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EVALUATION OF ANTIOXIDANT - PHYTOCHEMICAL COMPOUNDS IN *PUNICA GRANATUM*

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Keywords:

Pomogranate, Antioxidant activity, phenols, ascorbic acids, β - carotene

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ABSTRACT: The antioxidant activity of pomegranate fruit was evaluated using *in vitro* tests. Phenol content was more in G-137, 11.8±0.11 mg/g dry wt followed Arakta variety 10.1±0.05 mg/ g dry wt., respectively flavonol and flavonoid content was high in mrudula 3.5±0.05 mg/g dry wt, 7.0±0.05 mg/g dry wt., and proanthocyanin content was high in Bhagwa variety 19.62±0.05 mg/g dry wt, Anthocyanin content and Ascorbic acid content was high in Bhagwa variety. 40.8±0.3 %, 0.95±0.05 % Total carotenoid content, and β-carotene content was high in Bhagwa variety 10±0.1 mg/g fr wt.

INTRODUCTION: Pomegranate Known as in (English – Pomegranate, Hindi – anar, Telugu – danimma) for the present study five varieties have been used (Bhagwa, Arakta Mrudula G-137, Ganesh.) it is a fruit-bearing deciduous shrub or small tree growing between 5–8 meters (16–26 ft). There are over thousand cultivars of *Punica granatum* [1] originating from the Middle East, extending throughout the Mediterranean, eastward to china.

Pomegranate fruits were being used from ancient times and were being widely used in many different cultures and countries for thousands of years. Pomegranate fruit has gained a great deal of Popularity over the years.

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Punica granatum (pomegranate) has potential for prevention and treatment of inflammation and cancer [2-6] Quantification of phenols, flavonoids, anthocyanin in pomegranate including antioxidant capacity of different extracts from different parts of the plant [7-8] The pomegranate is a symbol of life, longevity, health, femininity, fecundity, knowledge, morality, immortality and spirituality, if not Divinity [9] In ayurvedic medicine the pomegranate is considered" a pharmacy unto itself," it shows antihelmintic and vermifuge properties [10] it possess various pharmacological and toxicological properties including antioxidant, anti-inflammatory by inhibiting pro-inflammatory cytokines), anti-cancer and anti-angiogenesis activities [11 -13]. The pomegranate juice are powerful astringent and cures diarrhea and oralaphthae, "refrigerant and "blood tonic" [14]

MATERIAL AND METHODS:

Chemicals and reagents:

Folin ciocalteau, aluminium chloride, was obtained from Sigma –Aldrich Co., St. Louis, USA. 2, 6 – dichlorophenolindophenol (DCIP), methanol Folin ciocalteau, phenol, sodium carbonate,

ethlenediaminetetra acetic acid disodium salt EDTA. Ascorbic acid, pyrogallol, metaphosphoric acid (MPA), acetic acid.

Experimental Plant material:
The experimental pomogranate plant materials were procured and collected from Horticultural Research Station, Sanga Reddy, Medak, District, Telangana state.

Extraction of phenols, flavonols, flavonoids and proanthocyanins:
Ten grams of sample (fruit) was thoroughly crushed and homogenized in mortar pestle with10 ml of 80% acetone and the extract was centrifuged at 10000 rpm for 15 min at 40^0C and and the supernatant was combined with initial extract, and this was used for the estimation of phenols [15]

Determination of phenols:
Total phenol contents in the extracts were determined by the modified Folin ciocalteau method 16 Wolfe (2003). An aliquot of the extract was mixed with 5ml folin-ciocalteau reagent (diluted with water 1:10 v/v) and 4ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30min at 40^0c for color development. Absorbance was then measured at 765 nm using Shimadzu 160A UV-VIS double beam spectrophotometer samples of the extract were evaluated at a final concentration of 0.1mg/ml. Total phenol content was expressed as mg/g gallic acid equivalent using the following equation based on the calibration curve: y= 0.126x, R2=0.9365, Where x was the absorbance and y was the Gallic acid equivalent (mg/g).

Determination of flavonols:
Total flavonols in the plant extracts were estimated using the method of [17]. To 2.0 ml of the sample, 2.0 ml of 2% AlCl, ethanol and 3.0ml (50g/l) sodium acetate solutions were added .The absorption was read at 440nm by Shimadzu 160A UV-VIS double beam Spectrophotometer was read after 2.5h at 20^0c. Extract samples were evaluated at a final concentration of 0.1mg /ml. The total flavonols content was calculated as quercetin (mg/g) using the following equation on the calibration curve: y=0.0255x, R^2=0.9812, where x is the absorbance and is the quercetin equivalent.

Determination of flavonoids:
Aluminium chloride colorimetric method was used for the determination of flavonoids [18]. Each plant extracts (0.5ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes: the absorbance of the reaction mixture was measured at 415nm with a Shimadzu 160A UV-Visible double beam spectrophotometer. Expressed in mg/g.

Determination of proanthocyanidins:
Determination of proanthocyanidins was based on the procedure reported by [19] A volume of 0.5 ml of 0.1mg /ml of the extract solution was mixed with 3ml of 4% vanillin-methanol solution and 1.5ml of HCl, the mixture was allowed to stand for 15min. The absorbance was measured by Shimadzu 160A UV-VIS double beam Spectrophotometer at 500nm. Extract Samples were evaluated at a final concentraton of 1.0mg/ml. Total proanthocyanidin content were expressed as catechin equivalents (mg/g) using the following equation based on the Calibration curve; y=0.5825x, R^2=0.9277, where x was the absorbance and y is the catechin equivalent (mg/g).

Estimation of anthocyanins:
Ten grams of the fresh sample was thoroughly mashed in 70% (v/v) ethanol in water, and kept overnight at room temperature. The extracts were filtered and centrifuged at 4°C at 8000 rpm for 10 minutes stored in refridgerator [19-20].

Estimation of ascorbic acid:
Ten millilitres of the sample (fruit) was titrated against standard 2, 6-dichlorophenolindophenol dye [21- 22] which was already standardized against standard ascorbic acid. Results were expressed in percentage

Estimation of total carotenoids:
The 10gm of the fruit or leaf was dehydrated at 60°C to constant moisture content. Moisture content was determined in dried samples according to [23] 1 g of the sample was dissolved in 20 mL petroleum ether and 3 ml chloroform mixture, and filtered and made to various concentration of supernatant by taking 1, 2, 3, 4 and 5 ml of the

filtrate in 100 ml volumetric flask and diluted with petroleum ether. The absorbance was measured by Shimadzu 160 A UV-VIS double beam Spectrophotometer at 648nm, 663nm and 452nm by using 1 ml chloroform and 20 ml petroleum ether as blank.

Estimation of β- carotene β:

Carotene was determined according to the method of [25] the dried methanolic extract (100mg) was vigorously shaken with 10ml of acetone – hexane mixture (4:6) for 1min. The absorbance of the filtrate was measured at λ = 453, 505, 645 and 663 nm by Shimadzu 116 A UV-VIS Spectrophotometer.

Statistical analysis:

All results are expressed as mean± Standard deviation. All results are means of three replicates. The data were correlated using Pearson correlation coefficient at $p<0.05$ Correlations among data obtained were calculated using Pearson's correlation coefficient (*r*) and $P<0.05$ was considered significantly different; SPSS 15 Version was used for the statistical analysis.

RESULTS AND DISCUSSIONS:

Phenol content in *Punica granatum* varieties:

Phenolic content of the five varieties *Punica granatum* species extract was highest in G-137 (11.8±0.11 mg/g dry wt.) followed by Arakta> Mrudula> Ganesh and the least phenolic content was observed in Bhagwa (4.0±0.3 mg/g dry wt) and significant difference was observed within the varieties. similar studies were done by 274mg/g [26-27] the values are significant ($p<0.05$) the relationship among FRAP, phenol and flavonoid in peel is not significant, therefore the high antioxidant activity in peel is not depended to the phenolic and flavonoid contents have reported the antioxidant activity of pomegranate peel in comparison with the pulp extract their report showed similar ratio antioxidant activity for peel-pulp extract.

And according to [28- 29] have reported a systematic evaluation of natural phenolic antioxidants from 133 medicinal plants in India, polyphenols are secondary metabolites which are derivatives of the pentose phosphate, shikimate and phenyl-propanoid pathways in plants, phenols are one of the most occurring phytochemicals in plants including fruits pericarp.

In addition to their contribution to color and sensory characteristics of fruits and vegetables, phenols play a very important role in providing protection against *in vivo* and *in vitro* oxidation the higher reducing power indicated presence of reductones which are able to break free radical chains by donating hydrogen atoms and thus converting them to a more stable non-reactive species since the reducing power was directly related to the phenol content (**Table 1: Fig. 1**)

TABLE 1: SHOWING ANTIOXIDANT CONTENT IN *PUNICA GRANATUM* VARIETIES IN mg/g dry wt.

S.NO	*Punica granatum* L. varieties	Phenols mg/g dry wt.	Flavonols mg/g dry wt.	Flavonoids mg/g dry wt.	Proanthocyanin mg/g dry wt.
1	Bhagwa	4.0±0.3	0.35±0.006	1.0±0.1	19.62±0.05
2	Arakta	10.1±0.05	1.0±±0.1	5.6±0.005	9.85±0.01
3	Ganesh	7.6±0.02	2.1±0.04	6.7±0.005	12.77±1.35
4	Mrudula	8.2±0.01	3.5±0.05	7.0±0.05	3.3±0.03
5	G-137	11.8±0.11	0.2±0.003	4.0±0.1	7.94±0.02

Each value in the table is represented as mean ±SE (n=3) of triplicates, statistically significant ($p<0.05$) and are expressed in mg/g dry wt.

Flavonol content in *Punica granatum* varieties:

Flavonoid content in five varieties of *Punica granatum* varieties highest was recorded in Mrudula (3.5±0.05 mg/g dry wt) followed by decreasing order and least in G-137 (0.2±0.003 mg/g dry wt). The values are significant ($p<0.05$). Similar studies was done by in berries and the results were recorded higher (**Table 1, Fig. 1**)

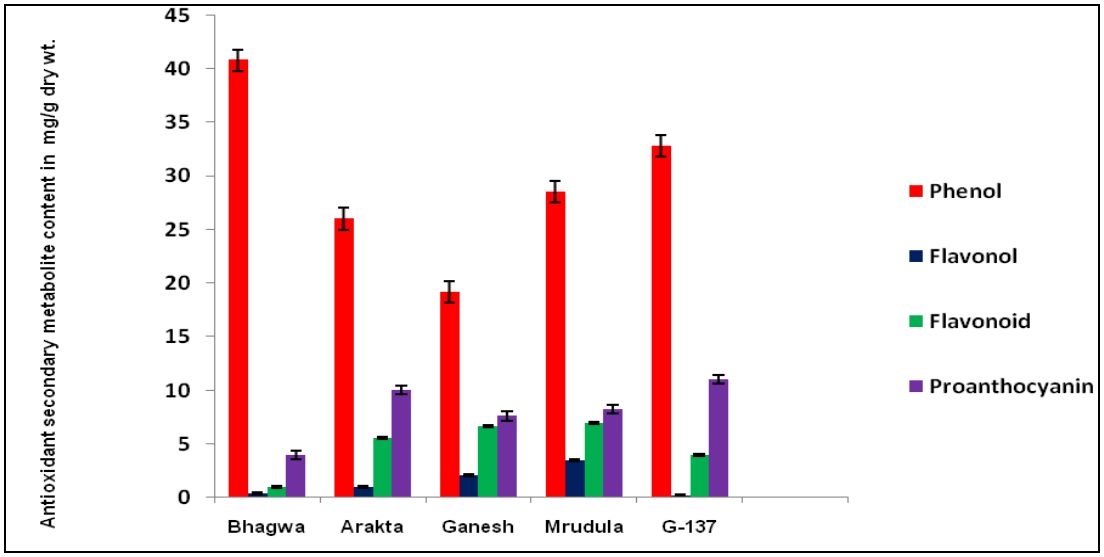

FIG.1: SHOWING CONTENT IN *PUNICA GRANATUM* VARIETIES IN mg/g dry wt

Proanthocyanin content in *Punica granatum* varieties:

Proanthocyanin content in five varieties of *Punica granatum* species varieties of extracts was highest in Bhagwa (19.62±0.05 mg/g dry wt) and least in Mrudula (3.3±0.03 mg/g dry wt) the values are significant p<0.05). Similar studies was done on juice and peels of pomogranate and results were maximum according to [33] (**Table 1, Fig. 1**)

Anthocyanin content in *Punica granatum* varieties:

Anthocyanin content in five varieties of *Punica granatum* species varieties of extracts was highest in Bhagwa (40.8±0.3 %) followed by least content in (19.62±0.05 %) (**Table 3) (Fig.3**), the values are significant (p<0.05). Similar studies were done on juice and peels of pomegranate according to [34] and the results were recorded higher. Anthocyanin studies were done on blueberries and strawberries and the results were recorded maximum. (**Table 2, Fig. 2**)

TABLE 2: SHOWING ANTHOCYANIN CONTENT IN *PUNICA GRANATUM* VARIETIES IN PERCENTAGE

S.NO	*Punica granatum* L.varieties	Anthocyanin percentage
1	Bhagwa	40.8±0.3
2	Arakta	26.08±0.52
3	Ganesh	19.62±0.05
4	Mrudula	28.57±0.173
5	G-137	32.86±0.2

Each value in the table is represented as mean ±SE (n=3) of triplicates, statistically significant (p<0.05) and are expressed in percentage

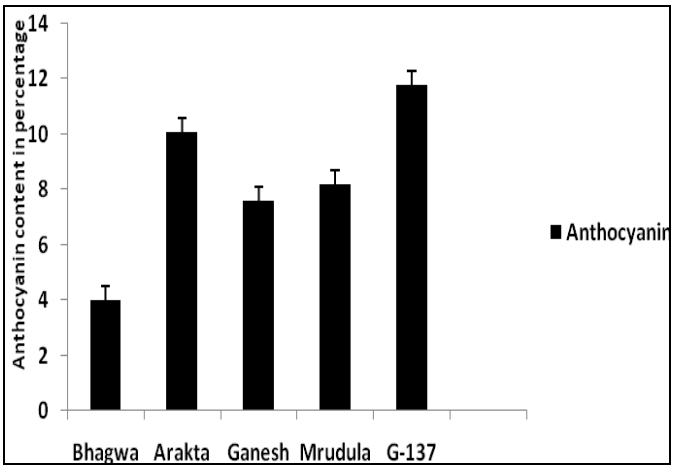

FIG.2: SHOWING ANTHOCYANIN CONTENT IN *PUNICA GRANATUM* VARIETIES IN PERCENTAGE

Ascorbic acid content in *Punica grantum* varieties:

The ascorbic acid content in the five varieties of *Punica granatum* varieties was highest in Bhagwa (0.95±0.586 %) followed by decrease in content of ascorbic, Ganesh, Arakta, Mrudula and the least content was observed in G-137 (0.51±0.05 %), similar studies were done in sour summer variety of pomegranate recorded more content 2.35 mg/ g, the values are significant (p<0.05), ascorbic acid an antioxidant vitamin plays a primary role to neutralize free radicals. Ascorbic acid is an water soluble compound, which helps to work both inside and outside the cells to control the free radical damage. Vitamin C is capable of neutralizing reactive oxygen species in the aqueous phase before lipid peroxidation is initiated. The possible ant carcinogenic effect of vitamin C appears to be related to its ability to detoxify carcinogens or

block carcinogenic processes through its action as an antioxidant or as a free radical scavenger .Reports suggest that vitamin c reduce the risk of chronic diseases such as cancer and cardiovascular disease and cataract, (**Table 3: Fig.3)**

TABLE 3: SHOWING ASCORBIC ACID CONTENT IN *PUNICA GRANTUM* VARIETIES IN PERCENTAGE

SNO	*Punica granatum L.* varieties	Ascorbic acid percentage
1	Bhagwa	0.95±0.586
2	Arakta	0.84±0.5
3	Ganesh	0.91±0.003
4	Mrudula	0.53±0.05
5	G-137	0.51±0.05

Each value in the table is represented as mean ±SE (n=3) of triplicates, statistically significant (p<0.05) and are expressed in percentage.

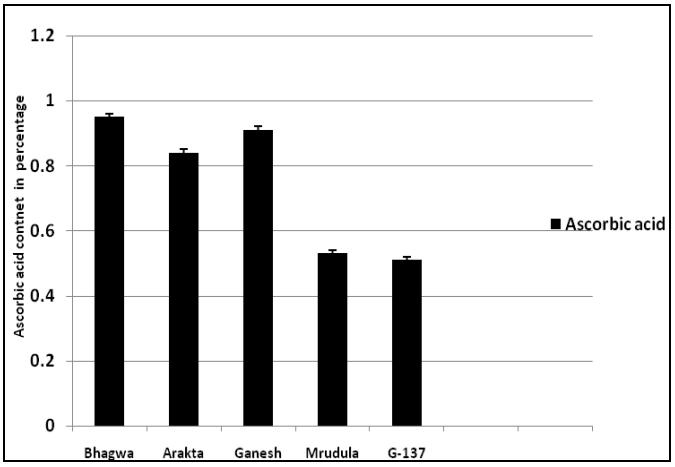

FIG.3: SHOWING ASCORBIC ACID CONTENT IN *PUNICA GRANTUM* VARIETIES IN PERCENTAGE

Total carotenoid content in *Punica granatum* varieties:

Total carotenoid content in the five varieties of *Punica granatum* the highest content was seen in, Mrudula (10.2±0.1mg/g Fr wt.) followed by Bhagwa, Arakta, Ganesh and the least was observed in G-137(0.46±0.05 mg/g Fr wt.) the values are significant (p<0.05). According to [35], similar work was done and the content was recorded less, carotenoids are important pigments which play a major role in the protection of plants against photo oxidative processes; they are efficient antioxidants scavenging singlet molecular oxygen and peroxyl radicals. In human beings carotenoids are again part of the antioxidant defense system. (**Table 4: Fig. 4**)

TABLE 4: SHOWING TOTAL CAROTENOID AND β CAROTENE CONTENT IN *PUNICA GRANATUM* VARIETIES IN mg/g F r wt.

S.NO	*Punica granatum L.* varieties	Total carotenoids mg/g Fr wt.	β carotene in mg/ g F r wt
1	Bhagwa	10.1±0.1	10.2 ±0.1
2	Arakta	7.03±0.02	7.03±0.02
3	Ganesh	1.09±0.2	1.2±0.2
4	Mrudula	10±0.1	10±0.1
5	G-137	0.46±0.05	0.46±0.05

Each value in the table is represented as mean ±SE (n=3) of triplicates, statistically significant (p<0.05) and are expressed in mg/g Fr wt.

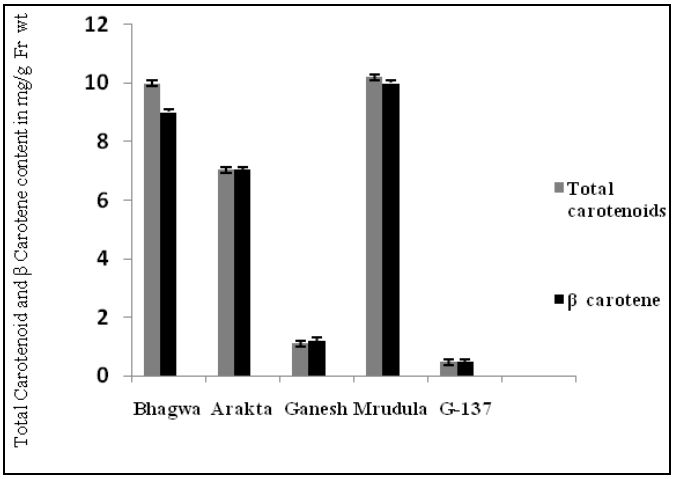

FIG.4: SHOWING TOTAL CAROTENOID AND β CAROTENE CONTENT IN *PUNICA GRANATUM* VARIETIES in mg/g F r wt.

CONCLUSIONS: Pomogranate is been used in many purposes for the human health point of view, and this study Bhagwa variety was showing maximum antioxidant activity, and phenol being highest ,phenol groups dominant in majority of plants ,and the varieties showed much variations in the antioxidant- phytochemical studies .The phytochemical compounds present in the fruit has to been evaluated and exploited within these varieties, pomegranate's chemistry and medicinal potential, the beginnings of a possible use for the fruit in cancer chemoprevention.

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