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ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF *PUNICA GRANATUM* FRUIT EXTRACT

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ABSTRACT

Pomegranate (*Punica granatum L*.) is one of the oldest known edible fruit tree, originating in central Asia prominent for its high nutritional and medicinal properties. In this study, the antioxidant activity and free-radical scavenging activity of the three different acetone extracts of pomegranate fruit: arils with seeds (R), rind with inner septal membrane (Y) and whole fruit (F) [mixture of both arils and rind], prepared were assessed. DPPH scavenging assay, FRAP, Nitric oxide radical scavenging assay were the methods adopted to study the antioxidant potential of the extracts. Results conclude that, of the three acetone extracts of the pomegranate fruit, acetone extract of the whole fruit (F) has the highest antioxidant capacity when compared to Aril seed Extract (R) and Rind Septum extract(Y). The free radical scavenging property of the acetone extracts clearly shows its potential application in disease management.

Keywords: **Pomegranate, antioxidant activity, Phytochemical, DPPH**

INTRODUCTION

Pomegranate is considered one of the oldest fruits and one of the earliest to appear in human diet. The pomegranate is native from the Himalayas in northern India to Iran but has been cultivated and naturalized since ancient times over the Mediterranean region [8]. Pomegranate has a long history of medicinal uses, the pharmaceutical and medicinal bioactivities of compounds from different parts of the pomegranate plant report hypolipidemic, antioxidant, antiviral, anti-neoplastic, anticancer, antibacterial, anti-diabetic, anti-diarrheal, helminthic, vascular and digestive protection, and immunomodulation effects. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. In Ayurvedic medicine, pomegranate is used as an antiparasitic agent, as a blood tonic and for healing ulcers, diarrhoea and to treat aphthae. Pomegranate also serves as a remedy for diabetes in the Unani system of medicine practiced in the Middle East and India [8]. The fruit contains many seeds (arils) separated by white, membranous pericarp, and each is surrounded by small amounts of tart, red juice. The juice contains a considerable amount of carbohydrates, ascorbic acid, vitamin B, pectin, cellulose and tannins [3]. The pericarp is a good source of polyphenols such as anthocyanin, leucoanthocyanins, catechins and flavonoids and contains about 30 % of tannins. Pomegranate fruit is characterized by its high phenolic contents and antioxidant properties. Pomegranate peel extracts exert anti-oxidant and anti-mutant activities in vitro due to their content of polyphenols (tannins, ellagic and gallic acids) [9].These substances have been used in the preparation of cosmetics and tinctures as well as in therapeutic formulas and food recipes. On the other hand, pomegranate syrup was reported to have anti-sclerotic effects on animal models in vitro [6][1][10]. Furthermore, human studies have shown that daily consumption of pomegranate juice lowers blood pressure in hypertensive subjects, delays the atherosclerotic process and increases the total antioxidant status of blood. Pomegranate juice has a remarkable ability to decrease oxidative stress by 40–80% [1]. Moreover, cholesterol homeostasis is improved by a decrease in LDL total cholesterol

levels especially in diabetics [7]. Studies have been conducted to analyze the antioxidant activity of peels and seeds, this study is intended to compare the antioxidant potential of acetone extract of the Whole fruit extract (F) Aril seed extract (R) and Rind septum extract(Y).

MATERIALS AND METHODS

Materials: All solvents/chemicals/reagents used were of analytical grade and obtained from Hi Media, Mumbai, India.

Methods

Preparation of pomegranate fruit extracts: Fresh Pomegranate (*Punica granatum*) fruits were obtained from local market. The fruits were peeled manually and separated as three parts: Rind and Septum, Aril and Whole fruit (Rind, Septum and Arils). The separated parts were crushed finely and squeezed in acetone and water, 70:30(v/v). The filtrate were filtered through Whatman no.1 filter paper, condensed and dried using rotary evaporator. The extracts were concentrated to a residue. The crude extract was used for analysis of phytochemical compounds and antioxidant properties. The dried extracts were stored as Whole Fruit Extract (F), Aril Extract (A) and Rind and Septum Extract (R) at 4˚C.

Preliminary phytochemical screening of pomegranate fruit extracts: The Whole Fruit Extract (F), Aril Extract (A) and Rind and Septum Extract (R) were screened for the presence of Alkaloids, Carbohydrates, Phytosterols, Saponins, Flavonoids, Proteins, Amino acids, Fixed oil and fats, Phenolic compound, Tannins and Glycosides using the standard procedure [11].

Evaluation of Antioxidant activity of pomegranate fruit extracts

DPPH radical-scavenging activity: The fruit extracts free radical scavenging activity was determined by the method adopted by Elizabeth and Rao. The reaction mixture was prepared using 0.3mM DPPH (diphenyl 2-picryl hydrazyl radical) in 50ml of methanol. 1ml of 0.3mM DPPH in methanol was added to 100µl of compound with concentrations ranging from 20µg to 100µg. DPPH solution with methanol was used as a positive control and methanol acted as blank. When DPPH reacts with antioxidants in the extracts, it was reduced and the colour of the mixture changed from dark violet to light yellow. The absorbance was measured at 517nm after thirty minutes and the radical scavenging activity was calculated as percentage inhibition using the formula

Percentage of Inhibition = $[(A_O - A_E) / A_O] \times 100$

A_O = Absorbance without Extract

A_E = Absorbance with extract / standard

FRAP assay: A modified method of Benzie and Strain *e*t a*l*., was adopted for the FRAP assay. The stock solutions included 300mM acetate buffer, pH 3.6, 10mM TPTZ (2, 4, 6-tripyridyl-Striazine) solution in 40mM HCl and 20mM $FeCl_3.6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $FeCl_3.6H_2O$. The temperature of the solution was raised to 37ºC before using. Fruit extracts (20-100µg/ml) were allowed to react with 900µl of FRAP solution and the solution was made upto 1ml with methanol. After four minutes, absorbance of ferrous tripyridyltriazine complex was measured at 593 nm. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the antioxidant concentration. Ascorbic acid was used as standard.

Nitric oxide radical scavenging assay: Nitric oxide radical scavenging activity of the extract was determined by the method reported by Garrat *e*t a*l*.,. Nitric oxide was generated from sodium nitro prusside in aqueous solution at physiological pH, which interacts with oxygen to produce nitric ions, which may be determined by the Griess Illosvoy reaction. 2ml of 10mM sodium nitro prusside and 0.5ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of extracts at different concentrations and the mixture was incubated at 25ºC for thirty minutes. From the incubated mixture, 1.5ml was taken and added to 1.5ml of griess reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% napthyl ethylene diamine di HCl) and incubated at room temperature for five minutes. The absorbance of the mixture was measured at 546nm. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the antioxidant concentration. Ascorbic acid was used as standard.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: The extracts were screened for qualitative analysis of phytochemicals. The results of the phytochemical screening are tabulated in Table 1. Whole fruit Extract (F), Aril Extract (R), Rind and Septum extract (Y) showed presence of Alkaloids, Carbohydrates, Phytosterols, Saponins, Flavonoids, Proteins, Amino acids, Phenolic compounds, Fixed oil and fats and Glycosides. Whereas Tannins was present in Whole fruit Extract (F) and Rind and Septum extract (Y) and was absent in Aril Extract (R).

Table 1: Preliminary phytochemical screening of pomegranate fruit extracts

Phytochemicals	Whole fruit extract (F)	Aril Extract (R)	Rind and septum extract (Y)
Alkaloids	++	++	++
Carbohydrates	++	++	++
Phytosterols	++	++	++
Saponins	++	++	++
Flavonoids	++	++	++
Proteins	++	++	++
Aminoacids	++	++	++
Fixed oil and fats	++	++	++
Phenolic compound	++	++	++
Tannins	++	--	++
Glycosides	++	++	++

++ denotes presence of phytochemicals and – denotes absence of phytochemicals

DPPH radical-scavenging activity: The DPPH radical scavenging activity of Whole fruit Extract (F), Rind and Septum extract (Y) and Aril Extract (R) is depicted in figure 1. Whole fruit Extract (F) shows greater inhibition at maximum concentration (100µg) than standard, Aril Extract (R) and Rind and Septum extract (Y). The fruit extracts has less scavenging activity at lower concentrations when compared to ascorbic acid, however as the concentration increases the fruit extracts has higher inhibition percentage than the standard ascorbic acid.

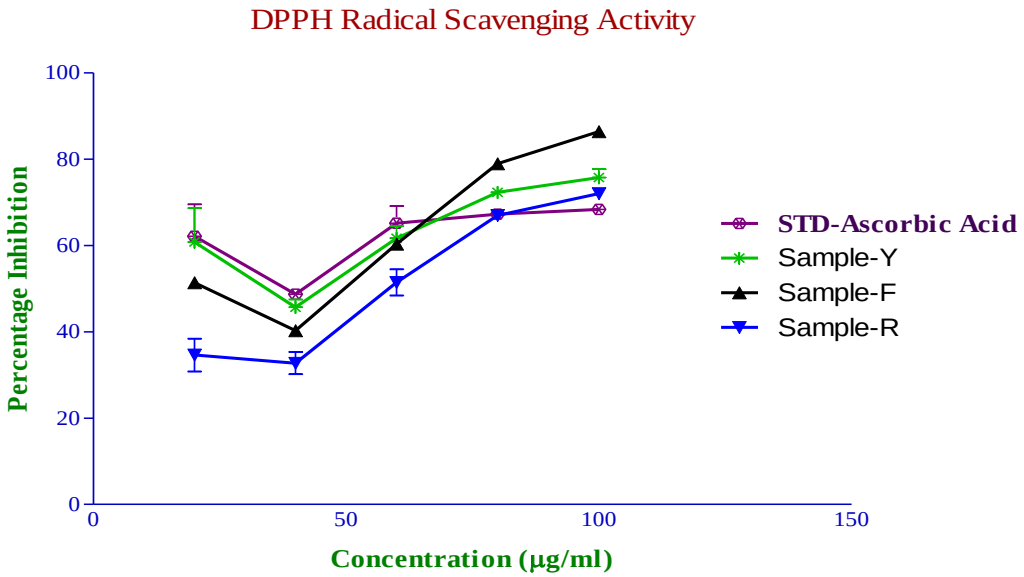

Figure1: DPPH Radical scavenging activity of pomegranate fruit extracts
Sample Y - Rind and septum extract (Y), Sample F- Whole fruit extract (F) Sample R- Aril Extract (R)

The Effective concentration of the fruit extracts on DPPH Radical scavenging activity are tabulated in Table 2. The EC_{50} values of Aril Extract (R), Rind and septum extract (Y) and Whole fruit extract (F) were 50.48±0.58, 29.69±0.17, 16.31±0.86 respectively. Effective concentration of ascorbic acid is found to be 7.443±0.79 which is less when compared to that of fruit extracts.

Table 2: EC_{50}values of DPPH Radical scavenging activity of pomegranate fruit extracts

DPPH EC_{50}	Ascorbic Acid Standard	Aril Extract (R)	Rind and septum extract (Y)	Whole fruit extract (F)
Concentration (µg/ml)	7.443±0.79	50.48±0.58	29.69±0.17	16.31±0.86

Each value presented as Mean ± SD (n=3)

FRAP Assay: Ferric ions reducing activity of standard ascorbic acid and the fruit extracts are shown in figure 2. The graph clearly shows that the reducing potential of the extracts increases as the concentration increases. However Rind and septum extract (Y) shows lowest inhibition percentage when compared to ascorbic acid and other two extracts. Rind and septum extract (Y) has low Ferric ions reducing activity. Similarly Aril Extract (R) showed good reducing activity at low concentration but as the concentration increases percentage inhibition of the Aril Extract (R) also decreases and is found to be lesser than Ascorbic acid and Whole fruit extract (F). Whole fruit extract (F) showed good ferric ion reducing activity at higher concentrations and has highest inhibition at maximum concentration than the standard and the other two extracts.

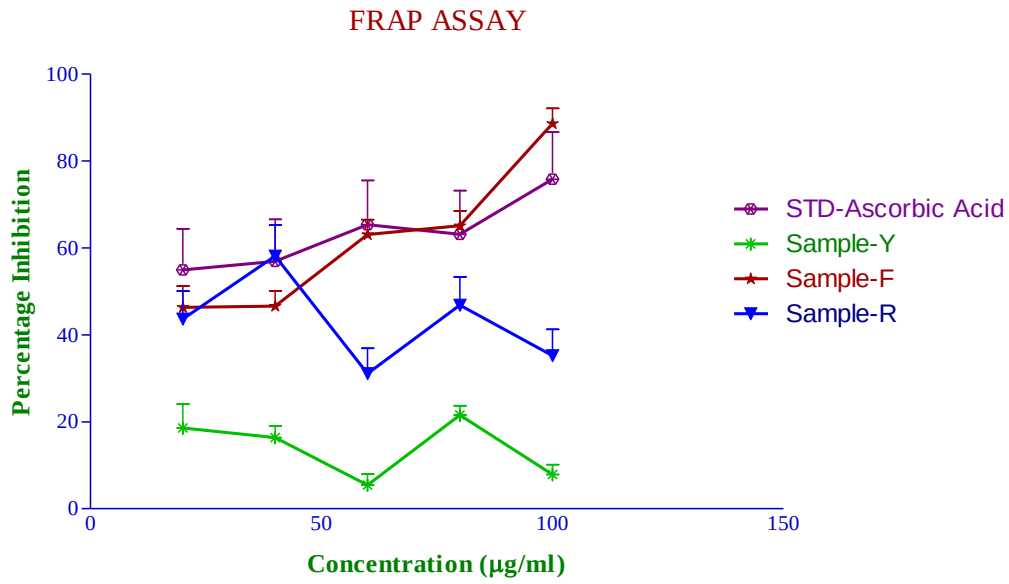

Figure 2: FRAP assay of pomegranate fruit extracts

Sample Y - Rind and septum extract (Y), Sample F- Whole fruit extract (F) Sample R- Aril Extract (R)
The effective concentration of FRAP assay is tabulated in Table 3. Rind and septum extract (Y) having the least EC_{50} value (0.3034±0.72) and Whole fruit extract (F) having the high EC_{50} value (31.2±1.08)

FRAP EC_{50}	Ascorbic Acid Standard	Aril Extract (R)	Rind and septum extract (Y)	Whole fruit extract (F)
Concentration (µg/ml)	16.11±2.90	16.23±1.84	0.3034±0.72	31.2±1.08

Table 3: EC_{50} values of FRAP Assay of pomegranate fruit extracts
Each value presented as Mean ± SD (n=3)

Nitric oxide radical scavenging assay: Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by the fruit extracts. Nitric oxide scavenging activity of the fruit extracts is shown in figure 3. Rind and septum extract (Y) and Whole fruit extract (F) has higher scavenging property than Aril Extract (R) and ascorbic acid. The scavenging activity was higher in low concentrations and inhibition percentage decreased gradually as concentration increases. The fruit extracts has higher scavenging activity than ascorbic acid.

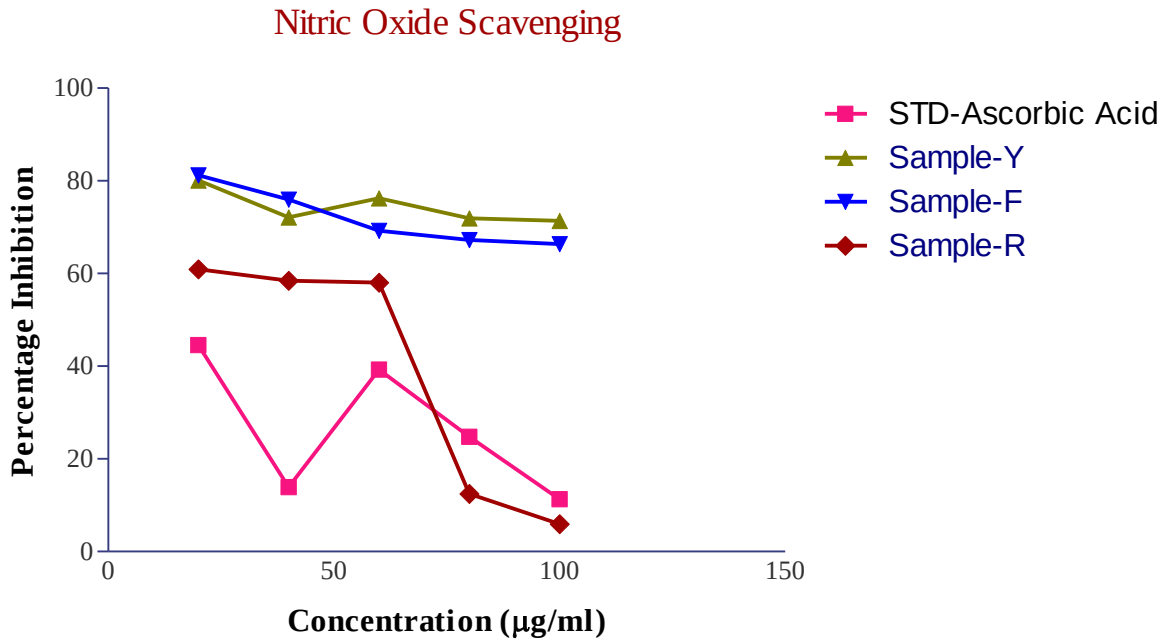

Figure 3: Nitric oxide radical scavenging activity of pomegranate fruit extracts
Sample Y - Rind and septum extract (Y), Sample F- Whole fruit extract (F) Sample R- Aril Extract (R)

The effective concentration EC_{50} of Nitric oxide radical scavenging assay of pomegranate fruit extracts is tabulated in table 4. Rind and septum extract (Y) having high EC_{50} value (3923±2.80) and Ascorbic Acid Standard having least EC_{50} value (11.71±0.64)

Table 4: EC_{50} values of Nitric oxide radical scavenging assay of pomegranate fruit extracts

Nitric oxide EC_{50}	Ascorbic Acid Standard	Aril Extract (R)	Rind and septum extract (Y)	Whole fruit extract (F)
Concentration (µg/ml)	11.71±0.64	39.93±0.77	3923±2.80	341.8±0.77

Each value presented as Mean ± SD (n=3)

CONCLUSION

The phytochemical screening and quantitative analysis of the pomegranate fruit extracts showed to be rich in phenols, flavonoids and tannins. The higher level of antioxidant activity of extracts were due to the presence of phenolic and flavonoid components. Flavonoids and polyphenols rich pomegranate fruit is considered as a good remedy for many ailments from ancient time. This study showed that the whole fruit extract has higher antioxidant activity due to the combined phytochemical effect of arils, rind and septum. Hence the whole fruit is considered to have high natural antioxidants and can be used in the management of oxidative stress due to disease conditions. Further studies are done for characterization and identification of chemical constituents, the high antioxidant activity in whole fruit may be attributed to the synergistic effect of the active ingredients which should be investigated in detail.

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