digestive system fats.

Punica grantum L. is small woody shrub that can grow up to 3-4.5 m tall. Leaves are opposite, oblong-lanceolate 3-8 cm and branches are spiny. Flowers are large trumpet-shaped, and bright orange-red in color. Fruit is globose wide waxy surface, tough leathery skin. Turn deep pink or red up on maturity, and contain numerous seeds with fleshy covering. Peels of pomegranate contain a wide variety of phytochemical compounds according to previous studies, like gallo-tannins, ellagic acid, gallic acid, phenolic compounds, including flavonoids, anthocyanins and tannins. Although previous studies stated clearly the antimicrobial and antioxidant activities of *punica grantum.*

Materials and Methods

Plant Material

Punica fruit peel was purchased from local market. Then powered and weighed and keep in dry container.

Plant extraction

500 g of *punica* grantum peel was extracted successively by using orbital shaker apparatus.

Phytochemical Screening:

Phytochemical screening of four extracts of *Guiera Senegalensis* leaves was performed using standard procedures as described by Sofowora, Trease and Evans and Harborne [1-3].

Alkaloid

a. Wagner's test: To 2ml of extract, 1 ml of Wagner's reagent was added, the appearance of reddish brown precipitate indicates presence of alkaloid.

b. Hager's: To 2ml of extract 1 ml Hager's reagent was added, the appearance of yellow precipitate indicates the presence of alkaloid.

c. Dragendroffs's: To 2ml of extract, 1ml of Dragendroffs reagent was added the appearance of brick red precipitate indicates presence of alkaloid.

Flavonoid

a. Ammonium solution test: To 2ml of filtrates 1ml of dilute ammonia solution 1% was added. The appearance of yellow color indicates presence of flavonoid.

b. Shinoda test: To 2ml of extract was dissolved in the ethanol then divided in to 2 test tube.

In the first test tube 1ml of sodium hydroxide 10% was added. The appearance of yellow color indicates the presence of flavonoid.

In the second test tube: 0.3g of magnesium powder, 5 drops of HCl was added then heated in water bath for 10 minutes the red or pink color was formed indicates the presence of flavonoid.

Tannins

a. Ferric chloride test: To 2ml of extract dissolved in ethanol then 0.5 ml of ferric chloride 5% was added. The blue-black color indicates the presence of tannins.

b. Legal test: 2ml of extract 0.3ml of lead acetate solution was added creamy gelatinous precipitates indicate the presence of tannins.

Glycosides

killer-killiani test: 2ml of extract 1ml of glacial acetic acid 3 drops 5% Ferric chloride and concentrated suphuric acid were added. The reddish brown color at the junction of two layers and bluish green in upper layer indicates the presence of glycosides.

Saponin

Foam test: The extracts was diluted with 2ml of distilled water and shaken vigorously and observed for persistent foam, which indicates the presence of saponin.

Terpenoids and sterols

a. Salkowaski test: To 2 l of extract, 2ml chloroform and concentrated sulphuric acid was added, shaken and allow to stand. Appearance of greenish blue color indicates the presence of terpenoids and sterols.

b. Liebermann bur chard test: To 2ml of methanolic plant extract mix with chloroform, 1-2ml of acetic anhydride was added. Then 2 drops of concentrated sulphuric acid was added from the sides of test tube, appearance of greenish blue color indicates the presence of terpenoids and sterols.

Antimicrobial activity test

Cup plate diffusion method

or hole diffusion method as modified by Ali was the standard method used to determine the antibacterial and antifungal activity of the bioactive compounds. Solving Briefly in this method: sterile nutrient agar powder was prepared by dissolving 12 agar in 250 ml distilled water, boiled to ensure complete dissolution and sterilized at 121 °C for 30 minutes and dispensed in to labeled petri-dishes and allowed to gel. The sterile agar plates were inoculated with the test culture by surface spreading uses wing mm sterile cork borer. 0.2 g of each crude extracts was weighed in to sample bottles and dissolved in to 100ul of DMSO and then the concentration 100 mg/ml, 50 mg/ml, 25mg/ml,12.5mg/l were sterilized dilution by DMSO and 5ul of DMSO was used for positive control. The agar was inoculated with the test organism using a sterile swab stick before incubating at 37°C for 24 hours. Zones of inhibition were determined by measuring the diameter of inhibition zone around the well in mm including the well diameter [4-8].

Free radical scavenging procedure (DPPH)

This method was carried out according to that described by Shyur with some modifications stock solution was prepared by dissolving 1 mg of the sample in 1 ml of absolute ethanol (98%). Stock solution was diluted to final concentration 100, 50, 25, 12.5, 6.25, 3.125.1.526ug/ml in ethanol. 0.9 ml tris-HCl and 1 ml of 0.1mm DPPH in methanol solution were added to each concentration and incubated at room temperature in dark for 30 minutes [9-15]. The absorbance of the resulting mixture was measured at 517nm and converted to percentage antioxidant activity using formula below:

$$Scavenging\ activity\ (\%) = \frac{A\ control\ -\ A\ sample}{A\ control} X100$$

Results and Discussion

Phytochemical screening of *punica grantum* was listed in **Table 1.**

Table 1: General phytochemical screening of *punica granatum* peel extracts.

Secondary Metabolites	Tests	Solvents for extraction		
		CH_3CL	MeOH	EtOH
alkaloids	Dragendroffs	+++	+++	+++
	Wagner's	+++	+++	+++
	Hager's	+++	+++	+++
flavonoids	Ammonia	+	+	+
	NaOH 10%	+	+	
	Mg/H_2SO_4	+	+	+
tannins	Ferric chloride	+++	++	+
	Lead acetate	+++	+++	+++
sterols	Salkowaski	+++	+++	+++
	Liebermann	-	-	-
triterpenes	Salkowaski	-	-	-
	Liebermann	+++	+++	+++
Saponins	Foam test	+	+	+
glycosides	Killer-Killiani	+	+	+

+++: means very high, +++: means moderate +: means found

-: means not detected.

CH_3Cl: chloroform; MeOH: Methanol; EtOH: Ethanol

Antimicrobial activity

In this study, Three extracts of *punica* granatum peels were screened against one Gramm positive bacterium *Staphyocous aureus* and one Gramm negative bacterium *Escherichia Coli* and one fungus *Candida albicans*, the results presented in **Table 2.** The high inhibition zone was found for ethanol extract against *Staphyocous aureus* (30mm). the high antifungal activity for methanol extract (22mm).

The antioxidant activity

In the present study three extracts were screened for antioxidant activity using DPPH free radical scavenging assay the result was tabulated in Table 3. The high inhibition was for methanolic extract with %RSA 70.9% in comparison with the standard propyl-gallate (77%).

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Table 2: Antimicrobial activity of punica grantum peel extracts.

Solvents	Concentrations mg/ml	Diameter of growth inhition zone with mm		
		S.a	*E.coli*	*Ca*
methanol	100	11	8	22
	50	8	7	5
	25	6	6	12
	12.5	5	5	10
ethanol	100	30	20	20
	50	22	17	12
	25	18	15	10
	12.5	12	12	6
chloroform	100	19	15	6
	50	12	11	5
	25	18	10	4
	12.5	10	8	4

Sa: *Staphyocous aureus;* E.coli: *Escherichia coli;* Ca : Candida albicans

Table 3: Antioxidant activity of *punica grantum* peel extracts.

Sample	% RSA
Ethanol	68.2%
Methanol	70.9%
Chloroform	63.6%

%RSA: Percentage radical scavenging activity

Note: Standard propyl-gallate = 77%

Conclusion

The present study provides more evidence on the importance and value of *punica granatum* peel, especially, *punica* peel usually considered as waste product. Phytochemical tests revealed that peel contain considered a mount of alkaloid, tannins, saponin, sterols and poly-phenolic compounds. The peel has good antimicrobial and antioxidant activities due to presence of these phytoconstituents which confirmed the folk use of this plant.

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