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COMPARITIVE STUDY OF ANTIOXIDANT AND PHYTOCHEMICAL LEVEL OF ORGANIC POMEGRANATE

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Abstract

Punica granatum L. (Pomegranate) has been used from ancient times for the treatment of many health disorders such as cardio vascular diseases, inflammation, diabetes, diarrhea, dysentery, intestinal infections and cancer. This study aims at providing an overview of the phytochemistry and antioxidant activity of pomegranate peel and pulp extract. For the study, organic pomegranate was selected and the peel and pulp extracts of the same were subjected to total flavonoid present in it. The antioxidant capacity tests were carried out using hydrogen peroxide test and reducing power assays for both peel and pulp. A positive and significant correlation was observed between parameters like total flavonoid content and antioxidant capacity at p = 0.01 level which proved to be 99% significant. Antioxidant capacity was significantly correlated to all the phytochemical parameters at p = 0.05 which proved at 95% level which showed that higher flavonoid concentration was present in both peel and pulp extract. But peel showed the highest concentration in comparison to pulp. It was clearly proved that greater the flavonoid content, higher was the antioxidant activity for both.

Keywords:

Punica Granatum L;
Antioxidant;
Phytochemcials;
Flavonoid;

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1. Introduction (12pt)

Consumption of fruit and vegetables has been a part of the Indian diet and it is strongly associated with reduced risk of cardiovascular disease, Cancer, Diabetes, Alzheimer disease, Cataracts, and other degenerative diseases. Fruits and vegetables contain bioactive compounds as a part of plant, which has secondary metabolites, produced by plants itself. They have pharmacological or toxicological effects in man when consumed as a part of daily diet. The antioxidants present in fruits and vegetables are rich in phytochemicals and bioactive compounds such as phenols, flavonoids, and tannins, play a very important role in the prevention of these diseases thus showing the importance of inclusion in the diet.

Punica Granatum L commonly known as Pomegranate which belongs to fruit family of Punicaceae are rich in bioactive compounds which contribute health effects as whole fruit, as well as its juices and peel extracts, have been studied in relation to a variety of chronic diseases. The major chemical components of the pomegranate peel and pulp are Icosanoic acid , Linolenic acid (Conjugated) , Linolenic acid (Alpha) , Oleic acid ,

Palmitic acid, Punicic acid, Stearic acid. Citric acid and Malic acid was the most abundantly found. Phenolic compounds like Gallic acid, Protocatechuic acid, Chlorogenic acid, Caffeicacid, Ferulic acid, o –Coumaric acid and p -Coumaric acids, Catechin, Phloridzin and Quercetin are the major bioactive compounds found in the peel. It yields the major antioxidants such as flavonoids, phenolics, and pro - anythocyanidins than what the pulp yields. Even the antioxidant activity test also showed the same result. With respect to these findings, it is proved that the antioxidant activity of the peels played a major role in protecting LDL cholesterol against oxidation than the pulp. The peels of pomegranates were once considered to be the unused part of the healthy fruit but various bioactive compounds present in it made it as an important source of phytochemicals to cure degenerative diseases.

2. Research Method

Procurement of Fruit

This is the pre preparation step, which includes selection of the type of fruit and procuring it from the market. For this study, the organic fruit was purchased online. On procurement of fruit sample, the fruit was cleaned and peeled.

Preparation of plant extract

In order to carry out the set of experiments, the pomegranate peel extract and pulp extract were prepared prior to perform the experiments. The first step involved was preparation of sample extract and a stock solution for peel and pulp separately.

Sample preparation

The fresh peel is used as the base of this plant extract, fresh peels were cleaned and ground into a course paste. This ground extract was dissolved in the ratio of 1:10, measuring 30 grams of freshly ground peel and 300 ml methanol. Macerating is a technique is used to mash the extract and dissolve in 300 ml methanol. This is stored in a closed conical flask. The pomegranate from which the peel was taken, 30 grams of fruit part was used and macerated extract was taken from the juice. The seeds were discarded. This juice was mixed with 300 ml methanol and stored in closed conical flask. The methanol extracts were allowed to sit for 72 hours, to allow the phytochemical components to dissolve in the methanol. After a period of 72 hours, the extracts were filtered separately and centrifuged at 3000 rpm for 15minutes. The sample is stored separately in a closed conical flask refrigerated at 4^0C. Small quantities were utilized for the experiments.

Aqueous extract

The fresh peel were boiled in water for a period of 15 minutes and cooled. This solution is used for further experiments .The fresh pomegranate fruit was crushed using mortar and pestle, the juice was collected separately discarding the seeds. The fresh juice was used for phytochemical screening.

Preliminary Phytochemical Screening

Phytochemical screening of *Punica granatum* peel and pulp extracts were assessed by standard method as described by *Savithramma et al.* (2011) and *Selvaraj et al.* (2014). Two solutions were used (methanolic extract and aqueous extract solution) to check the presence of phytochemicals. Test for the following phytochemicals such as Phenols,

Tannins, Flavonoids, Saponins and Terpenoids were carried out as a part of the preliminary phytochemical screening.

Quantitative Estimation of Flavonoid content

The quantitative experiments involved the estimation of major phytochemical content of organic pomegranate peel and pulp samples using various techniques and principles.

Total Flavonoid Content

The determination of Total flavonoid of the organic pomegranate peel and pulp samples were carried out using the Aluminium chloride calorimetric assay (Satish *et al.*,2008 and Pallab *et al.*,2013). In the experiment Aluminium chloride (AlCl3) forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols . In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids *(Bhaigyabati et al. 2014).* The experiments was carried out using the following reagents Sodium nitrite, Aluminium chloride and Sodium hydroxide. Quercitin solution was used as the standard reference.

Antioxidant Capacity Tests

In order to find out the antioxidant capacity ,the organic pomegranate peel and pulp samples were subjected to the following set of experiments using the principle of oxidation and reduction and the free radicals scavenging capacity.

Hydrogen Peroxide Test

The Hydrogen peroxide test (Ruch *et al.* 1989*)* were carried out for organic pomegranate peel and pulp separately. In the experiment, Hydrogen peroxide acts as a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H2O2 can probably react with Fe2+ and Cu2+ ions to form hydroxyl radical and this is the origin of many of its toxic effects. This test was carried out using Ascorbic acid solution as the standard reference and chemical reagents such as Hydrogen peroxide solution, and Phosphate buffer with specific pH and temperature were used to obtain results.

Reducing Power Assay

The estimation of reducing power of the organic pomegranate peel and pulp samples were carried out separately *(*Priyanaka *et al.,* 2013*).* In this experiment the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity by action of Ascorbic acid solution, Potassium ferricyanide, Phosphate buffer, Trichloro acetic acid and Ferric chloride solution to form a blue colour assay solution.

Statistical analysis

The Statistical methods used to analyze the data obtained through experimental trials were as follows, descriptive statistics (color intensity) and Inferential statistics (Correlation test).

3. Results and Analysis

Phytochemical Screening of Pomegranate Peel and pulp Extract

Table 1 below depicts the presence of phytochemicals like phenols, tannins, flavonoids, saponins and terpenoids in aqueous extract and methanol extracts of organic pomegranate peel and pulp. In case of flavonoids, it showed (+) result for aqueous extract whereas (++) for methanol extract of peel which depicts a

higher concentration in methanol extract. Phenol, tannin and flavonoid showed deep colour intensity for methanol extracts due to higher presence. Saponins and terpenoids showed (+) result, which shows presence in both the extracts.

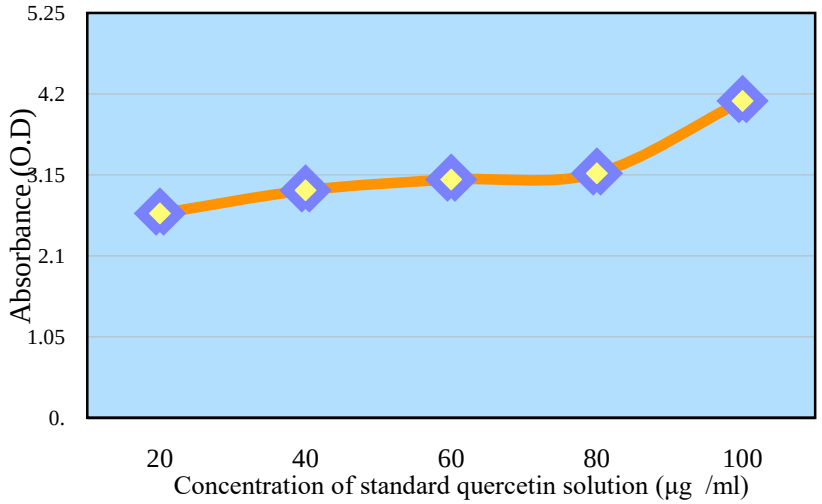
Table 1 : Peliminary phytochemical Test

SL.NO	PHYTOCHEMICAL TEST	AQUEOUS EXTRACT		METHANOL EXTRACT	
		Pulp	**Peel**	**Pulp**	**Peel**
1	Phenols	+	+ +	+	+ +
2	Tannins	+	+ +	+ +	+ +
3	Flavonoids	+	+	+	+ +
4	Saponins	+	+	+	+
5	Terpinoids	+	+	+	+

Absorbance of standard Quercetin solution (Total Flavonoids)

Estimation of total flavonoids were carried out using the Aluminium chloride assay method to obtain values for different concentrations. The Quercetin standard solution was used as standard to determine total flavonoids content and was expressed as (µg QE /ml).The standard curve was plotted by taking the Quercetin solution in different concentrations. The results obtained were used as reference value for analysing the concentration of total flavonoid content.

Figure 1 - Standard curve for Quercetin solution



The above standard curve (Figure 1) depicts the absorbance of Standard Quercetin solution against various concentration of 20, 40, 60, 80 and 100 (µg /ml).The concentration of the translucent s solution was observed to be directly proportionate to its absorbance as per principle of spectrophotometry. Therefore, it was inferred from the figure that when there was an increase in concentration of standard solution the absorbance also increased gradually.

Concentration of Total Flavonoids - Peel and Pulp Extracts

The bar graph (Figure 2) depicts the concentration of total flavonoids in the pomegranate peel and pulp extract in different volumes of 1.0, 1.5, 2.0, 2.5 and 3.0 (ml) and absorbance was recorded by spectrometer at 510 nm. Therefore it was evident that as the volume of peel extract was increased, the concentration of Total flavonoid also increased simultaneously.

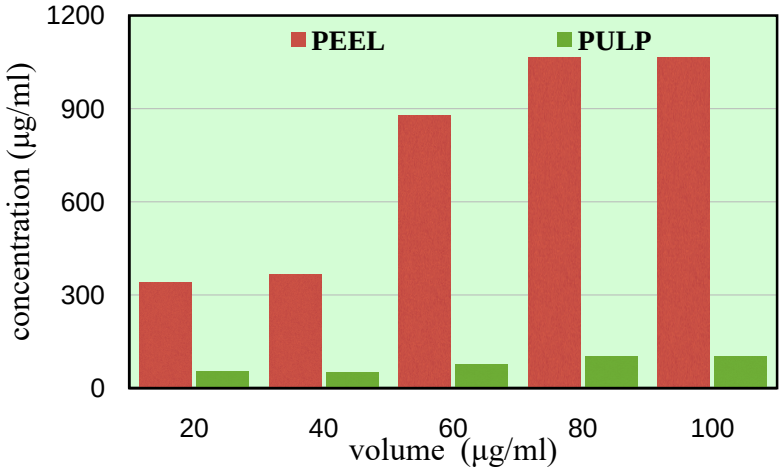


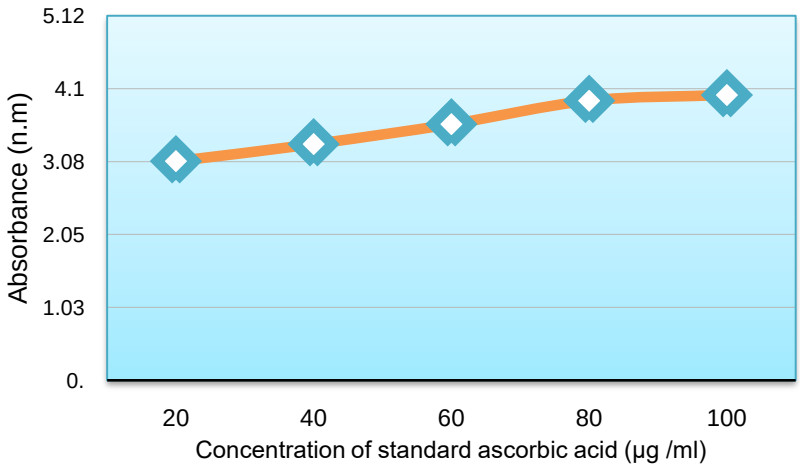
Figure 2: Comparison of Total Flavonoids concentration of Peel and Pulp Extract

The bar graph (Figure 2)above depicts the comparison of total flavonoid content of pomegranate peel and pulp extract .It can be inferred that when both extracts were taken in similar volume and measured for absorbance, the concentration of pomegranate peel extract showed higher values. Thus the graph clearly explains that the peel extracts had a higher concentration of total flavonoid than pulp extract.

Ascorbic acid standard concentration

For Hydrogen peroxide test and Reducing Power assay, ascorbic acid standard was used as reference**.** Graph 3depicts the different concentrations of standard Ascorbic acid taken as 20, 40, 60, 80 and 100 (µg /ml) and the corresponding absorbance at 230 nm. It was observed that there was an increase in absorbance value of standard Ascorbic acid as the concentration increased.

Figure 3:Standard curve for Ascorbic acid concentration

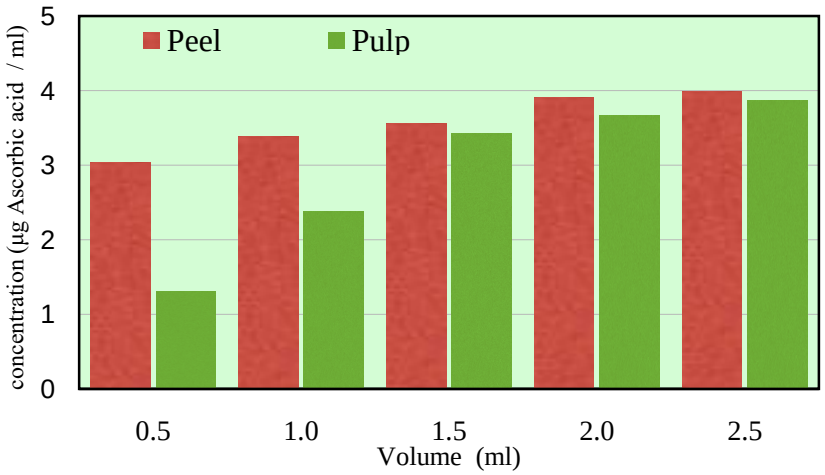


The standard curve (Figure 3) depicts the absorbance of Standard Ascorbic acid against various concentration-20, 40, 60 and 100 (µg /ml).

Hydrogen Peroxide Concentration - Peel and Pulp Extract

The bar graph (Figure 4) represents the concentration of Total Flavonoids in the Pomegranate peel and pulp extract in the different volumes of 1.0, 1.5, 2.0, 2.5 and 3.0 (ml) and the absorbance by spectrometer at 510 nm. It can be inferred from the table that the concentration of total flavonoids increased with the increase in volume of extract.

Figure 4 :Comparison of Hydrogen peroxide concentration in peel and pulp

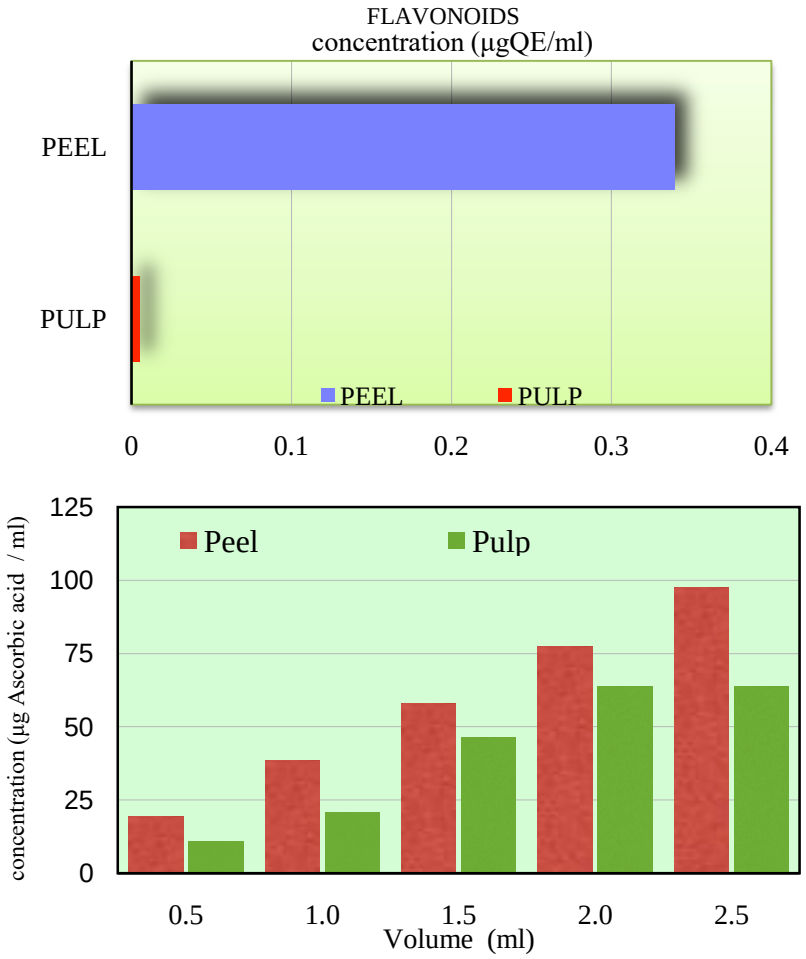


The graphical representation clearly showed that pomegranate peel extract has a higher concentration of Total flavonoid than the pulp extract, when they were measured at similar volumes.

Reducing Power Assay

The bar graph as shown in Figure 5 represents the concentration of Reducing power in the Pomegranate Peel and pulp extraction in the different volumes of 1.0, 1.5, 2.0, 2.5 and 3.0 (ml) and the absorbance by spectrometer at 510 nm. It can be inferred from the table that as the volume of the extract was increased the concentration of Reducing power were higher and tend to increase gradually.

Figure 5 : Comparison of reducing power of pomegranate peel and pulp extracts



The graphical representation shows the difference in reducing power of peel and pulp at the same volume of the extracts.

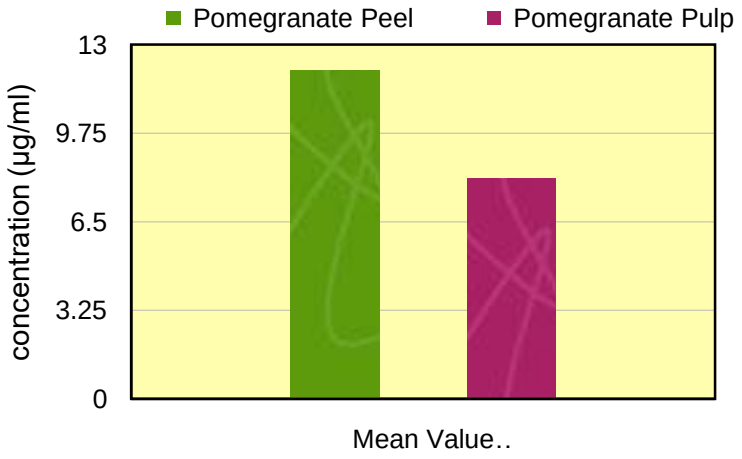
Figure 6: Concentration of total Flavonoids per gram of plant extract

Figure 6 represents Total flavonoids content expressed as Quercetin equivalence i.e. mg QE /g as Quercitin was used for standard reference to measure Total flavonoids. From the table (4.15a) 1.0 g of Pomegranate peel sample shows the presence of 0.34mg QE / g and 1.0 g of Pomegranate pulp sample shows the presence of 0.00533 mg QE / g. From these values it can be inferred that the Pomegranate peel has higher amounts of Total flavonoids than that of the Pomegranate pulp.

Mean Values - Total Flavonoids

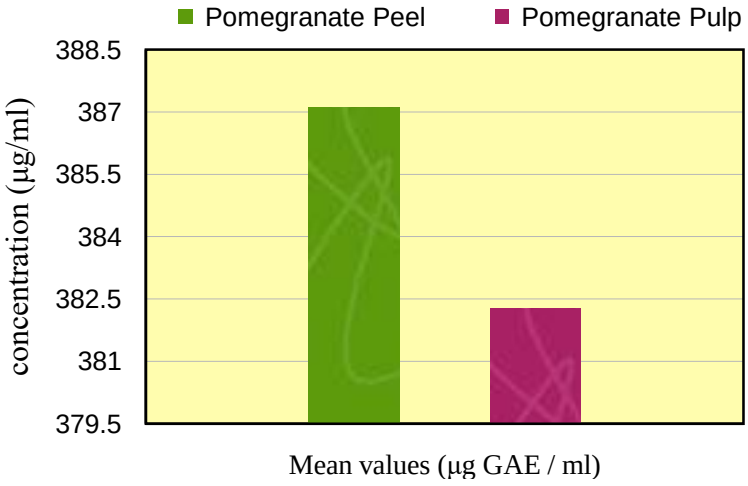
The experimental study was performed to obtain triplicate values and used to calculate the mean and standard deviation. This mean value is used to plot a graph to show the differences in mean value of the 2 samples respectively. Flavonoid values are considered for comparing the differences in mean. The mean of the concentrations shows the difference in the phytochemical profile of both the pomegranate peel and pulp. The graph (Figure 7)shows the change in mean values.

Figure 7: Mean values of Total Flavonoids



The mean values from the graphical representation show that there was a high flavonoid content in the pomegranate peel than the pomegranate pulp.

Figure 8: Graphical representation of Mean value of Anti Oxidant Activity



The mean values from the above graph (Figure 8)show that there is a high antioxidant power in the pomegranate peel than pomegranate pulp.

CORRELATION TEST

A correlation coefficient is a statistical measure of the degree to which changes to the value of one variable predict change to the value of another. In positively correlated variables, the value increases or decreases in

tandem. In negatively correlated variables, the value of one increases as the value of the other decreases.

Table 2 - Significance &Correlation of antioxidant parameters

The antioxidant power showed highest significance with flavonoid content with the correlation being significant at the 0,01 level (99 %). Of all the parameters it was inferred from the above table that flavonoid and antioxidant activity showed highest correlation with p = 0.000 and r = 0.990. Therefore it was concluded that when there was high flavonoid content in the plant sample, the antioxidant activity was greater. The correlation was thereby positive with p = 0.000 and r = 0.990. There was highest correlation between the flavonoid content and antioxidant power observed according to the statistical data.

CONCLUSION

The present study concluded that, the high concentration of phytochemicals was found in the peels of the pomegranate and had great antioxidant activity when compared to pulp. When the pomegranate peel was subjected to phytochemical and antioxidant tests ,the organic pomegranate peel showed highest antioxidant activity compared to organic pomegranate pulp. There are numerous nutritional benefits exhibited by the Organic pomegranate peel and is an excellent source of flavonoids. In this study, the phytochemicals from pomegranate peel was extracted, using a mixture of methanol and aqueous solution. The antioxidant properties of the peel extract were further investigated and compared with pulp extract. The contents of total flavonoids were estimated. According to the study by Singh *et al*., 2013 pomegranate indicated presence of compounds possessing antioxidant activity from peel. The present study supports the above fact that the peel was found to be an enriched source of the antioxidants exhibiting higher activity as compared to other parts of fruit. The difference in the antioxidant activity of the peel is due to their different phytochemical concentrations. Therefore it was concluded from this study that pomegranate peel extract had markedly higher antioxidant capacity than the pulp. Therefore pomegranate peels can be considered as an effective nutraceutical agent and used to treat medical conditions.

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PARAMETERS	r (Correlation)	P (Degree of significance)	SIGNIFICANCE
Antioxidant v/s Total Flavonoid	0.990**	0.000	**Significant**

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