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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *PUNICA GRANATUM* (L.) BARK AND PEEL EXTRACTS

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ABSTRACT

Punica granatum is the predominant member of Punicaceae family. The phytochemical screening was carried out using, Petroleum ether, ethyl acetate and 70% ehanol, The results showed the presence of alkaloids, saponins, Tannins, Phenols, Cardiac glycosides, Steroids, Coumarins and Anthraquinone glycoside. The different extracts of *Punica granatum* L. (bark and Peel) have been tested for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) by cup diffusion method. The antibacterial activity shows that all of the extracts were found to be active against all bacterial strains tested.

KEYWORDS: *Punica granatum;* pomegranate; Punicaceae; Peel; Bark; Anthraquinone glycoside.

1. INTRODUCTION

Since, ancient time's pomegranate (*Punica granatum* L.) has been grown for its appetizing fruits, attractive flowers, and color. It is native of Iran and belongs to Punicaceae family.[1,2,3] It is usually known as super fruits due to its high genetic diversification. It is also known as Chinese apple, Carthage Apple and Seeds Apple,[4] in various regions. *Punica granatum* L., is a native shrub of Asia and Mediterranean Europe that has a rich history of traditional use in medicine. For centuries, the barks, leaves, flowers, fruits, and seeds of this plant have been used in medicines.[5] The potential curative properties of pomegranate are wide ranging and include medication and prevention of cancer cardiovascular disease, diabetes.[6] Other potential applications include infant brain ischemia, Alzheimer's disease, male infertility, arthritis, and fatness,[4,7,8,9,10,11] It is widely reported that pomegranate exhibits antivirus, antioxidant, anticancer, and anti-proliferative activities.[12,13,14,15] The medicinal value of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and tannins,[16,17] *Punica granatum* is a rich source of bioactive compounds and contain a variety of secondary metabolites. The fruit is rich by tannins and other biochemical's.[18,10] The nutritional parameters are not limited to the edible part of the fruit, the vital role are played by the non- edible fractions of fruit and tree i.e. leaves, barks, buds, flower and peel. Although, these parts are considered to be waste, they contain enormous amount of nutritional value and biological active compounds compared to the edible portion of the fruit.[19] reported that Pomegranate peel is an inedible portion of the fruit, is found to have higher medicinal value and the attracts attention due to its apparent wound healing properties, immune modulatory activity and antibacterial activity, anti-atherosclerotic and anti-oxidative capacities. The entire plant has medicinal property such as the seeds and juice cure throat problems, eye diseases, gum bleeds, toning skin, cancer, cardiovascular disease, diabetes, infant brain ischemia and male infertility.[20]

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

Punica granatum (bark-Peel) was obtained from (Bara) North Kordofan State- Sudan. 2019.

2.2. Preparation of plant extracts

A weight of 100 grams of the coarsely powdered shade – dried sample was successively extracted using Continuous extraction methods.

2.2.1. Continuous extraction methods

The plant material sample was exhaustively extracted by Petroleum ether in a Soxhlet apparatus. The process took in average 24 hours .The Petroleum ether extracts were filtered and evaporated under reduced pressure using Rotary evaporator the residue was weighed and The yield percentage was determined the extracted plant materials were then air –dried placed in the soxhlet and exhaustively extracted with Ethyl acetate. The extract was filtered and evaporated under reduced pressure again using Rotary evaporator .The residue was weighed and the yield percentage was determined The extracted plant materials were then air – dried. The Ethyl acetate extracted and air –dried plant materials were then

extracted with Ethanol described above .The residue was weighed and the Yield percentage was determined.[21]

2.3. Preliminary phytochemical screening for the major constituents of the Plant extracts

General Phytochemical screening for the active ingredients was carried out using method described by.[21,22] with few modification.

2.3.1. Preparation of the ether extract

Twenty five gram of plant powdered was extracted with Petroleum ether in a continuous extraction apparatus (Soxhlet). The extract obtained was dried under reduced pressure using rotary evaporator apparatus. This extract was used for various phytochemical tests.[22]

2.3.1.1. Testing for the presence of Unsaturated Sterols and Triterpens

A sample (10 ml) of each extract prepared as above was evaporated to dryness on bath and the cooled residue was stirred several times with Petroleum ether to remove most of the coloring materials .the residue was then extracted with 10 ml chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous .5 ml portion of the chloroform solution was mixed with 0.5 ml of acetic anhydride following by 2 drops of concentrated sulphuric acid. The gradual appearance of green, blue & pink to purple color was taken as an evidence for the presence of sterols (green to blue) and Triterpense (pink to purple) in the sample.

2.3.1.2. Test for Anthraquinone glycoside

0.5g of thee extract was boiled with 10 ml of 0.5N KoH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene (S.D. Fine, India). To 5 ml of the benzene solution 3 ml of 10% ammonium hydroxide solution was added and the two layers were allowed to separate. Appearance of pink or red color in the lower layer indicated the presence of anthraquinones.

2.3.1.3. Testing for the presence of Alkaloids

0.5 ml of the prepared extract were dissolved in 2ml of 2N HCL and stirred while heating on the water bath for 10 minutes, then cooled, filtered and divided into two test tube. To first tube few drops of Mayer reagent were added. While to the other tube few drops of Hagar reagent were added to it. An opalescence or precipitate in either of the two test tube was taken as a presumptive evidence for the presence of alkaloids.

2.3.1.4. Testing for the presence of the Flavonoids

Three ml of the ether extract are evaporated till a residue is obtained. The residue is dissolved in (1 – 2 ml) of (50%) methanol in the heat. Metallic magnesium and 4-5 drops of concentrated Hydrochloric acid are added. A red or orange color indicates the presence of flavonic aglycones.

2.3.1.5. Testing for the presence of Coumarins

Three ml of the ether extract are evaporated to dryness. The residue is dissolved in hot water .after cooling the solution is divided in two tubes one tube will contain the reference, and the aqueous solution of the second tube is made alkaline with 0.5 ml of ammonia solution (10 %). The occurrence of an intense fluorescence under UV light indicates the presence of coumarins and derivatives.

2.3.2. Preparation of alcohol extract

After extraction with petroleum ether is extracted by refluxing, in a flask, two or three times, with alcohol for 20 -30 minutes. The filtered solution are combined and concentrated up to 50 ml. The chemically active extracted constituents can be identified by means of some specific reaction within the alcoholic or hydrolyzed extract.

2.3.2.1. Testing for the presence of Tannins

The alcohol extract (o.5 -1) is diluted with water (1-2 ml) and diluted solution of ferric chloride (light yellow, 2 – 3 drops) added the occurrence of a blackish blue color shows the presence of gallic tannin and a green blackish color indicates the presence of tannin.

2.3.2.2. Testing for the presence of reducing compounds

The alcohol extract (0.5 – 1 ml) is diluted with water (1 - 2 ml) add fehling's (1 + 11) (R) (0.5 -1 ml) solution and heat them. A brick –red precipitate indicates the presence of reducing compound.

2.3.3. Preparation of aqueous extract

The vegetable product extracted with ether and after wards with alcohol is dried and extracted in a conical flask with warm water for 20 minutes. The filtered solution are concentration up to a volume of 50 ml.

2.3.3.1. Testing for the presence of (pectins, mucilage, gums)

2 ml of the aqueous extract are added dropwise in a taste tube where (10 ml) of alcohol have already been placed. if a thick precipitate is formed if will be separated off by filtration and washed away with alcohol then stained with specific stains (hematoxylin toluidine blule, methylene blue, etc) the occurrence of a violet or blue precipitate dentose the presence of mucilages.

2.3.3.2. Testing for the presence of saponins

One g of the original dried powder plant material was placed in a clean test tube. 10 ml of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of honeycombs whose persistent appearance for at least an hour, was taken as an evidence for the presence of saponins.

2.4. Biological study

2.4.1. In-vitro testing of extracts for anti-bacterial activity

The antibacterial assay of plants extracts against different bacterial strains was conducted by cup diffusion method.

2.4.1.1. Testing of cup diffusion method

The cup-plate agar diffusion method,[23] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

One ml of the standardized bacterial stock suspension 10^8 –10^9 C.F.U/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars were left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each of the extracts dilutions in methanol using automatic micro-liter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

3. RESULTS

Phytochemical Screening of *Punica granatum* bark and Peel

The Phytochemical analysis of *Punica granatum* bark and Peel showed the presence of the following metabolites as in table (1).

Table (1): The results of Phytochemical screening were obtained as follows.

Part Used	Extracts	Metabolites								
		1	2	3	4	5	6	7	8	9
Bark	Petroleum ether	+	+	+	-	+	+	-	-	+
	70% Ethanol	+	+	+	+	-	-	+	+	-
	Aqueous	-	+	+	-	-	-	+	+	-
Peel	Petroleum ether	+	+	+	-	+	+	-	-	+
	70% Ethanol	+	+	+	+	-	-	+	+	-
	Aqueous	-	+	-	+	-	-	+	+	-

Key: 1= Flavonoids 2=Alkaloids, 3=Steroids 4= Phenols 5 = Coumarins 6 = Cardiac glycosides, 7=Tannins, 8 = Saponins, 9 = Anthraquinone glycoside. (+) = detected and (-) = not detected.

The Phytochemical screening of various extracts shows the presence of certain important components such as alkaloids, saponins, Tannins, Phenols, Cardiac glycosides, Steroids, Coumarins and Anthraquinone glycoside in *Punica granatum* (bark and Peel).

Antibacterial Activity of *Punica granatum* (Bark and Peel):

Table (2): Antibacterial Activity of *Punica granatum* (bark and Peel) against Standard bacterial strains at concentration 100mg/ml.

Part Used	Plant extract	Standard bacterial strains			
		E.c	*Ps.a*	*S.a*	*B.s*
Bark	Petroleum ether	9	12	11	12
	Ethyl acetate	13	12	16	21
	70% Ethanol	20	18	24	22
Peel	Petroleum ether	9	11	11	8
	Ethyl acetate	17	20	18	16
	70% Ethanol	14	12	10	10

Standard bacterial strains used; *E.c = Escherichia coli Ps.a = Pseudomonas aeruginosa, S.a =Staphylococcus aureus, B.s = Bacillus subtilis.*

The ethanol extract of *Punica granatum* bark reflected high activity against all bacterial strains tested. While the Petroleum ether extract of the plant showed low activity as compared to other extracts. On the other hand; the ethyl acetate extract of *Punica granatum* peel showed maximum activity against all bacterial strains tested. While the other extracts exhibited low activity.

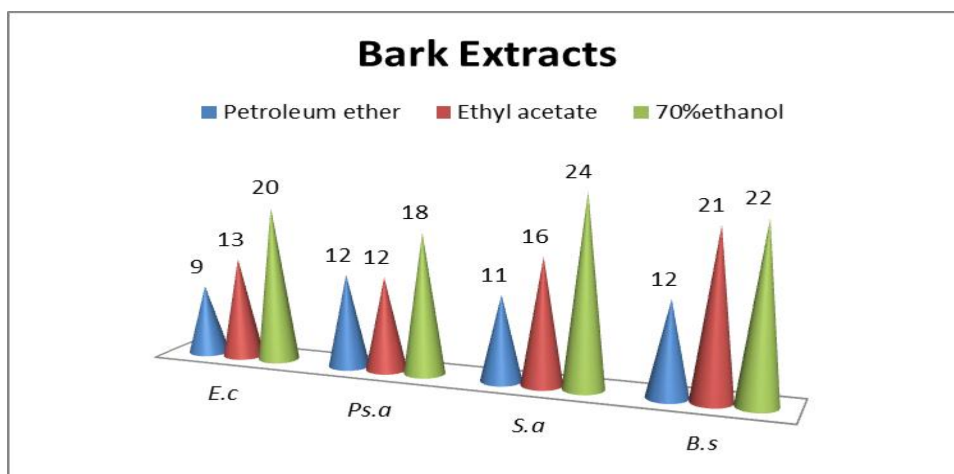

Figure 1: Antibacterial Activity of *Punica granatum* (bark).

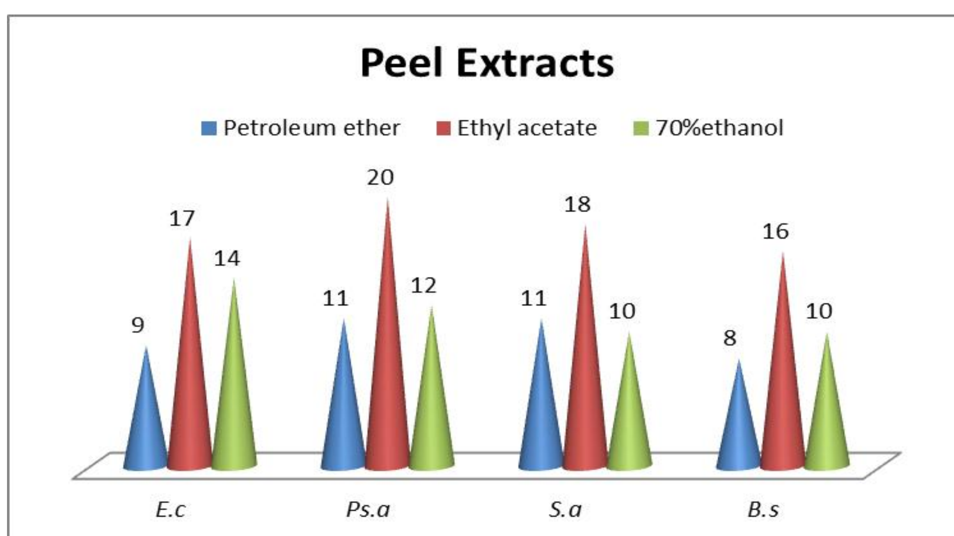

Figure 2: Antibacterial Activity of *Punica granatum* (Peel).

DISCUSSION

Phytochemical screening of *Punica granatum* (bark and Peel) showed the presence of alkaloids, saponins, Tannins, Phenols, Cardiac glycosides, Steroids, Coumarins and Anthraquinone glycoside. This result is on line with many authors who reported that *Punica granatum* contains alkaloids, sterols, tannins, flavnoids and saponins.[24,25,26] The strong antibacterial effect of plant could be due to flavonoids[27] that complex with extracellular and soluble proteins with bacterial cell walls.[28] Tannins and saponins also could be well correlated with the antimicrobial properties,[29] as tannins inactivate microbial adhesions enzymes, cell envelope transport proteins.[30] The Presence of alkaloids also justifies antimicrobial activity of plant against a number of microorganisms.[31]

CONCLUSION

Punica granatum is a rich source of bioactive compounds and contain a variety of secondary metabolites. These compounds can be used as drugs to prevent various diseases.

Conflicts of Interest

The authors declare no conflict of interest.

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