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The Profile of Bioactive Compounds in Seabuckthorn: Berries and Seed oil

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ABSTRACT: Seabuckthorn *(Hippophae rhamnoides*) is a deciduous shrub with yellow to orange small berries. It is a thorny shrub native to Eastern Europe and Asia. It is a well recognized source of traditional herbal medicines and modern vitamins and nutrients. Study on seabuckthorn (SBT) pulp and seed oil was carried out to evaluate its phytoconstituents. The fruit pulp was dried, powdered and was further used for the preparation of various extracts (100% methanolic, 70% ethanolic, 50% methanolic and 100% aqueous). Results showed that oil contains more vitamin E content (233.907±0.245mg/100g respectively) as compared to pulp. Among various pulp extracts and seed oil, the total phenolic, flavonoid and lycopene content was found to be higher in 70% ethanolic extract (14.408±0.16mg of gallic acid/gm, 6.794±0.30 mg of rutin/gm and 29.72±0.53 mg/100g, respectively). Whereas, vitamin C content was found to be highest in aqueous extract as compared to all other extracts and seed oil. β-carotene content in 100% methanolic , 70% ethanolic and 50% methanolic extracts of seabuckthorn pulp was found to be 359.046±2.07, 358.45±2.68, 355.143±2.014 µg/gm respectively, whereas in aqueous extract (100%) it was 351.56±1.73 µg/gm. In oil its content was 303.536±2.30 µg/gm.

Keywords: Seabuckthorn, Pulp, seed oil, total phenol, vitamins.

I. INTRODUCTION

Seabuckthorn (*Hippophae rhamnoides* L. Elaeagnaceae), a unique and valuable plant has recently gained worldwide attention, mainly for its medicinal and nutritional potential. It is a thorny nitrogen-fixing deciduous shrub of cold arid region native to Europe and Asia. It is currently domesticated in several parts of the world due to its nutritional and medicinal properties (Li, 2003). It has been used extensively for treatment of asthma, skin diseases, gastric ulcers and lung disorders. Current research is now beginning to understand and support the traditional uses of SBT. Various pharmacological effects of SBT have been recently reported, *viz*., antioxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective and tissue repair (Gao *et al.*, 2003; Gupta *et al.*, 2005; Basu *et al.*, 2007; Chawla *et al.*, 2007; Saggu *et al.*, 2007; Upadhyay *et al.*, 2009, 2011).

All parts of SBT plant are considered to possess large number of bioactive substances like vitamins (A, C, E, K, riboflavin, folic acid), carotenoids (α, β, δ-carotene, lycopene), phytosterols (ergosterol, stigmasterol, lansterol, amyrins), organic acids (malic acid, oxalic acid), polyunsaturated fatty acids and some essential amino acids (Yang *et al.*, 2001; Pintea *et al.*, 2005, Christaki, 2012). In the present study the main objective was to estimate the various bioactive substances in seabuckthorn pulp and oil of Keylong, distt. Lahaul and Spiti (H.P.) origin so as to get a clear concept over the compositional importance for the future nutritional research.

II. MATERIALS AND METHODS

A. Sample preparation

Seabuckthorn (*Hippophae rhamnoides*) berries and seeds were procured from Keylong, Distt. Lahaul Spiti, Himachal Pradesh (India) in the first week of October when the plant grows wildly under natural conditions. The berries were kept in plastic pots and transported to CSK HPKV, Palampur. The fresh fruits were then cleaned and pound to pieces with squeezer. The extract was filtered and the filtrate was stored at -20^{o} C in a refrigerator. Pulp was dried and powdered.

For analysis SBT pulp powder was extracted in different solvents **{**100% methanolic, 70% ethanolic (ETE), 50% methanolic and 100% aqueous (AQE)}. The extraction procedure involved adding of solvent to 25g of ground sample, keeping it for 24hrs with intermittent shaking. The extract so obtained was filtered, dried in Rota vapor (Buchi, Switzerland) and lyophilized (Alpha1-2 LD$_{plus}$, Martin Christ, Germany).

The lyophilized sample was used for *in vitro* studies. Whereas, oil from seeds was extracted by local oil mill. The oil obtained was used as such. *B. Chemoprofiling*

Total phenol: Total phenolic contents were determined by using procedure of the Folin-Ciocalteau method (FCR) of Gulcin, 2002. The extract (50µl) was added to FCR (0.5ml, 1N). Sodium carbonate solution (2.5ml, 20%) was then added to the mixture. After 40 min of reaction at ambient temperature the absorbance of the mixture was measured at 725nm against a blank of distilled water using UV-1800, Shimadzu spectrophotometer. Gallic acid was used as a standard and results expressed as gallic acid equivalent (GAE/gm). All analysis was performed in triplicate.

Total flavonoids: Total flavonoids were estimated spectrophotometrically by the method of Zhishen *et al.* 1999 with slight modification. To 1.0ml of extract (1mg/ml), 4.0ml of distilled water and 0.3 ml of 5% sodium nitrite was added. After 5 min, 0.3ml of aluminium chloride and 2.0ml of sodium hydroxide (1M NaOH) were added to the mixture and total volume was made up to 10.0 ml with distilled water. The solution was mixed thoroughly and absorbance was measured against reagent blank at 510nm. Rutin was used as a reference compound.

β-Carotenoids: β-carotenoids were estimated by the method of Gowenlock *et al.* 1988.To 1.0 ml aliquot of sample, 1.0 ml of ethanol and 3.0 ml of petroleum ether were added. The mixture was shaken vigorously and then centrifuged at 2500rpm for 15min. The absorbance was read at 450nm against petroleum ether as blank. Amount of β-carotenoid in extracts was caliberted using beta carotene standard curve.

D. Vitamin E

Vitamin E was estimated by the method given by Kayden *et al.* 1973 with slight modification. To the extract (1.8 ml), equal amount of absolute alcohol was added and mixed thoroughly. To standard (1.8 ml), equal amount of distilled water and to blank (1.8 ml) equal amount of absolute alcohol was mixed. To this 1.8 ml of purified xylene was added and vortex for 2 min followed by centrifugation at 800 g for 10 min. Xylene extract (0.4 ml)was carefully pipetted out and to this equal amount of bathophenanthroline reagent was added. Then 0.4 ml of ferric chloride was added followed by 0.4 ml of o-phosphoric acid and 1.0 ml of alcohol. Blank was also run sidewise. The contents were mixed thoroughly and absorbance was measured at 536 nm. Alpha-tocopherol was used as a reference material.

E. Vitamin C

Vitamin C was estimated by 2, 4 dinitro phenylhydrazine (DNPH) method by Baker and Frank 1968. To 2.0 ml of extract 6.0 ml of 5% TCA was added. The solution was mixed thoroughly and centrifuged at 2000 rpm for 10min. 2.0 ml of supernatant was pipetted in to each sample tube and 2.0 ml of ascorbate standard was taken in separate tubes .Then one drop of indophenol reagent was added and mixed well followed by addition of 0.5 ml of DT (2 gm DNPH + 1 gm thiourea in 9N H_2SO_4) mix. All the test tubes were incubated for 1hr in a water bath at 60ºC and cooled in ice water. After cooling 2.5 ml of 85% H_2SO_4 was added to tubes and mixed well .The optical density was recorded at 505 nm against distilled water. The vitamin C content in SBT pulp and seed oil was extrapolated from standard curve using ascorbic acid as a standard.

F. Lycopene

Lycopene content in Seabuckthorn byproducts were measured by the method of Ranganna 1976. Samples were extracted repeatedly with acetone using pestle and mortar until the residue was colorless. The acetone extracts were pooled and transferred to a separating funnel containing about 20ml petroleum ether and then 20ml of 5% sodium sulphate solution was added and shaken in the separating funnel. Twenty ml more petroleum ether was added to the separating funnel for clear separation of two layers. Two phases were separated and the lower aqueous phase was re-extracted with additional 20ml petroleum ether until the aqueous phase was colorless. The petroleum ether extracts were pooled little more distilled water. The washed petroleum ether extract containing lycopene was poured into a brown bottle containing about 10 g anhydrous sodium sulphate and kept aside for 30 min. The petroleum ether extract transferred into 100 ml volumetric flask through funnel containing cotton wool, the sodium sulphate slurry was then washed with petroleum ether until color less. The volume was made up to 100 ml with petroleum ether and absorbance read in a spectrophotometer at 503 nm using petroleum ether as blank.

Absorbance (1 unit) = 3.1206µg lycopene/ml

$$\text{mg lycopene in 100 g sample} = \frac{31.206 \times \text{Absorbance}}{\text{Wt. of Sample (g)}}$$

III. RESULTS AND DISCUSSION

The colour of fruit pulp was orange whereas that of seeds and seeds oil was brown and golden yellow respectively. The per cent recovery of different pulp extracts ranged between 13 to 18 per cent in different solvents. Results showed that ethanolic extract of pulp have more per cent recovery of phytoconstituents as compared to methanolic and aqueous extracts (Table 1).

Table 1. Colour, per cent recovery of the different pulp extracts.

Sample	Colour	% recovery
100% Methanolic	Reddish brown	16
70% Ethanolic	Reddish brown	18
50% Methanolic	Golden brown	14
100% Aqueous	Brown	13

Chemoprofiling

The total phenolic, flavonoid and lycopene contents were found to be more in 70% ETE (14.408±0.16 mg of gallic acid/gm, 6.794±0.30mg of rutin/gm and 29.72±0.53mg/100g, respectively) than in other extracts (*i.e.* 50% and 100% ME, 100% AQE) as well as seed oil (Fig. 1). Phenolic acids have been associated with color, sensory qualities, and nutritional and antioxidant properties of foods (Mega 1978). Phenolics behave as antioxidants, due to the reactivity of the phenol moiety (Shahidi and Wanasundara 1992). The phenolic acids composition in Seabuckthorn berries was reported to be in the ranged from 3570±282 to 4439 ± 405 mg/kg on a dry weight basis (Zadernowski *et al.* 2005). Seabuckthorn juice has flavonoid content of 1182 mg/L. Isorhamnetin-rutinoside (355 mg/L) and quercetin-glycoside (35 mg/L) have been identified as the main flavonoids present. Quercetin is the main flavonoid in European sea buckthorn fruit (Hakkinen *et al.* 1999). Flavonoids contained in all parts of Hippophae are mainly responsible for the antioxidant and anti-cancer effects. They protect cells from oxidative damage, consequent genetic mutation and ultimately cancer (Suryakumar and Gupta 2011, Gao *et al.* 2000, Zeb 2006).

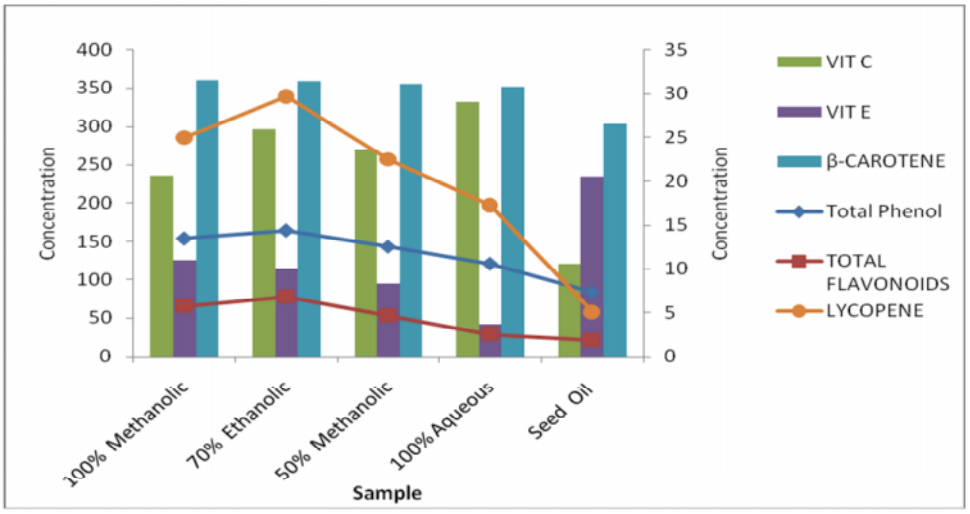

Fig.1. Total phenols (mg of GAE/gm), total flavonoids ((mg rutin equi./gm), β-crotenoids (µg/g), vitamin C (mg/100g), vitamin E(mg/100g) and lycopene content (mg /100 gm) in different SBT Pulp extracts and seed oil.

The vitamin C content was higher in 100% AQE (331.8±0.18mg/100g) as compared to other extracts and seed oil. In other extracts (*i.e.* 100% ME,70% ETE and 50% ME) it was found to be 234.4±0.44, 295.8±0.68, 269.6±0.46 mg/100g respectively, whereas, in seed oil its amount was found to be 120.20±0.19mg/100g (Fig.1).

Vitamin E content present in seed oil (233.907±0.245 mg/100g) was higher as compared to various pulp extracts. β-carotene content of 100% ME and 70% ETE was almost at par (359.046±2.07 and 358.45±2.68 µg/gm, respectively) and higher as compared to other extracts as shown in Fig.1. Yao and Tigerstedt (1992) reported that vitamin C concentration in berries varies from 360mg/100g of European subspecies *rhamnoides* to 2500mg/100g of berries for the Chinese sub species *sinensis* (Zhao *et al.* 1991). Vitamin E concentration in berries was reported to be 160mg/100g of berries (Zhang *et al.*, 1989).

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