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RESEARCH ARTICLE

PHYTOCHEMICAL AND ANTIOXIDANT INVESTIGATION OF PUNICAGRANATUM

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ABSTRACT

This review article is all about the Punicagranatum Pomegranate possessing the phytochemicals, their activity and effect in terms of benefits and uses. Although pomegranate has already been proven containing therapeutically active ingredients collectively called as antioxidants, the fruit holds great importance in many mythologies and cultures starting from Middle East to all over the world. This article is also about the myths and facts about the fruit. The facts coming out of scientific studies carried out before are also discussed in the article. However along with factual data the article is focused on phytoconstituents of Punicagranatum and their activity determination. Mainly the active ingredients present in Punicagranatum such as flavonoids, vitamins, punicalagins and minerals are discussed.

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INTRODUCTION

Medicinal plants play a major function in meeting the medicinal and health requirements of about 70 of populations in developed and developing countries for treatment of various maladies and illnesses Plants synthesize abroad range of chemical compounds which are classified based on their chemical structure, natural or biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites directly involved in growth and development while secondary metabolites aren't involved directly and they've been worked as biocatalysts. Primary metabolites are broadly distributed in nature, being in one form or another in virtually all organisms. They' chlorophyll, amino acids, nucleotides, and soon, which have main role in metabolic processes like as photo synthesis, respiration and nutrient assimilation. They're used as industrial raw materials and food additives. Secondary metabolites are synthesized during secondary metabolism of plants. They're the basic source for the establishment of several pharmaceutical industries since they've enormous medicinal properties (2). The most important secondary metabolites are alkaloids, tannins, flavonoids, phlobatannins, saponins and cardiac glycosides. All secondary metabolites have specific function similar as saponins have antifungal activity (3). Some alkaloid may be useful against AIDS (4). Flavonoids have Broad anticancer activity (5). And tannin have antimicrobial activity.

In the search for phytochemicals or phytoconstituents that may be of benefit to the pharmaceutical industry, investigators sometimes follow leads provided by local healers in a region (6). Following similar leads, plant parts are commonly screened for phytochemicals that may be present. The presence of an important phytochemical may lead to its further isolation, cleansing and characterization. Also it can be used as the basis for a new pharmaceutical product. Successful determination of biologically active compounds from plant material is largely dependent on the type of cleanser used in the extraction procedure (7). This thus underscores the need to try as major solvents as possible in screening plant parts for phytochemicals. Punica granatum belongs to the family Punicaceae, generally known as pomegranate or small tree (8). It's also plant in India and other arid regions of South East Asia (9). The East Indies, and tropical Africa. Forcenturies, the barks, leaves, disorders (10). The potential therapeutic Activities of pomegranate are wide- ranging and include treatment and prevention of cancers, cardio vascular disorder, diabetes, dental conditions, erectile dysfunction the pericarp of P. granatum is used to treat infections found in Human being sexual organs as, allergic dermatitis, tympani is, scalds, diarrhoea and dysentery (11). Considering all these in formation, the present study was designed to research the presence of various phytochemicals and their activity in the extract of Punica granatum leaf, a plant which evokes various remedial Properties.

Nutritional Content

Table 1. Nutritional Value of Pomegranate seeds

Sr. No.	Nutrition	Content
1	Energy	72Calories
2	Carbohydrates	16.3g
3	Fat	1g
4	Protein	1.5g
5	Fibres	3.5g
6	Sugar	11.9G
7	Vit-C	8.9mg(14.8%DV)
8	Vit-K	14.3mg(17.9%DV)
9	Foliate	33mg(8.3%DV)
10	Potssium	205mg(5.9%DV)
11	Phosphorus	31mg(3.1%DV)
12	Vit-B6	0.07mg(3.5%DV))

Traditional uses of pomegranate

Table 2. Uses of Drug

A)	Heart Problems
B)	Stomach disorder
C)	Dental Care
D)	Cancer
E)	Osteoarthritis
F)	Diabetes
G)	Anemia

MATERIALS AND METHODS

Preparation of leaf extracts: Leaves of pomegranate plant (*Punica granatum L.*) were obtained from around the Yeola city Maharashtra India. The Punicagranatum leaf was authenticated by Dr.Shaikh Farooq Ahmed, Professor of Department of Pharmacognosy at NSCOP College of Pharmacy, Vaijapur, Dist. Aurangabad, Maharashtra. The leaves of the plant were carefully removed and thoroughly washed with tap water and distilled water respectively to remove dust particles and dried in shade and makes the finely powdered.

Method of Extraction: Decoction Method: weigh 50 g of dried powder on weighing balance was extracted with 500 ml of deionized water or distilled water at 100°C for 40 min in a water bath. Filter the solution. The extracts were evaporated to dryness under controlled temperature (30-40 °C). The extracts were stored in air tight containers under in cool and dry place. These dried extracts were dissolved in respective solvents and used for further analysis. Qualitative and quantitative phytochemical screening.

Phytochemical screening: The pomegranate methanol leaf extract (PMLE) was subjected to preliminary phytochemical (qualitative and quantitative) screening/analyses for identification of the various classes of the plant chemical constituents.

Qualitative phytochemical screening

Test for anthraquinone: To take 1ml of plant extract, few drops of 1% HCl were added. Then produce red colour precipitate that indicates the presence of anthraquinone.

Figure 1. Decoction Assembly

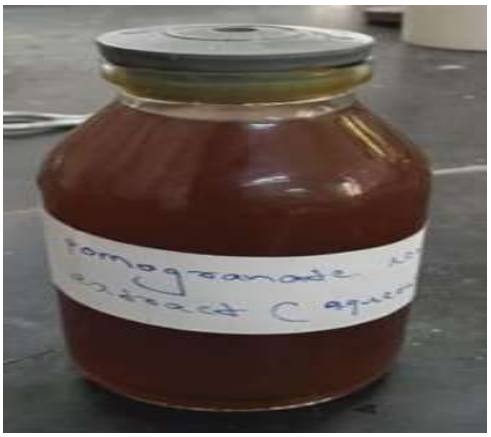

Figure 2. Leaves Extarct

Test for carbohydrates: To take 1ml of extract powder was mixed with α-napthol solution and then to the sides of the test tube conc. H_2SO_4 is added. Appearance of violet ring results that the presence of carbohydrates.

Test for reducing sugar: Take 1ml of plant extract was mixed with some drops of Benedict's reagent and keep in boiling water bath, then the formation of reddish brown precipitate. Positive result shows the presence of reducing sugar.

Test for flavonoids: To take 1ml of extracts was treated with few drops of 20%NaOH solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCL, indicates the presence of flavonoids.

Test for phenols: A portion of the extract was treated with aqueous 5% ferric chloride and formation of deep blue or black colour indicates the presence of phenols.

Test for proteins: From the extract,5ml of dist. Water was added & then heated with Biuret reagent and observe to the formation of Pink /violet colour.

Test for free amino acids: The extract was heated with 0.2% solution of Ninhydrin, which gives the result in the formation of purple colour, Which is indicate the presence of free amino acid.

Quantitative Phytochemical screening

Estimation of total phenolic content (TP): The total phenolic compound content in the extract of peel was determined by the Folin-Ciocalteau method. An aliquot of sample (0.2 ml) was mixed with 0.5 ml of Folin-Ciocalteau reagent (1: 2dilution) and 4 ml of sodium carbonate (1M) and allowed to stand steady for half hour at room temperature. The absorbance was measured at 750nm by the help of a spectrophotometer (Beckman, DU 7400 USA). TP content in the extract of peel was calculated and expressed as milligrams of Chlorogenic acid equivalent per gram of dry weight of extract (mgCGE/gDW).

Estimation of tannins: The tannin present in extracts of P. granatum was determined by using the method of Peril and Pompeii, 1971. 0.5 ml of extract was made upto 2 ml with addition of distilled water and 2 ml of water acts as blank. To this 0.5 ml of Folin ciocalteau phenol reagent (1:2 dilution) followed by the addition of 5 ml of 35 % sodium carbonate and kept steady at room temperature for 5 min. Blue colour formed was read at 640nm. A standard graph (representing Chlorogenic acid) was plotted, from which the tannin content was determined. The total tannin content was expressed in milligram CGE/g DW of extract.

Estimation of flavonoids: The TFC (total flavonoid content) of Punica granatum leaf extract was determined by using the aluminium chloride assay by colorimetry. An aliquot (0.5 millilitre) of extract was taken in different test tubes then 2 ml of distilled water was added right after the addition of 0.15 ml of sodium nitrite (5% NaNO2, W/V) and allowed to stand steady for 6 min, 0.15 ml of aluminium trichloride(10%AlCl3) was added and incubated for 6minutes, followed by the addition of 2ml of sodium hydroxide (NaOH, 4% W/V). The solution was well stirred and absorbance was measured in comparison with reagent blank at 510 nm. The total flavonoid content (mg/g) was determined from the calibration curve of quercet in and expressed as mg quartet in equivalents (mgQE/gDW).

Table 3. Flavonoid Content

Extracts	Flavonoid Content of Pomegranate Rind (mgRE/g)	Flavonoid Content of Pomegranate Aril (mg RE/g)
Petroleum ether	0.51±0.04	0.21±0.01
Dichloromethane	0.60 ±0.06	0.17±0.03
Ethylacetate	0.24 ±0.04	0.11±0.02
Methanol	14.59 ±1.12	1.08±0.01
Water	8.37 ±0.71	0.90±0.03

Estimation of total triterpenoids: Briefly 200 µl sample solution in a 10 ml volumetric flask was heated to evaporation in a waterbath,1mlof 5%(W/V)vanillin-acetic acid solution and 1.8ml sulphuric acid were added, mixed and incubated at 70 °C for 30 min. Then the solution was cooled and diluted to10 ml with acetic acid. The absorbance was measured at 573 nm against blank using spectrophotometer. The blank consisted of all reagents and solvents without sample solution. The triterpenoids was determined using the standard ursolic acid and expressed as milligram of ursolic acid equivalent/gram dry weight of extract (mg UE/g DW).

Estimation of Carbohydrates: 0.2ml of extract was made up to 1.0ml with addition of distilled water. 4.0ml of anthrone reagent, (0.2% anthrone in ice cold concentrated Sulphuric acid) was added and kept in boiling water bath for 8 min, cooled rapidly and read at 630 nm. The blank was consisted of all reagents without sample solution. D-glucose was used as standard. The total sugar content was expressed in terms of percentage of the dry weight.

Estimation of proteins: 0.2ml of extract was made up to 1.0ml with distilled water.5.0ml of alkaline copperre agent was added to all the tubes and allowed to stand steady for 10 min. Then 0.5 ml of Folin's Ciocalteau reagent was added and incubated in dark for 30minutes. The intensity of the color developed was read at 660 nm. The blank was consisted of all reagents without sample solution. The protein content was determined by the help of the standard Bovine serum albumin and expressed in terms of percentage of dry weight.

Estimation of total lipids: 10gm. of sample was used to extract lipids with 150ml of petroleum ether for 16hr.,at a solvent condensation rate of 2–3 drops/second. The obtained extract was concentrated and evaporated at the room temperature to dryness. The weight of extract gives the total lipid content which was expressed in terms of the percentage of dry weight.

Alkaloids were determination by harborne (1973) Method: 5grams of the sample was weighed and added to a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated by using water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was introduced drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle down and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried, weighed and expressed in terms of percentage of the dry weight.

TESTS AND RESULTS

Table 4. Qualitative Test

Phytochemicals Tested		Presence/Absence
Phenols		+
Flavonoids	Test(a): Flavones	+
Anthocyanin		+
Coumarin		+
Quinones		+
Anthraquionone		+
Steroids		+
Alkaloids	Dragendroff'sTest	+
	Mayer'sTest	+
(+) = PRESENCE (Test positive)(-)=ABSENCE (Test negative)		

In vitro antioxidant activity determination: Free radicals avenging activity of aqueous leaf extract of Punicagranatum Hydrogen peroxide (H2O2) scavenging activity of natural antioxidants present in plant extracts has been determined widely by measuring decrement of H_2O_2 in an incubation system containing H_2O_2 and the scavenger using the classical UV-method at 230 nm. If our extract is having an antioxidant activity the absorbance decreases. and the overall percentage scavenging activity is calculated by using following formula for screening of invitro antioxidant activity were quire, phosphate buffer 0.2M (pH7.5) and hydrocarbon peroxide 2mM. Now take two test tubes one is for blank and one is for standard.

$$\text{Scavenging activity (\%)} = \left(\frac{\text{Absorbance}^{\text{control}} - \text{Absorbance}^{\text{sample}}}{\text{Absorbance}^{\text{control}}} \right) \times 100$$

Add 600µL of hydrogen peroxide (H202) to each test tube to each test tube add 4ml of phosphate buffer, to the blank add 0.1ml phosphate buffer and to the sample add 0.1ml of extract incubate the se test tube at30°C for 30 minutes. Measure the absorbance at 230 nm. The absorbance of control should be more than sample. It means that our extract is having antioxidant activity.

Readings

Table 5. Reading

Sr. No.	Test	Absorbance	K*Absorbance
1	Control	3.510	35.104
2	Sample	2.130	21.295

CONCLUSION

Now a days herbs are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. The plant based compounds have the effective dosage response and minimal side effects when compared to the synthetic compounds Phytochemical screening of Punica granatum leaves reveals it as a valuable medicinal plant with numerous medicinal properties. Since the ethanolic extract of P. granatum leaves contains more constituents it can be considered beneficial for further investigation. Atypical research and developmental work needs to be carried out for its better therapeutic and commercial utilization. Punica granatum methanolic leaf extract has antioxidant property and hence it may be used for therapeutic purposes.

According to the formula of percent scavenging activity,

$$\textbf{Percent Scavenging activity} = \frac{3.510-2.130}{3.510} \times 100$$

$$= \frac{1.38}{3.51} \text{ x100}$$

$$= 0.3932\text{x}10$$

$$= 39.32$$

The percent scavenging activity was determined as 39.32%

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