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Evaluation of In-Vitro Antioxidant and Free Radical Scavenging Activities of Punica granatum Pericarp Extract

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ABSTRACT

Punica granatum belonging to family Punicaceae, one of the most well known plant to mankind and in India, it is mainly used for treatment of various ailments. All the parts of the plant that is fruits, leaves, seeds and pericarp are medicinally important. Punica granatum is available easily in almost all over the country and is cultivated largely for their fruits. Based on the literature survey it was found that Punica granatum showing many bioactive components and is of medicinal value without side effects. Hence, in the present study, an attempt was made to reveal the anti oxidant and free radical scavenging activities of isopropanol: n-butanol (1:1) extract of Punica granatum pericarp by five methods, Nitric oxide radical scavenging activity, Iron chelating activity, hydrogen peroxide radical scavenging activity, DPPH method and reducing power method. From this present investigation, it can be concluded that Punica granatum pericarp can be used as potent antioxidant for various oxidative stress induced disorders.

Key Words: Punica granatum, pericarp, nitrogen radical, hydrogen peroxide radical, DPPH.

INTRODUCTION

Alternative approach to drug discovery is through the medicinal plants. Many number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. Most of the people have faith in traditional medicine, particularly plant drugs for their primary healthcare[1]. Punica granatum belonging to family Punicaceae, one of the most well known plant to mankind and in India, it is mainly used for treatment of various ailments. All the parts of the plant that is fruits, leaves, seeds and pericarp are medicinally important[2]. Punica granatum is available easily in almost all over the country and is cultivated largely for their fruits. Based on the literature survey it was found that P. granatum showing many bioactive components and is of medicinal value without side effects. Hence, in the present study, an attempt was made to reveal the anti oxidant and free radical scavenging activities of isopropanol: n-butanol (1:1) extract of Punica granatum pericarp.

MATERIALS AND METHODS

Chemicals

Sodium nitroprusside, Sulphanilamide, Naphthyl ethylene diamine dihydrochloride, H_3PO_4, O-phenanthrolline, $FeCl_3$, Potassium ferricyanide [$K_3Fe(CN)_6$], Trichloroacetic acid, H_2O_2 were purchased from Sigma Chemicals. All other chemicals and reagents used are of analytical grade.

Collection of plant material

In the present study, Punica granatum fruits were purchased from local market, Anantapur, A.P., and botanically identified by Dept. of Botany, Balaji PG College, Anantapur, A.P., India. The pericarp was separated carefully and a voucher specimen (RRV/09/01) was stored in our institution for further reference. Pericarps were shade dried and powdered using mechanical grinder. This powder was used for further studies.

Extraction

Pericarp powder was extracted by continuous hot percolation method using Soxhlet apparatus and the solvent employed was isopropanol: n-butanol (1:1). The extract thus obtained (designated as IBPG) was evaporated in rotary evaporator, and stored in desiccator till further studies[3].

Preliminary phytochemical analysis

IBPG was subjected to battery of preliminary phytochemical analysis using standard procedures to find out the phytoconstituents present in the extract[4].

Antioxidant and free radical scavenging activity

Isopropanol: n-butanol (1:1) extract of Punica granatum (IBPG) at various concentrations ranging from 10-100µg/ml was used to perform different methods of antioxidant and free radical scavenging activities.

Determination of Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction (5, 6). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and IBPG and the reference compound in different concentrations (20, 40, 60, 80 and 100 µg) were incubated at 25°C for 150 min. After incubation 1.5ml of the Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546nm. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples.

Determination of Iron chelating activity

The method of Benzie and strain (1996) was adopted for the assay[5, 6]. The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. 10 mg of IBPG was dissolved in 10ml of methanol. Different concentrations (20µg, 40µg, 60µg, 80µg, 100µg) of test solution were prepared. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. Ascorbic acid was used as a standard.

Determination of Hydrogen peroxide radical scavenging activity

Scavenging activity of hydrogen peroxide by the plant extract was determined. IBPG (1 ml) prepared in distilled water at various concentration (20µg, 40µg, 60µg, 80µg, 100µg) was mixed with 2ml of 20mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm[6, 7].

Determination of reducing power

The reducing power of the IBPG was determined according to the method of Oyaizu (1986). 10 mg of IBPG was dissolved in 10ml of distilled water. Various concentrations of the IBPG (20, 40, 60, 80, 100µg/ml) in deionized water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium

ferricyanide (2.5 ml). The mixture was incubated at 50^{o}C for 20 min. Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 1036 x g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared $FeCl_3$ solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power[7, 8].

Determination of Total phenolic compounds

A known concentration of IBPG (20 µg) was prepared in methanol. 20µl of the above concentration was taken to it 100µl of folin-cio calteau reagent and 1.58 ml of distilled water and 300µl of sodium carbonate were added. The absorbance was measured at 765nm[8].

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The phytochemical screening test revealed that the pericarp extract obtained from isopropanol: n-butanol contains alkaloids, tannins, Phenolic compounds, Flavanoids, Flavanones, Saponins and Triterpenoids. The results are summarized in Table-1.

Anti oxidant activity

In present study we evaluated the in vitro antioxidant and free radical scavenging activity of IBPG on various in vitro models. The results were compared with the standard natural antioxidant Ascorbic acid. Percentage inhibition of IBPG and Standard at various concentrations for different methods is given in Table-2.

Determination of Nitric oxide radical scavenging activity

In the present study, Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by extract. Percentage scavenging of nitric oxide examined at different concentrations of IBPG was depicted in Table 2. Figure-1 illustrates the percentage inhibition of nitric oxide generation by IBPG. Ascorbic acid was used as a reference compound. The IC_{50} value of IBPG and standard was recorded.

Determination of Iron chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components. It causes lipid peroxidation through the Fenton and Haber-weiss reaction and decomposes the lipid hydroxide into peroxyl and Alkoxyl radicals that can perpetuate the chain reactions. Iron binding capacity of the IBPG and the standard at various concentrations were examined and presented in Table-2 and figure-2. The IC_{50} value of IBPG and standard was recorded.

Determination of Hydrogen peroxide radical scavenging activity

Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Percentage scavenging of Hydrogen peroxide examined at different concentrations of IBPG was depicted in Table-2. Figure 3 shows that IBPG has less scavenging activity (H_2O_2) than that of Ascorbic acid. The IC_{50} value for scavenging of H_2O_2 for extract and ascorbic acid were recorded.

Determination of DPPH assay

DPPH is a stable free radical having purple color. When free radical scavengers are added, DPPH is reduced and its color is changed to yellow, based on the efficacy of antioxidants. Percentage scavenging of DPPH examined at different concentrations of IBPG was depicted in Table 2. The IC_{50} value for scavenging of DPPH for extract and ascorbic acid were also recorded (Refer Table-4).

Determination of reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The plant extract could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of ascorbic acid. Increasing absorbance indicates an increase in reductive ability. Absorbance of different concentrations of IBPG and standard were depicted in Table 3. Figure 5 shows the reducing power potentials of the IBPG in comparison with a standard Ascorbic acid. The results show that there was increase in reducing power of the plant extract as the extract concentration increases.

Determination of Total Phenolic Compounds

The present study indicates that the IBPG contains 1.23 µg/ml equivalents of Gallic acid.

CONCLUSION

The present investigation shows that IBPG at various concentrations has good antioxidant capacity. The results were dose dependent and comparable to that of the standard ascorbic acid. From this present investigation, it can be concluded that IBPG can be used as potent antioxidant for various oxidative stress induced disorders. The phytochemical screening test revealed that the pericarp extract obtained from isopropanol: n-butanol contains Alkaloids, Tannins, Phenolic compounds, Flavanoids, Flavanones, Saponins and Triterpenoids. The presence of these variety of components as phytoconstituents may be responsible for the anti oxidant activity. Further studies are needed to isolate and purify compounds from Punica granatum showing anti oxidant activity.

Tables -1: Preliminary phytochemical screening of Punica granatum

TESTS	RESULT
TEST FOR ALKALOIDS	+ve
TEST FOR CARBOHYDRADATES	-ve
TEST FOR STEROIDS	-ve
TEST FOR PROTEINS	-ve
TEST FOR TANNINS	+ve
TEST FOR PHENOLIC COMPOUNDS	+ve
TEST FOR FLAVANOIDS	+ve
TEST FOR FLOVANONES	+ve
TEST FOR GLYCOSIDES	-ve
TEST FOR GUMS AND MUCILAGES	-ve
TEST FOR SAPONINS	+ve
TEST FOR TRITEPINOIDS	+ve

+ve = Positive, – ve= Negative

Table-2: Percentage inhibition of IBPG and Standard at various concentrations for different methods

Method	IBPG (µg/ml)					Standard (µg/ml)				
	20	**40**	**60**	**80**	**100**	**20**	**40**	**60**	**80**	**100**
Nitric oxide radical	4.01	8.3	12.2	20.3	31.3	6.2	15.9	21.6	35.5	42.2
Iron chelating activity	31.2	38.7	49.2	59.1	68.4	42	47	53	64	71
Hydrogen peroxide radical	6.2	10.3	21.2	39.3	69.1	10.8	14.6	31.1	58.5	81.2
DPPH	11.6	19.8	31.1	46.2	64.6	20.2	35.9	49.6	69.5	75.8

Table-2: Effect of IBPG on reducing power method

Concentration (µg/ml)	IBPG	Standard
20	0.16	0.19
40	0.18	0.36
60	0.21	0.59
80	0.28	0.76
100	0.38	0.88

Table-3: IC_{50} values of IBPG and standard for different in vitro antioxidant and free radical scavenging Models

Method	IBPG	Standard
Nitric oxide radical	147.32	169.33
Iron chelating activity	42.10	65.26
Hydrogen peroxide radical	39	45
DPPH	58.32	62.06

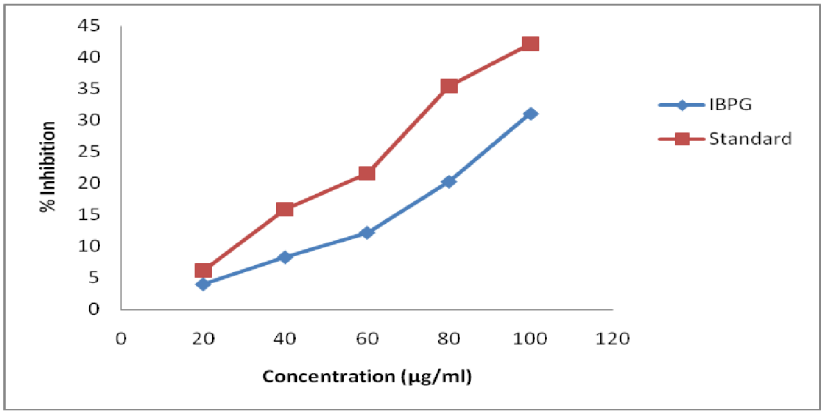

Fig. 1: Nitric oxide scavenging activity of IBPG

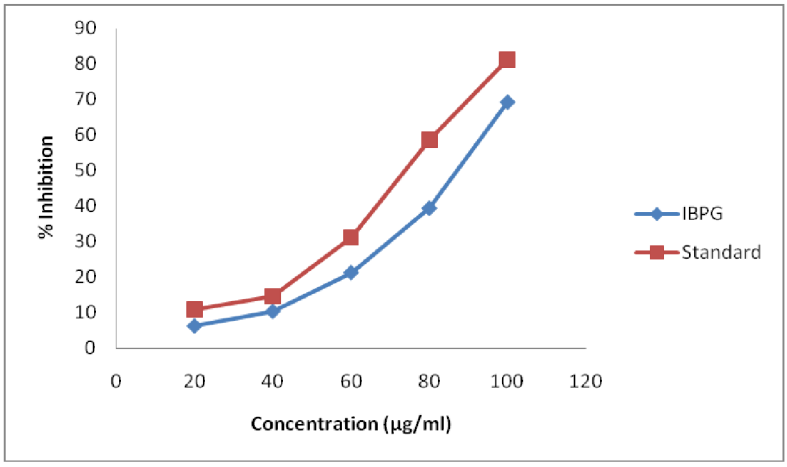

Fig. 2: Iron chelating activity of IBPG

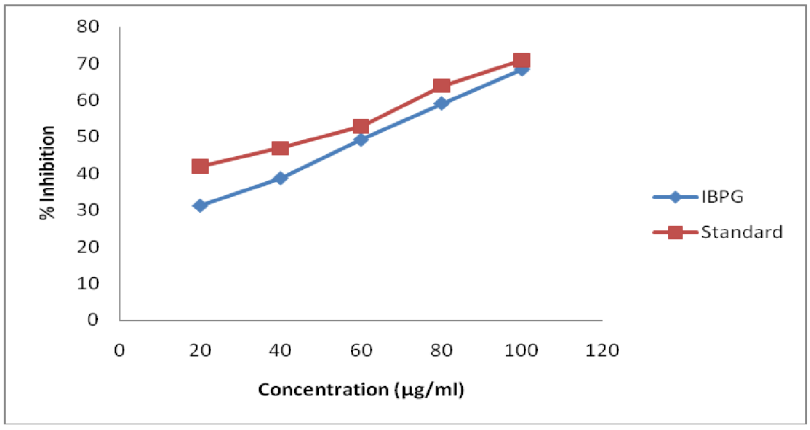

Fig. 3: Hydrogen peroxide scavenging activity

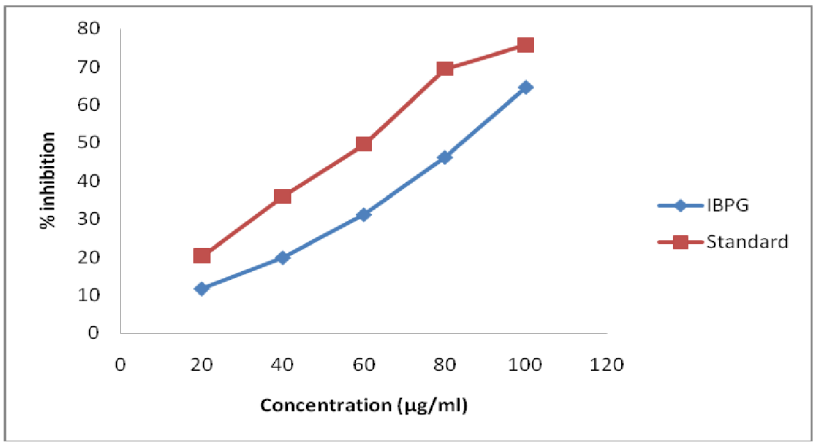

Fig. 4: DPPH activity of IBPG

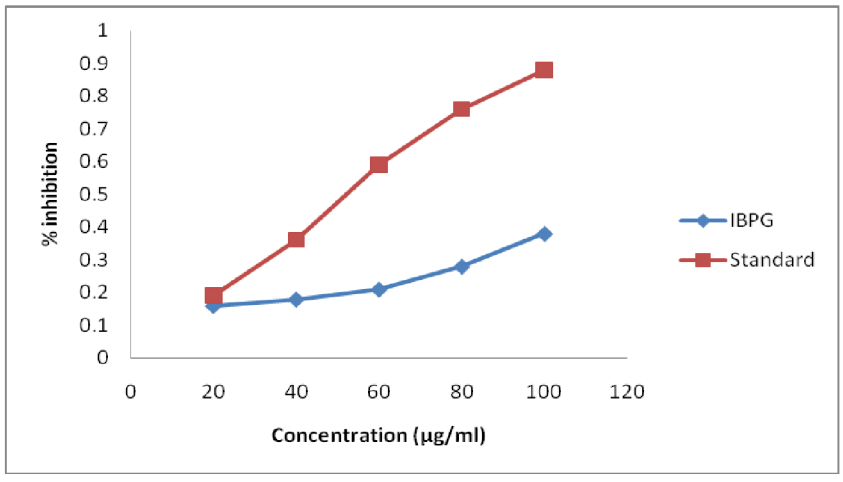

Fig. 5 Reducing power scavenging activity of IBPG

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