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Optimization and Validation of Maceration-Mediated Hydrodistillation to Extract Caryophyllene-Rich Essential Oil from Sea-Buckthorn Berries

Running head: Extraction of Essential Oil from Sea-Buckthorn Berries

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SUMMARY

Research background. Hydrodistillation (HD) is a convenient and economic method to extract essential oils but this technique has been precluded for limited recovery rates. Maceration-mediated hydrodistillation (MMHD) in comparison to conventional HD method could increase mass transfer and offer better control over extraction thermodynamics which will eventually preserve the aroma constituents and retain their antioxident activities.The present study describes an expedient and innovative modification in conventional HD by introducing a macerating agent Triton x-100 (Tx-100) and NaCl as an electrolyte to accelerate mass transfer for better recovery of caryophyllene-rich essential oil from sea-buckthorn berries.

Experimental approach. The parameters of maceration-mediated hydrodistillation (MMHD) including concentration of macerating agent, electrolyte, and withholding time were investigated within

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a wide range of 1−10 %, 1−10 g/100 mL, and 3−8 h respectively to enhance the oil yield (g/100 g). parameters were optimized following the desirability approach through response surface methodology.The essential oil obtained under optimum conditions was characterized for its antioxidant activities via *in vitro* antioxidant assays and aroma profile using gas chromatography hyphenated with mass spectrometery (GC-MS).

Results and conclusions. The optimized parameters for MMHD were observed at 4.22 mL of Tx-100, 4.03 g of NaCl for 5.61 h of extraction time offered (3.24±0.14) % essential oil compared to the conventional HD which produced 2.10 %. The essential oil produced via MMHD was found rich in (−)-β-caryophyllene (37.25 %) with good antioxidant activities in terms of free radical scavenging capacity (84.2 %), inhibition of linoleic-acid peroxidation (68.16 %), and Trolox equivalent antioxidant capacity (168 µmol Trolox equivalents/mL).

Novelty and scientific contribution. Tx-100 may disrupts the cell membranes to release the bioactives while, NaCl salt reduces the solubility of non-polar components of essential oil in the aqueous phase which ultimately can improve the extraction yield. The proposed approach can work with already present hydrodistillation setups with small modifications and seems to be more economical for the extraction of sea-buckthorn essential oil without compromising its antioxidant potential or valuable aroma constituents at industrial scale.

Keywords: sea-buckthorn berries; essential oil; caryophyllene; maceration mediated hydrodistillation; GC-MS; antioxidant activities

INTRODUCTION

The sea-buckthorn (*Hippophae rhamnoides* L.) also known as sea-berries and Siberian pineapple is an exceptionally important shrub of the Elaeagnaceae family (*1*). Sea-buckthorn (SBT) grows worldwide but moderate temperature zones of Pakistan, China and northern Afghanistan favor its high prevalence as wild shrub (*2*). During recent decades this shrub is attracting the medicinal chemists due to its interesting phytochemical profile and medicinal importance (*3*). Being abundant in terpenoids, palmitoleic acid, oleic acid, and tocopherols(*4*), sea-buckthorn essential oil (SBTEO) finds its applications in cosmetics, food and pharmaceutical industry (*5*) due to its interesting pharmacological properties (*2*). A wide number of reports claimed that SBTEO works as a promising solution of depression management (*6*), hypolipidemic agent as well as anti-inflammatory agent due to presence of caryophyllene (*1,7*).

A meteoric rise in the consumption of natural essential oils and flavoring agents in food, cosmetics and pharmaceutical industry was observed that calls for high quality and abundant feed.

Therefore, extraction of EO through conventional methods like hydrodistillation becomes a challenge to meet markete demands due to poor efficiency and compromised quality (*8,9*). An array of alternative approaches has been brought into practice by applying: (*i*) ultrasound-assisted extraction (*10*), (*ii*) microwave and thermal extraction (*11*), (*iii*) supercritical fluid (*12-14*), subcritical (*15*), and organic solvent extractions (*4*) and (*iv*) enzyme-assisted extraction (*16*). Although these approaches enhanced the extraction yield but face challenges related to cost and complexity when applied for the distillation of essential oils.

Hydrodistillation (HD) of essential oils is an expedient, inexpensive, and sustainable method nevertheless it has been ruled out for poor extraction efficiencies mainly due to limited mass transfer and heat-transfer rates (*17*). It has been observed that the addition of surfactants/macerating agents can accelerate the mass transfer (*18*). Likewise, the presence of electrolyte/salt in extraction media can help us to gain more control over the mass transfer rate and thermodynamics. Therefore, we planned to introduce the use of macerating agents (non-ionic surfactants) and salt (NaCl) for the enhanced recovery of SBTEO. The extracted SBTEO, under optimum maceration mediated hydrodistillation (MMHD) conditions was further subjected to gas chromatography coupled with mass spectrometry (GC-MS), *in vitro* antioxidant assays in aqueous and organic mediums to establish its antioxidant characteristics and major constituents, respectively.

MATERIALS AND METHODS

Preparation of sample

The SBT berries were collected from a local supplier (Akhter Corporation, Karachi, Pakistan), dried under vacuum till no further weight loss, and pulverized into coarse particles using household grinder AG-639 Deluxe (Anex Electrical Co Ltd, Hong Kong). The grinded sample was sieved and stored in zipper bag for further use.

Procurement of supplies

The Clevenger tube having 25 mL collector and different dimensions (Fig. S1) was prepared in a local glass-blowing workshop. The Sigma Aldrich Chemie (GmbH, Germany) supplied all the standards and reagents comprising ABTS (di-ammonium salt of 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), gallic acid, linoleic acid and BHT (butylated hydroxytoluene). The Triton x-100 (Tx-100) and anhydrous sodium sulfate (ethereal solution drying agent) were procured from Reagents Duksan (Ansan, South Korea). The analytical grade solvents and chemicals

like petroleum ether, methanol, ethanol, sodium phosphate buffer, ferrous chloride, NaCl, and ammonium thiocyanate were supplied by Merck (Darmstadt, Germany).

Extraction of SBTEO

For the extraction of SBTEO, accurately weighed 100 g of SBT berries powder was transferred to a round bottom flask already filled with 250 mL double distilled water containing a specific amount of salt along with macerating agent as per condition given in Table 1. The flask was connected to the Clevenger type apparatus (*19*) having different side arm lengths and dimensions (Fig. S1) in order to evaluate the effect of tube design and lengths. The whole set up was connected with condenser set at (10±2) °C by circulating a coolant through it. While conducting all experiments listed in Table 1, the temperature of extraction mixture (round bottom flask) was kept at 105 °C whereas pressure was kept at 101325 Pa by leaving the upper end of Clevenger tube open. Finally, the effect of withholding time, amount of maceration agent (Tx-100), and salt (NaCl) concentration was evaluated at five different levels (Table 1). The amount of SBTEO accumulated in the collector (Fig. S1) was transferred to pre-weighed ethereal solution bottles and weighed again to calculate the percentage yield (g of SBTEO/100 g of berries) according to the following equation:

$$Y = \left(\frac{m(\text{SBTEO})}{m(\text{SBT berries})}\right) \cdot 100 \qquad /1/$$

The organic layer was subsequently mixed with 5 g of anhydrous sodium sulphate to remove any moisture and stored under darkness ((10±2) °C) for further use.

Experimental layout

The preliminary screening experiments revealed that surfactant concentration, salt content and withholding time affect the recovery of aroma compounds from SBT berries. Based on these observations, surfactant ratio (A), salt content (B), and extraction time (C) were applied at five different levels coded as α, 1, 0, -1, and - α (Table 1) in a central composite option of experimental design in response surface methodology. A fully rotatable approach was followed, in which total of 20 runs were conducted comprising six replicates, the center point (coded as 0), eight runs for the axial point (coded +α, -α), and six runs for factorial points (coded as +1, -1).

Chromatographic analysis of SBTEO

The individual constituents in SBTEO, produced under optimum conditions were studied by Gas Chromatography hyphenated with quadrupole mass spectrometry (GC-MS). The analysis was carried out using Shimadzu GCMS-QP2010 Series (Kyoto, Japan) equipped with 30m×0.25 mm

capillary column (SH-Rxi®-5Sil MS, Shimadzu, Kyoto, Japan) of cross-bound 1,4-bis(diphenyl/dimethyl) polysiloxane (5/95) stationary phase of particle size 0.25 µm. The analysis conditions involve the use of helium (99.99 %) as the carrier gas. The flow rate, linear velocity, and purge flow were set at 1.5 mL/min, 55.5 cm/s, and 0.0 mL/min, respectively. The column oven temperature was programmed with 3.0 min hold at the start (60.0 °C) and end point (300 °C), with a ramp rate of 6.0 °C. The 3.0 µL of SBTEO produced via MMHD and conventional HD were injected manually at an injection volume of 3.0 µL with split-type injection mode at 150 kPa pressure and a split ratio of 80/20. The detection and quantification of SBTEO constituents were carried out by QP-MS under total ion collector (TIC) mode scanning from 40 to 500 *m*/*z*. The QP-MS operating parameters, viz. ion source temperature, interface temperature, and solvent cut-off time were set at 150 °C, 300 °C, and a 2.5 min, respectively. After analysis, the obtained mass fragmentations of each compound were compared with the National Institute of Standards and Technology (NIST) Library provided by Shimadzu (Japan) and the literature to authenticate the compounds present in SBTEO.

Antioxidant characterization of SBTEO

The antioxidant quality of SBT essential oil, extracted by thermodynamically modified MMHD and conventional HD, was monitored by measuring their ability to scavenge free radicals (FRSC) (*20*), linoleic acid peroxidation inhibition capacity (*15*) and Trolox equivalent antioxidant capacity (*21*).

Free radical scavenging capacity (FRSC)

The ability of SBTEO produced via MMHD and HD to scavenge 1,1-diphenyl 2-picrylhydrazyl free radicals (DPPH°) was established by simply incubating the SBTEO with DPPH° (*20*). Briefly, 500 µL of SBTEO was mixed with 500 µL of freshly prepared 1.0 ppm solution of DPPH° in HPLC grade methanol and held under darkness for 15 min. The absorbance of incubated solution (A_s) was noted at 517 nm against the original DPPH solution as control (A_c) by using a microplate reader (ELX 800, Bioteck, Winooski, USA) to calculate the inhibition percentage of DPPH by SBT essential oil:

$$\text{Inhibition} = \left(\frac{A_c - A_s}{A_c}\right) \cdot 100 \qquad /2/$$

Linoleic acid peroxidation inhibition capacity (LAPIC)

The ability of SBTEO to retard the formation of peroxides in linoleic acid was monitored following the method cited by Zheng *et al.* (*15*). The assay was slightly modified to apply in microliters of reagents involved, in short, 500 µL of SBTEO of both samples obtained via MMHD and simple HD separately was added into a mixture containing 100 µL of 10 % linoleic acid followed by addition of 500 µL of 0.2 M phosphate buffer (pH 7.0) and 750 µL of ethanol. The linoleic acid in the resultant

mixture was allowed to oxidize at 40 °C for 72 h. At the end of incubation time, 200 µL of the above mixture was further diluted with 500 µL of ethanol, treated with 30 % ammonium thiocyanate (1.0 mL) and 200 µL of $FeCl_2$ solution (20 mM) in HCl (3.5 %) was added and resultant mixture incubated for 3 min at 40 °C. The amount of peroxides produced in the solution was determined by estimating the formation of thiocyanates *i.e.* absorbance (A_s) measured at 500 nm applying phosphate buffer as control (A_c) following Eq. 2.

Trolox equivalent antioxidant capacity (TEAC)

The TEAC of SBTEO obtained by both methods was evaluated according to the TEAC assay as reported by Mushtaq *et al.* (*21*). The ABTS radical cations were produced by treating 100 mL of 7.0 mM diammonium salt of ABTS (2,2-azinobis(3-ethylbenzothiazo-line-6-sulphonic acid)) with 50 mL of 2.45 mM potassium hydrogen sulphate under dark at ambient conditions for 8.0 h. The resultant $ABTS^{\cdot+}$ were diluted in ethanol until the solution's absorbance dropped to 0.70±0.05 at 734 nm (λ_{max}). Now, equal volumes (100 µL) of SBTEO and $ABTS^{\cdot+}$ were mixed in wells of a 96-well plate, incubated for another 8.0 min and read at above-mentioned wavelength to calculate the percentage scavenging of ABTS (Eq. 2) by SBTEO (A_s). The synthetic antioxidant Trolox was used as positive control (A_c) to express the antioxidant capacity and results are reported as Trolox equivalent/mL of SBTEO.

Statistical analysis and optimization

The optimized yield of SBTEO by two methods were statistically analyzed through analysis of variance (ANOVA) version 12.0.0.3.0 of a statistical workstation Design Expert by Stat-Ease Inc., U.S.A (*27*). Experimental layout comprising six replicates was made at the center points to calculate mean square error (MSE) as well as any treatment (axial or factorial point) that significantly ($p \leq 0.05$) impact the yield was modulated following the regression equation (Eq. 3). Meanwhile, chi square test (χ^2 182 value ≥ 3.84) was used to measure any significant ($p \leq 0.05$) difference between treatments or variables. A probability (p) ≤0.01 was also applied to check the fitness of the model and agreement between observed and predicted values.

$$\text{SBTEO} = c_0 + \sum_{i=1}^{k} c_i X_i + \sum_{i=1}^{k} c_{ii} X^2 + \sum_{i>1}^{k} \sum_{j}^{k} c_{ij} X_i X_j + \varepsilon \qquad /3/$$

In Eq. 3, C_o, and ε denote noise (intercept) and pure error whereas X_i, X^2, and $X_i X_j$ refers to linear, quadratic, and interaction effects of MMHD conditions applied. All the factors (X_i) significantly ($p \leq 0.05$) affecting the recovery of SBTEO were further transformed into what is known as desirability (d) while considering the relative importance of factor and the conditions with the highest values of "d" were validated in a separate set of triplicate experiments.

RESULTS AND DISCUSSION

Preliminary screening of hydrodistillation conditions

In a preliminary screening of hydrodistillation experiments, it was found that an increase in water temperature increases the recovery rates, but it also increases the water content in the collected SBT essential oil. On the other hand, lowering the temperature below 60 °C couldn't produced significant recovery of essential oil from SBT berries. This finidng is supported by literature reports for experimental conditions of HD in laboratories that maintained temperature of extraction solvent near the boiling point of water. However the addition of non-volatile electrolyte can increase the boiling point of water and hence total heat content could facilitate the recovery rates.

Likewise, the dimensions of the Clevenger tube (Fig. S1) also affected the recovery of essential oils. We have investigated experimental outcomes with with different side arm lengths, angles (bend), and vertical column heights of Clevenger tubes and observed that Clevenger tube having a 150 mm long column with 175 mm bend tube (at 120° angle) can offer a better yield of SBTEO. Besides, the physicochemical characteristics of water,particularly heat capacity and polarity need to be optimized. Interestingly, addition of small amounts (about 10 %) of Triton X-100 and NaCl worked as activators by affecting colligative properties of extraction solvent that results in enhanced extraction of SBTEO. This increase in extraction through addition of activators could be associated with maceration and micellization potential of surfactant and change in heat capacities of the extraction solvents. It has been already observed that Tx-100 disrupts the cell membranes to release the bioactives by lowering the surface tension of water (*18,22,23*). Meanwhile, NaCl salt impacts the formation of suspension among oil and water to reduce the solubility of non-polar components of essential oil in the aqueous phase as well as alteration in cell wall structure that ultimately improve the extraction yield (*23-26*). Addition of these activators were further investigated at five different levels to optimize the extraction yield.

Optimization of maceration mediated hydrodistillation (MMHD)

The SBTEO yield obtained via modified hydrodistillation under various extraction parameters was investigated and optimized over a range of non-ionic surfactant concentrations (A), NaCl content (B), and extraction time (C) using the rotatable central composite design (Table 1). The analysis of variance (Table S1) in essential oil (g/100 g of SBT berries) reveals that the augmented factors affect the recovery of essential oil. Overall, the linear (A, B, and C), quadratic (A_2, B_2, and C_2), and interaction (AB and AC) effect of all these parameters on the recovery of essential oil from SBT was significant ($p \leq 0.05$) except interaction between NaCl added (B) and withhold time (C) with $p > 0.05$. Likewise, the high model F-value (795.54) indicates the model is significant and there are only 0.01 % changes that this large F-value happens due to noise.

The variation change in essential oil recovery from SBT in response to the addition of non-ionic surfactant (Tx-100) and NaCl, might be due to a change in plant micro-structure (maceration) and heat-capacity of water (*23*). Besides, the Tx-100 may solubilize the lipid bilayer by reducing the surface tension between the two phases, and as a result mass transfer increases. However, the increase in surfactant Tx-100 concentration leads to the formation of micelles which entrap the oil droplets. In this scenario, NaCl increases the diffusivity of the solvent, allowing it to penetrates the micelles that helps in the release of essential oil components. The amounts of Tx-100 and NaCl must be carefully proportioned, as an increase in the level of either NaCl or Tx-100 beyond 5-6 % *m*/*m* or *V*/*V* of the sample may adversely affect the yeild of essential oil. This affect on yield could be due to elevated boiling point of the extraction medium by addition of salt that improves the heat and mass transfer. In this way, the presence of NaCl or any other electrolyte may offer thermodynamic control over the extraction process which ultimately makes the process rapid and more economical. Hydrodistillation using water, with an elvated boiling point, can also extract those components which have boiling points higher than that of water (*23*). The withholding time ranks as the third important factor affecting the essential oil recovery from plant material (*27*).

The interactions between these three parameters (AB, AC, BC) can be better understand, from the data plotted in Fig. 1. The results in Fig. 1a and Fig. 1b indicates that an increase in the amount of Tx-100 up to 5.5 mL increases the recovery of essential oil while further addition of Tx-100 can adversely affect the yield of essential oil. However, the addition of NaCl along with Tx-100 improves the essential oil yield beyond 5.5 mL of Tx-100 to about 7.7 mL of Tx-100. These factors were optimized through statistical model to improve the yield and make the process more economical and rapid (*23*). The quadratic effect of all the extraction parameters (A^2, B^2, and C^2) was also significant (Table S1) on SBTEO yield. Moreover, the coefficient of determination (R_2) value 0.9986 confirms the agreement between observed and predicted values, and concordance between adjusted and predicted R_2 *i.e.* 0.9974 and 0.9930 indicates the absence of outliers. A coefficient of variation (CV) value of 4.00 % confirms the validity and reliability of observed data. Finally, Fig. S2 shows visual evidence for the agreement between the observed and predicted yield of SBTEO. Overall, the SBT essential oil yield can be modulated using the following equation:

$$Y(\text{SBTEO}) = +4.57 + 0.305 \cdot A + 0.1301 \cdot B - 0.2746 \cdot C - 0.750 \cdot AB - 0.3625 \cdot AC - 0.0750 \cdot BC - 1.40 \cdot A^2 - 1.32 \cdot B^2 - 0.6869 \cdot C^2 \quad /4/$$

Validation of optimum extraction parameters

To check the effect of various thermodynamically modified maceration parameters on SBTEO yield, a range of Tx-100 *i.e.* 2.8−10.0 mL, NaCl 2.8−10.0 g, and extraction time 5.0−8.0 h were

studied. It was observed that sea buckthorn berries when processed under above mentioned conditions offered higher SBTEO as compared to conventional hydrodistillation. The statistical predictions indicate the MMHD under the optimum extraction conditions, which are 4.22 mL Tx-100, 4.03 g NaCl, and 5.61 h of extraction time can recover about 3.1 % SBTEO. The suitability of the model equations for predicting the response values was confirmed by performing a validation experiment under these optimized conditions which yielded (3.24±0.14) % (g/100 g) of SBTEO (Table 2 (*28-30*)). It is obvious from the validation experiments that predicted optimum conditions offer higher SBTEO recovery rates. A keen review of previously published research indicates, no study has been undertaken to macerate the plant material, and modify the thermodynamics of water simultaneously as done in the present study. However, Cakir (*28*) extracted the SBT essential oil by conventional steam distillation with a yield of 0.1 % (*m*/*m*) which mainly consists of alcohols, terpenes, aliphatic esters, and hydrocarbons. Yue *et al.* (*29*) recovered 0.03, 1.26, and 0.37 % of essential oil from seed, pulp, and the leaf of sea buckthorn, respectively, by conventional hydrodistillation. Li *et al*. (*30*) extracted the SBT essential oil by combining ultrasound and microwave-assisted extraction with ionic liquids as extraction solvent and compared it with conventional hydrodistillation. These authors could recovered (0.095±0.004) % SBTEO by ionic liquid-based ultrasonic/microwave-assisted simultaneous distillation and extraction, and (0.089±0.003) % by conventional hydrodistillation, respectively. Interestingly these authors observed that extraction of essential oil via microwave, ultrasound, or conventional hydrodistillation contained same volatile constituents which seems to be difficult. In contrast, during present research, we have observed that essential oil obtained via MMHD comes with larger individual compounds and superior antioxidant quality as compared to that of produced via conventional hydrodistillation. The higher recovery rate, as observed during present research authenticate the effectiveness of TX-100 as macerating agent. Another, important reason behind the higher recovery rates of essential oil during present study as compared to the previous studies cited in Table 2 might be of controlled low temperature of the condenser (10 °C).

Antioxidant characteristics of SBTEO

The antioxidant activities of SBTEO extracted via MMHD and conventional hydrodistillation were established in terms of their free radical scavenging capacity, inhibition of linoleic peroxidation, and ability to neutralize ABTS radical cations. The results obtained from the antioxidant activities SBTEO showed a significant difference of both SBTEO extracted via MMHD and conventional method, results are given in Fig. 2. This difference in antioxidant activity could be attributed to increase in the relative abundance of caryophyllene in the essential oil produced by former techniques. Likewise, TEAC represents the most reliable assay to evaluate the antioxidant character of bioactive

in terms of their capacity to reduce the ABTS radical cations. The extracts obtained via MMHD and HD exhibited 168 and 150 µmol of Trolox equivalent/mL respectively. The free radical scavenging ability of both the SBT essential oils (produced via MMHD and HD) were also significantly different ($p \leq 0.05$). Moreover, the preservative potential of extracted oil was evaluated in terms of peroxidation inhibition of linoleic acid. The peroxide inhibition potential of SBTEO produced via MMHD and conventional HD in linoleic acid varied up to 68.16 and 65.11 % respectively. Overall, results indicate that MMHD produced the SBTEO with significantly higher antioxidant activities (FRSC, LAPIC, TEAC). The increase in the antioxidant character of SBTEO obtained via MMHD can be associated with hydrolytic/maceration potential of surfactant (Tx-100) and water thermodynamic in MMHD. Previously, no MMHD type extraction of SBTEO has been undertaken, however, conventional HD and solvent extraction reveals the presence of potential antioxidant bioactives in sea buckthorn berries (*3*).

GC-MS characterization

Metabolic profiling of SBTEO via Gas Chromatography coupled with Mass Spectrometry (GC-MS) revealed that MMHD can more effectively recover SBTEO. Fig. 3 compares the relative abundance of various bioactive compounds found in SBTEO produced via (a) MMHD and (b) conventional HD. The caryophyllene has been found as the most abundant volatile terpene (37.25 %) followed by (Z)-9-Octadecanoic acid methyl ester (12.74 %) and d-limonene (10.23 %) in SBTEO produced via MMHD. The relative abundance of (Z)-9-octadecanoic acid methyl ester in SBTEO obtained via conventional HD (20.70 %) was higher as compared to devised MMHD viz 12.74 %. Fig. 3 indicates the presence of eight valuable aroma compounds in the SBT essential oil produced in conventional HD (control) and MMHD (treatment) via hydrodistillation, respectively. The most abundant aroma compound detected in both control and treatment SBTEO was (Z)-9-octadecanoic acid methyl ester ($C_{19}H_{36}O_2$) with a relative abundance of 20.70 and 23.74 % at the retention time of 16.68 and 20.035 min, respectively. The least abundant aroma compounds were detected as 8,11-octadecadienoic acid methyl ester ($C_{19}H_{34}O_2$) and oleic acid ($C_{18}H_{34}O_2$) in both control and treatment. Besides, the SBTEO produced via either type of distillation can contain n-hexadecanoic acid, oleic acid, heptanal, nonanal and n-octanal (Table 3).

A careful study of the literature reveals that Cakir (*28*) extracted the SBT essential oil by steam distillation in which ethyl decanoate was present as a major component in 39.4 %. Previously, Yue *et al.* (*29*) recovered the n-hexadecanoic acid in abundance in all three extracted oils of seed, pulp, and the leaf of sea buckthorn with 36.64, 32.88 and 26.07 % by conventional hydrodistillation. Li *et al.* (*30*) extracted the myristic acid (10.24±0.15) and (Z)-8-dodecen-1-yl acetate (9.22±0.09) as main

components in sea buckthorn essential oil by ionic liquid-based ultrasonic/microwave-assisted simultaneous distillation and extraction (ILUMASDE) and conventional hydrodistillation, respectively. Recently, Sanwal *et al.* (*10*) reported methyl palmitate (25.85±0.01), as an abundant component in SBT essential oil at optimum by using ultrasound assisted extraction. However, the technique we presented for the extraction of essential oil from sea buckthorn berries simultaneously enhanced the recovery of essential oil and reduced the extraction time and associated cost. Besides this, the essential oil produced via MMHD was found to be rich in (−)-β-caryophyllene and other high value bioactives (Table 3).

CONCLUSIONS

Sea buckthorn essential oil (SBTEO) may work as a potential substitute for the food and pharmaceutical industries for the presence of bioactive compounds like terpenes and short-chain fatty acids esters. However, many of these valuable volatiles are lost or degraded during extraction/distillation and for similar reasons, recovery of essential oil has remained a challenging task. The present research established, that maceration-mediated hydrodistillation (MMHD) can work efficiently as compared to conventional hydrodistillation (HD) to extract sea buckthorn essential oil. The MMHD offered more than 54 percent higher yield of essential oil and this increase might be due to several known and undisclosed factors. Few of the former include: (*i*) better control over the extraction thermodynamics and (ii) it can fairly assumed that the incorporation of Tx-100 (non-ionic surfactant) improves the mass transfers while the presence of NaCl changes the thermodynamics of water which helps to capture the entrapped aroma. In contrast, the undisclosed factor response for the increase in essential oil recovery might be the design of tube, particularly the collector arm length and angle. It has been observed during the present research that both bend angle and arm length of Clevenger type tube needs to be further investigated. Conventional HD has been ruled out for limited recovery rates and it was observed that this innovative technique helps in the fast release of volatile compounds by simultaneously changing the boiling point and surface tension of the extraction solvent. The extracted SBTEO was found to be rich in (−)-β-caryophyllene (37 %) and esters of short-chain fatty acids.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Z. Liaqat conducted the experiments and prepared the manuscript draft; M.U Kamal and R. Ashraf helped in sample analysis and data acquisation. S. Akram and R. Naeem have revised the manuscript and M. Mushtaq has supervised the the research work.

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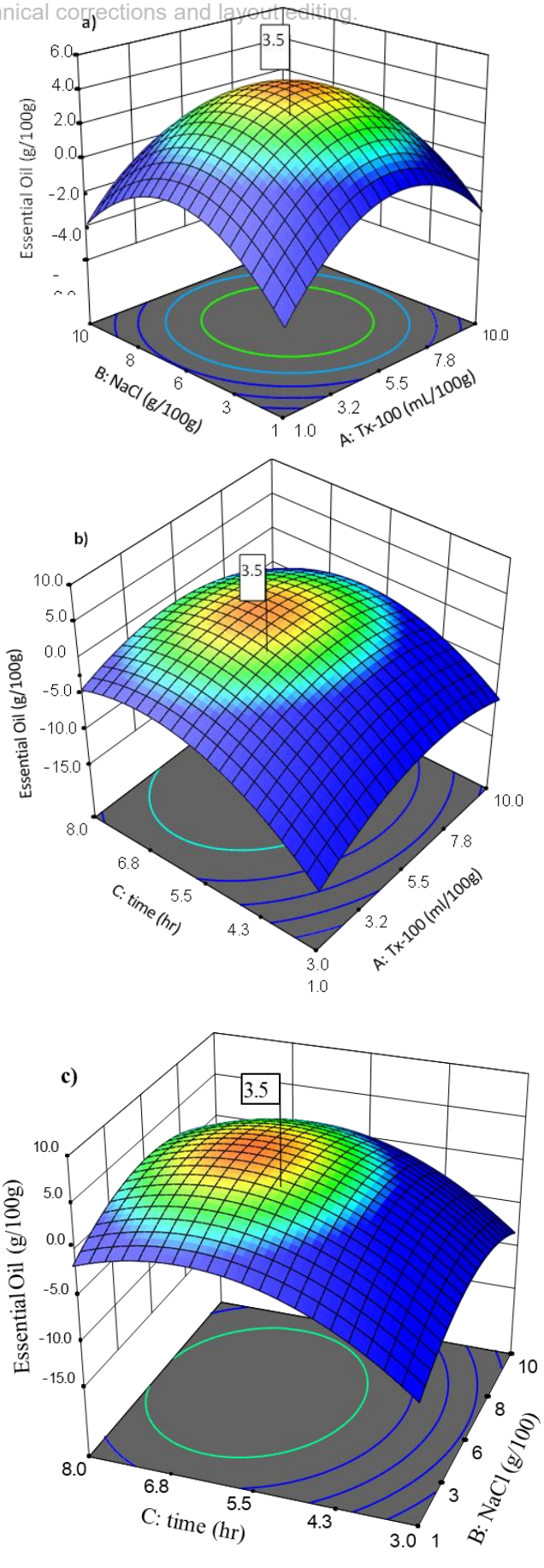


Fig. 1. Three-dimensional surface plots of the interaction between various extraction parameters: a) Tx-100 vs NaCl, b) Tx-100 vs time and c) NaCl vs time affecting the recovery of seabuckthorn essential oil

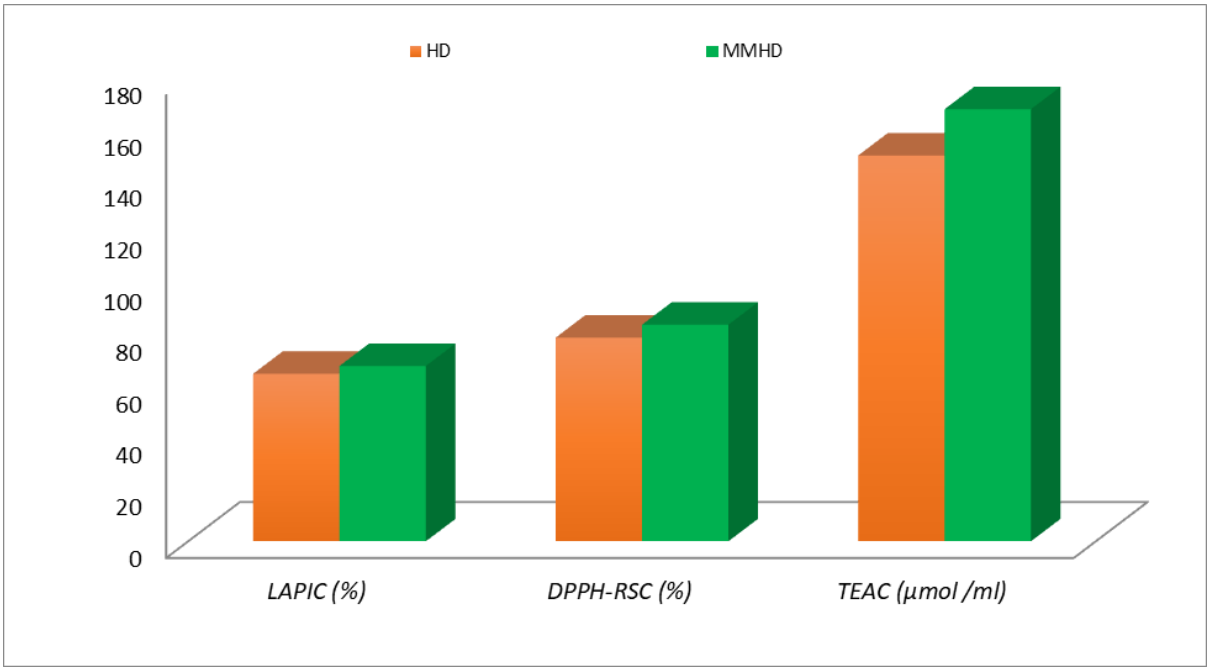


Fig. 1. Comparison of antioxidant activities of sea buckthorn essential Oil (SBTEO) extracted via maceration mediated hydrodistillation (MMHD) and conventional hydrodistillation (HD)

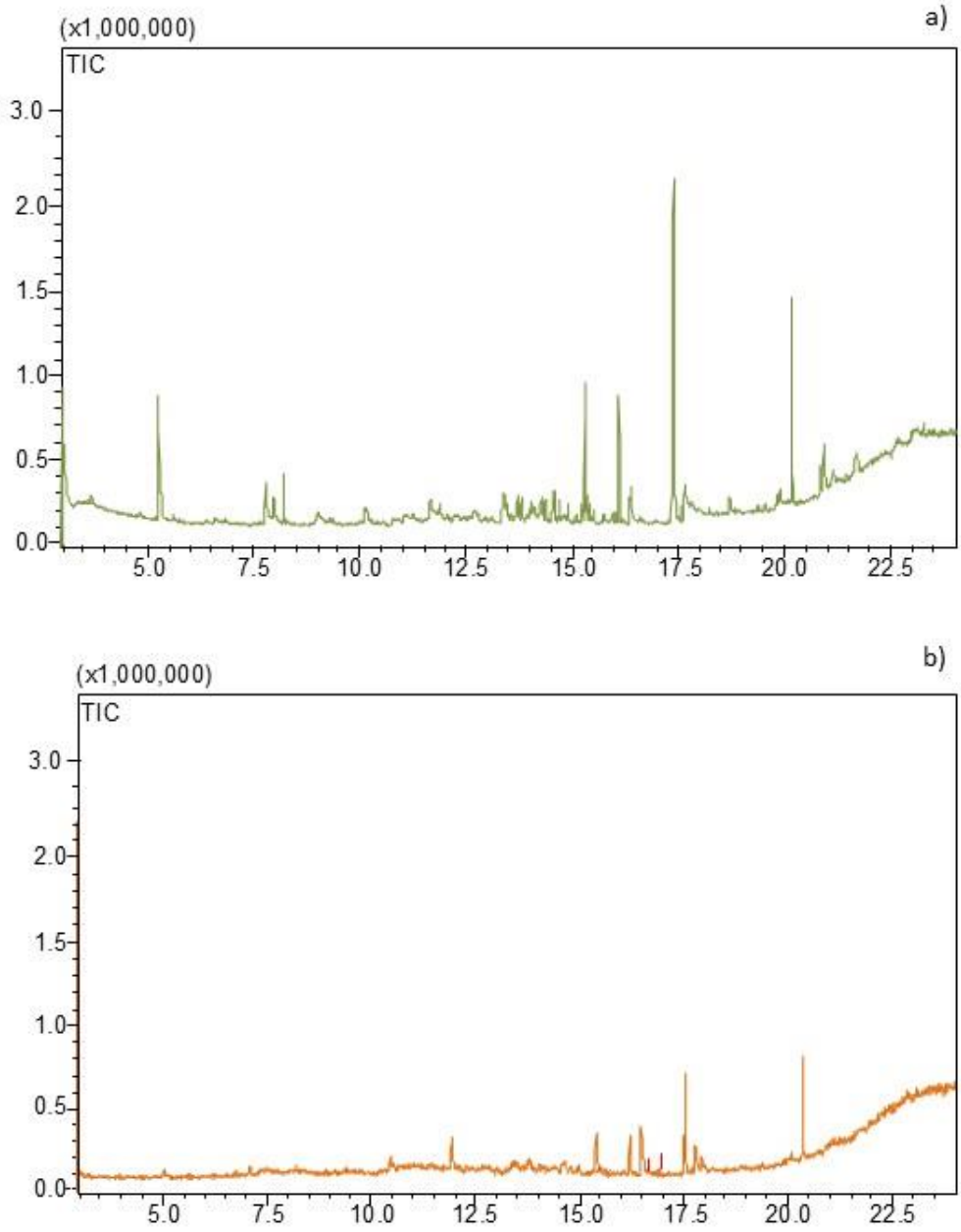


Fig. 2. Gas chromatogram indicating the constituents of sea buckthorn essential oil (SBTEO) produced via: a) maceration mediated hydrodistillation (MMHD) and b) conventional hydrodistillation (HD)

Table 1. The experimental conditions applied for maceration mediated hydrodistillation (MMHD) of seabuckthorn berries essential oil

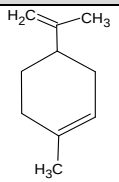
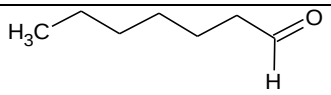
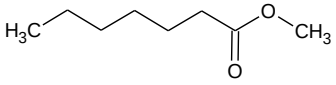
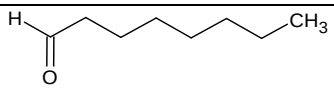
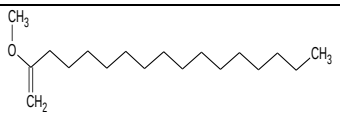
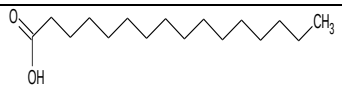
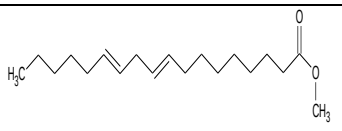
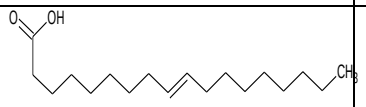
Factor	Central composite experimental design point				
	-*α*	-1	0	1	*α*
A: *V*(Tx-100)/mL	1	2.82	5.5	8.18	10
B: *m*(NaCl)/g	1	2.82	5.5	8.18	10
C: *t*/h	5	5.61	6.5	7.39	8

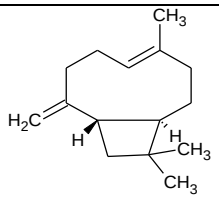
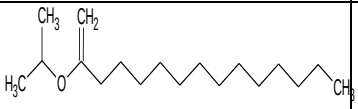
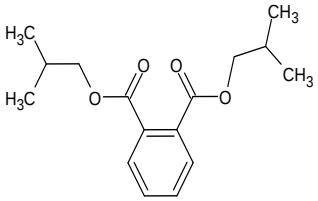
*(+*α* and –*α*), (+1 and −1), and (0) represent axial, factorial, and center points of the proposed experimental design

Table 2. The detail of validation experiments undertaken for maceration mediated hydrodistillation (MMHD) of seabuckthorn berries essential oil

Treatment condition			Hydrodistillation parameters		*Y*(essential oil)/(g/100 g)
A: (*V*(Tx100)/*m*(sample))/(mL/100 g)	B: *w*(NaCl)/(g/100 g)	C: *t*/h	Temperature (°C)	Condenser Temperature (°C)	
4.22	4.03	5.60	105	10	3.25
4.22	4.03	5.60	105	10	3.38
4.22	4.03	5.60	105	10	3.10
Experimental (mean±S.D.)					3.24±0.14
Predicted extraction (d≥0.05)					3.10
Conventional hydrodistillation (HD)		6.0	95	10	2.10
Increase in extraction efficiency					54.28 %
Literature report					
Cakir (*28*)		4.0	100	-	0.1 %
Yue *et al.* (*29*)		4.0	40	-	0.03 %
Li *et. al.* (*30*)		6.0	95	-	0.095 %

Table 3. GC-MS profile of SBTEO produced under optimum: a) MMHD conditions and b) conventional HD

Retention time	Compound name	Molecular formula	Molecular mass	Most probable structure	Relative abundance/%	
					HD	MMHD
5.735	Limonene	C_6H_{14}	72		1.25	10.23
7.789	Heptanal	$C_7H_{14}O$	114		ND	1.46
8.710	Heptanoic acid methyl ester	$C_8H_{16}O_2$	144		ND	3.92
9.218	Nonanal	$C_9H_{18}O$	142		ND	3.18
10.420	n-octanal	$C_8H_{16}O$	97		1.47	2.54
15.460	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270		7.73	8.25
15.700	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256		12.99	ND
16.649	8,11-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294		7.41	7.33
16.929	Oleic acid	$C_{18}H_{34}O_2$	282		8.27	2.75

17.451	Caryophyllene	$C_{15}H_{24}$	204		9.89	37.25
17.610	Isopropyl myristate	$C_{17}H_{34}O_2$	270		13.04	8.70
19.336	1,2-Benzenedicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	278		19.98	4.19
20.085	(Z)-9-Octadecanoic acid methyl ester	$C_{19}H_{36}O_2$	296		20.70	12.74

HD: hydrodistillation, MMHD: maceration-mediated hydrodistillation, ND: not detected