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Separation of bioactives from seabuckthorn seeds by supercritical carbon dioxide extraction methodology through solubility parameter approach

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ABSTRACT

Seabuckthorn (*Hippophae rhamnoids* L.) seed oil having high nutraceutical, cosmeceutical and therapeutic activity has been extracted from dried seabuckthorn (SBT) seed powder using supercritical carbon dioxide (SC-CO_2). The solubility parameter of SBT actives (tocopherols, lycopene and β-carotene), CO_2, and entrainer solvents was calculated and validated with experimental results. The free radical scavenging activity of the extract was evaluated in terms of DPPH· assay. Theoretically, pressure and temperature had significant effect on extraction of SBT actives. A maximum recovery of 77.2% tocopherol, 75.5% carotene and an EC_{50} of 49.9 mg/mL (from DPPH· assay) was obtained after SC-CO_2 extraction at 35 °C, 400 bar and run time of 60 min. Further optimization of the extraction conditions by Box–Behnken design increased the extraction efficiency. Use of 2-propanol as an entrainer at 30% v/w of dried SBT seed powder at optimized conditions showed a recovery of tocopherols and carotenes of 91.1% and 69.6%, respectively, while it showed EC_{50} of 38.9 mg/mL. The optimized conditions were a temperature of 35 °C, pressure of 305 bar and run time of 90 min.

1. Introduction

Seabuckthorn (*Hippophae rhamnoids* L.) (SBT), a plant of the Elaeagnaceae family is a deciduous spiny nitrogen fixing species native to Eurasia and has been domesticated in several countries including India, China, Nepal, Pakistan, Myanmar, Russia, Britain, Germany, Finland, Romania and France at a high altitude of 2500–4300 m. SBT is a protective natural agent to human health since all parts of the plant are considered to be a good source of a large number of bioactive substances [1]. Various parts of sea buckthorn, especially berries, have been used as raw materials for nutraceuticals and therapeutic properties. Traditionally SBT is used as herbal medicine for burns and wound healing, relieving cough and aiding digestion for centuries in China and Russia. Recently, the nutritional importance of the berries in the form of health drinks has been increasing in North America, Europe and India [2].

Over 200 bioactive components have been found in SBT, of which many are investigated in SBT berries. SBT contains a series of chemical compounds including carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid and tannins. These compounds are of interest not only from the chemical point of view, but also because many of them possess biological and therapeutic activity including antioxidant [3], antitumoral [4], hepatoprotective [5], antimicrobial [6], dry eye syndrome [7] and immunomodulatory [8] activity. The SBT berries are also rich in vitamins like B_1, B_2, K, P and phytosterols. SBT seeds constitute about 17–20% of the berries, which are a byproduct of the SBT berry processing industries. SBT seed has 8–10% oil which has a high percentage of essential unsaturated fatty acids like oleic acid, palmitoleic acid, palmitic acid and linoleic acid, of which palmitoleic acid is a principal constituent of skin fat. SBT extract is recommended for skin softening and antiwrinkle products. This fatty acid can also nourish skin if taken in adequate quantities; this is a useful method for treating systemic skin disorders such as atopic dermatitis. SBT seed oil also contains tocopherols, carotenoids and phytosterols which are valuable bioactives that could be extracted from a product otherwise considered as waste. The carotenoids protect the skin against sun damage. Tocopherols and tocotrienols are powerful antioxidants that protect against sun damage as well. Phytosterols replace cholesterol in the skin, by doing this they improve the reduced barrier functions of the skin and besides it also soothen irritation [9].

The ever-increasing concern about the environmental pollution attributed to several chemical wastes has paved the way for the introduction of "green chemistry". Chemists are now becoming exceedingly careful about the use of chemicals and solvents, and are putting significant efforts in designing environment-friendly research protocols. Extraction and isolation of natural products from various sources conventionally generates large amount of

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Nomenclature

ΔE_v or ΔE_{vap}	summation of all cohesive energy (cal/mol)
P_c	critical pressure (bar)
T_c	critical temperature (°C)
V	molar volume
Δv	summation of all molar volume (cm^3/mol)
$\rho_{r.SF}$	reduced density of the supercritical fluid (g/cm^3)
$\rho_{r.L}$	reduced density of liquid state (g/cm^3)
δ_1	solubility parameter of solute at temperature T_1 $(cal/cm^3)^{1/2}$
δ_2	solubility parameter of solute at temperature T_2 $(cal/cm^3)^{1/2}$
ΔH_{vap}	heat of vaporization of solvent
R	gas constant = 1.987 cal/mol K, 8.314 J/mol K, 0.08205 L atm/K mol

waste organic solvents. An eco-friendly alternative to the use of organic solvents in natural product extraction is the application of supercritical fluid extraction (SFE) protocol. The emerging stricter environmental regulations concerning the use of common industrial solvents, most of which are hazardous to human health, have led to the increasing popularity and growth of the SFE technologies, especially those employing supercritical CO_2 (SC-CO_2).

The extraction of nutraceuticals using SFE technique covers principles of green technology as it is solvent-free and safe [10]. Supercritical fluids are advantageous due to simplicity and less degradation of thermolabile compounds [11]. As reported by Van der Velde et al. [12] SFE competes with conventional organic solvent extraction. SFE requires less time than conventional extraction due to higher mass transfer rates in supercritical fluids than in liquid solvents. Reports on basic thermodynamic properties of different species in supercritical fluids like phase equilibrium [13], solubilities [14], spectroscopic studies [15], density measurements [16], determination of partial molar volumes [17] and binary diffusion coefficients [18] are available. Ibañez et al. [19] estimated the solubility parameter of β-carotene using group contribution method in order to elucidate the elution conditions for separation of β-carotene and lycopene using CO_2. Sajilata et al. [14] estimated the solubility parameter in order to make an appropriate choice of solvent for extraction of zeaxanthin. Ajchariyapagorn et al. [20] predicted the extraction yield of nimbin in supercritical CO_2 using group contribution model, equation of state and shrinking core model, while Gerszt et al. [21] studied the group contribution methods for sterols and vitamins including tocopherols.

To the best of our knowledge, no reports are available on correlation of solubility parameters of SBT actives, SC-CO_2/entrainer and its extrapolation to experimental results. The present work reports on determination of solubility parameters for SBT actives, SC-CO_2/entrainer and development of an efficient protocol for SFE for SBT actives from SBT seeds.

2. Materials and methods

2.1. Materials

The seeds of SBT (*Hippophae* spp.) were procured from Sindhu fruit processing industry, Leh, India. They were ground in a mill fitted with 40 mesh to an average particle size of 225 µm. All the chemicals and solvents used in the present study were of AR grade and purchased from Sigma-Aldrich and s.d. Fine-Chem. Ltd, Mumbai, India. CO_2 cylinders were supplied by Bombay Carbon Dioxide Gas Company, Mumbai, India.

2.2. Estimation of solubility parameters for SBT actives, CO_2 and entrainer

One of the ways to have a detailed insight into the solubility of a solute in supercritical fluid is the estimation of its solubility parameter [22,23]. The solubility parameter estimation provides a semi-quantitative evaluation of experimental conditions to be selected for optimized extraction conditions. The solubility parameter, δ, of a supercritical fluid can be estimated by using the following equation [24]:

$$\delta\ (cal/cm^3)^{1/2} = 1.25\sqrt{P_c}\frac{\rho_{r.SF}}{\rho_{r.L}} = 0.47\rho_{r.SF}\sqrt{P_c} \quad (1)$$

where P_c is the critical pressure (bar), $\rho_{r.SF}$ is the reduced density (g/cm^3) of the supercritical fluid, and $\rho_{r.L}$ is the reduced density of liquid state. This equation reflects the variation of the solvent power of the supercritical fluid as a function of density.

Solubility parameter (δ) of a given solute can be estimated by using the Fedors group contribution method when the solute molecular structure is known [25]. Table 1 illustrates the procedure to estimate the solubility parameter of β-carotene by using the Fedors method. The solubility parameters of β-carotene, lycopene, α-, β-, γ- and δ-tocopherol were calculated using the following equation:

$$\delta\ (cal/cm^3)^{1/2} = \sqrt{\frac{\sum_i(\Delta E_v)_i}{\sum_i(\Delta v)_i}} \quad (2)$$

where $\sum(\Delta E_v)_i$ is the summation of cohesive energies (cal/mol) and $\sum(\Delta v)_i$ is the summation of molar volumes (cm^3/mol).

Using the above equation, $\delta_{\beta\text{-carotene}}$ for β-carotene at 25 °C was found to be 9.237 $(cal/cm^3)^{½}$ or 18.895 $MPa^{½}$. The δ values for β-carotene, lycopene, α, β, γ and δ-tocopherols at other temperatures are shown in Table 2. The critical temperature of SBT actives were calculated using the equation: $T_c = 535\log\sum\Delta T_i$, where ΔT_i is the summation of the critical temperatures of the contributing groups.

The Fedors method was found to be useful for estimating the extraction potential of complex molecules using supercritical fluids [26]. Table 1 lists solubility parameter contributions of β-carotene molecule. The solubility of solute in SC-CO_2 as a function of temperature can be described as follows [27]:

$$\delta\ (cal/cm^3)^{1/2} = \delta_1\left(\frac{V_1}{V_2}\right)^{1.13} = \delta_1\left(\frac{\rho_2}{\rho_1}\right)^{1.13} = \delta_1\left(\frac{T_c - T_2}{T_c - T_1}\right)^{0.33} \quad (3)$$

Table 1
Solubility parameter of β-carotene using Fedors group contribution.

Group	No.	Δe_i	Δv_i	ΔT_i
$-CH_3$	10	1125	33.5	1.79
$-CH_2-$	6	1180	16.1	1.34
>C<	2	350	−19.2	−0.22
>C=	8	1030	−5.5	0.89
=CH−	14	1030	13.5	1.40
C6 ring	2	250	16.0	2.68
Conjugation	11	400	−2.2	0.13
Total ($\sum$)		46,590	546	59.01