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BIOCHEMICAL AND NUTRITIONAL EVALUATIONS OF SEA BUCKTHORN (*HYPPOPHAE RHAMNOIDES* L. SPP. *TURKESTANICA*) FROM DIFFERENT LOCATIONS OF PAKISTAN

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Abstract

Sea buckthorn (*Hyppophae rhamnoides* L.) is a hardy shrub belonging to the family Elaeagnaceae. It bears yellow to orange berries, which have been used for centuries in Europe and Asia for beneficial purposes. It has attracted considerable attraction in the world mainly for its nutritional and medicinal value. Currently it has been cultivated in the various parts of the world. The abundant naturally growing Sea buckthorn ecotypes are of diverse genetic make up with diverse biochemical and nutritional expressions. To study the biochemical and nutritional values in 10 selected Sea buckthorn ecotypes from variable locations in northern areas, a research was conducted at the Faculty of Agriculture Rawalakot Azad Kashmir Pakistan. In the present study ascorbic acid was determined by indo phenol dye method and oil contents from the berries were analyzed by using diethyl ether as solvent in Soxhlet apparatus for 6 hours at 30-40^{o}C. The minerals iron and phosphorus were estimated using spectrophotometer. Comparison on the basis of biochemical analysis provided data that vitamin C had the range (191-295.6mg/100g). The high content of Vitamin C reflects the significance of Sea buckthorn in northern areas. Results have shown the values of oil content in the range of 18.2-43.5% and 7.03-12.86% in Sea buckthorn berry pulp and seeds respectively. The huge difference in the range of oil content is due to altitude variations and genetics make of Sea buckthorn ecotypes as well. The minerals, iron and phosphates were in the range of 2.6-7 and 34.3-466.6 mg/100g respectively which again reflects the variations among the ecotypes. The study provides the evidence of the presence of genetically diverse ecotypes of Sea buckthorn in northern areas with tremendous biochemical and nutritional values.

Introduction

Mountain areas show distinct signs of un-sustainability, decreasing soil fertility and a high degree of instability. There is a trend of abandoning agricultural land and this sharply contrasts with the decrease of land/man ratio in cultivated areas. The reduced flexibility and diversity of agriculture (i.e., complex of land based activities) and resource generative processes, that helped to sustain natural resource use systems in a low demand situation, are other visible manifestations of the emerging scenario in most parts of mountain regions (Jodha *et al.*, 1992).

Therefore this is the need of time to explore the hidden potentials of northern areas and Azad Kashmir so that the conventional agricultural system can be diversified and more economic opportunities can be discovered (Ahmad & Shah, 1999). Sea buckthorn (*Hyppophae rhamonides* L.), a berry bearing hardy bush of the family Elaegnaceae, is an excellent specie that has great potential for satisfying the biochemical and nutritional requirements. It is resistant to cold, drought, salt and alkali. The vigorous vegetative reproduction and the strong complex root system with nitrogen-fixing nodules make sea buckthorn an optimal pioneer plant in soil and water conservation and reforestation for eroded areas (Yang & Kallio, 2002).

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Studies have shown that the fruit of Sea buckthorn is a storehouse of Vitamins and important bioactive substances (Xurong *et al.,* 2001). The vitamin C content is 5 to 100 times higher than any other fruit or vegetable known. The pulp also contains high quality oil which is regarded to be very important for its medicinal value (Rongsen, 1992; Lebeda, 2004). The highest content of seed oil is 17.85% in *Hyppophae rhamnoides* sub spp. *tibetana* and lowest one is 7.80% in *Hyppophae neurocarpa* (Lebeda, 2004). It was concluded that oil content of seeds in other genotypes were in the range of 9.50 to 23.36%. Oil content of Sea buckthorn from pulp of fresh berries form different origins was not higher than that of seeds (Rongsen, 2004). The highest content of oil in pulp was 18.75% in *Hyppophae neurocarpa* and the lowest one is 2.46% in spp. *Yunaanesis*. The Vitamin C in *turkestanice* was analyzed and found to be up to 128.5-130mg/100g (Rongsen, 2004). Maximum vitamin C content was found in spp. *Salcifolia* (1784.7 mg/100g). The 100 berry weight, berry color and 100 seeds weight was also estimated and it as found that maximum 100 berry weight was of spp. *mongolica* (42.0 g), *Turkestanica* had 12.0-16.9 g) for 100 berry weight (Rongsen, 2004). The mineral contents of Sea buckthorn make the shrub most important. There is an ample quantity of quality vitamins in Sea buckthorn fruit and leaves. Iron content of Sea buckthorn genotypes of different origins was variables in the range of 22mg/Kg to 282 mg/kg (Yang & Kallio, 2001). Reported studies revealed a range of Vitamin C concentrations of 29-293.3mg% (mg Vitamin C/100g berries in Finish bushes) (Rousi & Aulin, 1977; Yao, 1994). Iron content was in a range of 40-300 mg in Russain cultivars (Plekhanove, 1988). On the other hand, a range of 200-250mg% of vitamin C has been found on Chinese bushes (Yao, 1994; Zhang & Songi 1992). Tang, *et al.,* (2001) reported that Berry size was measured in Sea buckthorn genotypes to study the biochemical variations (Zhang *et al.,* 1989; Xurong *et al.,* 2001). Sea buckthorn is a native plant species of Northern Pakistan, with about 3000 hectares of wild forests annually producing 1200-2250 tons of Sea buckthorn fruit (Sabir e*t al.,* 2003). The only sub spp. found in Northern Areas is (*Hyppophae rhamonides* spp. *turkestanice*) which is widely distributed in central and western Asia including Northern India. Northern Areas of previous state of Jammu Kashmir are the center of origin of spp. *Turkestanice* (Ahmad & Kamal, 2003).

Present study was designed in view of the possible biochemical/ nutritional role and properties of Sea buckthorn in the world and underutilization of its tremendous potential in Pakistan and Azad Kashmir. This study might be helpful to explore the potentials of Sea buckthorn in northern areas and to introduce Sea buckthorn as a vital tool to initiate the economic activity for its commercial exploitation on sustainable and international basis.

Materials and Methods

Berries collection: Sea buckthorn (*Hyppophae rhamonides* spp. *turkestanice*) berries from Gilgit, Nagar Hunza, Murtazaabad, Hussain Abad, Shigar and Khaploo areas of Northern areas of Pakistan were collected in the 1st week of October when they were fully matured. These berries were kept in plastic pots and transported to University College of Agriculture, Rawalakot, Azad Kashmir and were subjected to deep freezer on –20°C.

Biochemical analysis

Oil content of berries: Oil content from the berries of different populations of Sea buckthorn were extracted in Soxhelt apparatus (Anon., 1983). Samples were oven dried at 105ºCfor 6-12 hours and 10g of dried samples was used for extraction of oil in Soxhelt apparatus (30-40ºC) for 6 hour using diethyl ether as solvent. The solvent was removed under vacuum and residual oil dried over anhydrous Na_2So_4. Three replications were conducted for each determination. Analytical grade chemicals were used for extraction of the oil.

Vitamin C: Determination of ascorbic acid was carried out under standard conditions using Phenol indophenol Dye method (Anon., 1984). Thawed berries (10g) were blended with metaphosphoric acid extracting solution to homogenous slurry and 5 ml of the filtrate extract was then titrated with standard indophenol to pink end point. Three replications were taken for each determination. All the reagents were of analytical grade.

Estimation of mineral elements

Preparation of acid digest: Weighed amount of powder sample with a known quantity (0.5 to 1.0g) was used. After transferring it to a digestion tube 5 ml of concentrated HNO_3 was added with vigorous shaking. The tube were kept in Treater Digester for half an hour at 70ºC then the temperature was raised to 140ºC so that nitrous acid fumes come out. Sample tubes were cooled and then 3 ml of HNO_3:$HClO_4$ (1:1) mixture was added in the medium. Again the tubes were re-heated upto maximum temperature of 200^0C so that white dense fumes of perchloric acid ($HClO_4$) disappeared. Tubes were allowed to become cool and all content was transferred to a 50 ml volumetric flask. Volume was made upto the desired mark with deionized water and the digest was kept in a refrigerator and used for mineral determination.

Determination of iron: Iron contents of berries were determind by treatment of sample with Potassium thiocynate. Potassium thiocyanate 5 ml (1.5 M) and 2 M HCl were added to 3 ml mineral extract. A red complex of iron (FeSCN) was formed which was estimated at 477 nm Spectronic 20-D (Milton Roy Company). The results were drawn by plotting a calibration curve using different concentrations of standard iron against absorbance.

Determination of phosphorus: Phosphorus was determined by the method as described earlier (Anon., 1984) by treating with 0.25% KH_2 PO_4 ammonium vandate and 5% ammonium molybdate. The inorganic phosphorus reacts with ammonium molybdate to produce a yellow color complex of molybdenum which was estimated at 470nm on a spectrophotometer. The results were drawn by plotting a calibration using different concentrations of standard phosphorus against absorbance.

Statistical analysis: The results of the biochemical analysis are presented as a mean of three determinations ± SD. and analysis of variance (ANOVA).

Results and Discussion

Biochemical analysis: The ecotypes differed significantly and expressed minimum content of vitamin C as 246.3 mg/100g and the maximum 295.6 mg/100g. The ecotypes expressed variations for vitamin C (Table 1). The oil content from pulp of the ecotypes also varied. When ranged form 18.22 to 43.5%. The ranking order regarding oil content was found to be E_2, E_{10}, E_8, E_5, E_9, E_1, E_6 and E_7 respectively. Iron content among different ecotypes also showed variations. and ranged from 2.6 mg/100 g as minimum to 7 mg/100g as maximum. When the ecotypes were compared on the basis of mineral phosphorus the maximum phosphorus was found in ecotype E_9 with 466mg/100g. The minimum value for phosphorus was found to be in E_4 with 343.3mg100g^{-1}. The phosphorus content was variable in different ecotypes.

The mean values for the physical characteristics of the berries for 10 ecotypes of Sea buckthorn were compared in (Table 1).

There was significant variations among the traits. It is depicted that the ecotypes of Sea buckthorn form different areas of Northern Pakistan vary greatly among themselves The weight of 100 berries of 10 ecotypes has been observed in the range of 2.6 to 15.16g/100 berries.

The vitamin C content in Sea buckthorn ecotypes was found in the range of 170 mg/ 100g to 250 mg/100g by Sabir *et al.*, (2003) and in the range of 92 mg/100g to 461 mg/100 g by Xurong *et al.*, (2001). These results are in agreement with our studies with Vitamin C range from 188mg/100g to 290 mg/100 (Table 1). The variations among the ecotypes for Vitamin C content is showing the biological diversity in Sea buckthorn ecotypes in Northern Areas. It has been reported that spp. *Turekestanica* is distributed in Central and west Asia including Afghanistan, Tajikistan, Turkmenistan, Uzbekistan, Kirghisistan, Xinjang Province of China, Northern India and Pakistan. (Rongsen, 1992; Sabir *et al.*, 2003). These areas are geographically connected with land but are isolated by high mountain peaks, rivers and variable climatic conditions. During its spread and establishment, many changes took place in the genetic make of the sub spp *Turkestanica* due to natural selection and adaptation (Ahmad & Kamal, 2003). Northern Areas of Pakistan also vary in topography and microclimate; therefore the variation in different traits among the populations could be due to the some physiological factors. The oil content of Sea buckthorn berries of different areas also varied in different ecotypes. The highest concentration of oil content was detected in E4 with 43% oil in pulp while the minimum oil was observed in the ecotype E2 with 18.4%. The oil content of remaining 8 ecotypes ranged form 18.55 to 35.5%. The oil content in seeds of Sea buckthorn varied also. The oil content ranged from 7.03 to 12.86%. Sea buckthorn seed oil was reported as 5.3 to 15.7% (Xurong, 2001) .Our observations are in consistent with the reported literature.

Our studies on oil content in pulp of Sea buckthorn findings do not agree with the observations of (Rongsen, 1992) who reported 1-4.5% oil in pulp. The investigations also did not match with the reports of Sabir *et al.*, (2003) who observed 1-1.4% oil in Sea buckthorn populations collected form Skardu and Kahploo areas of Northern Pakistan. The difference of results of present studies and previous is due to the fact that the berries in present investigation were collected from more diverse areas of different altitudes and agro climatic conditions. The oil content was reported as 2% and 8% for pulp and seeds respectively, Bernath *et al.*, (1990) which also does not match with our studies. The huge oil content in Sea buckthorn berries of Northern Areas could be exploited for commercial purposes as the oil of Sea buckthorn has rich medicinal and edible value. Sea buckthorn ecotypes were also compared on the basis of Iron and Phosphorus content. The Phosphorus was estimated in the range 343.3 mg /100g to 466.6 mg /100g. The ecotypes presented variation in Iron content from the berries. This variation is again due to the diverse berries collected from diverse areas.

Similarly the phosphorus content was also estimated in the range of 7.5% to 12.86%. Earlier studies reported the oil content in the seed of Sea buckthorn spp. *turkestanica* .

The present investigation suggests that Sea buckthorn of different locations of Pakistan and specifically Northern Areas is of diverse nature in biochemical and nutritional constituents due to diverse genetic make up and establishment on variable altitudes. This diversity in Sea buckthorn germplasm is a very good source for plant breeders to evolve more productive and commercial varieties of Sea buckthorn which can bring a positive economic change with its commercial cultivation and management on large scale. The study also provided an insight about the importance of Sea buckthorn ecotypes in Northern Areas of Pakistan in relation to its medicinal, biochemical and nutritional aspects and indeed, in the line with these observations, more issues will be addressed in future studies.

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