Antioxidant Activities in Methanolic Extracts of *Olea Ferruginea* Royle Fruits

R. K. Sharma, N. Sharma, S. S. Samant, S. K. Nandi, and L. M. S. Palni

***Abstract*—*Olea ferruginea* Royle is an important multipurpose tree and an underutilized fruit tree crop of Himachal Pradesh, India. The antioxidant potential of fruits of this species has not been properly investigated; therefore, in the present study, total phenolic content and antioxidant capacity of methanolic extracts of fruits of *O. ferruginea* from five populations were investigated. Mature fruits from three different trees of approximately same height and age from various populations (Thalaut, Sapangi, Suind, Kolibehar and Kais) were collected. One gram of fresh fruits was homogenized with 10 ml of 80% (v/v) methanol and extracts were analyzed for total phenolic content (mg tannic acid equivalent (TAE)/g fw) and antioxidant capacity [mM of ascorbic acid equivalent (AAE)/g fw of fruit] using 3 *in-vitro* assays, namely, 1, 1-diphenyl-2-pycrylhydrazyl (DPPH), 2,2'–azinobis, 3-ethylbenzothiazoline-6-sulphonic acid radical scavenging (ABTS) and ferric reducing antioxidant power (FRAP). The total phenolic content in the methanolic fruit extracts among different populations varied between 2.30-3.41 TAE/g fw, and their antioxidant activities using DPPH, FRAP and ABTS assayes among the populations ranged from 0.15 - 0.24, 28.02 - 31.4 and 0.0019 - 0.0138 AAE/g fw. The study further showed that ripe fruits of *O. ferruginea* possess significantly higher phenolic content, DPPH and ABTS radical scavenging potential as compared to the raw fruits. On the other hand, raw fruits have significantly higher FRAP activity in comparison to ripe fruits. The study reveals that the ripe fruits of *O. ferruginea* are a rich source of natural antioxidants and can be used as nutraceuticals and should be exploited for commercial purposes.**

***Index Terms*—Total phenolics antioxidant capacity, Indian olive, population, fruits.**

I. Introduction

Interest in recent years in natural antioxidants from plants is increasing due to their free radical scavenging potential. Several plant based extracts have been screened for investigating their antioxidant and radical scavenging activities [1], [2]. Distributions of phenolics and flavonoids in nature as antioxidants have been reported worldwide [2], [3].

Antioxidants have therapeutic importance as they possess neuroprotective and neurodegerative roles. The main characteristic of an antioxidant is the ability to trap free radicals. Antioxidants are categorized into two groups, i.e., exogenous and endogenous. The exogenous group includes dietary phytochemicals (polyphenols, quinones, flavonoids, chatechins, coumarins, terpenoids, etc.) and smaller molecules (ascorbic acid, alpha-tocopherol, beta-carotene, etc.). The endogenous group includes enzyme (superoxide dismutase, catalase, glutathione peroxide, etc.) and trace metals (Cu, Zn, Mn, Se, etc.)

Indian Himalayan Region is one of the richest biodiversity hotspots on globe and supports a large number of multi-purpose plant species [4]. *Olea ferruginea* Royle (family: Oleaceae) is a native species of the Himalaya region and grows naturally in the agro-ecosystems. Being one of the six species of *Olea*, known as Indian Olive (locally known as Kahoo), *O. ferruginea* is found in Afghanistan, west Nepal, Jammu & Kashmir and Uttrakhand between 500-2000 m asl. It is a multipurpose, zero-waste and evergreen fruit tree crop providing various uses such as quality fodder, fuel wood, edible fruits and treatment for various ailments. According to Joshi [5] the content of mono-unsaturated fatty acid, particularly oleic acid, ranged from 64.4 - 67.2% in seed oil of Indian Olive, slightly higher than its fruit oil.

Therefore, the present study has been carried out to assess the antioxidant activities in methanolic extracts of the fruits of *O. ferruginea* growing in Kullu, Himachal Pradesh and also to compare the antioxidant activities of both raw and ripe fruits.

II. Materials and Methods

The present study was carried out in Kullu district of Himachal Pradesh, India during August/September 2011. Five populations of Indian Olive, namely Thalaut, Sapangi, Suind, Kolibehar and Kais were selected and fruit samples from three different trees of the same height and age (each population) were collected in poly bags. The samples of both raw and ripe fruits were also collected from the same tree of Kolibehar population. The samples were brought back to the laboratory and kept at 4^0C in the refrigerator for 24 hours. All samples were washed thrice using running tap water to remove dust particles and chopped into small pieces. One gram of fruits was weighted accurately and crushed in 10 ml of 80% (v/v) methanol using mortar and pestle. The extract was further kept in the refrigerator at 4^0C for 48 h and then centrifuged at 10,000g; the supernatant was collected and used for the analysis of total phenolic content and antioxidant activities. Similarly, the extracts were prepared from the raw and ripe fruits.

The total phenolic content in methanolic extract of fruits of Indian Olive was determined spectrophotometrically using

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the modified method of Wolfe et al. [6]. One ml aliquot of the extract was mixed with 1 ml of Folin-Ciocalteu Phenol Reagent and 2 ml of 2% (w/v) sodium carbonate; the total volume was made up to 10 ml using double distilled water. The mixture was then heated at 80^0C till blue color appeared and after cooling, the absorbance of blue color was measured at 650nm using UV-visible spectrophotometer (Ultrospec 2100 Pro, Healthcare Biosciences AB, Uppsala, Sweden). The content of total phenolics in extracts was quantified using standard curve prepared with different concentrations of tannic acid. The results were expressed as mg tannic acid equivalent (TAE)/g fw of fruit.

The antioxidant activities in methanol extracts of fruit was measured in terms of 2, 2'–azinobis (3-ethylbenzothiazoline-6-sulphonic acid) [ABTS)], 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) using the methods of Re et al. [7], Liyana-Pathirama et al. [8] and Benzie and Strain [9], respectively. For the determination of DPPH radical scavenging, 1 ml of extract was mixed with 5 ml of 0.04% (w/v) DPPH in methanol, mixed properly and the reaction mixture was kept in dark at room temperature for half an hour. The absorbance of mixture was then measured at 517 nm using spectrophotometer and the results were expressed in mM ascorbic acid equivalent (AAE)/g fw of fruit.

An equal volume of ABTS salt (7 µM) and potassium persulphate (2.45 µM) were added for the production of ABTS cation and kept in the dark at room temperature for 16 h. ABTS cation solution was then diluted by using 80% (v/v) methanol till an absorbance of 0.70 ±0.001 was obtained. The diluted ABTS cation (1ml) was properly mixed with 1ml of the extract and kept in the dark for 7 min. The absorbance of this mixture was measured at 734 nm against methanol blank. A standard curve of various concentrations of ascorbic acid was prepared in 80% (v/v) methanol for quantification of antioxidant potential with respect to ascorbic acid. The results were expressed in mM AAE/g fw of fruit.

FRAP assay was arrived out by adding 10 ml of 300 mM acetate buffer (3.1g of sodium acetate and 16 ml glacial acetic acid per liter), 1ml of 10 mM 2,4,6-tri-2pyridyl-1,3,5-triazine (TPTZ) in 40 mM HCl and 1 ml of 20 mM ferric chloride. The mixture was pre-warmed at 35^0C. Three ml of mixture was added to 1ml of extract and kept at room temperature for 10 min. The absorbance of resulting mixture was read at 593 nm and the results expressed as mM AAE/g fw of fruit.

The data thus obtained were subjected to SPSS analysis (in triplicates) and mean ± standard error was obtained. The significant differences between populations and between the raw and ripe fruits were analyzed using Duncan Multiple Range Test and Student's T-test, respectively. The statistical analyses were performed using SPSS software, version 12.

III. Results and Discussion

The results of the present study are shown in Table I and Figure 1. The total phenolic content expressed as mg TAE/g fw was maximum in Kais, followed by Kolibehar, Suind, Sapangi and least in Thalaut population. The effect of population on total phenolic content in methanol extracts of fruit was also found significant at $p \leq 0.001$ (Table 1). The results further showed that changes in the total phenolic content in ripe and raw fruits were insignificant at $p \leq 0.05$ (Figure 1).

A number of studies have shown that high total phenolic content is associated with greater antioxidant activity [10]-[12]. The variation in total phenolic content and antioxidant activities in fruit extract in the present study may be ascribed to variation in altitude, habitat, vehicular and other emissions. Sharma et al. [2] have reported that plants of the medicinal herb *Withania somnifera* growing near the roadside contained higher total phenolics than those in the forest. Significant variation in the total phenolic content has been reported in fruits of a wild edible plant *Myrica esculenta*, and linked to altitudinal variation [13].

TABLE I: Total Phenolic Content and Antioxidant Capacity in Methanolic Fruit Extracts of Indian Olive from Selected Populations

Populations	**Total phenolic (mg TAE /g fw)**	**Antioxidant capacity (mM AAE/ g fw)**		
		DPPH	FRAP	ABTS
Thalaut	2.30^{c}	0.20^{b}	30.54^{b}	0.0041^{b}
Sapangi	2.33^{c}	0.21^{b}	31.43^{a}	0.0026^{c}
Suind	2.87^{b}	0.15^{c}	30.01^{ab}	0.0138^{a}
Kolibehar	3.24^{a}	0.17^{c}	28.02^{b}	0.0019^{d}
Kais	3.41^{a}	0.24^{a}	29.98^{ab}	0.0045^{b}
Average	2.83	0.19	30.00	0.0054
F-value	16.55	17.46	3.34	584.53
p	≤ 0.001	≤ 0.001	≥ 0.05	≤ 0.001

Values are mean ± SE of three replicates. Values with different letters (a-c) are statistically different at $p \leq 0.05$ probability level (Duncan's Multiple Range Test).

The source population and age have significant effect on the antioxidant properties of extracts in ABTS and DPPH assays, whereas, no significant variation in FRAP assay due to population was recorded (Table I and Figure 1). Among populations, antioxidant activities (mM AAE/g fw) in methanolic fruit extracts ranged from 0.15 - 0.24, 28.02 - 31.4 and 0.0019 - 0.0138 in DPPH, FRAP and ABTS assays, respectively. The results further showed that DPPH, FRAP and ABTS inhibiting potential of fruit extract were highest in Kais, Sapangi and Suind populations, respectively and lowest in Kolibehar population. Rawat et al. [13] have also reported significant variation in antioxidant activities in the methanolic fruits extract collected from *M. esculenta* populations growing at different altitudes. The present study further showed that DPPH and ABTS activities in methanolic extracts were significantly higher ($P \leq 0.01$) in ripe fruits as compared to raw fruits, whereas FRAP activity was higher ($P \leq 0.001$) in raw fruits in comparison to ripe fruit. This study clearly indicates that both the source population and fruit maturity significantly influence the total phenolic content and antioxidant activities in Indian Olive.

Plant antioxidants have played an important role in maintaining health and providing protection against coronary heart diseases, cancer, etc. Thus, researches on natural antioxidants of plant origin have attracted scientists, food manufacturers and consumers as potential source of functional foods.

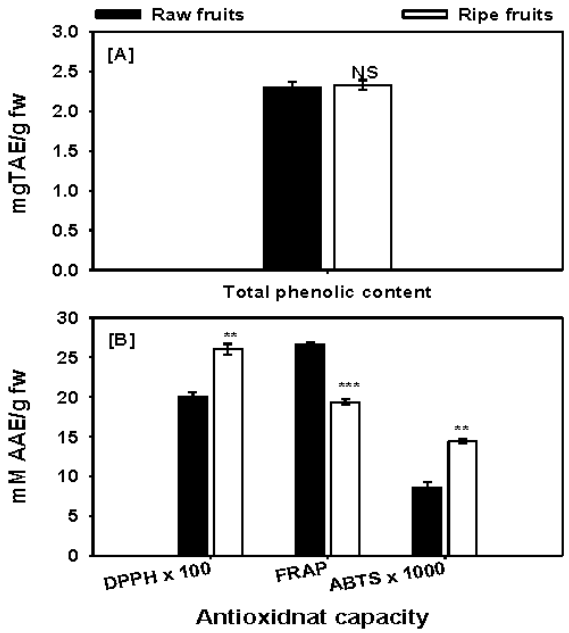

Fig. 1. Total phenolic content [A] and antioxidant capacity [B] of methanolic extract of raw and ripe fruit of Indian Olive. Bar with stars in each group is statistically significant (Student's T-test, NS=not significant, **p<0.01; ***p<0.001)

IV. Conclusion

The study concludes that ripened fruits of Indian Olive can serve as a source of natural antioxidants for the local population and can also be exploited for commercial purposes. However, further investigations on individual phenolic compounds in different plant parts of this species are needed.

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