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Original Article

PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS OF PUNICA GRANATUM PEEL, WHOLE FRUIT AND SEEDS

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Abstract :

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The objective of the present study was to investigate the presence of various phytochemicals from the ethanolic, aqueous and chloroform extracts of Punica granatum peel, whole fruit and seeds. The three different extracts from peel were found to contain Triterpenoids, Steroids, Glycosides, Flavonoids, Tannins, Carbohydrate & Vitamin C. The three different extracts from whole fruit were found to contain Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Carbohydrate & Vitamin C. The three different extracts from seeds were found to contain Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Tannins, Carbohydrate & Vitamin C.

The generated data from the three different extracts of Punica granatum peel, whole fruit & seeds provided the basis for its wide uses in the traditional & folk medicines.

Keywords : Phytochemical, Punica granatum, Flavonoids.

Introduction :

The therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal medicinal practitioners.[1] Natural products are the source of synthetic and traditional herbal medicine.

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. The ripe pomegranate fruit can be up to five inches wide with a deep red, leathery skin, is grenade-shaped, and crowned by the pointed calyx. The fruit contains many seeds (arils) separated by white, membranous pericarp, and each is surrounded by small amounts of tart, red juice. The pomegranate is native from the Himalayas in northern India to Iran but has been cultivated and naturalized since ancient times over the entire Mediterranean region. It is also found in India and more arid regions of Southeast Asia, the East Indies, and tropical Africa.[2]

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The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, and protection from ultraviolet (UV) radiation. Other potential applications include infant brain ischemia, Alzheimer's disease, male infertility, arthritis, and obesity.[3-7] The potent antioxidant capacity of pomegranate and its components has been reported by numerous scientists using multiple in vitro assay systems. [8]

The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins,

volatile oils, gums and tannins, etc. The active principles usually remain concentrated in the storage organs of the plants.[9]

Considering all these facts, the present study was designed to investigate the presence of various phytochemicals in the three different extracts of Punica granatum peel, whole fruit and seeds, a plant which evokes various therapeutic effects.

Materials and Methods

Collection of Plant Material

The fruits of Punica granatum were collected from the local market, Mangalore, and the specimens were identified.

Preparation of Plant Extracts

Ethanolic & chloroform extract

The peel, whole fruit and seeds of P. granatum was dried in hot air oven at 40°-50°C for a week. The dried plant material was powdered using mixer grinder, and subjected to soxhlet extraction with 99% ethanol and chloroform for 24 hours. The mixture was evaporated to dryness in a rotary flash evaporator and stored in refrigerator. The condensed extracts were used for preliminary screening of phytochemicals.

Aqueous extract

The peel, whole fruit and seeds powder was boiled in distilled water for 15-20 minutes, kept in room temperature overnight and filtered. The filtrate was evaporated to dryness in hot air oven and stored in refrigerator. The condensed extracts were used for preliminary screening of phytochemicals.

Phytochemical analysis of different Crude extracts

Extracts were tested for the presence of active principles such as Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C.

Following standard procedures were used. [10-11]

Test for Steroids and Triterpenoids:

Liebermann Burchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

Test for Glycosides:

Keller Killiani Test – Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Bromine water test - Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Test for Saponins:

Foam Test – Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Alkaloids:

Hager's Test – Test solution was treated with few drops of Hager's reagent (saturated picric acid solution).Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Test for Flavonoids:

Ferric chloride test – Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test – Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test – Test solution when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

Test for Tannins:
Gelatin Test – Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

Test for Proteins:
Biuret Test – Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

Test for Free Amino Acids:
Ninhydrin Test – Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

Test for Carbohydrate:
Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Vitamin C:
DNPH Test – Test solution was treated with Dinitrophenyl hydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

Results
The curative properties of medicinal plants are perhaps due to the presence of various secondary

metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The various extracts of peel, whole fruit and seeds of Punica granatum have revealed the presence of Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Carbohydrate & Vitamin C. Proteins and free amino acids were found to be absent in all the extracts. Saponins and alkaloids were present only in the whole fruit and seeds extract while flavonoids were found to be present only in the peel and whole fruit extract. From this analysis, ethanolic extract of whole fruit was found to have more constituents compared to peel and seeds extracts.

The results of preliminary phytochemical analysis are shown in Table 1.

Discussion :
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. [12]

The phytochemical screening in the present study, has revealed the presence of triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrate and vitamin C in the peel extract; triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrate and vitamin C in the whole fruit extract and triterpenoids, steroids, glycosides, saponins, alkaloids, tannins, carbohydrate and vitamin C in the seeds extract. Further the presence of different phytoconstituents in the three different extracts may be responsible for the therapeutic properties of pomegranate.

Flavonoids and tannins are phenolic compounds and plant phenolics 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 (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example, saponins have hypotensive and cardiodepressant properties [13]. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia [14].The presence of saponins in whole fruit and seeds extract and glycosides in all the extracts might play a role in the cardioprotective potential of pomegranate.

Since the whole fruit extract contains the components of both peel and seeds, it was found to contain more

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

constituents. It would thus mean that in this study, the whole fruit extract had the highest number of bioactive compounds. Since the yield of bioactive metabolites in a plant extract also varies considerably with the method/solvent of extraction [15, 16] it is plausible that the ethanolic extracts were generally more potent than the aqueous extracts probably because the active principles in the plant dissolved more readily in and were better extracted by a less polar solvent (ethanol) than water. This is in agreement with many literatures reporting of differences in the activities of extracts obtained from the same morphological part of a plant using different solvents. For instance, the methanolic extract of the fruits of *Tetrapleura tetraptera* is more potent than the aqueous extract [17]. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound.

Conclusion :

The presence of phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. In the present study, we have found that most of the biologically active phytochemicals were present in the ethanolic, aqueous and chloroform extracts of *Punica granatum* peel, whole fruit and seeds. Since the ethanolic extract of whole fruit contains more constituents it can be considered beneficial for further investigation. The medicinal properties of *Punica granatum* peel, whole fruit and seeds extract may be due to the presence of above mentioned phytochemicals.

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