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Effect of Aqueous Peel Extract of Prunus Dulcis on In Vitro Antioxidant Activity

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Abstract: *Prunus dulcis commonly called as almond belongs to the family Rosaceae. We all know that from our ancestors' that the almonds are having rich antioxidants. So in this study shell or peel of prunus dulcis was selected and make it as an aqueous form. To evaluate the in vitro antioxidant activity of aqueous extract of Prunus dulcis by using several in vitro models. The APPD was evaluated by using DPPH radical scavenging (1, 1-diphenyl-2picrylhydrazyl), reducing power, nitric oxide radical scavenging and phosphomolybdinum radical scavenging assay was done. The antioxidant activity was represented in terms of inhibitory concentration. The results indicated that the APPD possesses the notable antioxidant activity against the free radicals in a concentration dependent manner compared with standard ascorbic acid. The IC-50 (50% of inhibitory concentration) of APPD showed that 542.283mg of DPPH radical, 17.567 mg of reducing power activity, 359.722mg of nitric oxide free radical, and 34.544 mg of phosphomolybdinum radical scavenging assay than compared with standard ascorbic acid. The free radical scavenging activity of APPD might be due to the presence of polyphenols, those are flavonols, flavan-3-ols, hydroxybenzoic acid, flavanones and tannins. These chemical constituents show beneficial to maintain balance and lowering oxidative stress in humans.*

Keywords: *Prunus dulcis (Almond),in-vitro methods, free radicals, antioxidants.*

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I. Introduction

Nuts are considered to be one of the most nutritionally complete feed for human wellness due to their high nutritional contents such as carbohydrates, protein, unsaturated fatty acids, vitamins and essential minerals (Welna M., 2008). Nut consumption reduced the risk of cardiovascular disease (CHD), which may be partly explained by the cholesterol-lowering effect and oxidative stress(Fraser GE., 1992;Yang J., 2009). Oxidative stress is a biological process occurs due to the formation free radicals. Free radicals either environmentally or internally produced can be neutralized by anti-oxidants, caused by an imbalance between reactive oxygen species (ROS) and antioxidants, in favor of the ROS. This imbalance leads to oxidative damage to lipids, proteins and DNA and ultimately cell death, thus giving rise to a variety of diseases. Excessive ROS generated could attack the cellular proteins, lipids and nucleic acids leading to cellular dysfunction including loss of energy metabolism, altered cell signaling and cell cycle control, genetic mutations, altered cellular transport mechanisms and overall decreased biological activity, immune activation and inflammation. Many evidences from experiments have given link between diabetes and oxidative stress by measuring various biomarkers that include DNA damage biomarkers and lipid peroxidation products. It is believed that in the onset and progression of late diabetic complication, free radicals have got a major role due to their ability to damage lipids, proteins and DNA. A variety of pathological conditions are induced by oxidative stress such as Rheumatoid arthritis, Diabetes mellitus and cancer. Biomarkers of oxidative stress in diabetes mellitus include proteins, lipids, and vitamins, enzymatic and non-enzymatic antioxidants (Jebur AB1, 2016).

Fig 1: prunus dulcis (almonds and outer layer)

Almonds are useful food remedy for anaemia, as they contain copper, iron and vitamins, polyphenols, includingflavonols (kaempferol, isorhamnetin, and quercetin), flavanols (catechin and epicatechin), flavanones(naringenin), anthocyanins (cyanidin and delphinidin), and procyanidins (B2 and B3), as well as simple phenolicacids (caffeic cid, ferulic acid, pcoumaric acid, protocatechuic acid, and vanillic acid)(Amarowicz.R,Troszynska. A, Shahidi,2005, Frison-Norrie, S.: Sporns, 2002, Milbury, P.2006, Monagas, M,2007, Sang, S,2002) present in Prunus dulcis.Hence in the present study aqueous peel extract of prunus dulcis was screened to evaluate the in vitro antioxidant activity of DPPH radical scavenging activity, reducing power, nitric oxide radical scavenging activity, phosphomolybdenum method.

II. Materials And Methods

2.1. Chemicals:

1,1diphenyl2picrylhydrazyl (DPPH), potassium ferric cyanide, trichloroacetic acid, ferric chloride, potassium di-hydrogen phosphate, sodium hydroxide, sodium nitropruside, sulfanilamide, napthyl ethylene diamine hydrochloride, phosphoric acid, sulfuric acid, sodium phosphate, ammonium molybdate.

2.2. Preparation of plant extract:

Fresh fruits of Prunus dulcis seeds were collected and outer brownish layers (skin) were separated and shade dried. The dry peel was making into powder.

2.3. In vitro antioxidant studies:

2.3.1. Dpph Scavenging Activity:

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. According to Manzocco et al., 1998 the sample extract (0.2 mL) is diluted with methanol and 2 mL of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below:

$$\%\ \text{inhibition of } \mathbf{DPPH} \text{ radical} = ([A_{\text{br}} - A_{\text{ar}}]/A_{\text{br}}) \times 100$$

The molecule 1, 1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-β-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

2.3.2. Reducing power scavenging activity:

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicate greater the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measure at 700 nm. Increase in absorbance of the reaction mixture indicate the reducing power of the sample (jayaprakash et al.,2001) The reducing power of AEPD was determined according to the method of Oyaizu et al., 1986; extract in 1 ml of distilled water mixed with phosphate buffer and potassium ferricyanide. The resulting mixture is incubated at 50°C for 20 min, followed by the addition of 2.5 ml of Trichloro acetic acid (TCA, 10% w/v).the mixture is centrifuge at 3000 rpm for 10 min and collect the upper layer of the solution. 2.5 ml of Upper layer is mixed with 2.5ml distilled water and add 0.5 ml of ferric chloride finally observe the optical density values on visible spectrophotometer at 700 nm against blank solution.

2.3.3. Nitric oxide scavenging activity:

The process is based on the principal that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interact with oxygen to produce nitrite ions that can be estimated by using Griess reagent (Marcocci et al., 1994). Formation nitric oxide via a five electron oxidative reaction (David ,1999; Ghafourifar and Cadenas, 2005; and Virginia et al.,2003) Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM), in phosphate buffer saline, was mixed with different concentrations of AEPD and incubated at room temperature for 150 min along with control (without extract). After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% $H_3\ PO_4$ and 0.1% of N-(1-naphthyl) ethylenediamine hydrochloride) was added. The absorbance of chromophore formed was read at 546 nm.

2.3.4. Phosphomolybdenum method:

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH (Md. Nur Alam., 2013). Different concentrations of test sample (5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml) and standard (1.0µg/ml, 2.5µg/ml, 5.0µg/ml) Gallic acid were prepared using a suitable solvent. 0.3ml of each concentration of test sample and the standard was mixed with 3.0ml of reagent (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were capped and incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695nm using UV-visible spectrophotometer after cooling to room temperature. Distilled water (0.3 ml) was used as blank in place of test sample.

2.3.5. Results and discussion:

Several studies reported that seeds of *prunus dulcis* containing therapeutically active compounds like flavanoids, phenolic and tannins, helps to reduce free radicals and oxidative stress. Daily intake of seeds can act as an anti inflammatory and antimicrobial agent and reduces the cardiovascular, diabetics, cancer like diseases. Seeds of *prunus dulcis* used for study are commonly available all over the India. Required material was collected once before starting the work, so as to maintain uniformity. Aqueous peel extract of *prunus dulcis* was used for experimental work. During extraction process fine powder of peel was used. Various concentrations of the APPD were tested for antioxidant activity in different *in vitro* models.

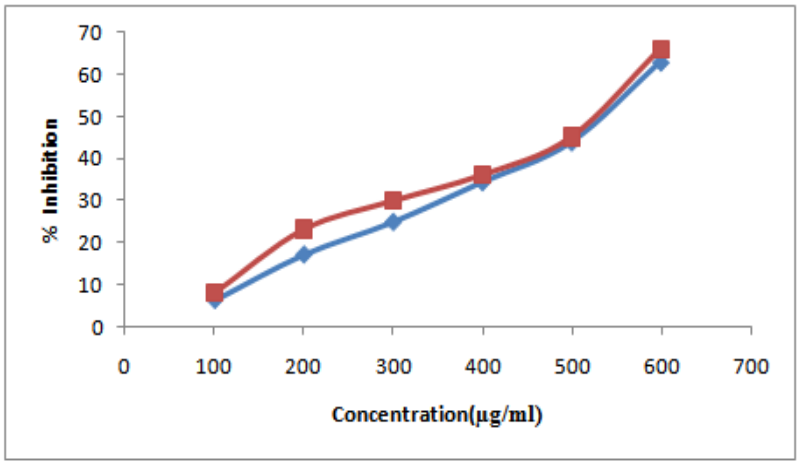
2.4. Evaluation of In-Vitro Antioxidant Activity

2.4.1. 2,2- Diphenyl-1-Picrylhydrazyl Activity:

Aqueous peel extract of *prunus dulcis* (APPD) exhibited a comparable antioxidant activitywith that of the standard ascorbic acid at various concentrations (100, 200, 300, 400, 500, 600 µg/ml). There was a dose-dependent increasing the percentage antioxidant activity for all the concentrations tested. Ascorbic acid was used as a standard drug for the determination of antioxidant activity by DPPH method. The concentration of ascorbic acid varied from 100-600 µg/ml. Ascorbic acid at a concentration 100 µg/ml exhibited a percentage inhibition of 10.99 % and for 600 µg/ml is 65.99 % [Table: 1]. As compared with the APPD to the ascorbic acid, APPD observed better antioxidant activity i.e.,542.283% IC-50. Graph-1 shows that dose dependent % inhibition of both APPD and ascorbic acid. It is observed that APPD is having significant DPPH radical scavenging property.

Table 1: Effect of APPD on DPPH Radical Scavenging Activity

Concentration(µg/ml)	APPD	Ascorbic acid
100	6.50±1.62	10.99±1.92
200	17.24±2.51	23.17±3.05
300	24.99±1.13	29.95±2.05
400	34.46±1.65	36.09±1.37
500	43.93±3.36	45.09±2.39
600	62.97±1.19	65.99±3.19
IC-50	**542.283**	**485.436**



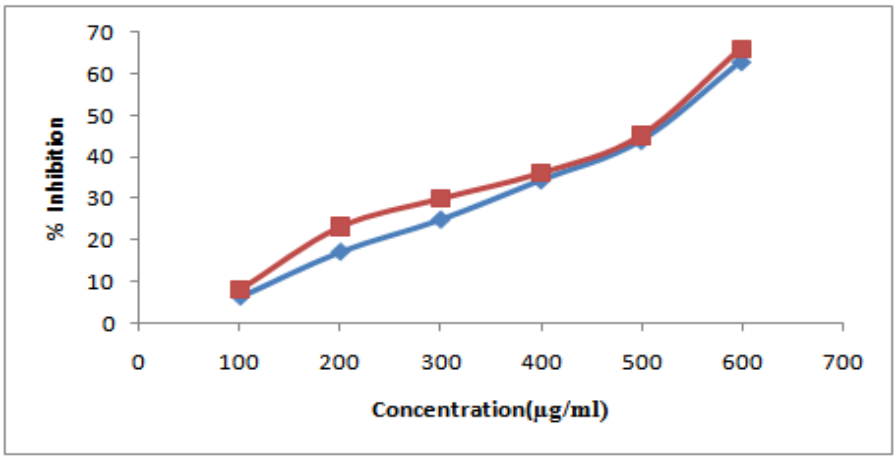
Graph 1: Effect of APPD on DPPH Radical Scavenging Activity

2.4.2. Reducing power:

Reducing power method is based on the principle that substances, which have reduced potential activity, that react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then react with the ferric chloride to form ferric-ferrous complex that has absorption at 700 nm. Graph 2 shows that how the APPD increases with the increase with the amount of sample [Table: 2]. Percentage inhibition of APPD is linear with that of standard ascorbic acid. The IC-50 of APPD is 17.56 and ascorbic acid is 19.17. It represent that APPD is having more antioxidant activity than ascorbic acid.

Table 2: Effect of APPD on Reducing Power Method

Concentration (µg/ml)	APPD	Ascorbic acid
10	23.33±3.33	32.07±1.97
20	53.33±6.66	66.87±4.13
40	120.00±11.54	125.55±3.28
60	153.33±6.66	161.00±2.50
80	180.00±5.77	196.24±4.50
100	243.33±14.52	255.97±5.07
IC-50	17.560	19.171



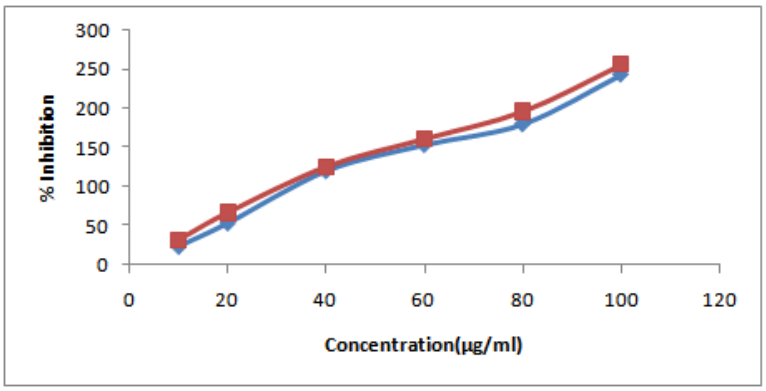
Graph 2: Effect of APPD on Reducing Power Method

2.4.3. Nitric Oxide Scavenging Activity:

Aqueous extract also showed a good nitric-oxide scavenging activity between 100 and 500 µg/ml in a dose dependent manner as showed in table: 3. The plant (or) plant products may have the property to counteract the effect of NO formation and in turns may be of considerable interest in preventing the ill effects of excessive NO generation in human body. The % inhibition was increased with increasing concentration i.e., IC-50 of APPD is 359.72 %. APPD is compared with standard ascorbic acid IC-50 is 247.52 % and results represented that APPD is having strong antioxidant activity.

Table 3: Effect of APPD on Nitric Oxide radical Scavenging Activity

Concentration (µg/ml)	APPD	Ascorbic acid
100	15.8±4.79	20.09±2.00
200	29.33±4.80	42.27±3.78
300	49.33±2.66	53.36±1.73
400	57.33±1.33	77.99±2.01
500	94.66±1.33	105.22±1.70
IC-50	359.722	247.52



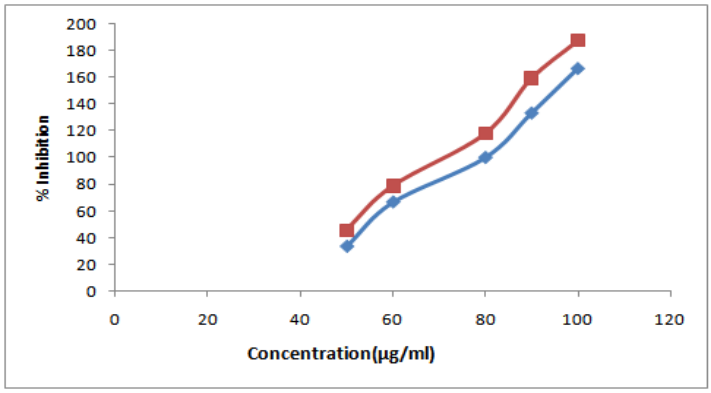
Graph 3: Effect of APPD on Nitric Oxide radical Scavenging Activity

2.4.4. Phosphomolybdenum Assay:

The total antioxidant assay based on reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V). The incubation of extracts with the Molybdenum (VI) will tell you the presence of antioxidant components in the extract, which can be assessed by recording the absorbance at 695 nm (to detect the reduced green molybdenum complex). So, this assay is very useful to predict the antioxidant activity of crude extracts on the total basis. So here table 4 results represented that APPD is having antioxidant activity when compared with the standard ascorbic acid. Inhibition concentration of APPD was 34.55 and ascorbic acid was 84, it represents APPD values. The antioxidant capacity was expressed as ascorbic acid equivalent by using the standard ascorbic acid graph.

Table 4: Effect of APPD on Phosphomolybdenum method

Concentration (µg/ml)	APPD	Ascorbic acid
50	33.33±2.33	45.97±1.99
60	66.66±4.62	71.09±2.59
80	100.00±5.16	110.89±5.04
90	133.3±5.94	140.09±3.95
100	166.66±4.97	187.21±6.44
IC50	34.554	84.00



Graph 4: Effect of APPD on phosphomolybdenum method

III. Discussion

In order to ascertain the applications of antioxidant activity of prunus dulcis, different methods were employed to evaluate the free radical scavenging and antioxidant activities of aqueous peel extract of prunus dulcis (APPD). We evaluated the DPPH free radical scavenging, reducing power, nitric oxide radicals, phosphomolibdinum assay.Generally plant studies we are carried out due to rich antioxidant properties. In fact, there are several pathological conditions which are correlated with perturbation of intracellular redox status such as cancers, cardiovascular diseases, diabetics, osteoporosis, and neurodegenerative diseases. An overproduction of oxidants or free radicals to an extent that overcomes the endogenous antioxidant system brings about a period of oxidative stress which results in a disturbance of signal transductions and consequently biological processes (M. Schieber, 2014.P.D. Ray, 2012). Antioxidants are beneficial compounds that protect cells against oxidative

damage by controlling free radical formation. When availability of antioxidants is limited, oxidative damage such as lipid peroxidation, DNA degradation, protein modification, and inflammation become cumulative and threaten human health. Therefore, antioxidants that scavenge reactive oxygen species or chelate metal transition ions have great value in preventing the onset and propagation of oxidative diseases (J.S.F. Swaran, 2009). One of the study reported that shell extract results provide a great therapeutic potential against infection of scalp caused by Tinea capitis and provides scientific evidence for clinical efficacy against dermatologic disorders (Nasreen Thebo, 2014).The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann, 1979).DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, violet color DPPH solution is reduced to yellow colored product, diphenylpicryl hydrazine, by the addition of the extract in a concentration dependent manner. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. Our results revealed that the aqueous peel extract of prunus dulcis had a similar free radical scavenging activity when compared with standard ascorbic acid. The results obtained in this study suggest that APPD is showed radical scavenging activity by their electron transfer or hydrogen donating ability. So, APPD is having high anti oxidant activity than standard ascorbic acid.The reducing power is generally associated with the presence of reductants, which exert antioxidant action by breaking the free radical chains by donating a hydrogen atom. In this assay, the presence of reductants in the antioxidant sample reduces Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form. Thus, the reducing power of the sample can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oktay M, 2003). In this study, the iron reducing capacity of the aqueous extract of prunus dulcis was estimated as ability to reduce the Fe^{3+}-ferricyanide complex to the ferrous form by donating an electron. The percentage inhibition of APPD was 17.560µg/ml and compared with standard ascorbic acid was 19.171µg/ml (Table: 2). The extract showed a good reducing power capacity, which was concentration-dependent (Graph: 2). Here, we assume that the antioxidant activity and reducing power capacity of the extract was likely due to the presence of flavanoids, phenolic and tannins which can act as free radicals scavengers by donating an electron or hydrogen.Nitric oxide is a very unstable species and reacting with oxygen molecule produce stable nitrate and nitrite which can be estimated by using Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid will decrease which can be measured at 546 nm. Aqueous peel extract of prunus dulcis has potent nitric oxide scavenging activity and IC-50 value is 359.722µg/ml (Table: 3).The scavenging of NO by the extract was increased in dose dependent manner. Graph-3 illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The APPD shows maximum activity of 94.66 % at 500 µg /ml, whereas ascorbic acid at the same concentration exhibited 247.52 % inhibition respectively.Phosphomolybdenum assay used to determine the total antioxidant capacity of fractions which is based on formation of phosphate/Mo (V) complex because of reduction of Mo (VI) by the fraction. Figure 4 and Graph 4 illustrates the antioxidative capacities of various concentrations of factions. The APPD presented a strong total antioxidant activity. The antioxidant activity might be attributed to the presence of antioxidant phytochemicals such as flavonoids, phenolic and tannins compounds.

IV. Conclusion

In the past few years, interest in the search of new natural antioxidants has grown because reactive oxygen species (ROS) production and oxidative stress is linked to many diseases. The use of synthetic antioxidants generally leads to problems of toxicity.The present investigation shows that *prunus dulcis* has antioxidant properties by virtue of its ability to serve as a reducing agent, free radical scavenger. *Prunus dulcis* showed strong antioxidant activity by inhibiting DPPH, Reducing power, nitric oxide and phosphomolibdinum radical scavenging activities when compared with standard ascorbic acid. It is easily accessible source of antioxidant. However the chemical constituent may present in the extract such as alkaloids, flavonoids, and phenolicand tannin contents. It is using as a food remedy in India since from ancestors'.

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