Morphological and biochemical comparison of *Hippophae rhamnoides*, *Elaeagnus umbellata* and *Crataegus oxyacantha* intra- and interspecifically

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The hilly areas of Pakistan are very rich in medicinal plants. Among these, *Hippophae rhamnoides* ssp *turkestanica* L., *Elaeagnus umbellata* Thunb. and *Crataegus oxyacantha* L. are native plants of Northern Pakistan. Intra- and interspecific comparisons were made to investigate their morphological and biochemical composition using morpho-molecular techniques. The comparisons indicated large genetic and biochemical variation among the populations of each species and between the different species. The sodium dodecyl sulphate polycrylamide gel electrophoresis comparisons using total seed proteins of *Hippophae rhamnoides*, *Elaeagnus umbellata* and *Crataegus oxyacantha* indicated many common proteins and some variable proteins among the species. The biochemical comparison of Vitamin C, fatty oil and phytosterol content was also found to be variable among the species. Vitamin C and fatty oil contents were highest in *Hippophae rhamnoides* while phytosterol content was highest in *Crataegus oxyacantha* when compared with the other species tested in this study. Significant variation in morphological characters including plant height, number of branches per plant, number of thorns, plant canopy and berry weight was also observed in these three plant species.

**Introduction**

Hilly areas of Pakistan including Baluchistan, North Western Frontier Province, Azad Kashmir and northern Areas have a very rich and diverse flora due to their diverse climate, soil conditions and multiple ecological regions. The medicinal plant resources are not only abundant but are also rich in genetic diversity and biochemical composition. The herbs are extensively used locally for treating many diseases; however, their commercial exploitation is limited because of the lack of a scientific basis for their use (Hussain and Khaliq 1996). The farmers in the area are very poor and the cultivated land plots are either very small or unmanageable due to soil degradation and specific topography. Quite recently an initiative has been undertaken to characterise the medicinal plants for genetic and biochemical variation in order to improve medicinal plants for commercial purposes. Three plant species, Sea buckthorn (*Hippophae rhamnoides* ssp *turkestanica* L.), Autumn olive (*Elaeagnus umbellata* Thunb.) and Hawthorn (*Crataegus oxyacantha* L.), native to Azad Kashmir and northern areas of Pakistan, were investigated intra- and inter specifically for genetic and biochemical composition using morpho-molecular and biochemical techniques (Ahmad and Kamal 2002, Ahmad et al. 2003).

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**Hippophae rhamnoides**

*Hippophae rhamnoides* is a shrub or small tree of the genus *Hippophae*. The genus belongs to the family Elaeagnaceae that consists of six species and 10 sub-species, among which the most economically important one is *Hippophae rhamnoides* Linn., commonly known as Sea buckthorn (Rongsen 1992). The only sub-species found in the northern areas of Pakistan is *Hippophae rhamnoides* ssp. *turkestanica*, which is widespread in Central Asia and West Asia; that includes Afghanistan, Tajikistan, Turkmenistan, Uzbekistan, Kirghisistan, Xinjiang Province of China and Northern India. It is the only sub-species that can withstand the harsh bio-physical conditions characterised by arid, hot summers and cold winters (Rongsen 1992). *Hippophae rhamnoides* fruit contains 60 to 80% juice rich in sugar, organic acids, amino acids and vitamins. Vitamin C content is 200 to 1 500mg/100g, which is 5–100 times higher than any other known fruit or vegetable (Ahmad and Kamal 2002). The oil content ranges from 1.5–3.5% in fruit pulp and about 9.9–19.5% in seeds (Rongsen 1992). Oil from the juice and pulp is rich in palmitic and palmitoleic acids, while the oil from the seed contains the essential fatty acids, linoleic acid and linolenic acid. The oil from the seed and juice also contains Vitamin E and carotene (Bernath and Foldesi 1992, Ma and Cui 1989).
Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of total seed protein/allozymes revealed genetic diversity among the different individuals belonging to *Hippophae rhamnoides* ssp. *turkestanica* (Ahmad and Kamal 2002). Four woody species of the Elaeagnaceae family including *Hippophae rhamnoides*, Russian olive, buffalo berry and silverberry have been compared by the Random Amplification of Polymorphic DNA (RAPD) markers technique, which showed phenotypic diversity among these species (Chowdhury et al. 2000).

**Elaeagnus umbellata**

*Elaeagnus umbellata* is a rapid-growing large shrub that has been widely planted for shelterbelts, for food, cover for wildlife, for roadside reclamation, and for soil stabilisation. The plant’s tolerance to high pH soils, drought, pollutants and its ability for nitrogen fixation makes this shrub a very successful invasive species. The fruits are 1.25cm to 1.5cm in size, appearing light green in mid-summer and turning to red in the autumn (Dirr 1998). *Elaeagnus umbellata* fruit contains about 8.3% sugars, 4.5% protein and 1% ash. Its Vitamin C content is about 12mg/100g. The *Elaeagnus umbellata* berry is an excellent source of minerals, vitamins (A, C, E), flavonoids, essential fatty acids and other bioactive compounds (Graham 1964). Nutritionists have found that the red berries contain high concentrations of lycopene, the pigment that colours tomatoes red. Ripe tomatoes are currently being used as a source of lycopene (7.85mg/100g) but *Elaeagnus umbellata* contains very high amounts of lycopene, i.e. 53.96mg/100g (Deman 1980).

Lycopene is one of the most potent antioxidants and has been suggested to prevent carcinogenesis and atherogenesis by protecting critical biomolecules including lipids, low density lipoproteins (LDL), proteins and DNA (Agarwal and Rao 1998). The seeds and flowers of *Elaeagnus umbellata* are used for treating coughs and essential oil for pulmonary infections. The flowers are also used as an astringent and for the treatment of cardiac ailments (Chopra et al. 1986). *Elaeagnus umbellata* has been proved to be useful as a deterrent to heart disease and for cervix and gastrointestinal tract cancer (Dirr 1998).

**Crataegus oxyacantha**

*Crataegus*, a genus belonging to the rose family, is now known to have about 280 species found in northern temperate regions, including North America, Europe and northern Asia. The parts of *Crataegus oxyacantha* used as medicines are flowers, leaves and berries. In *Crataegus oxyacantha* these contain a variety of bioflavonoid-like complexes, oligomeric procyanidins (OPC), vitexin, quercetin and hyperoside (Hamon 1988). Since the 17th century, *Crataegus oxyacantha* has been used to treat various heart conditions and today is also believed to lower blood pressure (Occhiuto and Circosta 1986). Clinical trials have confirmed *Crataegus oxyacantha* to be beneficial for persons with Stage 2 (mild) congestive heart failure. *Crataegus oxyacantha* improves the blood and oxygen supply to the heart by dilating the coronary vessels (Weihmeyr and Ernst 1996).

All of the above-mentioned species are berry-producing plants, having similar morphological characters (plant height, size of berries, stem girth and size of thorns), and they were therefore tested to investigate their evolutionary relationships.

**Material and Methods**

**Plant collection**

Plant collections – of four populations each of *Hippophae rhamnoides*, *Elaeagnus umbellata* and *Crataegus oxyacantha* – were made in September 2002. These plants grow as wild plants in Northern Pakistan and Azad Kashmir. Four populations of *Hippophae rhamnoides* were collected from the district of Skardu, whereas *Elaeagnus umbellata* and *Crataegus oxyacantha* plants were collected from the district of Rawalakot Azad Kashmir.

**Morphological analysis**

Morphological characters – including plant height, number of main branches per plant, number of sub-branches per main branch of the plant, number of thorns per main branch of the plant, stem girth, plant canopy and 100 berries’ weight – were compared from five randomly-selected plants (in triplicate for each population) and mean values were taken for analysis using the ANOVA method of Steel and Torrie (1980).

**Molecular studies**

**Extraction of total proteins**

Total protein was extracted from 1g of seed of 12 plants, including 10 populations of *Hippophae rhamnoides* and one plant each of *Crataegus oxyacantha* and *Elaeagnus umbellata*, with 0.125M tris/borate, pH 8.9 by the method described by Laemmli (1970). Sodium dodecyl sulphate polyacrylamide gels contain 12 wells for electrophoresis; therefore 10 protein samples of *Hippophae rhamnoides* and one protein sample of each *Crataegus oxyacantha* and *Elaeagnus umbellata* were loaded for electrophoresis. All the obtained extracts were kept at 4°C for 24h and then centrifuged at 10 000rpm for 20min. The supernatants were used for electrophoresis.

**Determination of protein content**

Protein levels of cytosols were determined using a Spectronic 20-D (Milton Roy Company) spectrophotometer at 595nm, using Coomassie blue G-250 as a protein-binding dye (Bradford 1976). Bovine serum albumin was used as a protein standard.

**Protein electrophoresis**

Sodium dodecyl sulphate polyacrylamide gel electrophoresis was carried out according to the method described by Laemmli (1970), using 12.5% acrylamide concentration. Before electrophoresis each sample (protein extract) was heated at 100°C for 2min in 10mM Tris HCl buffer (pH 7) containing 2% (w/v) sodium dodecyl sulphate, 2% 2-mercaptoethanol and 5% (w/v) glycerol. A 30µl aliquot of the protein was loaded per well after adding one drop
of bromophenol blue and glycerine. A constant current of 250mA was applied for 3h. After the termination of this process the gel was removed from the apparatus and protein sub-units were stained with Coomassie blue R-250, using standard techniques. Finally, the gel was scanned using Jel-Pro-Analyzer version 3.3 (Media Cybermetics, 93–97). The Rf values of the protein bands were calculated as follows: Rf = distance moved by the protein band/distance moved by solvent front.

**Biochemical analysis**

Fruits/berries were collected from the same plants for biochemical analysis (i.e. four populations each of *Hippophae rhamnoides*, *Crataegus oxyacantha* and *Elaeagnus umbellata* were selected for biochemical analysis).

**Vitamin C content of fruits**

Vitamin C was determined using the phenol indophenol dye method described by the Association of Official Analytical Chemists (1984). Ten grams of the fresh berries/fruits were blended with metaphosphoric-acetic acid-extracting solution, which acts as a stabilising medium for Vitamin C. Five millimetres of the filtrate extract was then titrated against standard indophenol dye to the pink end point. The experiment was repeated three times.

**Lipid content of berries**

Oil from the berries/fruits of different plants was extracted according to standard methods described by the American Association of Cereal Chemists (1983). Fruits were dried in an oven at 105°C for 6–12h to constant weight. Ten grams of dried samples were extracted for oil in Soxhlet apparatus (30–40°C) for 6h using diethyl ether as solvent. The solvent was removed under vacuum and the residual oil was dried over anhydrous Na2SO4. The experiment was repeated three times. Analytical grade chemicals (Merck) were used for extraction of oil.

**Sterol determinations**

Sterol estimation was carried out by the Lieberman-Burchard method (Said et al. 1995). One gram samples of oil – obtained after Soxhlet extraction – were diluted with chloroform to 10ml. Three ml of diluted sample solutions were taken and their absorbance was determined after adding 2ml of chloroform and Lieberman-Burchard reagent, containing 0.5ml of sulphuric acid dissolved in 10ml of acetic anhydride. Lieberman-Burchard reagent reacts with the sterol to produce a characteristic green colour, the absorbance of which was determined on a Spectronic 20-D (Milton Roy Company) spectrophotometer at 640nm. Ten mg of standard cholesterol was dissolved in 10ml chloroform. Choles-5-en-3-β-ol was used as standard cholesterol, the minimum purity of which was 95%.

**Statistical analyses**

All the comparisons between the different parameters of morphological characters were done following ANOVA. The results of biochemical analysis were expressed as a mean of three determinations ±SD.

| Traits                  | SBT-1       | SBT-2       | SBT-3       | SBT-4       | HT-1       | HT-2       | HT-3       | HT-4       | AO-1       | AO-2       | AO-3       | AO-4       | Students' level of significance |
|-------------------------|-------------|-------------|-------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|-----------------------------|
| Plant height (cm)       | 77.2 ± 8.5  | 65.5 ± 5.2  | 93.9 ± 7.4  | 106.6 ± 6   | 125.3 ± 3  | 21.9 ± 2.2 | 30.9 ± 2.2 | 28.4 ± 1.9 | 90.2 ± 6.1 | 61.9 ± 4.8 | 46.9 ± 4.8 | 38.1 ± 3.2 | 43.9 ± 5.1  |
| Number of main branches | 3.6 ± 1.14  | 3 ± 0.707   | 2.4 ± 0.54  | 4.2 ± 0.99  | 3 ± 0.99   | 2.8 ± 0.7  | 2.5 ± 0.6  | 2.9 ± 0.8  | 6 ± 0.2    | 4 ± 0.3    | 3 ± 0.5    | 5.3 ± 0.1  | ** NS       |
| Number of sub-brances/note | 36.2 ± 1.9 | 37.2 ± 1.9  | 37.2 ± 1.9  | 37.2 ± 1.9  | 37.2 ± 1.9 | 37.2 ± 1.9 | 37.2 ± 1.9 | 37.2 ± 1.9 | 37.2 ± 1.9 | 37.2 ± 1.9 | 37.2 ± 1.9 | 37.2 ± 1.9 | ** NS       |
| Number of main thorns/branch | 304.9 ± 1.9 | 258.8 ± 5.63 | 5.6 ± 0.8  | 4.2 ± 4.99  | 13 ± 1.5   | 12 ± 1.9   | 7 ± 0.8    | 11 ± 0.3   | 20 ± 0.5   | 19 ± 0.04  | 16 ± 0.1   | 17.2 ± 0.4  | ** NS       |
| Stem girth (cm)         | 69.2 ± 6.46 | 64.6 ± 4.15 | 66.8 ± 4.98 | 66.8 ± 4.98 | 66.8 ± 4.98 | 66.8 ± 4.98 | 66.8 ± 4.98 | 66.8 ± 4.98 | 66.8 ± 4.98 | 66.8 ± 4.98 | 66.8 ± 4.98 | 66.8 ± 4.98 | ** NS       |
| Plant canopy (cm)       | 46.2 ± 1.64 | 36.2 ± 1.07 | 43.2 ± 0.4  | 43.2 ± 0.4  | 43.2 ± 0.4  | 43.2 ± 0.4  | 43.2 ± 0.4  | 43.2 ± 0.4  | 43.2 ± 0.4  | 43.2 ± 0.4  | 43.2 ± 0.4  | 43.2 ± 0.4  | ** NS       |
| 100 berries' weight     | 0.25 ± 0.01 | 0.03 ± 0.02 | 0.81 ± 0.06 | 0.49 ± 0.01 | 0.91 ± 0.01 | 0.91 ± 0.01 | 0.91 ± 0.01 | 0.91 ± 0.01 | 0.91 ± 0.01 | 0.91 ± 0.01 | 0.91 ± 0.01 | 0.91 ± 0.01 | ** NS       |

SBT = Sea buckthorn (*Hippophae rhamnoides*)

HT = Hawthorn (*Crataegus oxyacantha*)

AO = Autumn olive (*Elaeagnus umbellata*)

SL = level of significance

** = significant at 0.05 level of probability

*** = significant at 0.01 level of probability

NS = non-significant at 0.01 level of probability
Results and Discussions

Morphological analysis

Seven morphological parameters were investigated; results were analysed statistically and are compared in Table 1. The populations of *Hippophae rhamnoides*, *Crataegus oxyacantha* and *Elaeagnus umbellata* indicated intra- and interspecific variability (*P* ≤ 0.01). The plant height was the highest among the *Hippophae rhamnoides* populations while it was the lowest among the *Crataegus oxyacantha* populations. The average number of main branches per plant was highest in *Elaeagnus umbellata* (6), variable in *Hippophae rhamnoides* (2–5) and lowest in *Crataegus oxyacantha* (3). Similar variability in *Hippophae rhamnoides* plant height was recorded in earlier investigations (Sabir et al. 2003). Rouvi (1971) and Yao and Tigerstedt (1994) reported *Hippophae rhamnoides* as extremely variable in height, from a small bush less than 50cm to a tree more than 20m high, whereas *Elaeagnus umbellata* was found to be a large, spreading, spiny-branched shrub often obtaining 3.5 to 5.5m in height and 3.5 to 5.5m in width (Dirr 1998). The number of sub-branches per main branch of the plant varied significantly (*P* ≤ 0.01) among the populations of *Hippophae rhamnoides*, *Crataegus oxyacantha* and *Elaeagnus umbellata*, as shown in Table 1. *Hippophae rhamnoides* had the highest number of sub-branches per main branch (30–42) while *Crataegus oxyacantha* had the lowest number of sub-branches (13), with an intermediate number of sub-branches in *Elaeagnus umbellata* (20). Variation in plant canopy was also found to be highly significant among the populations of *Hippophae rhamnoides*, *Crataegus oxyacantha* and *Elaeagnus umbellata*. *Elaeagnus umbellata* had the highest plant canopy (145cm) followed by *Crataegus oxyacantha* (79.8cm), while in *Hippophae rhamnoides* it ranged from 35cm to 50cm. The number of thorns ranged from 50–67 per main branch among *Hippophae rhamnoides* populations. In *Crataegus oxyacantha* the number was also found to be in the same range (66). The overall comparison was highly significant, indicating large variability among the individual populations. However, in *Elaeagnus umbellata*, no thorns were recorded on the main branch but thorns were present on the lateral branches. When stem girth was compared, no significant variation was found among the populations compared, indicating the similar nature of these plant species. The comparison of 100 berries’ weight indicated significant variation between the species. The weight of 100 berries was the highest (3.961g) in *Crataegus oxyacantha*, intermediate (1.971g) in *Elaeagnus umbellata* and the lowest (0.56g) in *Hippophae rhamnoides*. The observation on *Hippophae rhamnoides* berries differs from earlier observations (Yao 1994), in which the weight varied from 4–60g/100 berries among different strains within natural populations, and exceeded 60g in some Russian cultivars.

Molecular studies

In order to observe variability at a genetic level, the gene products (seed proteins) were compared from 10 populations of *Hippophae rhamnoides* and one species each of *Crataegus oxyacantha* and *Elaeagnus umbellata*, by the fractionation of total seed proteins in sodium dodecyl sulphate polyacrylamide gel electrophoresis. In the absence of standard protein markers, comparisons among different genotypes were made on the basis of banding pattern or electrophoretic mobility within the gel. Photographs were taken by placing the measuring scale along one side of the gel, starting from the top of the wells where protein was loaded, in order to note the distance covered by each protein band. A total of 64 protein bands appeared in the gel with different Rf values showing the great genetic variability among the populations. The Rf values of different protein bands are shown in Table 2. Table 2 showed that only two bands are present on the lateral branches. When stem girth was no thorns were recorded on the main branch but thorns were present on the lateral branches. When stem girth was compared, no significant variation was found among the populations compared, indicating the similar nature of these plant species. The comparison of 100 berries’ weight indicated significant variation between the species. The weight of 100 berries was the highest (3.961g) in *Crataegus oxyacantha*, intermediate (1.971g) in *Elaeagnus umbellata* and the lowest (0.56g) in *Hippophae rhamnoides*. The observation on *Hippophae rhamnoides* berries differs from earlier observations (Yao 1994), in which the weight varied from 4–60g/100 berries among different strains within natural populations, and exceeded 60g in some Russian cultivars.

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Table 2: Rf values of different bands in sodium dodecyl sulphate polyacrylamide gel electrophoresis of *Hippophae rhamnoides*, *Crataegus oxyacantha* and *Elaeagnus umbellata*

| Number of protein bands | SBT-1 | SBT-2 | SBT-3 | SBT-4 | SBT-5 | SBT-6 | SBT-7 | SBT-8 | SBT-9 | SBT-10 | HT | AO |
|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|----|
| 1                       | 0.25  | 0.34  | 0.34  | 0.22  | 0.34  | 0.34  | 0.34  | 0.34  | 0.34  | 0.34  | 0.34| 0.25|
| 2                       | 0.34  | 0.43  | 0.43  | 0.34  | 0.43  | 0.43  | 0.43  | 0.43  | 0.43  | 0.43  | 0.43| 0.34|
| 3                       | 0.43  | 0.51  | 0.51  | 0.43  | 0.51  | 0.51  | 0.51  | 0.51  | 0.51  | 0.51  | 0.51| 0.43|
| 4                       | 0.60  | 0.68  | 0.68  | 0.51  | 0.60  | 0.60  | 0.60  | 0.60  | 0.60  | 0.60  | 0.60| 0.51|
| 5                       | 0.68  | 0.77  | 0.68  | 0.65  | 0.68  | 0.65  | 0.65  | 0.65  | 0.65  | 0.65  | 0.65| 0.77|
| 6                       | 0.74  | 0.77  | 0.82  | 0.68  | 0.74  | 0.68  | 0.68  | 0.66  | 0.66  | 0.66  | 0.82| 0.77|
| 7                       |       |       |       |       |       |       |       |       |       | 0.74  |    |    |
| 8                       |       |       |       |       |       |       |       |       |       | 0.77  |    |    |

SBT = Sea buckthorn (*Hippophae rhamnoides*)
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Baltistan and reported different $R_f$ values for protein bands showing different seed proteins. Ahmed and Kamal (2002) reported the maximum $R_f$ value of proteins to be 0.98, whereas the maximum value in the present investigation was lower ($R_f = 0.86$). Isozyme analysis has also shown large genetic diversity at the species, sub-species, and population level in *Hippophae rhamnoides* (Yao and Tigerstedt 1993).

The *Crataegus oxyacantha* and *Elaeagnus umbellata* seed proteins were also loaded along with the *Hippophae rhamnoides* in the sodium dodecyl sulphate polyacrylamide gel electrophoresis, in order to make a biochemical (evolutionary) comparison between these berry-producing plants and *Hippophae rhamnoides*. Table 2 shows the $R_f$ values of protein bands extracted from seeds of *Crataegus oxyacantha* and *Elaeagnus umbellata*. Both *Crataegus oxyacantha* and *Elaeagnus umbellata* showed two bands with $R_f$ values of 0.34 and 0.43, which were common with the $R_f$ values of *Hippophae rhamnoides* populations. *Hippophae rhamnoides* and *Elaeagnus umbellata* belong to the same family (Elaeagnaceae) and the resemblance of their banding pattern could be explained by this taxonomic relationship. However, the resemblance in the banding pattern of *Crataegus oxyacantha* with *Hippophae rhamnoides* and *Elaeagnus umbellata* was not expected. This does not necessarily mean that the same proteins exist in their seeds. It may mean that different proteins with similar charges have produced similar banding patterns.

**Biochemical analysis**

When the populations of *Hippophae rhamnoides* were compared biochemically on the basis of Vitamin C, fatty oils and phytosterol contents, a wide range of variation was observed among the populations (Table 3). The average concentrations of Vitamin C were found to be 196.25mg/100g in *Hippophae rhamnoides*, 109.62mg/100g in *Crataegus oxyacantha* and 11.47mg/100g in *Elaeagnus umbellata* (as shown in Table 4). The concentration of Vitamin C in *Hippophae rhamnoides* was highly variable among the populations as reported earlier (Karhu et al. 1999) but the concentration in our investigation were low. Rongsen (1992) reported Vitamin C concentrations from 200–1500mg/100g in *Hippophae rhamnoides* fruits. The lower concentration of Vitamin C in the present investigation may be due to the specific geographic nature of the area, where a short reproductive season prevails (Yao and Tigerstedt 1995).

The average concentrations of oil in the pulp were found to be 3.85g/100g in *Hippophae rhamnoides*, 0.34g/100g in *Crataegus oxyacantha*, and 1.62g/100g in *Elaeagnus umbellata* (Table 4). The range of fatty oil content – from 3.4 to 4.5% – found in the present investigation was the highest reported so far. Rongsen (1992) reported oil contents from 1.5–3.5% in fruit pulp. The higher concentrations of oil in ssp. *turkestanica* may be very important in regard to its use in medicines.

Phytosterols are plant sterols with structures related to cholesterol, which are capable of lowering plasma cholesterol in humans. Elevated blood cholesterol is one of the well-established risk factors for coronary heart disease.
Table 4: Average concentration of Vitamin C, oil and phytosterol in *Hippophae rhamnoides*, *Crataegus oxyacantha* and *Elaeagnus umbellata* populations

| Biochemical characters | SBT         | HT             | AO           |
|------------------------|-------------|----------------|--------------|
| Vitamin C (mg/100g)    | 196.25 ± 0.37 | 109.62 ± 0.24  | 11.47 ± 0.125 |
| Oil (g/100g)           | 3.85 ± 0.6   | 0.34 ± 0.17    | 1.62 ± 1.45  |
| Sterol (mg/g)          | 14.22 ± 0.08 | 115.2 ± 1.42   | 1.45 ± 0.12  |

SBT = Sea buckthorn (*Hippophae rhamnoides*)
HT = Hawthorn (*Crataegus oxyacantha*)
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(Thurnham 1999). Phytosterols are the major constituents of the unsaponifiable fraction of *Hippophae rhamnoides* oils. The major phytosterol in *Hippophae rhamnoides* oil is β-sitosterol, with 5-avenasterol second in quantitative importance. Other phytosterols are present in relatively minor quantities. The total concentration of phytosterol is quite high in *Hippophae rhamnoides* and may exceed that of soybean oil by 4–20 times. The average concentrations of sterol were found to be 14.2mg/g in *Hippophae rhamnoides*, 115.2mg/g in *Crataegus oxyacantha* and 1.45mg/g in *Elaeagnus umbellata* (Table 4).

Conclusions

This investigation is based on morphological, molecular and biochemical characterisation of berries/fruits of *Hippophae rhamnoides*, *Crataegus oxyacantha* and *Elaeagnus umbellata*. Intra- and interspecific variability among the plants will help to breed better varieties, using conventional methods of breeding. The high concentration of oil found in *Hippophae rhamnoides* and *Elaeagnus umbellata* will have commercial importance and will help the local community in marketing their farm produce. Due to the high content of Vitamin C these berries can be used in making fruit juices and beverages. As *Hippophae rhamnoides* and *Crataegus oxyacantha* oil are concentrated sources of phytosterols, which compete with cholesterol in terms of absorption into in the body, use of oil from these plants might be helpful in preventing heart diseases.

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