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SHORT COMMUNICATION

Antioxidant Activity Vis-a-Vis Phenolic Content in Leaves of Seabuckthorn from Kargil District (J&K, India): A Preliminary Study

Deepak Gupta[1] · **Veenu Kaul**[1]

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Abstract *Hippophae rhamnoides* L. commonly called as seabuckthorn belongs to family Elaeagnaceae and grows in abundance in Ladakh, the eastern-most part of the state of Jammu and Kashmir (India). In the present study, phenolic content and antioxidant activity was determined in shade dried leaves of ten seabuckthorn populations of Kargil. Total phenolic content varied from 42.8 to 72.4 mg GAE and antioxidant activity quantified by DPPH assay varied between 37.4 to 89 %. Karl Pearson's coefficient of correlation was calculated to ascertain whether any relationship exists between antioxidant activity and phenolic content.

Keywords Antioxidant activity · India · Phenolics · Seabuckthorn

Chemo profiling of plants for natural antioxidants is of immense interest and has gained importance in food industry after the emergence of report that synthetic antioxidants like BHA (butylated hydroxyl anisole), BHT (butylated hydroxyl toluene), PG (propyl gallate), TBHQ (tert-butyl hydroxyl quinine) etc. are carcinogenic [1]. Among the various medicinal taxa, Seabuckthorn has acquired the status of food and medicinal fad of the next century. Found to contain more than 190 bioactive substances including flavonoids, phenols, carotenoids, vitamins (A, E, K, C, B_1, B_2, Folic acid), pigments, fatty acids (Omega 3, 6, 7, 9), organic acids, tocopherols, terpenes, sterols, tannins and minerals [2, 3], the plant is extensively used in the traditional system of medicine in many Asian, Chinese, Mongolian and Tibetan countries. In Ladakh, use of seabuckthorn in Amchi system of medicine has been in vogue since times immemorial despite the locals not knowing about its active constituents [4]. A recent report from Russia showed that oil extracted from leaves and fruits of seabuckthorn are used for wound-healing, as antibacterial, anti-ulcer, anti-inflammatory and as a multivitamin product [5]. The leaves are extensively used in the treatment of diarrhoea, rheumatoid arthritis and dermatological disorders [6]. Similarly, the polyphenols in seabuckthorn have multiple uses ranging from protection to human body against free radicals, in plants to infection by pathogens and defence against herbivory [7]. In order to assess antioxidative potential of plants growing in Kargil district (J&K, India), shade dried leaves were chosen as study material.

Collections were made in October from male and female plants from each of ten different populations growing in their natural habitats during the field visits to different parts of Kargil. Kargil lies between 34″30′N and 76″13′E longitude and these populations were located at Sankoo, Shilikchey, Pashkum, Mingee, Andoo, Shargol, Barootsog, Akchamal, Kanoor and Chanigund. For each population, sample size remained constant ($n = 5$) with the exception of Chanigund since this population was devoid of female plants. The leaves were collected, shade dried at room temperature (20–25 °C) in Ladakh and then stored in deep freezer at −20 °C (Vestfrost) at Department of Botany, University of Jammu. Phenols were extracted, as described by ISO [8] with slight modification. Total phenolic content of 70 % methanolic extract was determined by spectrophotometry using gallic acid as standard and expressed as mg GAE [9, 10].

✉ Veenu Kaul
veenukaul@yahoo.co.in

[1] Department of Botany, University of Jammu, Jammu 180006, India

A freshly prepared DPPH (1, 1-diphenyl-2-picryl hydrazyl) solution has a deep purple color with maximum absorption at 517 nm. The purple color generally fades/disappears completely when an antioxidant is present in the medium. Thus, antioxidants present in the extract can quench DPPH free radicals and convert them to colorless form resulting in decrease in absorbance at 517 nm. Hence, more rapid decrease in absorbance more potent the antioxidant activity of that extract. DPPH radical scavenging activity of 70 % methanolic extract was determined according to the method of Blois [11]. The absorbance was determined at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Gallic acid was used as positive control. DPPH radical scavenging activity was calculated using the following formula:

$$\% = A_0 - A_1/A_0 \times 100$$

A_0-Absorbance of the control
A_1-Absorbance of extract/Standard sample.

Results were expressed as mean ±standard error and data were analyzed by two-way ANOVA to determine the effects of population type and plant sex on phenolic content as well as antioxidant activity.

Existence of relationship between antioxidant activity and different antioxidants were calculated by Karl Pearson's method [12] and t-test was applied for checking significance of relation.

The total phenolic content of shade dried leaves in 70% methanolic extract is presented in Fig. 1a. Across populations, the average phenols ranged between 42.8 and 72.4 mg. In males, the highest quantity was found in plants of Barootsog (72.4) and lowest in those of Pashkum (42.8). Females of Barootsog showed a reverse trend; they had lowest quantity among those across populations. Statistically significant differences occurred among sexes ($F_{(1, 8)} = 6.06$; $P < 0.05$) but not between populations ($F_{(8, 8)} = 0.139$; $P > 0.05$). With the exception of Barootsog and Mingee, majority of the populations had female plants with greater phenolic compounds than their male counterparts. The significant differences in the quantity of bioactive compounds between male and female plants can be attributed to several reasons some of which are purely genetic and others ecological in nature. Reproductive functions associated with each sex also impose resource constraints, which inturn, affect the quantitative profile of the bioactive compounds [13]. Critical and crucial among these are the different resource needs and constraints encountered during different phases of life cycle like growth, defence and reproduction [14]. This is because each phase has its own physiological demands and accordingly seasonal changes are required for regulation and production of appropriate biochemical constituents, both qualitative and quantitative. These together

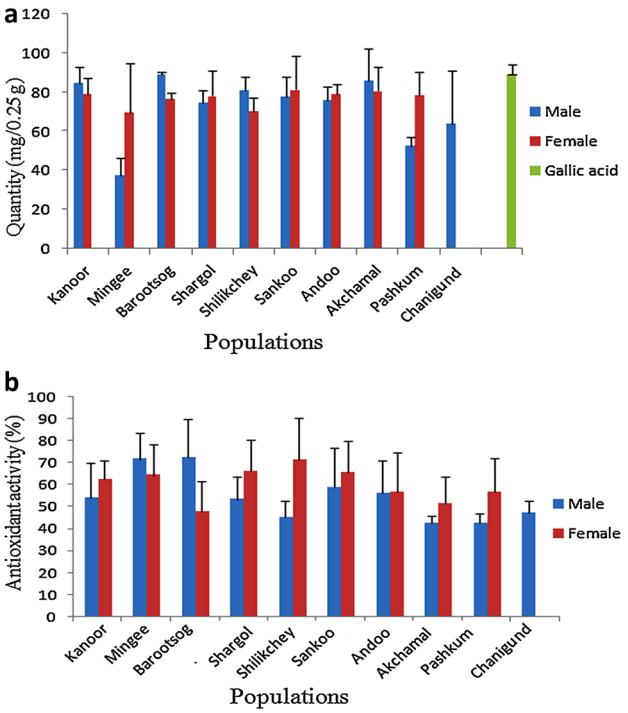

Fig. 1 Multiple bar diagrams depicting sex and between population difference in **a** total phenolic content in 70 % methanolic extracts, **b** antioxidant activity (as determined by DPPH method) of leaf extract *H. rhamnoides*. *Note* the comparable acitivity with Gallic acid

with environmental stresses contribute to variations within sexes and among populations [15]. In dioecious plants, female plants allocate more resources to reproduction than male plants. Therefore, it is expected that asymmetrical allocation to reproduction may lead to a reproduction-growth tradeoff, whereby female plants grow less than male plants, but invest more in defenses and thus experience lower herbivory than male plants. In the present study, collections were made in October when fruits were still attached to the plant, ready to harvest and if not collected, persist on the parent plant (non-deciduous) and remain available to the foragers. Plants have to face the severe winters ahead. Presence of greater amount of phenols in females around this time could be a strategy to ensure survival by being as defensive as possible and sufficient to discourage herbivory and prevent damage to the fruits but less enough to be desirable as food for humans.

Leaves collected from male as well as female plants showed comparable levels of radical scavenging activity, with respective averages ranging from 37.4 to 89 % and 69.7 to 81.3 %. The percentage inhibition was maximum in male plants of Barootsog (89 %) and least in those of Mingee (37.4 %). Among female plants the maximum populational average of 81.3 was recorded for Sankoo and least i.e. 69.7 for Mingee. Figure 1b enlists antioxidant activity values for each population. Overall, females had a

narrower range (52.0–95) than the male plants (6.3–96.3).The average DPPH radical scavenging activity in males and females was 72.3 and 77 % respectively. Gallic acid used as standard is equally good as far as its antioxidant activity is concerned; it was equivalent to males of Barootsog (Fig. 1b). Results of two-way ANOVA reveal significant variation in antioxidant activity between sexes ($F_{(1, 8)} = 12.36$; $P < 0.05$) which, however, doesn't change among various populations ($F_{(8, 8)} = 0.064$; $P > 0.05$).

Both positive and negative correlations were found between phenols and antioxidant activity with respect to different populations which is rather intriguing. Relationships of phenols with antioxidant activity were significant only in males of Shargol, Shilikchey and Andoo. The r-values were accordingly significant (Table 1). Additionally perfect positive correlations were found in males of Barootsog and Pashkum and in females of Mingee and Barootsog; while perfect negative correlation was specific to Akchamal males and Shilikchey females.

The amount of total phenols varied between 42.8 and 72.4 mg in plants of different populations irrespective of sexes. However, between sexes, the average quantity of phenols exceeds in females. On the contrary, Attrey et al. [16] found more phenols in males than females in 70% alcoholic extract. A trend similar to the present study i.e. Aqueous > Alcohol exists but the yield reported for male (both in aqueous and alcoholic) and female (aqueous only) plants is very high. At the population level, highest total phenolic content was found in plants of Barootsog followed by those of Kanoor, Sankoo and Shilikchey.

Results of DPPH radical scavenging decolourization assay reveal high degree of antioxidant capacity in 70 % methanolic extracts of all the populations studied. Recent investigations have shown that many phytochemicals e.g. phenols are more potent antioxidants than vitamin C and E due to their efficiency in scavenging peroxy radicals [17].

The antioxidant capacity of seabuckthorn is largely accrued to the plants because of the ample quantity of bioactive substances present in its leaves. This is also evident from a positive correlation between the different antioxidants and their antioxidant activity. However, correlation coefficients between different compounds and their antioxidant activity do not show a consistent trend across populations. Some coefficients are perfect, and some strong and others too less to suggest any relationship. Within and between sexes, similar trend was observed. Except for Barootsog and Shilikchey where the correlations within and between sexes were perfect or near perfect, all others vary. As of now, the relationship between the different antioxidants and their antioxidant activity in seabuckthorn is rather difficult to generalize or interpret by statistical methods. Goyal et al. [7] found a significant positive linear correlation between the phenolic content and DPPH activity in *Hippophae salicifolia*. Many other workers have also reported a direct significant relationship between the antioxidant activity and total phenolic content in several plants like tea, pineapple, vegetables, etc. [18, 19] although contributions of other secondary metabolites like volatile oils, proteins, vitamins, carbohydrates, tannins, etc. has not been ruled out completely [7, 20–22]. In buckwheat, two out of three methods i.e. Rancimat or beta-carotene bleaching showed no correlation between antioxidant activity and total phenolic content while DPPH method revealed a strong and significant positive correlation [23]. Similar results have been obtained in *Chenopodium quinoa*, *Amaranthus* spp., *Zizyphus jujube* and many other taxa [24–26].

Several reasons have been attributed to the ambivalent relationship between antioxidant activity and total phenolic content by Sun and Ho [23] in buckwheat. One, that total phenols exclude many antioxidants like ascorbic acid, carotenoids and tocopherols. Second, that antioxidant activity does not depend exclusively upon the concentration of antioxidants but also on their structure and various interactions between them. Third, different methods of estimation are likely to lead to different conclusions. Among these, first two seem plausible to the present results since only one method was used to determine antioxidant activity (DPPH free radical scavenging assay). Because total phenols were estimated instead of polyphenols, therefore these do not include antioxidants like flavonoids, ascorbic acid, carotenoids and tocopherol and antioxidant activity could be conferred by synergistic interaction between different substances of antioxidative potential. Howsoever, one cannot rule out the antagonism among

Table 1 Correlation between antioxidant activity and total phenolic content in males and females of different populations

S.No.	Populations	r-value	
		Male	Female
1	Kanoor	0.56	0.45
2	Mingee	0.03	1
3	Barootsog	1	1
4	Shargol	0.81*	0.46
5	Shilikchey	0.99**	−1
6	Sankoo	0.67	0.18
7	Andoo	0.98*	−0.38
8	Akchamal	−1	0.56
9	Pashkum	1	−0.50
10	Chanigund	−0.48	–

* $P < 0.01$, 0.05 (Significant)

** $P < 0.01$ (Significant)

some antioxidants as well. That is why probably some correlations were negative.

In conclusion, result of the present study suggests that methanolic extracts of shade dried leaves of different populations of Seabuckthorn is a potential source of antioxidants, which could be used as a natural preservative and in the development of nutraceutical formulations to overcome adverse affects of synthetics.

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