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Research Article

PRELIMINARY AND PHARMACOGNOSTIC INVESTIGATION ON LEAVES OF *PUNICA GRANATUM*

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ABSTRACT
Among the medicinal plants *Punica granatum* commonly known as pomegranate is one of the important ethno medicinal plants used in traditional and folklore therapies. For the preparation of aqueous extract decoction method, methanolic and hexane extract soxhlet distillation methods were used. The methanolic crude extract show higher degree of inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. Phyto chemical analysis conducted on whole leaf extracts of *Punica granatum* revealed the presence of various chemical constituents having medicinal values. Through proximate analysis identification of plant material and to ascertain it's quality and purity. In the present study morphology, macroscopic, microscopic, physico chemical, proximate, phytochemical screening of powdered crude drug were carried out as referential information for identification and characterization of *Punica granatum* leaf and it's extracts.
Keywords: Antibacterial activity, phytochemical screening, powder analysis, *Punica granatum,*

INTRODUCTION

The therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal medicinal practitioners. Natural products are the source of synthetic and traditional herbal medicine[1,2]. The pomegranate, *Punica granatum*, an ancient, mystical, and highly distinctive fruit, is the predominant member of Punicaceae family. The pomegranate tree typically grows 12-16 feet, has many spiny branches Flavonoids and tannins are phenolic compounds and plant phenolic are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of pomegranate. The secondary metabolites (phytochemical) and other chemical constituents of medicinal plants account for their medicinal value. For example, Saponins have hypotensive and cardio depressant properties[3]. Glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia[4]. The presence of saponins in whole fruit and seeds extract and glycosides in all the extracts might play a role in the cardio protective potential of pomegranate. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds[5].

Plant profile

Botanical name: *Punica granatum* L.

Taxonomical classification

- Kingdom - Plantae
- Division - Magnoliophyta
- Class - Magnoliopsida
- Sub class - Rosidae
- Order - Myrtales
- Family - Punicaceae

Common name-pomegranate

Vernacular name

- Gujarati : dadam
- Hindi : anar, anardana
- Tamil : madulam,
- Telugu : dhanimmapandu[6],

MATERIALS AND METHODS

Chemicals and Instruments

All the Solvents and reagents used in the work are of analytical grade and were obtained from Nice, Qualikems, Thomas baker, Muffle Furnace of Rolex. Glassware of Borosilicate glass; leaf constants were determined by using camera lusida. Microscopic photographs were taken using a binocular projection microscope with USB 2.0 camera.

Collection of plant

Fresh plant leaves of *Punica granatum* were collected from Guntur, Andhra Pradesh, India, Chalapathi institute of pharmaceutical sciences. The leaves were washed thoroughly with normal tap water followed by distilled water.

Identification and Authentification

Collected plant material were subjected to preparation of Herbarium and sent for identification.

Herbarium of *Punica granatum*

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Myrtales
- Genus: Punica
- Species: *Punica granatum*
- Family: Punicaceae
- Locality: lam, Guntur
- Habit: Tree
- Vernacular name: pomegranate, dhanimmapandu,

- Remark: Tree
- Collected by: M.Santhosh Aruna
- Identified by: Prof. Z. Vishnuvardhan

The herbarium of the plant specimen has been deposited at Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India. The voucher specimen No-ANU/CIPS/Identi./Tech/2013/26

Drying
Then leaves were dried under shaded condition at room temperature. Leaves were crushed to powder using grinding machine. Powder was stored at 4^{o}C in tight air container.

Sample preparation for phytochemical screening
50 g powdered sample was weighed and taken separately. The powder was moistening with ammonia and evaporated to dryness. Dried sample was extracted with chloroform and filtered. After filtration, extract the chloroform layer with 10 % sulfuric acid using separating funnel. And separate aqueous layer adjust with pH 8 with ammonia; after adjusting pH extract this solution with chloroform which organic extract obtained were evaporate to concentrate by kept open room temperature. However, aqueous extraction was evaporated to dryness by heating in water bath to obtain semi solid mass. Dried extract was stored in refrigerator for their future use in phytochemical analysis.

Evaluation
Macroscopic Evaluation
The macroscopic of a drug includes its visual appearance by the naked eye. For the anatomical studies fresh leaves were collected from the plant and investigated in different organoleptic features by repeated observations. Macroscopic identity of a medicinal plant material is based on shape, size, color, taste, apex, surface, base, margin, venation, texture, fracture and odour[7].

Microscopic Evaluation
The Microscopic examination of section and powder drugs aided by stains help in distinction of anatomy in. adulterants. Microscopically examination of epidermal trichomes, calcium oxalate crystals is extremely valuable, especially in powdered drugs. The size shape and relative positions of the different cells and tissues, chemical nature of the cell walls and of the cell contents are determined[8].

Physico-Chemical Analysis
Proximate analysis i.e., percentage ash values and extractive values were determined for the quality and purity of the crude drugs according to the official methods described. Loss on drying, swelling index, foaming index[9,10].

Ash value
The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it in the form of adulterant. This value is used to determine quality and purity if the drug.

Procedure
Crude powdered drug was taken into silica crucible, support the crucible on a pipe clay triangle placed on a ring of retard stand. Heat with a burner using a flame about 2 cm height and supporting the dish about 7 cm above the flame, heat till vapors almost seize to be evolved, then lower the dish and heat more strongly until all the carbon is burnt off . Cool in a decicator weigh the ash and calculate the % total ash with reference to the air dried sample of crude drug.

Alcohol and water extractive values
These values are useful for the evaluation of a crude drug and give idea about the nature of the chemical constituents present in a drug; useful for the estimation of specific constituents, soluble in that particular solvent used for the extraction.

Procedure
Take powdered drug and transfer to 250 ml conical flask. Fill a 100 ml graduated flask to delivery mark with the alcohol. Cork the flask and set aside for 24 hours with frequent shaking. Filter and filtrate transfer to porcelain dish as used for the ash value determination. Evaporate dryness on a water bath and complete the drying in an oven at 100^{0}C and cool in decicator and weigh. Calculate the % w/w of extractive with reference to the air dried drug.

Loss on drying
This value is useful for the determination of moisture content.

Procedure
Powdered drug was taken into porcelain dish. Dried in oven at 100^{0}C.cooled in a decicator; the loss in weight is usually recorded as moisture.

Determination of leaf constants
Stomatal Index
The % portion of the ultimate division of the epidermis surface of leaf converted into stomata is called as the Stomatal index.

$$S.I = S \div S+E \times 100$$

Slide Preparation
Set camera Lucida on microscope. Trace the divisions of stage micrometer on drawing paper and draw a square of specific surface area. Peel the epidermis and add chloral hydrate. Mount the tissue with glycerin water mixture and observe the slide under microscope.

Vein islet number and Vein termination number
It is defined as the no of vein islets/sq. mm of the leaf surface mid way between the mid rib and margin. It is constant for the given species of plant. It is used as characteristic for the identification of the allied species.

Slide Preparation
Cut the leaf midway between midrib and margin to get a square shaped piece of leaf. Boil it in chloral hydrate solution until chlorophyll is removed. Take decolorized leaf piece on to glass slide and mount it with glycerin-water mixture. Trace the vein islets and vein islet terminations onto the square which was done /drawn on a drawing paper. Count the vein islets and terminations on any two sides of square which arrive half of its portion inside and half outside.

Powder Analysis

Calibration of one division eye piece micrometer using stage micrometer

One division of stage micrometer = 0.01 mm/10 micrometer

$$Calibration\ factor = \frac{No\ of\ divisions\ of\ stage\ micrometer}{No\ of\ divisions\ of\ eye\ piece\ micrometer} \times 10$$

Determination of diameter of calcium oxalate crystals

Slide Preparation

Take a small quantity of leaf powdered drug into a clean glass slide. Add 1-2 drops of glycerin – water mixture. Place the cover slip and observe under high power magnification; measured the length of calcium oxalate crystals by focusing them onto the lines of eye piece micrometer.

Determination of shape and diameter of starch grains

Slide Preparation

Take small quantity of given powdered crude drug into a watch glass. Add few drops of iodine solution. Mix the contents properly. Mount the slide with cover slip. Observe under the microscope.

Phytochemical Screening

Qualitative screening for the presence of various phytochemical compounds was performed using the methanolic extract. Presence of carbohydrates and reducing sugars was determined by Molish's test, Benedict's test and Fehling's test. Presence of glycosides was detected by Borntrager's test. Alkaloids in the extracts were evaluated by Mayer's test. The presence of phytosterols was indicated by Salkowski's test. Deoxy sugars were detected by Killer Kiliani's test. The saponins were analyzed by Froth's test. The occurrence of phenolic compounds and tannins were confirmed by ferric chloride test and gelatin test, respectively. The presence of flavonoids was investigated by lead acetate test. The occurrence of amino acids in the extract was assessed by Ninhydrin's test while the possibility of gums was studied by conducting borax test[11].

Preliminary Phytochemical Screening

For the preliminary phytochemical analysis, the dried powdered material was extracted with methanol (40-60°C), hexane, and, using Soxhlet apparatus. The aqueous extract was prepared by cold maceration. The extracts were filtered and concentrated under reduced pressure, dried and weighed. Each extract was tested for the presence of different phytoconstituents, viz. alkaloids, Flavonoids, Saponins, steroids, tannins, coumarins, triterpenoids and glycosides by usual prescribed methods[12].

Test for Alkaloid

3 ml aqueous extract was stirred with 3 ml of 1 % HCl on steam bath. Mayer and Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

Test for Tannins

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ Solution were added; formation of green precipitate indication of presence of tannins.

Test for Saponins

5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of Saponins.

Test for Phlobatannins

About 2 ml of aqueous extract was added to 2 ml of 1 % HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of Phlobatannins.

Test for Flavonoids

To 1 ml of aqueous extract, 1 ml of 10 % lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for Flavonoids.

Test for Terpenoids

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 minutes. Development of a grayish color indicates the presence of Terpenoids.

Tests for glycosides (Liebermann's test)

2 ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A color change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycon portion of glycoside).

Tests for steroids

- A red color produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids.
- Development of a greenish color when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

Assay of Antibacterial Activity

Antibacterial activities of the extracts were studied by agar well diffusion method. Test cultures of the bacterial pathogens were prepared by transferring a loop full of bacteria from nutrient agar slants into Mueller Hinton broth and incubated at 37°C. Lawn cultures of the test pathogens were prepared by swabbing sterile Mueller Hinton agar plates with 24 h old bacterial broth. Wells were punched with a sterile cork borer (6 mm internal diameter) and 35 µl of the extract was added to each well. Controls were maintained with respective solvents. Ampicillin and streptomycin (50 mg/ml) were used as standard antibiotics for gram positive and gram negative bacteria, respectively. Following incubation at 37°C for 24 h, diameters of the inhibitory zones were measured to the nearest millimeter.

RESULTS AND DISCUSSION

Macroscopic Evaluation

Punica leaves are dark green in color, 3-7 cm in length and 2 cm in width. These are glossy and lathery leaves that are narrow and lanced shape. Upper and lower surfaces of leaves were in Figure 1. Macroscopic features were represented in Table 1.

Table 1: Macroscopic Features

S. No.	Features	Observations
1	Color	Dark green
2	Odor	Characteristic
3	Taste	Bitter
4	Size	3-7 cm long, 3 cm broad
5	Shape	Narrow and lance shape
6	Leaf margin	Entire and curled
7	Leaf surface	Leathery and glossy
8	Leaf apices	Tapered
10	Texture	Pubescent

Table 2: Proximate analysis of leaves

Parameters	% Values
Total ash	8
Acid insoluble ash	0.5
Water insoluble ash	2
Loss on drying	8 %
Alcohol extractive value (cold)	3 %
Water extractive value (hot)	28 %
Swelling index	0
Foaming index	120.8

Table 3: Quantitative details of microscopic characteristics of leaves

S. No.	Leaf Constants	Value (mm^2)
1	Stomata number	500 cells
2	Stomatal index : upper epidermis Lower epidermis	23.81 22.12
3	Vein islet no	29.5
4	Vein termination no	44.37

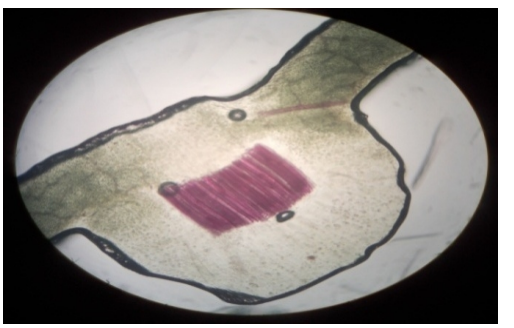
Table 4: Preliminary phytochemical investigation of *Punica granatum* leaves

Chemical tests	Aqueous extract	Ethanol extract	Hexane extract
I. Triterpenoids and Steroids			
Liebermann Burchard Test	-	+	+
II. Glycosides			
Keller Killiani Test	+	+	+
Bromine water	+	+	+
III. Saponins			
Foam test	-	+	-
IV. Alkaloids			
Hager's Test	-	-	+
V. Flavonoids			
Ferric Chloride test	+	+	-
Alkaline reagent test	+	+	-
Lead Acetate Solution test	+	+	-
VI. Tannins			
Gelatin Test	+	+	+
VII. Proteins			
Biuret test	+	-	-
VIII. Free amino acids			
Ninhydrin's Test	+	-	-
IX. Carbohydrates			
Benedict's Test	+	-	+
X. Vitamin C			
DNPH test	+	-	-

Table 5: Antibacterial activity of leaves

S. No.	Organism	Concentration	Methanol Extract	Aqueous extract
1	*E. coli*	20 %	7.5	2.8
		30 %	10	4.5
		50 %	15	5.0
2	*Staphylococcus aureus*	20 %	12.0	7.3
		30 %	12.8	7.1
		50 %	14	8.3
3	*Salmonella typhi*	20 %	9.0	4.5
		30 %	10.9	4.9
		50 %	12	6.9

Figure 1: Upper and Lower Surface of leaves



Figures 2: transverse section of leaf

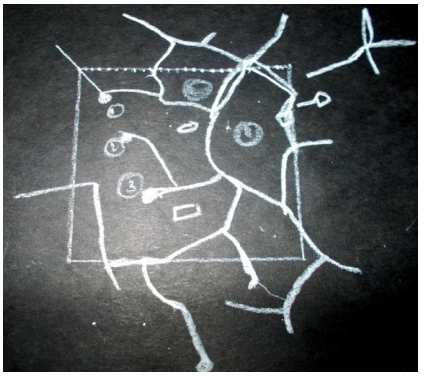


Figure 3: Stomatal index, vein islet /termination number

Figure 4: Powder microscopy

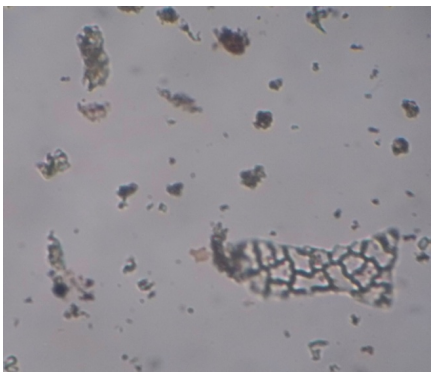


Figure 5: Calcium oxalate crystals

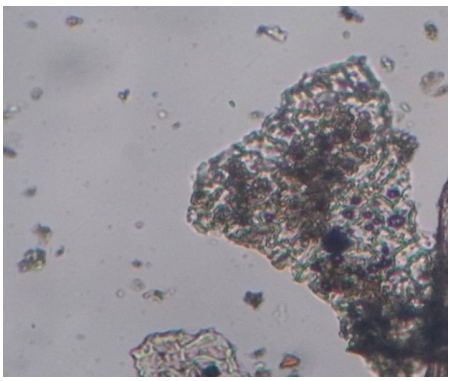


Figure 6: Starch grains

Microscopic Evaluation

Transverse section of leaf

In transverse section, the lamina shows an adaxial/upper epidermis consists of a single layer of cells found on the upper surface of leaf. It is covered by a thick waxy cuticle. The adaxial/lower epidermis made up of rectangular / barrel shaped cells with thick cuticle which distinctly forms on outer and inner ledges on the guard cells and the subsidiary cells of the stomata. Mesophyll region is distinctly differentiated into palisade and spongy tissues. Cells of palisade parenchyma are very compactly arranged in 5 -6 layers and were shown in Figure 2

Proximate Analysis

Proximate analysis helps to set up certain standards for dried drugs in order to avoid batch to- batch variation and to judge their quality and purity. Results of proximate analysis of leaves are show in Table 2.

Leaf constants: Surface Preparation of Leaf

Both upper and lower epidermises comprises of polygonal cells. Trichomes are simple, unicellular and with a very thick wall. Each stoma is surrounded by 4-5 subsidiary cells. Stomatal index of lower epidermis is less than the upper epidermis. The microscopic characteristics examination of the leaf is represented in Figure 3 and quantitative details are represented in Table 3.

Powder Microscopy

The leaf powder is dark green in color with an aromatic odor. On microscopical examination, powder of leaves showed the presence of phloem, vessels, parenchymatous layer, fibers, stomata and subsidiary cells of stomata Figure 4.

Calcium oxalate crystals

Simple and cluster crystals of rosettes were (58.80 93.68 micrometer and clusters were 235.20 to 519.20 micrometer) observed and their shape was shown in the Figure 5.

Shape and diameter of starch grains

The diameter of starch grains is 16 to 48 micrometer and shape was shown in the below Figure 6.

Phytochemical analysis of Successive extracts

Wide variety of natural compounds like alkaloids, glycosides, saponins, phytosterols, phenolics, terpenoids, Flavonoids, coumarins and tannins which exert physiological activity as synthesized in plants. Results of phytochemical analysis on various successive extracts are summarized in the following Table 4.

Antibacterial Activity

Results of antibacterial tests were given in Table 5.

CONCLUSION

The present study of the extracts from leaves of *Punica granatum* Linn. Will provide useful information for the identification. Morphological, microscopic and physicochemical standards discussed here, can be considered as identifying parameters to substantiate and authenticate the drug. This will provide a basis for the pharmacognostic standardization of the plant drug. Preliminary Phytochemical study of methanolic extract of the leaves is found to contain carbohydrates, steroids, Flavonoids, tannin, phenolic compounds and triterpenoids are present. The purified compound may be more potential and significant against the selected microorganism. Further studies require isolation and

individual characterization of each bioactive compound for the pharmaceutical use. The antimicrobial potential and antioxidant property of the plant may be attributed to the various bioactive compounds present in the crude extracts.

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REFERENCES

1. Bharat Gami, MH Parabia. Pharmacognostic Evaluation of Bark and Seeds of *Mimusops elengi*. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(4): 110-113.
2. Naqvi SA, Khan MS, Vohora SB. Antibacterial, antifungal, and antihelminthic investigations on Indian medicinal plants. Fitoterapia 1991; 62: 221-228.
3. Olaleye MT. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. Journal of Medicinal Plants Research 2007; 1: 9–13.
4. Brian FH, Thomas Bigger J, Goodman G. The Pharmacological Basis of Therapeutics, Macmillan, New York: NY, USA; 1985. p. 7
5. P Varadarajan, G Rathinaswamy and Asirvatahm D. Antimicrobial properties and phytochemical constituents of Rheo discolor. Ethno botanical Leaflet 2008; 12: 841–845.
6. National Plant Database. *Punica granatum* L; 2004.
7. Khandelwal KP. Pharmacognosy, 10th Edition, Nirali Prakashan, Pune; 2003. p. 162-168.
8. Evans WC. Trease and Evans Pharmacognosy, 14th Edition, Hartcourt Brace and Company, Asia Pvt. Ltd., Singapore; 1997. p. 226-227. PMid:9292354
9. Kokate CK. Practical Pharmacognosy. 1sted. New Delhi: Vallabh Prakashan; 1986.
10. Anonymous: Indian Pharmacopoeia 4thEd, Government of India, Ministry of Health and Welfare, Controller of Publications, New Delhi 1996; 2: A53.
11. PK Mukherjee. Quality Control Herbal Drugs–An approach to evaluation of botanicals. Business Horizons Pharmaceutical Publishers. New Delhi; 2005. p. 131-196.
12. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, New Delhi; 1994. p. 15–30.

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