

## Sea buckthorn leaves – a better substitute for green tea

Flavonoids are a broad group of secondary metabolites known to be of importance in plant physiology and metabolism. They play a crucial role in sexual reproduction by promoting pollen-tube development, assisting in plant–microbe interactions and providing protection against damage caused by pathogen attack, wounding and UV light<sup>1,2</sup>. Due to their free-radical scavenging activity they are of novel interest. Knowledge of plants having substantial quantity of flavonoids is not only desirable but of prime importance as well, since awareness about the carcinogenic effects of the synthetic antioxidants has been increasing day by day.

*Hippophae* L., belonging to the family Elaeagnaceae, is one such bioresource. Species of this genus are commonly called as sea buckthorn, sandthorn and seaberry. Besides having high nutritional value, its leaves, fruits and seeds have been used in Amchi, Chinese, Mongolian and Tibetan traditional medicine for more than a thousand years<sup>3–5</sup>. This is due to the presence of antioxidants in various parts of the plant. Leaves of *Hippophae rhamnoides*, *H. salicifolia* and *H. tibetana* are a rich source of antioxidants like phenols, flavonoids, sterols, tannins, vitamin C, saponins, etc.<sup>6,7</sup>. The five major flavonoids reported in sea buckthorn leaves are catechin, rutin, quercetin, kaempferol and isorhamnetin<sup>7</sup>. On account of the presence of these health-promoting agents, the leaves are being endorsed on a commercial scale for production of extracts, herbal tea, animal feed, pharmaceuticals and cosmetics<sup>7–9</sup>.

India ranks fifth in the world in terms of area under sea buckthorn cover<sup>10</sup>. Despite growing in five states – three in the northwest (Himachal Pradesh, Uttarakhand, and Jammu and Kashmir (J&K)) and two in the North East (Sikkim and Arunachal Pradesh), India lags far behind in tapping this natural resource. With this background, work on sea buckthorn was initiated<sup>11</sup>.

Plants belonging to 10 populations from Kargil district, Ladakh (J&K) selected for the study exhibit enormous variability in several ecological and morphological traits and are, therefore, expected to vary at the biochemical level also. Plants are diverse in habit, ranging

from woody herbs to shrubs and even trees (Figure 1).

The leaves belonging to male and female plants of each population were collected in October 2010 and processed according to the method of Gupta and Kaul<sup>6</sup>. The fresh leaves collected in April 2011 and green tea (Himalaya) served the control. The aqueous extracts were prepared according to the maceration technique<sup>6</sup>. Flavonoid content was determined spectrophotometrically<sup>12</sup> using Rutin as standard and expressed as mg RE.

The flavonoids vary between 32.6 and 45.8 mg RE in shade-dried leaves of different plants. Overall, Pashkum males have highest quantity and Shargol females the lowest. In general, the amount is greater in males with average content only marginally different between the sexes. Results of two-way ANOVA substantiate this observation. Among populations, flavonoids do not vary significantly ( $F_{(8,8)} = 0.130$ ;  $P > 0.05$ ), but between sexes the differences are highly

significant ( $F_{(1,8)} = 8.12$ ;  $P < 0.05$ ). In six of the nine populations, flavonoid content in males exceeds that in females, whereas in the remaining three the reverse is true.

The two controls, namely green tea and fresh leaves of *H. rhamnoides* contain 34.4 and 17.2 mg RE flavonoids respectively. This indicates that shade-dried leaves of *H. rhamnoides* are qualitatively as good as green tea and can be promoted effectively on a commercial scale. Similarly, fresh leaves of sea buckthorn contain less amount of flavonoids compared to their shade-dried counterparts. This may be due to differences in the age of leaves and/or plants and time of collection, even though extract solvent and preparation, time and temperature of extraction, and collection of sample were largely uniform. Nevertheless, there is a possibility that the fresh leaves collected in April may have been juvenile. Second, by this time the plants are in reproductive phase and thereby invest major part



**Figure 1.** *a*, *Hippophae rhamnoides* growing in its native habitat; note the tree-like habit. *b*, Leaves belonging to plants of different populations of sea buckthorn. Note the extent of variation in their shape, size and colour; the ones on the left have abaxial surface up and those on the right adaxial.

of their resources to differentiation of sexes rather than for synthesis of secondary metabolites. Third, shade drying may have induced the synthesis of more flavonoids in response to stress, like in *Cosmos caudatus*. Leaves of this species registered a substantial increase in flavonoid content during boiling in water<sup>13</sup>. This increase was attributed to the endogenous biotransformation of precursor or intermediate into flavonoids; likely to be applicable in the present case also. Variation in quantity may also arise due to subtle changes in environmental factors and, selection and usage of analytical techniques<sup>13-15</sup>.

Highly significant between-sex differences are a consistent trend across populations. Reasons for these differences, either purely genetic or ecological in nature, are being worked out<sup>16-18</sup>.

However, no definite pattern seems to emerge at the populational level. Despite the differences being insignificant, work is worth pursuing if the elite genotypes are to be identified and isolated for further application. At the moment it is difficult to choose the best plant, since each has a different quantity of flavonoids. Populations with maximum content are from Pashkum, followed by Kanoor and Mingee. They are likely to have the potential to sustain more than one industry – pharma-, cosma- and nutraceutical nature.

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