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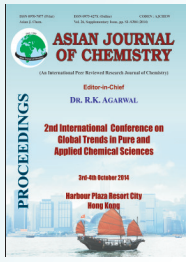

Phytochemical Analysis and Correlation of Antioxidant Activities of Methanolic Fruit Extracts of Family *Rosaceae*

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Main objective of this study was to quantify the total phenolics and flavonoid contents and to determine antioxidant activity in peel and pulp of *Prunus avium*, *Prunus domestica* and *Prunus persica* fruits of family *Rosaceae*. Antioxidant activities of three fruit extracts were determined and compared by DPPH, FRAP (ferrous reducing antioxidant power) and NO (nitric oxide) free radical scavenging assays. Total phenolics and flavonoid contents of those samples were also assessed and correlated. According to DPPH method *Prunus avium* had (91%) antioxidant activity, *Prunus domestica* (87%) and *Prunus persica* (81 %). Ferrous reducing antioxidant power assay of three fruit extracts was 84.4, 81.1 and 76.6 %, respectively. Nitric oxide scavenging activity of three fruit extracts was 85, 73 and 71 %, respectively. Total phenolic contents in fruits were 195.12; 167.05 and 150.13 (mg/g of extract), respectively. Flavonoid contents in all fruits varied from 68.42, 57.96 and 45.19 (mg/g), respectively. A good correlation between the antioxidant activities and total phenolic contents was found among various fruit extracts of family *Rosaceae*. *Prunus avium* extract was found to be enriched in flavonoids and phenolic contents, more than *Prunus domestica* and *Prunus persica*. Flavonoid contents are significantly related with antioxidant activity; whereas total phenolic contents are the major contributor to the antioxidant activity which varies considerably among different fruits of the family *Rosaceae*.

Keywords: *Prunus avium*, *Prunus domestica*, *Prunus persica*, Total Phenolics, DPPH.

INTRODUCTION

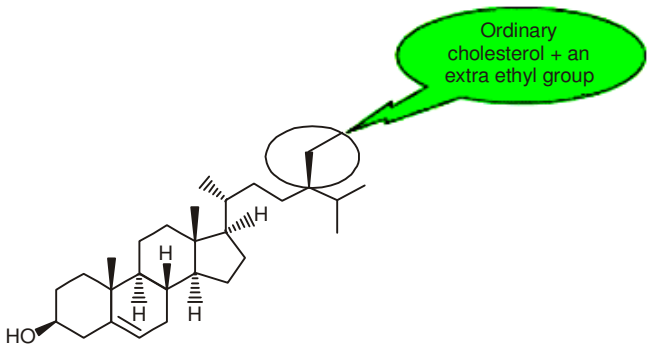
Botanical antioxidants have been shown to be associated with reduced incidence of ROS[1]. Antioxidant activity measurement is based on the scavenging free oxygen radical species[2]. Medicinal plants having phytoconstituents have gained an important position in the socio-cultural progress of people[3]. Novel research is mainly focused on plants for the discovery of natural antioxidant agents[4,5] mainly polyphenols[6]. It elucidates the importance of extracting antioxidant constituents from plants and fruits.

Cherry fruit obtained from the plant of genus *Prunus*, family *Rosaceae*. Cherries are packed with powerful antioxidants. A novel research published in the journal "Food Chemistry" predicts that when crude extract of cherry is used; its different ingredients work collectively to provide a strong antiradical blow[7]. Cherries contain important antioxidant compounds like lycopene and β-carotene. They contain a phytochemical β-sitostrerol, a plant sterol that has been linked to lower blood-cholesterol levels[8]. Plums contain fibre, potassium, vitamin A, calcium, magnesium, iron, minerals, and vitamin C, polyphenolic compounds, flavonols and anthocyanins. They are skin cleansers and reduce wrinkles so widely used as antiaging agents[9]. Peach is enriched in a variety of phytochemical constituents especially ascorbic acid. It contains minerals and electrolytes. Peach contains potassium which helps to maintain water balance. It is a fibrous fruit with plenty of water; used as a skin moisturizer and help us to stay younger for longer period of time due to its powerful antioxidant constituents[10].

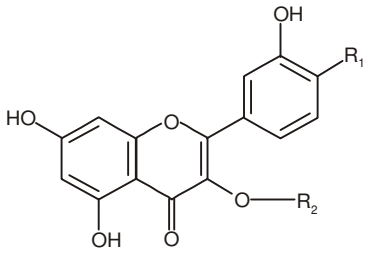
EXPERIMENTAL

Selection and identification of fruit Samples: Fruits of *Prunus domestica*, *Prunus persica*, *Prunus avium* were collected from local market and their herbarium numbers were obtained from Quaid e Azam University Islamabad Pakistan *i.e.*, *Prunus avium* ISL-83356, *Prunus domestica* ISL-22471 and *Prunus persica* ISL-39629.

Preparation of methanolic extracts of various fruits: Methanol (Merck KGaA Darmstadt, Germany) was used as extraction solvent alone and in combination with water in different ratios to extract the active constituents of the required activity and their activity was measured accordingly. Maximum

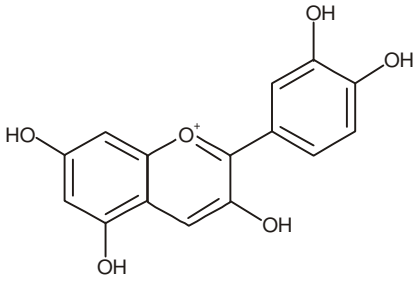


β-Sitostrerol

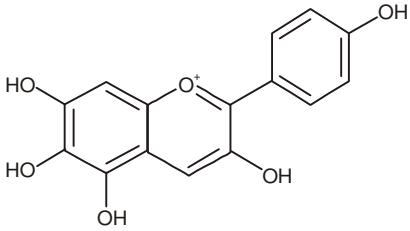


Flavonol	R_1	R_2
Rutin	OH	Rutinose
Querceitrin	OH	Rhamnose
Quercetin	OH	H
Kacmpferol	H	H
Isorhamnetin	OCH_3	H

Flavonoids



Cyanidin



Aurantinidin

Fig. 1. Chemical structures of phytochemicals and polyphenolic constituents in cherry, plum and peach

yield was obtained with 80:20 ratio of methanol and water, respectively and 1 mL of 0.5 % hydrochloric acid (Merck KGaA Darmstadt, Germany) through the process of maceration. *Prunus avium*, *Prunus demestica* and *Prunus persica* fruits (100 g each) were shade dried whole (without peeling but after carefully removing the stones). Then grounded to get powder and then this powder was soaked in 400 mL of above mentioned methanolic mixture and kept for 48 h in dark. It was then filtered by cloth (muslin) and then through Whattman No.1 filter paper for removing any coarse particles present in the extract. The solvent was then evaporated off under vacuum at 40 °C to obtain concentrated extracts using rotary evaporator (Eyela, Co. Ltd. Japan).

Determination of total phenolic contents: Total phenolic contents were determined according to the method of Ismail *et al.*[11] with slight modifications. A total assay volume of 200 µL contained 90 µL Folin-Ciocalteu reagent (Sigma, USA) and 20 µL test compound. The contents were mixed and pre-read at 725 nm and incubated for 5 min at 25 °C. The reaction was initiated by the addition of 90 µL (6 % w/v Na_2CO_3). The change in absorbance was observed after 30 min at 725 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

Gallic acid was used as standard: Different concentrations of gallic acid were prepared in methanol. The calibration curve was prepared by plotting absorbance *vs*. concentration and it was found to be linear over this concentration range of 50-500 µg/mL.

The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Based on the measured absorbance, the concentration of phenolics contents was read (µg/mL) from the calibration line; then the phenolics contents in extracts were expressed in terms of gallic acid equivalent (µg of GAE/mg of extract) and were calculated using the following equation:

$$C = cV/m$$

where C is the total content of phenolic compounds, µg GAE/mg dry extract (dE); c the concentration of gallic acid obtained from the calibration curve, µg/mL; V the volume of extract, mL and m is the weight (mg) of extract.

Determination of total flavonoid contents: The quantity of flavonoid content (TFC) in a crude extract was determined by the method of Zengin with a few modifications. A total volume of 200 µL contained 80 µL deionized water, 20 µL test compound and 16 µL $NaNO_3$ (5 % w/v). The contents were mixed, pre-incubated for 6 min at 25 °C and then pre-read at 510 nm. The reaction was initiated by the addition of 16 µL $AlCl_3$ (10 % w/v) and 68 µL NaOH solutions (4 %). The change in absorbance was observed after 20 min at 510 nm. The absorbance was determined using UV spectrophotometer. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

Rutin was used as standard: Different concentrations of rutin were prepared in methanol. The calibration curve was prepared by plotting absorbance *vs*. concentration and it was

found to be linear over this concentration range of 50-500 µg/mL.

Based on the measured absorbance, the concentration of flavonoid contents was read (µg/mL) from the calibration line; then the flavonoid contents in extracts was expressed in terms of rutin equivalents[12].

Determination of antioxidant activity: Antioxidant activity of *Prunus avium*, *Prunus domestica* and *Prunus persica* fruit extracts was determined by using DPPH (diphenyl-picryl-hydrazyl) assay; FRAP/ferric reducing antioxidant power assay and NO (nitric oxide) radical scavenging assay.

Statistical analysis: The data obtained was expressed as X ± SD. At 0.05 level of significance data was compared to assess the significance of difference using Graph Pad Prism (GPP) version 5.01.

RESULTS AND DISCUSSION

2,2-Diphenyl-1-picrylhydrazyl assay: 100 µL mixture of sample solution (10 µL) and 100 µM methanolic DPPH (Sigma, USA) (90 µL) solution was added in a 96 wells plate. Synergy HT BioTek® USA microplate reader was used to determine the reduction in absorbance (at 517 nm). Control used was ascorbic acid or vitamin C. The data was taken as three concordant readings to calculate the mean. Different dilutions were prepared to take the values of IC_{50} and their absorbance was taken by using UV-spectrophotometer of Shimadzu, Japan[13]. Less was the absorbance higher was the radical scavenging activity and it was calculated with given formula of the measurement of percentage inhibition. The absorbance of control shows the total enzymatic capacity without any inhibitor and absorbance of the sample is the measure of activity of test compound.

$$\%\text{age inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test solution})}{\text{Absorbance of control}} \times 100$$

Antioxidant activity of phytochemical extracts by DPPH method is shown in Table-1.

TABLE-1
ANTIOXIDANT ACTIVITY OF
PHYTOCHEMICAL EXTRACTS BY DPPH METHOD

DPPH scavenging assay		
Phytochemical extract	Inhibition (%)	IC_{50} (µg/mL)
Prunus avium	91 ± 0.99	17.8 ± 0.41
Prunus domestica	87 ± 1.54	19.1 ± 0.27
Prunus persica	81 ± 1.71	22.1 ± 0.92

Values are given as X ± SD (where, n = 3 and P ≥ 0.05)

Prunus avium fruit extract showed 91 % DPPH antiradical capacity; with vitamin-C as control. However, percentage inhibition of *Prunus domestica* and *Prunus persica* extracts were 87 and 81%, respectively.

Ferrous reducing antioxidant power (FRAP) assay: A simple and widely used method for antioxidant activity evaluation is ferrous reducing assay which is a measure of the capacity of antioxidants for the reduction of ferric to ferrous ions[14,15]. In Elisa microplate reader well equal volumes (25 µL) of the test solution and phosphate buffer were mixed. For incubation at 50 °C; one percent solution of $K_4[Fe(CN)_6]\cdot 3H_2O$ (50 µL) was taken; after that 25 µL 10 % trichloro-acetic acid and 100 µL of distilled water were added. With UV-spectrophotometer absorbance was measured at 540 nm. Then, 0.2 % $FeCl_3$ solution (25 µL) was added in the above solution and its absorbance was taken at 700 nm. Standard used was vitamin C.

$$\%\text{age inhibition} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Antioxidant activity of phytochemical extracts by FRAP method is shown in Table-2.

TABLE-2
ANTIOXIDANT ACTIVITY OF
PHYTOCHEMICAL EXTRACTS BY FRAP METHOD

FRAP ASSAY		
Phytochemical extract	Inhibition (%)	IC_{50} (µg/mL)
Prunus avium	84.4 ± 1.04	19.1 ± 0.41
Prunus domestica	81.1 ± 1.62	21.2 ± 0.27
Prunus persica	76.6 ± 0.12	24.1 ± 0.92

Values are given as X ± SD (where, n = 3 and P ≥ 0.05).

FRAP activity of *Prunus avium* and *Prunus domestica* is greater than *Prunus persica*.

NO (nitric oxide) radical scavenging assay: NO anti-radical activities of various fruits extracts were assayed in accordance with Marcocci *et al.*[16] by slight alteration. Total assay volume (250 µL); contained 45 µL methanol 5 µL test compound and 150 µL sodium nitroprusside solution. Mixture was incubated (2 h at 37 °C) then measured the absorbance as pre-read at 540 nm. 50 µL griess reagent was added to initiate the reaction and absorbance was measured by UV spectrophotometer at 540 nm (after 10 min). Control used was quercetin. Less the absorbance higher was the NO scavenging activity; calculated with the given formula of percentage inhibition.

$$\%\text{age inhibition} = \frac{(\text{Absorbance of standard} - \text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

Results for NO scavenging activity of fruits of family *Rosaceae* are shown in Table-3.

TABLE-3
ANTIOXIDANT ACTIVITY OF PHYTOCHEMICAL
EXTRACTS BY NO RADICAL SCAVENGING METHOD

NO Scavenging assay		
Phytochemical extract	Inhibition (%)	IC_{50} (µg/mL)
Prunus avium	85 ± 0.99	19.2 ± 0.41
Prunus domestica	73 ± 1.71	23.6 ± 0.27
Prunus persica	71 ± 2.12	27.4 ± 0.92

Values are given as X ± SD (where, n = 3 and P ≥ 0.05)

Prunus avium methanolic (80 %) extract showed greater NO scavenging activity. *Prunus domestica* and *Prunus persica* also showed good radical scavenging activity by nitric oxide assay.

Phytochemical analysis (Table-4) shows that the extracts of various fruit samples have many antioxidant phyto-constituents like polyphenols, anthocyanins, ascorbic acid, carotenoids and lycopene *etc*.

In present study, the phenolic contents (Table-5) and flavonoids (Table-6) of extracts from dry pulp and peel of cherry fruit were superior to that from plum; which contains

TABLE-4
PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS

Phytochemical test	Inference	Phytochemical test	Inference
Flavonoid	+ + +	Anthocyanins	+ + +
Vitamin C	+ + +	Lycopene	+ +
Alkaloids	+	Carotenoids	+ +
Phenolic contents	+ + +	Anthraquinones	+

TABLE-5
TOTAL PHENOLICS (µg) OF GALLIC ACID EQUIVALENT PER mg OF PULP WITH PEEL EXTRACT of *Prunus avium*, *Prunus domestica* AND *Prunus persica*

Total phenolics		
Prunus avium	*Prunus domestica*	*Prunus persica*
195.12 ± 0.32	167.05 ± 0.75	150.13 ± 0.57

Values are given as X ± SD (where, n = 3 and P ≥ 0.05)

TABLE-6
TOTAL PHENOLICS (µg) OF RUTIN EQUIVALENT PER mg OF PULP WITH PEEL EXTRACT OF *Prunus avium*, *Prunus domestica* AND *Prunus persica*

Total flavonoids		
Prunus avium	*Prunus domestica*	*Prunus persica*
68.42 ± 2.01	57.96 ± 1.04	45.19 ± 1.34

Values are given as X ± SD (where, n = 3 and P ≥ 0.05)

more quantity than peach. All the fruits contain an adequate amount of these phytochemicals which is directly related to their antioxidant capacity.

Conclusion

It was concluded that 80 % methanol (w/v) fruit extract of *Prunus avium* extract showed maximum free radical scavenging activity. The other two varieties which were *Prunus domestica* and *Prunus persica* also showed good antioxidant activity and contain active biological constituents. Cherry, plum and peach fruits should be used without peeling to get maximum benefits for consumer such as to lower the blood cholesterol level, anti-cancer agents, whitening agents and antiaging effects in topical formulations. Therefore further evaluation directs to isolation of responsible components which will provide new lead compounds in future.

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