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Phytochemical Screening and Fluorescence Analysis of Methanolic Flower Extracts of *Punica granatum* L.

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Abstract: To determine various phytoconstituents present in flowers of *Punic granatum*. Qualitative phytochemical analysis and fluorescence analysis was done by standard methods. The Phytochemical tests were carried out on the extracts using standard procedures to identify the constituents. The preliminary Phytochemical tests of methanolic flowers extract of *P. granatum* (L.) were screened for the presence of active principles such as carbohydrates, glycosides, fatty acids, protein and amino acids, saponins, tannins, phenolic compounds, β-sitosterols, triterpenoids, anthocyanins and flavonoids using standard procedures. The result of fluorescence analysis showed that in visible light, the plant powder exhibit various shades of green and brown fluorescence and various shades of green, blue and brown were found in under UV light. The results of the present study suggest that flowers have various phytoconstituents that can act as a source of natural antioxidants and can be used for the development of plant based drugs.
Keywords: *Punic granatum*, Secondary metabolites, phytochemical analysis, fluorescence analysis.

INTRODUCTION

The therapeutic use of herbal medicines is gaining considerable momentum in the world during the past decade. According to World Health Organization (WHO) "Any plant and its organs containing any substance that can be used therapeutically, or can be used as raw material for chemical/pharmaceutical synthesis" is classified as drugs. Medicinal plants are still used by about 80% of the world population, mainly in developing countries, for primary health care because of better tolerability, compatibility and fewer side effects [1]. Medicinal plant products are also of great importance in the process of drug discovery because of great diversity, permitting the identification of lead molecules for the development of new therapeutic agents. There is an increase in worldwide interest in the use of plant based pharmaceuticals as a complementary or alternative medicine, either to prevent or to ameliorate many diseases [2, 3, 4].The Indian region, with a vast heritage of diverse ethnic cultures and rich biodiversity is said to be a great emporium of ethnobotanical wealth [5]. Most of the flowering plants are used in alternative system of medicines like *Ayurveda, Siddha, Unani* etc [6].The plant derived medicines have been used as first line of defense to maintain health and combat diseases [7,8]. Elaborate research on the chemistry, pharmacology and clinical therapeutics of herbal products are mandatory which may eventually lead to the development and discovery of medicines that can be used in the treatment of various diseases [9].

Punica granatum L. (Punicaceae), known as pomegranate, is a deciduous small tree, up to 8 m in height with attractive reddish scarlet edible fruits. The species originated in Iran, Afghanistan and Baluchistan, found wild in the warm valleys of the Himalayas and is cultivated throughout India [10]. The dried flowers, known as Gulnar, are efficacious to treat haematuria, haemoptysis, diarrhoea, dysentery, nasal hemorrhage [11] and in Unani literature as a remedy for diabetes [12, 13]. Flower juice is recommended as a gargle for sore throat, in leucorrhoea, hemorrhages and ulcers of the uterus and rectum. The root bark and stem bark of the plant are astringent and used as anthelmintic especially against tapeworms. Fruit rind is valued as an astringent in diarrhea and dysentery. The powdered flower buds are useful in bronchitis. The seeds are reputed as stomachic and the pulp as cardiac and stomachic. The green leaf paste is applied to relieve conjunctivitis [14]. The aqueous-ethanol (50%, v/v) extract of the flowers leads to significant blood glucose lowering effect in normal, glucose-fed hyperglycemic and alloxan-induced diabetic rats [15]. In Chinese

medicine these flower are also used for the treatment of injuries from falls and grey hair of young man [16]. In addition *Punica granatum* is considered as "a pharmacy unto itself" in ayurvedic medicine and is used as an antiparasitic agent, a blood tonic, and to ulcers [17]. The results obtained in the present study thus suggest that the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Fig-1: *Punica granatum* L.

***Punica granatum* L.**

The description of the flowers is given below.

Family: Punicaceae

Vernacular name: pomegranate

Description: Deciduous small tree, up to 8 m in height with attractive reddish scarlet edible fruits and produce bright red flowers.

Distribution: Widely cultivated throughout India and also cultivated in Iran, Afghanistan and Baluchistan.

Constituents: Polyphenols including Flavonoid, tannins and organic acids.

Action/Uses: In Iran whose flowers are used as an astringent, haemostatic, antibacterial, antifungal, antiviral and as remedy for cut wound, bronchitis, diarrhea, digestive problems, men sex power reconstituent, dermal infected wounds and diabetes in Unani medicine (Iranian Traditional Medicine) literature.

MATERIALS AND METHODS

Collection and authentication of plant material

The flowers of *Punica granatum* were collected from in and around the Mannargudi, Thiruvarur DT, Tamil nadu, India. They were identified and authenticated by Dr. S. John Britto, Department of Botany, Rabiant Herbarium and center for Modular Systematics, St. Joseph's College, Trichurappalli, Tamil nadu, India.

Preparation of Plant Material

Collected plant material were thoroughly washed with distilled water and then dried under shade at room temperature for few days. The dried plant samples were ground well into a fine powder using blender. The powdered samples were then stored in airtight containers for further use at room temperature.

Preparation of extract

The residue was exhaustively extracted in a Soxhelt apparatus for at least 12 h with alcohol (methanol) and extract was used for experiment. The solvent from extract was removed under reduced pressure and controlled temperature (40-50 ºC). The yield of the extract was 12.28% w/w. The extract was kept in tightly closed container in refrigerator till further analysis.

Phytochemical screening of Methanolic Crude Extract

The Phytochemical tests were carried out on the extracts using standard procedures to identify the constituents as described by Harborne (1983) [18].

Physicochemical parameters

Foreign matter analysis

Foreign matter presence may be due to faulty collection of crude drug or due to deliberated mixing. It was separated from the drug so that results obtained from analysis of the drug gives accuracy. Its percentage in the crude drug was calculated [19].

Determination of moisture

10g of accurately weighed fresh flowers of the test plant (without preliminary drying) was taken in a china dish. It was incubated at 105^0c for 5 hours. The content of the dish after incubation was weighted and the values were noted. Drying and weighing was continued till the difference between two successive values corresponds to not more that 0.25%. The percentage of total moisture content of the drug was finally calculated.

Moisture content (%) = (Initial weight sample –final weight sample) / weight of sample x 100

Determination of extractive values

10g of the air dried plant drug was transferred to an extraction thimble, extracted with various solvents in the order of increasing polarity (Hexane, Chloroform, Ethyl acetate) by using Soxhlets extraction apparatus (for 6 hours). The extract was filtered into a tarred evaporating dish and the solvent was evaporated on a water bath. The residue was dried at 105^0C to constant weight. The percentage of extractive values for various solvents was calculated with reference to the air-dried drug.

Determination of alcohol soluble extractive values

5g of the air-dried drug macerated with 100ml of alcohol (ethanol and methanol) in a closed flask for 24hrs was frequently shaken during first 6hrs and

allowed to stand for 18hrs. It was rapidly filtered taking precautions against loss of solvents. 25ml of filtrate was evaporated to dryness in a tarred flat bottomed china dish and dried at 105^0C until constant weight was obtained. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

Determination of water soluble extractive values

5g of the air-dried drug macerated with 100ml of water in a closed flask for 24hrs was frequently shaken during first 6hrs and allowed to stand for 18hrs. Rapidly filtered and evaporated 25ml of filtrate to dryness in tarred flat-bottomed china dish and dried at 105^0C until constant weight was obtained. The percentage of water- soluble extractive was calculated with reference to the air-dried drug [20].

Fluorescence analysis

Fluorescence analysis of the drug was observed under day and UV light using various solvent extracts as well as acids and alkaline treated with solutions of the drug. The powder was treated with neutral solvents like hexane, benzene, chloroform, methanol, ethyl acetate, alcohol, acetone and acids like 1N Hydrochloric acid, 50% Sulphuric acid and alkaline solutions like aqueous and alcoholic 1N NaoH [21].

RESULTS AND DISCUSSION

In the present study the powder of crude drug was investigated for its macroscopic characteristics i.e. colour, odour, and taste are as shown in table-1. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [20]. The physicochemical analysis of powder exposed the foreign matter, moisture content (loss on drying), water soluble extractives, chloroform soluble extractives, ethanol extractives, methanol extractives, ethyl acetate extractives and hexane soluble extractives are as shown in table- 2. The fluorescence analysis of powdered flower material was subjected to analysis under long ultra violet light after treatment with various chemical and organic reagents. The fluorescence's behavior was noted as in table-3. Phytochemical evaluation was performed for qualitative detection of various chemical constituents which aid in tracing the presence of active entity that elicit a major pharmacological response. Analysis of the plant extracts revealed the presence of active principles such as carbohydrates, glycosides, fatty acids, protein and amino acids, saponins, tannins, phenolic compounds, β-sitosterols, triterpenoids, anthocyanins and flavonoids using standard procedures, which were tabulated in Table-3. Flavonoids have a wide range of biological and pharmacological activities according to in vitro studies, which includes biological activities like anti-hyperlipidemic, anti-inflammatory, antioxidant and anti-allergic properties.

Table-1: Organoleptic / macroscopic evaluation:

S.No.	Plant species	Local name	Part used	Description		
				Colour	Odour	Taste
1.	*Punica garnatum*	Anar	Flower	Brick red	Odourless	Slightly bitter

Table-2: Physicochemical analysis of *Punica granatum L.*

S. No.	Parameters	% Of Concentration
1.	Hexane	0.748
2.	Chloroform	1.872
3.	Ethyl acetate	0.900
4.	Ethanol	36.58
5.	Methanol	64.4
6.	Water	60.7
7.	Moisture	8.51
8.	Foreign matter	1.00

Table-3: Phytochemicals analysis of flowers of *Punica granatum L.*

Plant constituents	*Punic granatum* extract
Proteins	+
Carbohydrates	+
Alkaloids	-
Steroids	-
Triterpenoids	+
Glycosides	+
Saponins	+
Flavonoids	+
Tannins	+
Polyphenols	+
Anthocyanins	+
Fatty acids	+
β-sitosterol	+
Amino acids	+

Table-4: Fluorescence analysis of flowers of *Punica granatum*

S. No	Test	0 hours		24 hours		48 hours	
		Day Light	UV Light	Day light	UV light	Day light	UV light
1	Drug + Aqueous	Brown	Brown	Brown	Brown	Brown	Brown
2	Drug + methanol	Brown	Dark brown	Red	Reddish brown	Dark brown	Black
3	Drug + Alcoholic 1N N_a OH	Brown	Brown	Ash	Ash	White	Pale green
4	Drug + 1N N_a OH	Dark brown	Red	Orange	Orange	Pale orange	Orange
5	Drug + 50% HCL	Dark brown	Brown	Brown	Brown	Dark brown	Brown
6	Drug + Hexane	Light brown	Brown	Brown	pink	Pale pink	Light brown
7	Drug + CH CL_3	Brown	Light brown	Brown	Brown	Light brown	Brown
8	Drug+ Ethyl acetate	Light brown	Brown	Dark brown	Brown	precipitate	Brown
9	Drug + Acetone	Yellow	Yellow	Yellowish brown	Yellow	Yellow	Pale yellow
10	Drug+ Benzene	Brown	Brown	Brown	Brown	Brown	Pale green
11	Drug+ Alcohol	Yellow	Yellow	Yellowish brown	yellow	Pale yellow	Light brown
12	Drug+ water	Orange	Pale orange	Pale orange	orange	Pale orange	Pale orange

CONCLUSION

The selected medicinal plant was the source of the secondary metabolites i.e carbohydrates, glycosides, fatty acids, protein and amino acids, saponins, tannins, phenolic compounds, β-sitosterols, triterpenoids, anthocyanins and flavonoids. Researches in bioactive substances might lead to the discovery of new compounds that could be used to formulate new and most potent antihyperlipidemic drugs to overcome the problem of resistant to the currently available allopathic drugs. The phytochemical assessment suggests that the screened pomegranate flower and its associated bioactive compounds may possess a strong potential as a chemo preventive and possibly as new tools for preventing various human diseases. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases.

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