



Preliminary Phytochemical Screening, Photoluminance Study And TLC Of Leaf Extracts Of *Punica Granatum*.

CHINU KUMARI, DEV PRAKASH DAHIYA, NIKHIL RANA*, KANIKA, RAHUL SHARMA

Abstract: - Throughout history, herbal medicines have been the primary treatment in traditional medicine. *Punica granatum* is renowned for its medicinal properties attributed to its diverse chemical composition. Pomegranates have been used for ages to treat a wide range of illnesses in various medical systems. Examining the presence of different phytochemicals in *Punica granatum* leaf methanolic extracts was the aim of the current study. The contents of the leaf extracts were discovered to include carbohydrates, alkaloids, flavonoids, steroids, glycosides, saponins, and tannins. *Punica granatum* methanolic leaf extracts contained alkaloids, flavonoids, terpenoids, tannins, steroids, glycosides, and carbohydrates, as shown by the phytochemical screening. These bioactive substances may be involved in *Punica granatum* therapeutic effects. They are well-known for their therapeutic qualities. Additionally, the photoluminescence investigation shed light on the fluorescence properties of *Punica granatum* leaves under varied light conditions and wavelengths. Understanding *Punica granatum* chemical makeup and possible uses in various fields is made easier by this analysis.

Keywords: - *Punica granatum*, phytochemical screening, Photoluminance study, thin layer chromatography.

Introduction:

Punica granatum, commonly known as pomegranate, has been used in various traditional medicines for its anti-inflammatory properties.(1) The active compounds in *Punica granatum* that exhibit anti-inflammatory effects include ellagic acid, gallic acid, and punicalagin A&B. *Punica granatum* has been used by Egyptians as a remedy for multiple infections and in Unani medicine as a treatment against diabetes.(2) The fruit, bark, and seeds of pomegranate have been used to treat dysentery, diarrhea, intestinal parasites, and throat infections. Pomegranate peels have been widely used for the treatment of different pathologies such as inflammation, ulcers, infections, and brain ischemia.(3)

Punica granatum exhibits anti-inflammatory properties that have shown promise in the treatment of atopic dermatitis. Several research studies support the topical anti-inflammatory potential of *Punica granatum* extracts.(4) Studies have highlighted the anti-inflammatory activity of *Punica granatum* rind extracts when applied topically to skin, showcasing their effectiveness in reducing inflammation.(5) Polyphenols found in *Punica granatum*, such as punicalagin, have been shown to possess immune-modulating and anti-inflammatory activities, which can help alleviate the symptoms of atopic dermatitis.(6) Additionally, ellagic acid, gallic acid, and punicalagin A&B, components of pomegranate, have demonstrated inhibitory effects on inflammatory pathways, making them potential alternatives for treating atopic dermatitis.(7,8)

PLANT PROFILE: -

COMMON NAME – pomegranate (Anar).

TAXONOMICAL CLASSIFICATION

The taxonomic classification of *Punica granatum* is as follows:

Kingdom	-	Plantae
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Order	-	Myrtales
Family	-	Punicaceae
Genus: <i>Punica</i>	Species	- <i>granatum</i>

ECOLOGY: -

Pomegranates can withstand soil compaction, drought, and seasonal waterlogging but are vulnerable to fire, frost (at -11 degrees C, damage to trees is irreversible), and strongly alkaline soils.(9)

BOTANIC DESCRIPTION

Its native range is from northern India to southern Europe. It is a small tree or shrub that reaches a height of five to ten meters. Its canopy is open and it has many prickly branches.

The surface of the bark is smooth and gray, and the stem is hard and spiky. Simple, oppositely oriented, glabrous, oblong or obovate, and short-petioled, leaves measure 2 to 8 cm in length.

The orange-to-red blooms are striking. The fruit is spherical and has a dry outer layer, or husk, composed of two layers: the mesocarp, which is a soft inner layer, and the epicarp, which is a hard outer layer.

The *Punica granatum*, or pomegranate plant, is a large shrub or small tree that reaches a maximum height of thirty feet. It possesses, Leaves - Smooth, evergreen, oppositely oriented, oblong or obovate, 2–8 cm long, short petioled, and shining.

Flowers - Orange to red, regular, solitary or in fascicles at apices, 4-6 cm, petals lanceolate, 5-7, wrinkled, brilliant orange-red Fruit- Hexagonal in shape, 6–12 cm wide, 200 g in weight, with a thick tubular calyx atop. The rind is red, tough, and leathery, and it has 600 arils—seed casings—under its thick skin.

PHYTOGRAPHY

In India, flowering is seen from mid-April to mid-May. Fruits take six to seven months to mature, with fruiting starting in the seventh or eighth year. Fruit production can range from 20–25 for young trees 100–150 for trees that are 10 years old and even 200–250 for older trees. Tree sizes affect yield.(10,11)

PHYTOCHEMISTRY

- Vitamins, organic acids, fatty acids, pectins, ascorbate, polyphenols, polysaccharides, and malate are present in Fruit.
- Juice contains 85.4% water, 10.6% sugars, 1.4% pectin, and roughly 1% polyphenols.
- Peel: cardiolites, steroids, tanins, flavonoids, quinones, saponins, and terpenoids.
- Hydrolyzable tannins and flavonoids found in leaf extracts.

Punicalagin, gallic acid, cinnamic acid, quercetin, protocatechuic acid, and p-coumaric acid are extracts from pomegranate peels.

Pharmacological Activity

Antimicrobial activity: - Antimicrobial agents can be found in abundance in these medicinal plants. This Plants are a source of many strong and potent drugs that are used medicinally in various countries. Punicalagin, a compound found in pomegranate fruit peels, has antibacterial activity against *S. aureus* and *P. aeruginosa*.

Healing Activity: - According to Ayurveda literature this plant has also given the name of wranvishapaka which means that it has the property of healing all types of wounds.(12) Similarly, The primary components of *Punica granatum* that are in charge of the healing properties are gallic acid and catechin.

Anti-inflammatory Activity: - Pungenic acid, the main component of pomegranate fatty acids, is a well-known anti-inflammatory compound that prevents inflammation from starting by preventing prostaglandin from being synthesized.

The majority of the anti-inflammatory substances came from the seeds. The findings showed that fatty acids and polyphenols were the main anti-inflammatory ingredients.

Anti-diabetic Activity: The current diabetes pandemic is a global health concern, and there is a need for easily accessible, efficient treatments or preventative measures. One natural product with such potential in Indian traditional folk medicine is the pomegranate (*Punica granatum*), whose juice, seeds, and flowers have a unique hypoglycemic property. Pomegranate compounds that have been linked to diabetes prevention include gallic, ursolic, and oleanolic acids.(13)

Pomegranate flowers are utilized in many countries as a dietary supplement and as an antidiabetic in Unani medicine.(14,15)

Materials and Methods:

ETHNOMEDICINAL STUDIES:

Within the field of medical anthropology, ethnomedicine examines the causes, origins, and treatment of diseases as they relate to particular populations. The field of ethnomedicine emerged in accordance with the evolution of human civilization. Numerous terminologies are derived from ethnomedicine in the field of medical anthropology. Although folk medicine and primitive medicine are terms frequently used to describe this field, ethnomedicine is thought to be a more accurate term. To gather information about *Punica granatum* medicinal applications, various methodology was employed including-

- Interaction with the local folks
- Ethnobotanical Surveying and Fieldwork
- Data collection
- Research focus/Literature studies
- Pharmacological Assessment
- Publication of Research Findings
- Analysis and reporting

PLANT MATERIAL

Collection and Authentication of Sample:

The plant was harvested from its natural habitat in the mid of September at Baggi, Mandi Himanchal, Pradesh. The plant specimen was authenticated by Dr. Pankaj Sharma, Sr. Scientific Professional Himachal Pradesh State Biodiversity Board, Shimla, Himachal Pradesh Letter no.- HPSBB/271.

MACROSCOPIC STUDY

Morphological characteristics:

Morphological characteristics of *Punica granatum* such as plant structure, leaves, flowers, fruit, texture, colour and peeling sections were studied by visual observations and verified with standard taxonomical books.

Organoleptic study:

Organoleptic properties such as odour, taste and touch were visually and sensory observed. The collected data was precisely recorded and documented.(16)

Preparation Of Plant Material:

Punica granatum leaves were cleaned, shade-dried, and then coarsely ground. *Punica granatum* leaves were then powdered after being shade-dried.

Extraction of Plant material:

Extraction of *Punica granatum* leaves was done by Soxhlet extraction method. The powdered plant material was extracted with methanol. 20gm of plant powder were extracted at 60 degrees using 200 ml. The extract was then evaporated using the water bath at 60°C until it had reduced to one-third of its original volume. The extracts were then allowed to cool and stored for further use.(17)

PHOTOLUMINESCENCE STUDIES:

Photoluminescence, or photoluminescence spectroscopy, is a method in which light energy causes a material's photons to emit. By shining light on a sample, this non-contact, non-destructive technique produces photo-excitation, which raises the material's electronic state. Photoluminescence is a luminescence caused by photons released by the material as it relaxes back to a lower energy level.

The importance of photoluminescence studies lies in their ability to provide valuable insights into material properties, including:

Impurity levels and defect detection: - At low sample temperatures, the photoluminescence spectroscopy spectrum frequently displays spectral peaks linked to impurities present in the host material. This technique's high sensitivity makes it possible to detect incredibly low concentrations of both intentional and unintentional impurities, which can have a significant impact on the quality of the material.

Recombination mechanisms: - The relative amounts of radiative and nonradiative recombination rates are directly correlated with the amount of photoluminescence spectroscopy that a material emits. Since nonradiative rates are usually linked to impurities, this method can qualitatively track how growth and processing conditions affect the quality of the material.

PHYTOCHEMICAL SCREENING:

1). TEST FOR ALKALOIDS: -

Mayers Test: – take 1 ml of extract and transfer to a test tube. Then add 1ml of Mayers Reagent (potassium mercuric iodide solution) and shake well. The appearance of white, creamy, precipitate indicates the presence of alkaloids.(18)

Dragendroffs test – take 1 ml of extract solution in the test tube and add dragendroffs reagent (potassium bismuth iodide solution) shake well. Appearance of orange–red precipitate indicates the presence of alkaloids.(19)

Wagner's test: A test tube will be filled with 1 ml of extract. Wagner's reagent, 1 mL of potassium iodide, will be then added and shaken. A reddish-brown precipitate's appearance indicates the presence of alkaloids.(20)

2). TEST FOR TANNINS: -

Ferric chloride (FeCl₃) Test- About 0.5 mg of dried powder of plant extract taken in test tube, add 20 ml water in a test tube, boiled, and filtered. We'll add a few drops of a 0.1% ferric chloride solution. Tannins will be visible as a blue-black or brownish-green coloration.

Gelatin Test: A test tube will be filled with 1ml of extract. Next, add the sodium chloride-containing 1% gelatin solution and shake. The presence of tannins is indicated by the appearance of white precipitate.(21)

Borntrangers Test: Add 0.1 g of the drug, 5 ml of dilute HCl, and 5 ml of 5% solution of ferric chloride. Boil for a few minutes. Cool and filter. Shake the filtered part with benzene. The separated benzene layer and equal volume of dilute solution of ammonia shows pink color.(22,23)

3). TESTS FOR FLAVONOIDS:

lead acetate test - 1ml of the extract will be taken and put into a test tube in order to look for flavonoids. After that, shake well and add a few drops of lead acetate. When a yellow precipitate forms, flavonoids are present.(24)

Alkaline reagent test- A test tube will be filled with 1 mL of the extract. Subsequently, a small amount of sodium hydroxide solution will be added and agitated. The presence of flavonoids is implied by the appearance of an intense yellow colour that becomes colourless when diluted acid is added.(25)

4). TEST FOR GLYCOSIDES

legals test: After taking 1 ml of the extract, 1ml of sodium nitroprusside, a small amount of sodium hydroxide solution, and shaking were added. A pink to blood red precipitate's formation indicates the presence of a glycoside.(26)

5). TEST FOR TERPENOIDS

Salkowski's test: - 5ml Extract was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish-brown coloration of the inter face was formed to show positive results for the presence of terpenoids.(27)

6). TEST FOR REDUCING SUGARS

Fehling's test: - Take 1 ml of a given sample in a clean, dry test tube. Add about 2-3 drops of Fehling's reagent to both the tubes and mix them in a vortex. Keep the test tubes in the water bath for 1-2 minutes.

Observe the appearance of color in the test tubes. Note down the appearance of color seen in the test tubes.(28)

Benedict's test: - One millilitre of the analyte sample must be mixed with 2 millilitres of Benedict's reagent and heated in a bath of boiling water for 3 to 5 minutes. The development of a brick-red coloured precipitate of cuprous oxide confirms the presence of reducing sugars in the analyte.(29)

7). Test for carbohydrates

Molisch test: - Sample volume is 2 millilitres in a test tube. You now add two drops of Molisch's reagent to the sample and stir. Preparing Molisch's Reagent: Naphthol is added to 95% ethanol to create Molisch's reagent., add it gradually while tilting the test tube by its sides and avoiding vigorous mixing.(30)

THIN LAYER CHROMATOGRAPHY

Preparation of Stationary Phase: - The sample was applied to TLC-Precoated Plates that had silica gel G F254 (a fluorescent indicator) on them, 1cm above the baseline.(31)

Development of Chromatographic Chamber: - A glass container with a water and methanol (1:1) solvent system was used to create the chromatographic chamber.

To eliminate the edge effect, this chromatographic chamber was pre-saturated.

Development of Chromatogram: - The stationary plate containing the sample was put into the pre-saturated chromatographic chamber so that the solvent or mobile phase was below the sample application spot.

The sample was eluted through the TLC plate by the capillary action mechanism.(32)

Scanning and detection of the spots: - The TLC plates were left to air dry. Using a UV chamber exposed to UV-visible radiation, including both near- and far-UV radiation, the spots were scanned and found.(33)

We computed the R_f value of spots using the following formula:

$$R_f = \frac{\text{Distance travelled by spot from origin}}{\text{Distance travelled by solvent front}}$$

RESULT AND DISSCUSSION: -

MACROSCOPY STUDY:

Morphological Study:

Botanic Description of plant

- Plant Structure: *Punica granatum* is a small multi-stemmed shrub or tree that can grow 5-10 meters tall with an open canopy and a low base crown
- Leaves: The leaves are simple, 2-8 cm long, oblong or obovate, glabrous, oppositely placed, and have a shining surface
- Flowers: The flowers are regular, solitary or in fascicles at apices, 4-6 cm in size, with lanceolate petals that are wrinkled and brilliant orange-red
- Fruit: The fruit of *Punica granatum* is a round berry, 5-12 cm in size, with a leathery pericarp. The interior of the fruit is compartmentalized with pink-red sections of pulp-like tissue, each containing a seed grain

Organoleptic study:

Table No. 1: Organoleptic properties of *Punica granatum* leaves are:

S. No	Parameters	Observation
1	Taste and Flavour	mildly astringent
2	Colour	Dark green to pale yellow
3	Texture	smooth and slightly waxy texture
4	Aroma	Subtle, slightly astringent

PHOTOLUMINESCENCE STUDY:

Under visible and ultraviolet light, the fluorescence of a powdered plant material with different chemical reagents and extracts was observed.

The following is a list of the results obtained:

Table No. 2: Fluorescence characteristics of *Punica granatum* leaves:

Sr. No.	Treatment	Visible light	UV Light 254nm (Shorter Wavelength)	UV Light 365nm (Long Wavelength)
1.	Leaves Powder	Dark green	Greenish	Dark brown
2.	Leaves Powder + 1N NaOH	Black	Blood red	Dark purple
3.	Leaves Powder + 1N HCl	Pale Yellow	Light green	Greenish
4.	Leaves Powder + 1N H ₂ SO ₄	Pale Yellow	Light green	Dark green
5.	Methanolic extract	Dark green	Reddish Brown	Purplish

PHYTOCHEMICAL SCREENING:

Various phytochemical tests were performed and obtained results are as follow:

Table No. 3: Qualitative analysis of *Punica granatum* leaves in methanol.

Sr No.	Phytochemicals	Phytochemical Test	Methanolic Extract
1.	Alkaloids	Mayer's test	+
		Dragendroffs test	+
		Wagner's test	+
2.	Flavonoids	Lead acetate test	+
		Alkaline reagent test	+
3.	Terpenoids	Salkowski's test	+
4.	Saponins	Froth test	-
5.	Tannins	Ferric chloride test	+
		Gelatine test	-
		Borntrager's test	+
6.	Reducing Sugars	Fehling's test	-
		Benedict's test	+
7.	Steroids	Salkowski's test	+

8.	Glycosides	Legal's test	-
9.	Carbohydrates	Molisch's test	+

+ = Present, - = Absent

Thin Layer Chromatography:

The methanol and distilled water extract was subjected to qualitative chromatography using methanol: distilled water (1:1) (MeOH: DW) as the mobile phase. Four spots in the chromatogram of the methanolic extract were detected under 254 nm Ultra-Violet radiation.

Table4: Rf value calculation in thin layer chromatography of *Punica granatum* leaves under 254nm.

Extract	Mobile Phase	Total run(cm)	Number of spots	Distance of Solvent front from origin	Distance of spot from origin (Sample application site)	Rf value
Methanolic extract	MeOH + DW (1:1)	3	4	7	4.8	0.685
					5.2	0.742
					5.5	0.785
					6.4	0.914

CONCLUSION:

The initial research indicates that *Punica granatum* leaves contain a wide range of bioactive substances that may have pharmacological implications. In conclusion, the preliminary phytochemical screening, Photoluminance study, and TLC analysis of leaf extracts from *Punica granatum* reveal a rich diversity of bioactive compounds with potential medicinal and industrial applications. *Punica granatum* leaves have the potential to be medicinal due to the presence of numerous phytochemicals, which calls for more research into their pharmacological characteristics. The Photoluminance study also emphasizes *Punica granatum* possible use in optoelectronic applications. *Punica granatum* continues to reveal its significance in both traditional and modern scientific contexts through careful examination and exploration of its contents, opening up new possibilities for future research and development in the fields of nutraceuticals, pharmaceuticals, and beyond.

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