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PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES ON THE FLOWERS OF *PUNICA GRANATUM* (L)

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ABSTRACT

Punica granatum L. (Punicaceae), known as pomegranate, is small tree that is common in Mediterranean region, as far north as the Himalayas and Southeast Asia. The flower has numerous pharmacological activities such as an astringent, haemostatic, antidiabetic, antioxidant and hepatoprotective. Flowering part of this plant has been recommended in Unani literature as a remedy for diabetes. Pharmacognostic evaluation including organoleptic characters, physicochemical parameters, and powder analysis, determination of pH and bitterness value, and extractive values were carried out. Phytochemical screenings including qualitative chemical examinations were performed. Phytochemicals such as steroids, saponins, glycosides, carbohydrate, alkaloids, flavanoids, tannins, protein and amino acid are reported. Phytoconstituents in various extracts gives us clue for further investigation.

Key Words : *Punica granatum*, Pharmacognostic, Physiochemical studies, Phytoconstituents etc.

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INTRODUCTION

The pomegranate is native in the region from Iran to northern India. Since ancient times the pomegranate tree was cultivated and naturalized throughout the Mediterranean region of Asia, Africa and Europe. The pomegranate tree is a fruit bearing usually deciduous shrub or small tree between five and eight meters tall. The most plausible explanation for the name pomegranate is its derivation from the Latin word *pomum* meaning apple and *granatus*, which means seeded. It is said that the genus name *Punica* is named after the

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people of the Phoenicians who were active in broadening its cultivation¹. The Latin name is *Punica granatum* L. The tree belongs to the family *Punicaceae*. The bark and roots of *Punica granatum* are believed to have anthelmintic and vermifuge properties in Ayurvedic medicinal system. The pomegranate known locally as "Golnar-e-farsi" is an important medicinal plant in Iran whose flowers are used as astringent, hemostatic, antibacterial, and antifungal, antiviral and as a remedy for cut wound, bronchitis, diarrhea, digestive problems, man sex power

reconstituent, dermal infected wounds^{2,3}. This flower was also used for the treatment of injuries from falls and grey hair of young man in the traditional Chinese medicine⁴. Decoction of the flower is used in cases of oral and throat inflammations. In Unani medicinal system, the flower parts serve as a remedy for diabetes mellitus either as a single drug or in polyherbal formulations⁵. The ripe fruit is tonic, astringent, laxative, diuretic, used in brain diseases and chest troubles. It is useful in the treatment of dyspepsia and leprosy^{6,8}.

The chemical constituents from this herbal medicine, several fatty acids, sterols, triterpenes, anthocyanins, flavonoids, and tannins were identified and isolated from the juice, pericarps leaves and seeds^{7,8}. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics⁹. The objective of the present study is to evaluate various pharmacognostic standards like macroscopy, ash values, extractive values, microscopical characteristics of powdered flower and providing information on the type of secondary metabolites contained in different extracts the flowers. Since the plant, *Punica granatum* (Linn), is useful in traditional medicine for the treatment of several ailments, it is important to standardize it for use as a drug.

MATERIALS AND METHODS

Collection and authentication of plant material

The flowers of *Punica granatum* L. (Punicaceae) were collected from local areas of Jabalpur and Available online on www.ijprd.com

authenticated by Dr. S. Rao, Professor & Head Deptt. of Plant Physiology J.N.K.V.V. Jabalpur. All voucher specimens were deposited in *Department of Crop & Herbal Physiology*, J.N.K.V.V., Jabalpur, (M.P.) for future reference. The part of plant was shade dried at room temperature. They were reduced to a coarse powder, separately in a grinder and passed through a 40 # sieve for desired particle size and used for standardization.

Organoleptic / macroscopic evaluation

In the present study the powder of crude drug was investigated for its macroscopic characteristics i.e. colour, odour, taste⁹.

PHYSICOCHEMICAL PARAMETERS

Ash values

Total ash, acid-insoluble ash and water-soluble ash, values of the flower powder were done as per the reported methods^{10,11}.

Extractive values

Extracts were prepared with various solvents. Percentages of the extractive values were calculated with reference to air-dried drug^{10,11}.

FLUORESCENCE ANALYSIS

Many drugs fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents is reported^{10,12}.

pH VALUE

The pH value of the solutions was determined by means of standard glass electrode, a reference electrode and a digital pH meter. First the apparatus was calibrated using buffer of 4, 9 and 7 pH. 5 g powdered drug were taken and dissolved in 100 ml demineralized water (5%w/v solution) and 10 g powdered drug were taken and dissolved in 100 ml demineralized water (10 % w/v solution). The electrodes were immersed in the solution and the pH was measured¹³.

DETERMINATION OF BITTERNESS VALUE

The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride R.

The bitterness value is expressed in unit's equivalent to the bitterness of a solution containing 1g of quinine hydrochloride R in 2000 ml^{14, 15}.

Preparation of Stock and diluted quinine hydrochloride solutions

Accurately weighed 0.100g of quinine hydrochloride was dissolved in sufficient safe

Table 1: Serial dilution for the initial test

	Tube no.								
	1	2	3	4	5	6	7	8	9
S_q (ml) stock solution of quinine hydrochloride	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
Safe drinking water (ml)	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
Quinine hydrochloride in 10 ml of solution (= c) (mg)	0.042	0.044	0.046	0.048	0.050	0.052	0.054	0.056	0.058

Table 2: serial dilution for the second test

	Tube no.									
	1	2	3	4	5	6	7	8	9	10
S_T (ml)	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.0
Safe drinking- water (ml)	9.00	8.00	7.00	6.00	5.00	4.00	3.00	2.00	1.00	-

S_T, stock solution of the plant material being examined.

Preparation of Stock and diluted solutions of the plant material

The solution of 10 mg/ml concentration of each plant material was prepared. The serial dilution was done Using 10 test-tubes for the serial dilution for the test as indicated in Table 2.

Method

After rinsing the mouth with safe drinking-water, 10ml of the most dilute solution was tasted and was swirled in the mouth mainly near the base of the tongue for 30 seconds. If the bitter sensation is no longer felt in the mouth after 30 seconds, then the solution was spitted out and after waiting for 1 minute it was ascertained whether this was due to delayed sensitivity. Then mouth was rinsed with safe drinking-water. The next highest concentration was not tasted until at least 10 minutes have passed. The threshold bitter concentration was the lowest concentration at which a material continues to provoke a bitter sensation after 30 seconds. After the first series of tests, the mouth was rinsed thoroughly with safe

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drinking-water to produce 100 ml. Further 5 ml of this solution was diluted to 500 ml with safe drinking water. This stock solution of quinine hydrochloride (S_q) contains 0.01 mg/ml. Use nine test-tubes for the serial dilution for the initial test as indicated in Table 1.

drinking-water until no bitter sensation remains. At least 10 minutes was waited before carrying out the second test.

The bitterness value was calculated in units per g using the following formula:

$$\frac{2000 \times c}{a \times b}$$

a x b

Where a = the concentration of the stock solution (S_T) (mg/ml),

b = the volume of S_T (in ml) in the tube with the threshold bitter concentration,

c = the quantity of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration

EXTRACTION OF PLANT MATERIAL

The air-dried powdered flowers (400g) were subjected to successive hot continuous extraction (soxhlet) with petroleum ether, chloroform, ethyl acetate and ethanol. Finally crude drug was macerated with water. Each time before extracting with the next solvent the powder material dried in a hot air oven at 50^oc for one hour. After the effective extraction, solvents were concentrated

using rotary flash evaporator finally dried in dessicator over anhydrous sodium sulphate and the extract obtained with each solvent was weighed. Percentage yield of obtained extracts were calculated. The residue was stored at 0-4 °C for subsequent experiments^{16, 17}.

PHYTOCHEMICAL INVESTIGATION

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as sterols and triterpenoids (Salkowski; Libermann-Burchard and Libermann's test), Carbohydrates (Molisch's ; Fehling's solution; Benedict's and Barfoed's test) Tannins and phenol compounds (Ferric chloride; Lead acetate and Gelatin solution test), glycosides (Keller-Killiani and Baljet's test), alkaloids (Mayer's; Dragendorff's; Wagner's and 1% picric acid reagents), Saponin (frothing and haemolysis tests), Proteins (Biuret; Million's and Xanthoprotein test) , Amino Acids (Ninhydrin test) and Flavonoids (Shinoda test)^{16,17,18}.

RESULTS

Organoleptic evaluation of plant material are shown in table 1. The physico-chemical analysis of powder exposed the moisture content (loss on drying), total ash, acid insoluble ash, water soluble, alcohol soluble extractives, water soluble extractives, chloroform soluble extractives and petroleum ether soluble extractives are as shown in table 2. The fluorescence analysis of powdered flower material was subjected to analyse under Long Ultra Violet light after treatment with various chemical and organic reagents. The florescence behavior was noted as in table 3. pH of powdered drug solution of different concentration were found to be acidic are shown in table 4. Result of

bitterness value of plant material is shown in table 5.

The dried powder of *Punica Granatum* flowers were subjected to extraction with petroleum ether, chloroform, ethanol and water. the percentage yield obtained from the nonpolar solvents i.e. petroleum ether and chloroform (3.30%, 5.50% respectively) less polar solvent i.e. ethyl acetate (1.24%) were less as compared to the yield obtained from the polar solvents (10%, 16% respectively). The percentage yield obtained from the successive extraction are given in table 6. The Preliminary phytochemical screening of various extracts of *Punica Granatum* flowers revealed the presence of various phytoconstituents in each extracts. It showed the presence of steroids, saponins, glycosides, carbohydrate, alkaloids, flavanoids, tannins, protein and amino acid. Results of phytochemical screening of various extracts of *Punica Granatum* flowers are given in table 7.

Photo: *Pomegranate flowers and fruit*



Table 1: Organoleptic Characters

S No.	Name of plants	Part used	Description		
			Colour	Odour	Taste
1	<i>Punica Granatum</i>	Flower	Brick red	Odourless	Slightly bitter

Table 2: Physio-chemical parameters of *Punica granatum*

S. No.	Parameters studied	Values obtained on dry weight basis(w/w)
1	Loss on drying	12.15 % w/w
2	Total Ash	4.4 % w/w
3	Acid insoluble Ash	0.9 % w/w
4	Water soluble Ash	2.82 % w/w
5	Alcohol soluble extractives	19.23 % w/w
6	Water soluble extractives	28.16 % w/w
7	Chloroform soluble extractives	0.21 % w/w
8	Pet-Ether soluble extractives	0.83 % w/w

Table3: Florescence analysis of crude drug (*Puniica Granatum flowers*)

S No.	Solvent used	Ordinary light	UV light 254 nm	UV light 366 nm
1	Powder as such	Brick red	Red	Brick red
2	Powder + dil Hcl	Yellow brown	Light brown	Light black
3	Powder + dil HNO ₃	Yellow	Light green	Orange
4	Powder + dil H ₂ SO ₄	Brown	Light green	Green
5	Powder + glacial acetic acid	Brick red	Light green	Brick red
6	Powder + 5% NaOH	Orange yellow	Dark green	Orange yellow
7	Powder + 5%KOH	Yellowish black	Dark brown	Brown
8	Powder + 5%FeCl ₃	Black	Greenish black	Black
9	Powder + ammonia	Orange	Dark orange	orange brown
10	Powder + Pet Ether	Light pink	Transparent	Violet
11	Powder + benzene	Buff	Transparent	Violet
12	Powder + acetone	Light pink	Buff	White
13	Powder + ethyl acetate	Pink	Dirty	Violet
14	Powder + ethanol (95%v/v)	Light pink	Light green	Light yellow
15	Powder + water	Pink	Light brown	Light black

Table 4: pH of crude drugs

S. No.	Solution of different concentration	pH values (<i>Punica granatum</i> flowers)
1	pH of 5% solution	3.60
2	pH of 10% solution	3.72

Table 5: Determination of Bitterness Value

S.No.	Name of plant materials	Bitterness value (units/g)
1	<i>Punica granatum</i> flowers	1.20

Table 6: Percentage yield of various extracts of *Punica Granatum* flowers

Sr. No.	Solvent	Nature of extracts	Color	% Yield (w/w)
1	Pet-ether(60-80°C)	Solid	Yellowish green	7.30
2	Chloroform	Semisolid	Brown	5.50
3	Ethyl acetate	Semisolid	Reddish Light Brown	3.12
4	Ethanol(95% v/v)	Semisolid	Reddish Brown	10
5	Chloroform water IP	Semisolid	Reddish Brown	16

Table7: Preliminary phytochemical investigations of extracts of *Punica Granatum* flowers

Sr. No	Phytoconstituents	Pet-ether (60-80°C)	Chloroform	Ethyl acetate	Ethanol (95% v/v)	Chloroform water IP
1	Steroids	+	-	-	-	-
2	Triterpenoids	+	-	-	-	-
3	Saponins	-	-	-	+	+
4	Glycosides	-	+	-	-	-
5	Carbohydrates	-	-	-	-	-
6	Alkaloids	-	-	-	-	-
7	Flavanoids	-	-	-	+	+
8	Phenolic Compounds	-	-	+	+	-
9	Tannins	-	-	-	+	-
10	Proteins	-	-	+	-	+

+ Present**- Absent****CONCLUSION**

The presence of these phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physiochemical parameters. The above studies provide information in respect of their identification, chemical constituents & physicochemical characters which may be useful for setting standards for crude drugs. The

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pharmacognostic constants and phytochemical evaluation for the flower of this plant reported in this work could be useful for the compilation of a suitable monograph. And further work aiming towards tracing out of phytochemicals present in it and pharmacological activities are in progress.

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