Research Article

Evaluation of Free Radical Scavenging Activity of '*Prunus Avium*'

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ABSTRACT

The present study aims to evaluate antioxidant activity of ethanolic extract of *Prunus avium* (family: Rosaceae) fruits in terms of free radicals scavenging assays. Ethanolic extract of the fruit was prepared by using maceration (70% C_2H_5OH + 30% H_2O) for 10 days followed by solvent evaporation by rotary vacuum evaporator. Phyto-chemical screening, total phenolic and total flavonoid content was determined by using standard procedures followed by NO, H_2O_2 Scavenging assay and reducing ability tests were performed to evaluate scavenging activity. Ethanolic extract of the fruit shows presence of alkaloid, flavonoid, amino acid, carbohydrate, glycoside and high pyrocatechol (51.7 µg/ml) and Gallic acid content (11.13 µg/ml) and quercetin content is almost negligible. IC_{50} value towards H_2O_2 of ethanolic extract was found as 22.84 ± 2.54 µg/ml having percentage inhibition 69% at 100µg/ml whereas IC_{50} for NO scavenging assay was 21.1 ± 2.31 µg/ml having percentage inhibition 67% at 100µg/ml. Reductive ability of the extract was found to increase in a concentration dependent manner. Results indicates that ethanolic extract of *Prunus avium* can be a potential source of antioxidant and future studies can be performed to isolate the bioactive principles.

Keywords: Antioxidant activity, *Prunus avium*, No scavenging assay, H_2O_2 scavenging assay.

INTRODUCTION

Oxidative stress is the common pathological problem in inflammation, neurodegenerative disorder, neoplasm, Alzheimers' disease and ageing. Oxidative stress is the disturbance between production of reactive oxygen and nitrogen species (ROS, RNS) and anti-oxidant defense[1]. Oxidative stress is responsible for production of free radicals – which can damage the components of cells like protein, DNA, mitochondria and ultimately leads to the death of the cells. Protective mechanisms against oxidation include prevention of formation of reactive oxygen species (ROS), scavenging of various forms of ROS, and repair of oxidized cellular contents. In general any partial defect in any of these systems can cause damage to the cells and promote senescence. Cells contain a number of antioxidant defenses to decrease the production of ROS, but production of ROS in certain cases exceeds the cell's antioxidant capacity and as a result oxidative stress is generated. Host survival depends upon the ability of cells and tissues to adapt to or resist the stress, and repair or remove damaged molecules or cells. A number of stress response mechanisms have been evolved for these purposes, and they are rapidly activated in response to oxidative insults. Some of the pathways are preferentially linked to enhanced survival, while others are more frequently associated with cell death[2-3]. Recently it has been found that, inborn defects of the antioxidant system interfere with the various protective mechanisms of the RBC cells and effects of several pure flavonoids has good membrane protective activity against damage of the cells[4]. Antioxidants play important role in the management of oxidative stress. An antioxidant is the molecule that inhibits oxidation of other molecules by removal of free radical intermediates and as a result formation of oxidative stress is prevented.

But all the antioxidants do not work by the same mechanism. Some of them work by free radical scavenging assay (Example: Vitamin C, Vitamin E), by inhibition of free radical formation (Example: Flavonoids) and by cell damage repair[5].

Prunus avium is commonly known as 'sweet cherry' – which is a native of Europe and Western Asia and now the species is widely cultivated at the himachal and the western Himalayan region at high altitude[6]. Sweet cherries are thought to be alleviating the pain associated with gout and arthritis and composition of the fruit varies from one region to another depending upon the climatic condition[7-8]. Ethanolic extract of the plant already shows good amount of anti-microbial and radio protective activity[9] whereas in some cases it has been found as an activator of the human sperm samples[10].

The objective of our work is to reduce oxidative stress of cells by using ethanolic extract of *Prunus avium* fruit (sweet cherry).

MATERIALS AND METHODS

Chemicals and Reagents used

Commonly used chemicals are ethanol, nitric oxide, hydrogen peroxide, potassium ferro-cyanide etc. and all the chemicals and reagents used were high analytical grade.

Preparation of Ethanolic Fruit Extract

Ethanolic extract of the fruit was prepared by using maceration (70% C_2H_5OH + 30% H_2O) for 10 days with intermediate shaking followed by filtration with Al_2CO_3. Solvent evaporation was done by rotary vacuum evaporator at 35°C followed by heating in water bath to get the ethanolic fruit extract[11].

Phytochemical screening

Extracts were subjected to screen for various phyto-constituents such as Flavonoids, glycosides, alkaloids, amino acids, carbohydrates, tannins and phenols present in them by using standard protocols[12-14].

Pyrocatechol, gallic acid and quercetin content of the ethanolic extract were determined by using standard protocols[15].

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates Nitrite oxide which interacts with oxygen to produce Nitrite ions and these were treated by using various concentration of the fruit extract and Percentage inhibition was measured at 550nm by spectrophotometer[16-18].

H_2O_2 Scavenging Assay

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4)[19,20].

Extracts in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage inhibition of hydrogen peroxide scavenging assay of the ethanolic extracts were calculated[21,22].

Reducing Ability

Reducing ability of the extract was determined by the slight modification of the method of Oyaizu, 1986[23]. Substances having reduction potential react with the Potassium ferricyanide to form Potassium ferrocyanide–which reacts with the ferric chloride to form ferrous complex–which was found in spectrophotometer at 700nm[24]. Various concentrations of the plant extract were mixed with 2.5 ml of buffer solution and 2.5 ml potassium ferrocyanide solution and was kept in water bath for 20 minutes. After cooling 2.5 ml 10% Tri-chloro-acetic acid was added and centrifused at 3000 rpm for 10 minutes followed by decantation of the upper layer and was mixed with 2.5 ml distilled water. Absorbance was measured in uv-spectrophotometer at 700 nm.

Statistical Analysis

Results are expressed as mean ± SEM & it was calculated by using Grap Pad Prism version 4.03 software.

RESULTS

Results of Phyto-chemical screening

Ethanolic extract of the fruit shows the presence of alkaloid, flavoinoid, amino acid, carbohydrate and glycoside in the sample and absence of tannin, saponin and lignin in the sample.

Test for	Result of the Test
Alkaloid	Present
Flavonoid	Present
Tannin	*Absent*
Saponin	*Absent*
Amino acid	Present
Carbohydrate	Present
Glycoside	Present
Lignin	*Absent*

Figure 1

Result of Total phenolic and total Flavonoid content

Ethanolic extract of the fruit shows high pyrocatechol content (51.7 µg/ml) and Gallic acid content (11.13 µg/ml) and negligible amount of quercetin.

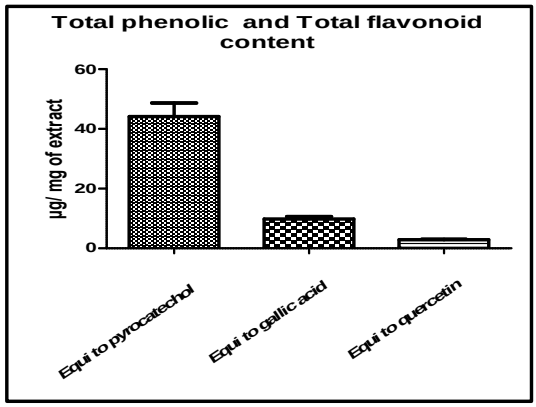

Figure 2: Ethanolic extract of the fruit shows high pyrocatechol content (51.7 µg/ml) and Gallic acid content (11.13 µg/ml) and negligible amount of quercetin.]

Result of NO Scavenging Assay

Ethanolic extract of the fruit showing increase of Percentage inhibition value in concentration dependent manner (Figure 3) having percentage inhibition 67% at 100µg/ml and IC_{50} for NO scavenging assay was reported as 21.1 ± 2.31 µg/ml – which proves presence of high antioxidant potential of the extract.

Result of H_2O_2 Scavenging Assay

Ethanolic extract of the fruit showing increase of Percentage inhibition value in concentration dependent manner (Figure 4) having percentage inhibition 69% at 100µg/ml and IC_{50} for H_2O_2 scavenging assay was reported as 22.84 ± 2.54 µg/ml – which proves presence of high antioxidant potential of the extract.

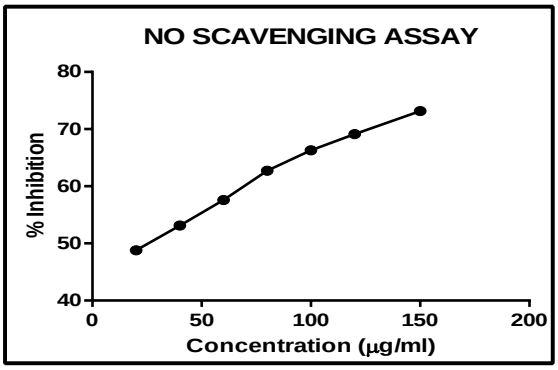

Figure 3: Ethanolic extract of the fruit shows increase of Percentage inhibition value in concentration dependent manner. Its percentage inhibition 67% at 100µg/ml and IC_{50} for NO scavenging assay was reported as 21.1 ± 2.31 µg/ml. Nitrite ions diazotize with sulphanilamide acid and couple with naphthyl ethylenediamine and forms pink color - which was measured by spectrophotometer.

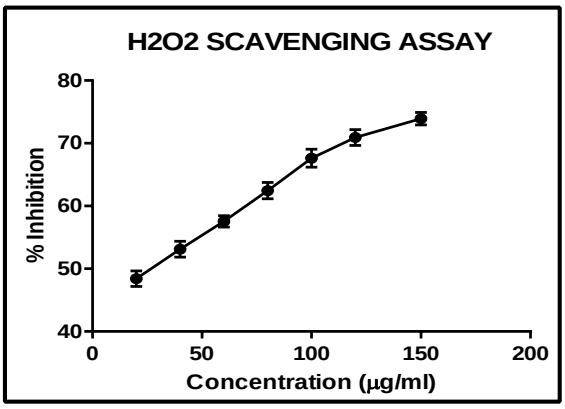

Figure 4: Ethanolic extract of the fruit shows increase of Percentage inhibition value in concentration dependent manner. Its percentage inhibition 69% at 100µg/ml and IC_{50} for H_2O_2 scavenging assay was reported as 22.84 ± 2.54 µg/ml µg/ml.]

Result of Reducing Ability Assay

Reducing Ability Assay shows that (Figure 5) gradual increase of spectrophometric absorbance reading with the increase of the concentration and it almost reaches a plateau phase when concentration of the extract becomes higher than 100µg/ml.

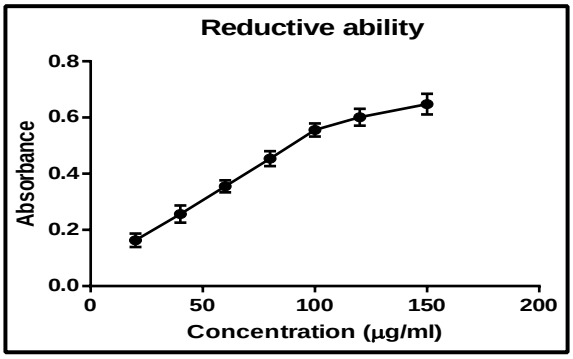

Figure 5: Reducing Ability Assay shows that gradual increase of spectrophometric absorbance reading with the increase of the concentration and it almost reaches a plateau phase when concentration of the extract becomes higher than 100µg/ml. Presence of reducers causes the conversion of Fe^{3+}/ ferrcyanide complex to the ferrous form and this change of color is determined by using spectrophotometer.

DISCUSSION AND CONCLUSION

The most abundant ROS represented in living inflammatory cells, is super-oxide, as well as hydrogen peroxide and highly toxic hydroxyl radicals and all of them are highly reactive to the living cells. Natural systems, like reduced glutathione, vitamins, and free fatty acids are considered an essential pool of antioxidants.[26] Oxidative stress is the situation where the production of oxidants exceeds the capacity to neutralize them & leads to damage of cell membranes, lipids, nucleic acids, proteins, and constituents of the extra-cellular matrix, such as proteoglycans and collagens.[21] There are different therapeutic approaches can be used to decrease the oxidative stress–which includes scavenging of free radicals, inhibition of free radical producing enzymes, enhancing the antioxidant system or targeting the signaling routes involved in the inflammatory cascade. Amongst the intracellular ROS generated, superoxide plays a pivotal role in inflammation[27]. Various plant-derived extracts were used as an important source of anti-oxidant activity and fruit of '*Prunus avium*' also shows the same.

Nitric oxide (NO) is generated from amino acid L-arginine by vascular endothelial cells, phagocytes, and certain cells of the brain. Nitric oxide is considered as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion ($ONOO^-$)[28]. Antioxidants from natural sources could be the alternative to synthetic antioxidants in counteracting oxidative stress associated diseases. A great number of naturally occurring substances have been recognized to have antioxidant abilities and various in vitro methods have been used to assess their free radical scavenging and antioxidant activity[17]. Therefore, in the present study, *Prunus avium* at different concentrations were assessed for their nitrite free radical scavenging activity in an in vitro model. Nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The nitrite ions diazotize with sulphanilamide acid and couple with naphthyl ethylenediamine and forms pink color - which was measured by spectrophotometer[29]. As antioxidants donate protons to the nitrite radical, the absorbance was changed and this variation of absorbance was measured by nitrite radical scavenging assay[30]. In the present study the result shows increase of Percentage inhibition value in concentration dependent manner and IC_{50} value was found to be as 21.10 ± 2.31 µg/ml.

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly and once inside the cell, H_2O_2 reacts with $Fe2^+$, and $Cu2^+$ ions to form hydroxyl radical and this is the cause of many toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate[31].

In our present study, different concentration of *Prunus avium* extracts were used to treat the H_2O_2 scavenging assay and the result shows increase of Percentage inhibition value in concentration dependent manner and IC_{50} value was found to be as 22.84 ± 2.54 µg/ml

Reducing power is associated with antioxidant activity and may serve as a significant reflection of antioxidant activity[32,33].

A compound having reducing power indicates that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process, so that they can act as primary and secondary antioxidants[34]. Presence of reducers causes the conversion of Fe^{3+}/ ferrcyanide complex to the ferrous form and this change of color is determined by using spectrophotometer. In our present study, different concentration of *Prunus avium* extracts were used to treat the Reducing Ability assay and the result shows increase of Percentage inhibition value in concentration dependent manner.

Taken together, the study has established that Ethanolic extract of '*Prunus avium'* shows good free radical scavenging activity. However *in vivo* experimentations are required for ultimate conclusion.

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