WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 16, 1286-1297. Research Article ISSN 2277– 7105

ANTIOXIDANT PROFILING AND FATTY ACID COMPOSITION OF LIPIDS PRESENT IN *HIPPOPHAE SALICIFOLIA* GROWN IN HIGHER ALTITUDE OF UTTARAKHAND REGION

Rakshit Pathak*, Shweta Goyal, Anjali Kumari and Sanjay K. Dwivedi

Defence Institute of Bio-Energy Research (DRDO) Haldwani, Uttarakhand.

Article Received on
13 July 2018,

Revised on 03 August 2018,
Accepted on 24 August 2018,

DOI: 10.20959/wjpr201816-13236

***Corresponding Author**
Rakshit Pathak
Defence Institute of Bio-Energy Research (DRDO)
Haldwani, Uttarakhand.

ABSTRACT

Seabuckthorn (Hippophae spp.) is widely distributed shrub of the cold Himalaya region with multiple medicinal properties. In the present article, we investigate some physicochemical parameters of the *H. Salicifolia* grown in the higher altitude of Himalaya where plants were grown in natural condition. We evaluate the oil content from its fruit via Soxhlet extraction and studied its parameters such as Acid value, Free fatty acid %, Density, refractive index and Total soluble sugar, Vitamin C concentration and Carotenoid content. Besides that, esterification of oil was also done to analyze the chemical components present in its oil via GC MS. For this purpose seeds were collected from the DIBER field station Auli (Joshimath), Oil percentage was almost 11% using hexane as a solvent which has the acid value of 9.23 mg KOH/g. The density of oil was 853 Kg/m^3, Refractive index was found 1.82 these two are specific stable identification for the oil. Further to this, the GC-MS study revealed that important chemical constituents were found are Docosanol, ß Sitosterol, Phytol and cis-linoleic acid. The result revealed the capability of *H. salicifolia* seed oil for medicinal purpose and for the food purpose.

KEYWORDS: Seabuckthorn; *Hippophae salicifolia D. Don;* esterification; Fatty acid methyl esters.

1. INTRODUCTION

A high altitude Himalayan valley always attracts the human being due to its beauty, natural habitat and different culture. Himalaya region has also very vast biodiversity with the occurrence of various wonder plants. Sea buckthorn (*Hippophae* species) is one of those

miraculous plants which found in cold Himalaya region. It grows widely in the world including Asia, America and Europe. *Hippophae* spp. has such type of bioactive components which makes it a "super fruit" at a commercial level.[1] Seabuckthorn (SBT) plant not only contains high antioxidant value but also fixes the nitrogen at an effective level as well as its strong root system stops the soil erosion.[2] Every part of this plant is very important due to the presence of various bioactive components.

Hippophae salicifolia D. Don is the plant belongs to the family "*Elaeagnaceae"* commonly known as Seabuckthorn, Chharama Chuk, Amli and Kalabis.[3] SBT is a decideious spiky willow like small tree with rusty scaly branches. Leaves are broad (4 to 10 mm) and green (less silvery), berries are yellow in colour. These features of the shrub distinguished it from *H. rhamnoides* which is another well known species belongs to this family. Flowering and fruiting season of SBT is May and September.[4] The whole plant is highly rich in antioxidant potential due to the presence of various bioactive substances such as phenolics, flavonoid, Caretenoids, vitamins C, E and K and some essential amino acids.[5] Because of such properties it is used as a traditional medicine from long time in hilly region for the treatment of cough, diarrhea, asthma, gastric ulcers, skin diseases, lung disorders, menstrual disorder etc.[6]

SBT's one of the species i.e. *H. rhamnoides* is well explored and highly studied by the Scientific communities, but in the case of *Hippophae salicifolia* D. Don there is need to do more efforts to understand the importance of the unexplored part of this spp. So the present investigation was aimed to estimate some physicochemical properties and chemical composition of *H. salicifolia* fruit via GC MS to know the fatty acid composition present in the oil.

2. MATERIAL AND METHOD

2.1. Material

The fruit part of *H. salicifolia* was harvested in the month of October 2017 at DIBER High Altitude Research Station, Auli, Joshimath (Chamoli district), Uttarakhand, where the plants were cultivated under natural conditions. Fruits were dried in air oven at 40°C, crushed and used for oil extraction. The collected fruit were washed thoroughly with distill water and dried in shed for 3 days than 24 hours dried in hot air oven at 35°C, Dried seeds were grounded in mixer grinder to form a homogeneous powder. (REMI, India). All other

chemicals required for the experiment was purchased from the local vendor and used without further purification.

2.2. Method

Physicochemical parameters of the lipid

2.2.1. Extraction of oil: 50 g of the seed powder were placed inside the thimble and extraction was carried out using hexane as a non polar solvent (400 ml) in soxhlet apparatus.[7] The choice of solvent was Hexane because the lipids are non polar in chemical nature so Hexane (μ = 0.08 Debye) was used on the basis of "Like Dissolve Like". The solvent was separated using rotary evaporator (IKA RV10, Germany). The oil collected after solvent removal was weighed for percentage oil estimation in seed and stored in sealed glass tube for further analyses.

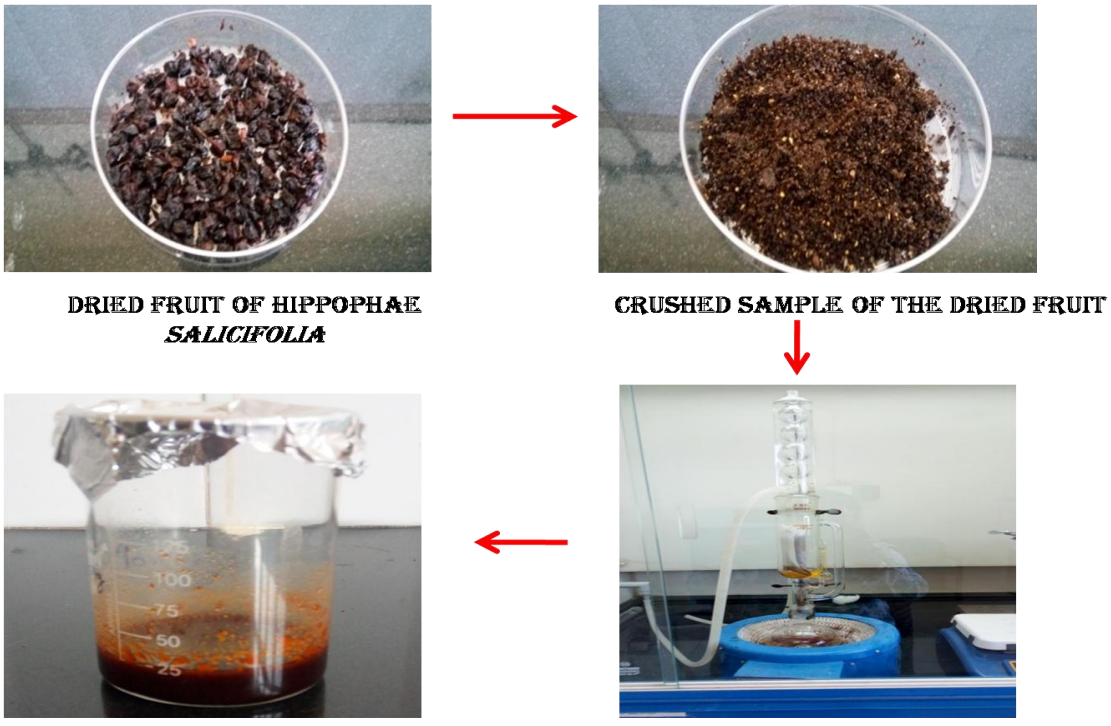


FIG : SHOWING THE EXTRACTION OF OIL FORM SEABUCKTHORN SEEDS

2.2.2. Determination of moisture content

Moisture content was determined by the air oven method, generally this method is applied for the oils and fats which has less content of moisture (>1%) 2 g of the oil was weighed in previously dried moisture dish (prepared by aluminum sheet). Dish was placed in the air-oven for approximately half an hour at 105 ± 1°C. Dish was removed and placed inside the

desiccators and weighed. Procedure was repeated till the two successive weighing does not exceed one milligram.

Percentage moisture content of the Seabuckthorn oil was calculated by the formula

$= (W_1\text{-}W_2/W_1) \times 100\%$

Where,

W_1 = Weight of the sample before drying.

W_2 = Weight of the sample after drying.

2.2.3. Density determination

Density of the oil was determined using Portable Density/ Specific Gravity Meter *DA- 130N*.

2.2.4. Refractive index

Refractive index of the oil was calculated by Kyoto electronics Refractometer, RA - 600.

2.2.5. Total soluble sugar estimation

Total soluble sugar was calculated with the help of Refractometer (HI 96801), an optical instrument that employs the measurement of refractive index to determine the % Brix of sugar in aqueous solution.

2.2.6. Determination of Acid value and FFA%

The number of milligram of KOH required neutralizing the free fatty acids present in one gram of fat or oil. It is relative measure of rancidity as free fatty acids are generally formed during the decomposition of oil. The value is also expressed as percent of free fatty acid calculated as oleic acid. Acid value and FFA% was calculated according to the methods described in IS 548: 1964 part - I method of Bureau of Indian standard.[8]

Oil was weighed accurately in a 250 ml conical flask and neutralizes with adding hot ethyl alcohol, 2 to 3 drops of phenolphthalein was added to this solution and titrate against KOH.

Calculation

AV= 56.1 * V * N/W

FFA% (in terms of oleic acid) = 28.2*V*N/W

Where,

V= Volume in ml of standard potassium hydroxide or sodium hydroxide solution used

N = Normality of standard potassium hydroxide solution.

W = Weight in g of the material taken for the test.

2.2.7. Carotenoid content estimation

Carotenoids belong to the category of tetraterpenoid (C_{40}), it exist as Hydrocarbons or oxygenated derivatives. Carotenoid content was determined by the method of (Harbone, J.B., 1973).[9] 0.1 g of fruit sample was treated with 10 ml of Acetone in mortar pestle, followed by centrifugation (Eppendorf centrifuge 5804 R) at 3000 rpm, supernatant was collected and its volume makes up to 10 ml and O.D. was taken at 480 nm.

Total Carotenoid (mg/g) = 4* OD value * total vol. of sample/wt. of plant tissue

2.2.8. Ascorbic acid estimation (Vitamin C)

Ascorbic acid also known as vitamin C is an antiscorbutic. Mostly present in all fresh fruits and vegetables. Vitamin C is a water soluble and heat labile vitamin. Ascorbic acid concentration was determined by volumetric estimation.[10] Ascorbic acid reduces the 2,6 dichlorophenol indophenols dye to a colourless leuco base. The ascorbic acid gets oxidized to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is an appearance of pink colour. Oxalic acid is used as the titrating medium.

2.2.9. FAME composition by GC MS

GC MS separates chemical mixture and identifies the components at a molecular level. The GC works on the principle that a mixture will separate into individual substances when heated, applicability of this instrument is only for volatile organic component so the esterification process was done to bring sample components into their vaporized form.

Sample Preparation: - 0.5 g of lipid sample was taken in a test tube and vortexed it with adding 5 ml of petroleum ether. After that 0.5% methanolic KOH was added to it and further vortex and mixed well. The reaction mixture passed into the water bath (maintained at 70°C) for 5 minutes. Upper layer was taken out in beaker and was evaporated till dry. 2 ml of n-hexane was added to residue and mixed well. This sample was sealed tightly in eppendorf vortex tube and stored at refrigerator for GC MS analysis.

Instrumentation

GC MS analysis was done at AIRF, JNU, New Delhi. Sample was placed in GC–MS QP-2010 model (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a CombiPAL

AOC-20i+s] autosampler (CTC Analytics, Zwingen, Switzerland). The compounds were separated on a Rxi®-5Sil-MS capillary column (30 x 0.25 mm ID and 0.25 µm film thickness). The carrier gas was helium, 16.3 mL/min, split ratio 10, injector temperature 260.00°C. The column oven temperature program used was: 50.0°C (hold for 4 min) to 280.0°C at 10°C/min hold for 23 min. The ion source temperature and interface temperature were set at 230.00 °C and 270.00 °C respectively and the MS mode was electron impact (EI). The compounds were separated by GC and further fragmented by Mass spectrometer and identified by comparing the mass spectra obtained with NIST14 and WILEY8.LIB from the US National Institute of Technology and Standards (NIST) mass spectra libraries. For calculating the total area percentage of peak and relative percentage, we considered the peaks which were repeatedly present in at least two TIC.

3. RESULTS AND DISCUSSIONS

3.1. Physicochemical parameters of the lipid

Oil percentage via soxhlet method is showing that the oil yield in *H. salicifolia* fruit was approximately 10.68%, which was the maximum from this region and it was also highest in case of *H. salicifolia*.[11] From the observation it can be concluded that the Hexane is appropriate solvent for extraction of oil from SBT. Moisture content was under the limit which does not influence very much on the other physicochemical parameters. Density and refractive index are also the specific stable parameters for the identification of the oil. Results of density and refractive index were as per the quality parameters. These parameters are also helpful for the quality of any product and the obtained result revealed the quality as per the reported literature.[12,13] Soluble sugars are almost present in every plant tissues, they are very important for living organism. They work as a primary precursor in the formation of many secondary metabolites such as Phenolics and Phytoalexins.[14] High sugar content also help in stress tolerance. In case of SBT fruit it contains 21.6% (in % Brix) of soluble sugar. Acid value is the measurement of the rancidity of the oil, higher the acid value more will be the formation of free fatty acids. In case of SBT fruit acid value 9.23 mg KOH/g and FFA% was 4.61(in terms of Oleic acid). Caretenoids are the pigments which help in photosynthesis and give a characteristic colour to the plant part; this pigment occurs in different chemical forms and has different functions. In case of SBT fruit Caretenoid content was 0.118 mg/g, due to this the obtained oil colour is red. Human being cannot produce Caretenoid itself so it should be taken in the diet therefore SBT can be a good source of food for intake the caretenoids. Ascorbic acid content was found 575mg/100g. It shows that SBT fruit has one of the highest

content of Vitamin C among other fruits such as Lemon and Amla. Ascorbic acid plays a vital role in enhancing the nutritional benefits of SBT fruit. The sour taste of SBT fruit is also due to the high content of ascorbic acid.

Table 1: Showing the Physicochemical parameters of the SBT fruit.

Sr. No.	Parameters	Unit	Observation
1.	Fruit Size	mm	5.28
2.	Colour of Fruit	-	Red
3.	Taste	-	Sour
4.	Oil	%	10.68
5.	Moisture	%	7.88
6.	Density At 15 ° C	Kg/m3	853
7.	Refractive Index	-	1.82
8.	Total Soluble Sugar	% Brix	21.6
9.	Acid Value	Mg KOH/g	9.23
10.	FFA	%	4.61
11.	Caretenoid Content	Mg/g	0.118
12.	Ascorbic Acid Content	Mg/100g	575

3.2. Chemical composition of *H. salicifolia* via GC MS study

GC MS analysis shows the presence of volatile matters such as long chain, branched, alcohols and various chemical compounds. In case of SBT fruit GC MS chromatogram confirmed the presence of 30 compounds, almost 15 compounds found in noticeable concentration (more than 1%).

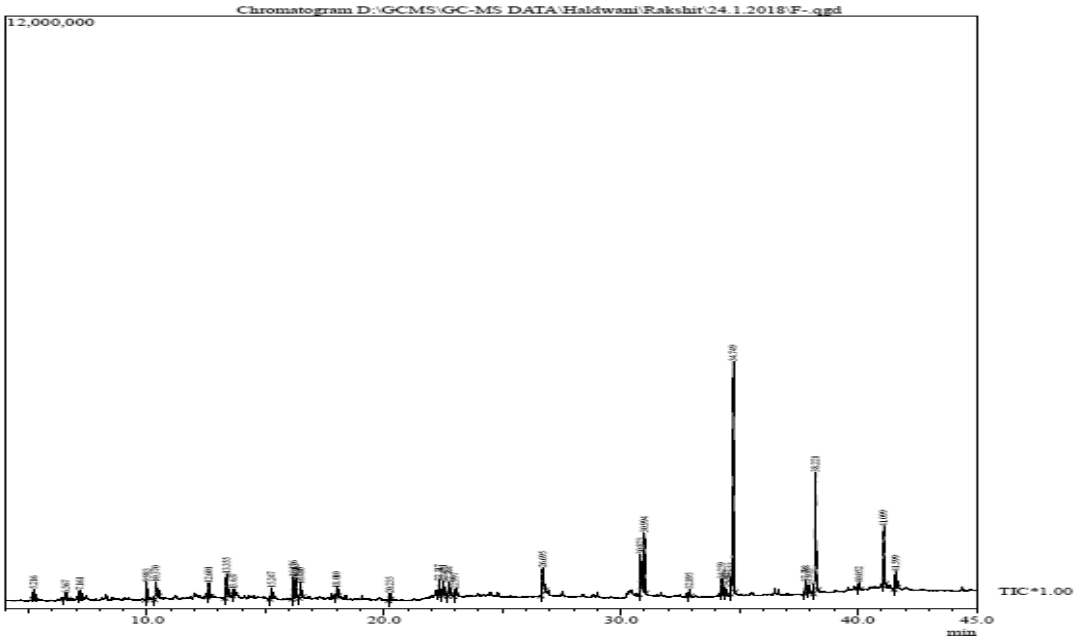


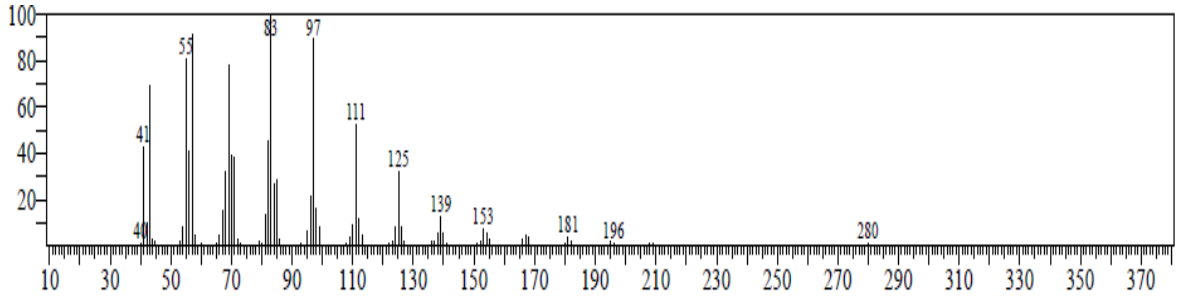
Fig: GC MS Chromatogram of *Hippophae salicifolia* seed showing retention time of different chemical compounds.

Table 2: Identified chemical compounds of SBT fruit via GC MS.

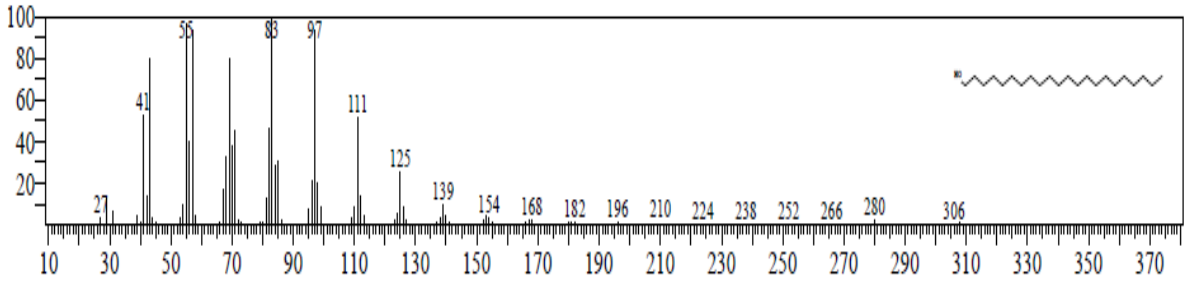
Sr. No.	Compound	Structural formula	Mol. weight	Weight %
1.	Tetracontane	$C_{40}H_{82}$	562	43.13
2.	Docosanol	$C_{22}H_{46}O$	326	10.50
3.	ß Sitosterol	$C_{29}H_{50}O$	414	7.54
4.	Tetrateracontane	$C_{44}H_{90}$	619	6.22
5.	3-Ethyl-4-hydroxydihydro-2(3H)-furanone	$C_6H_{10}O_3$	130	2.81
6.	3,7,11,15-tetramethyl-hexadecan-1-ol	$C_{20}H_{42}O$	298	2.81
7.	Eicosane	$C_{20}H_{42}$	282	2.52
8.	Hexadecane	$C_{16}H_{34}$	226	2.09
9.	6,10,14- trimethyl-2-pentadecanol	$C_{18}H_{38}O$	270	2.00
10.	Phytol acetate	$C_{22}H_{42}O_2$	338	1.99
11.	Phytol	$C_{20}H_{40}O$	296	1.99
12.	Elaidic acid methyl ester	$C_{19}H_{36}O_2$	296	1.52
13.	6,10,14-trimethylPentadecan-2-one	$C_{18}H_{36}O$	268	1.45
14.	Cis-linoleic acid methyl ester	$C_{19}H_{34}O2$	294	1.39
15.	1-chloro- Octadecane	$C_{18}H_{37}Cl$	288	1.00
16.	Others	-	-	17.35

Fragmentation pattern of nutraceutically important compounds found in SBT Fruit

1. Docosanol

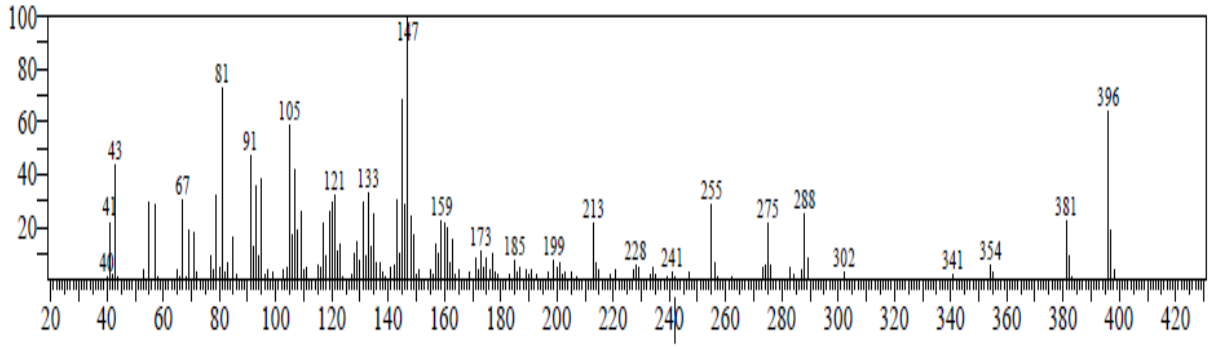


Fragmentation pattern found in esterified SBT oil.

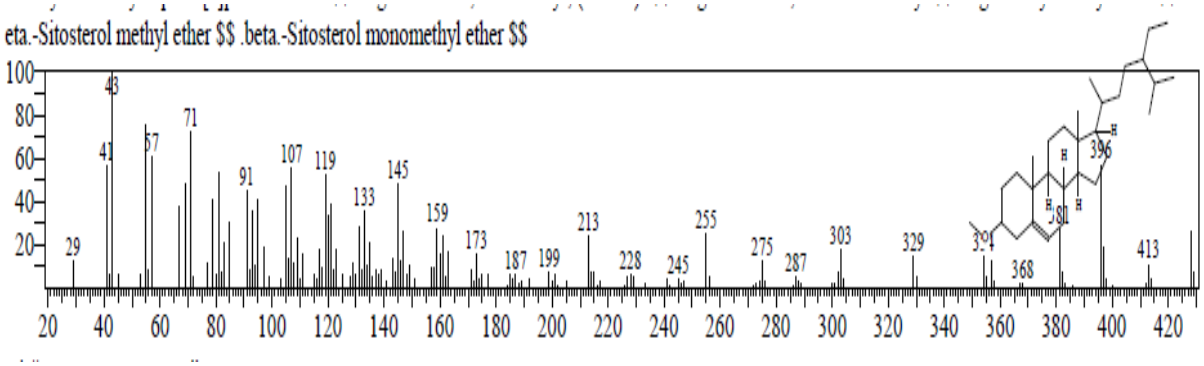


Docosanol (NIST library reference)

2. ß Sitosterol

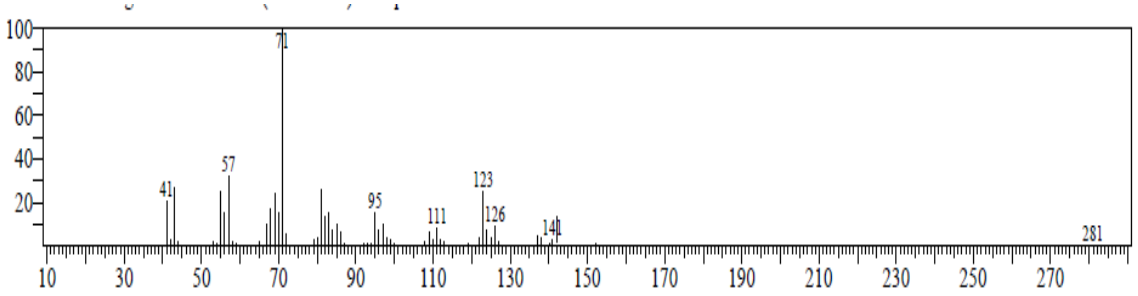


Fragmentation pattern found in SBT esterified oil.

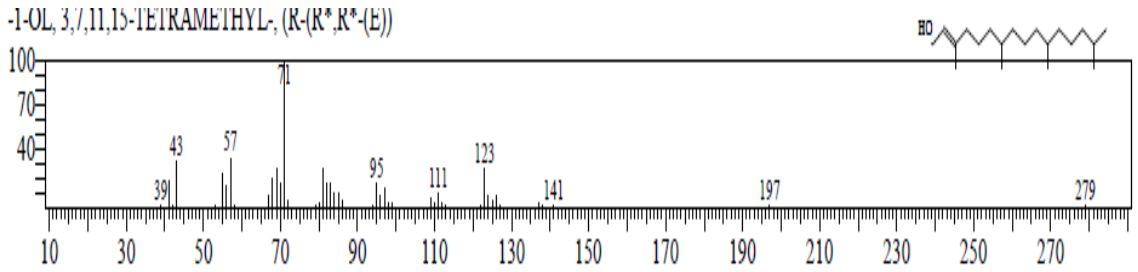


ß Sitosterol (NIST library reference).

3. Phytol

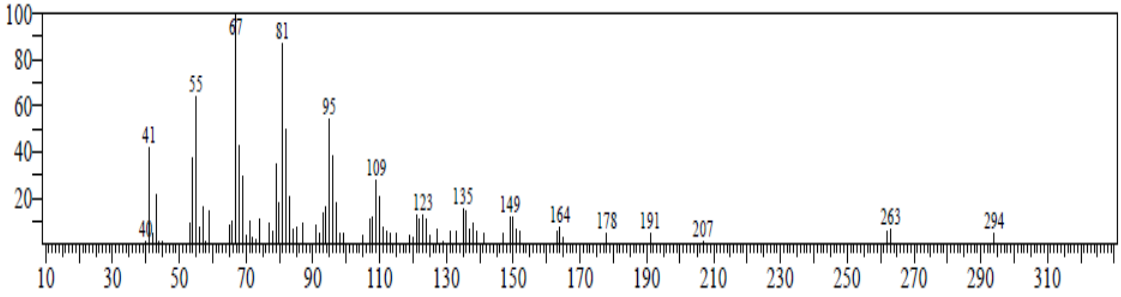


Fragmentation pattern found in SBT esterified oil.

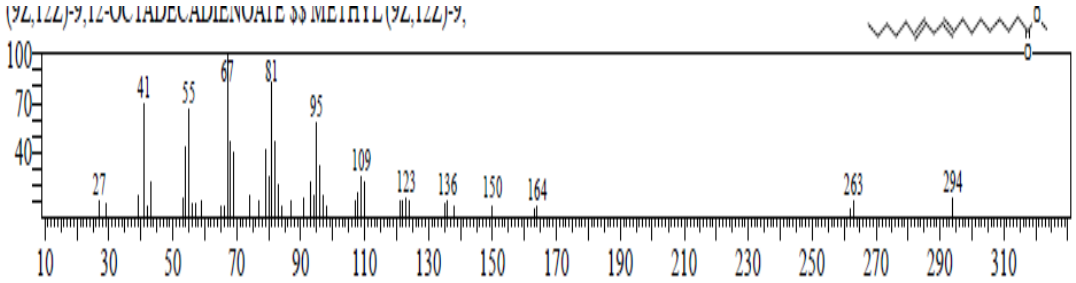


Phytol (NIST library reference)

4. Linoleic acid methyl ester



Fragmentation pattern found in SBT esterified oil



Linoleic acid methyl ester (NIST library reference)

The compound with highest concentration was tetracontane which 43%, it is higher alkane due to which viscosity of the SBT fruit oil is high. The presence of Docosanol is 10.5% which is a saturated 22 carbon alcohol with antiviral activity. It has a specific mode of action for inhibiting the fusion between plasma membrane and herpes simplex virus envelope, by which it help to prevent viral entry into cells.[15] Docosanol also help to reduce the pain and it heals sores fast. Another chemical compound which has been found in SBT fruit is ß Sitosterol (7.54%), also known as "plant sterol ester". This phytosterol is useful for the formation of various types of drugs. It is a dietary sterol and has the potential to prevent the human cancer.[16] It contains a double bond in its structure and susceptible to oxidation. It has distinct properties of anti – carcinogenic and anti-atherogenic properties**.** Phytol (1.99%) is one of the also very nutritional chemical components present in SBT fruit. Structurally, it is linear diterpene alcohol used in the preparation of Vit E and Vit K_1, also it is a decomposition product of chlorophyll. It is an oily liquid that is nearly insoluble in water, but soluble in most organic solvents. Cis-linoleic acid methyl ester (1.39%) is lipid-soluble form of linoleic acid. It is an essential fatty acid found in the western diet. The conjugate linoleic acid (CLA) shows positive effects on weight loss and increased the lean body mass as well as it is potential in improving the immune system of the body.[17]

3.3. CONCLUSION

There is no wonder that Sea buckthorn possess some extra potential and capability to meet the requirement of nutrition and health benefits for the human being. It has a wide scope as a remedy for various diseases. The oil of SBT fruit can be used as Cardiotonic, antioxidant, antifungal and anti inflammatory. It also shows the great potential as Vitamin C source. In the higher altitude of Himalaya SBT is cultivated and harvested by various agencies but there are very few reports are present regarding to the work on "*Hippophae salicifolia"* in Indian climatic condition. From the present investigation, it can be concluded that *H. Salicifolia* is rich in its antioxidant property and it has such bioactive components which can be used for the formulation of various drugs.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Narendra Singh Rawat, STA'B' for their support in the collection of seeds from the (Auli) Joshimath field station. Author is also highly thankful to Director DIBER- DRDO for providing logistic support and research facility for successful conduction of the research work. Financial assistance received by Rakshit Pathak from Defence research and Development Organization (DRDO), Ministry of Defence, New Delhi, India is duly acknowledged.

BIBLIOGRAPHY

1. Rajwant K, Rohtas S, Rai MK, Gyan P, Sharbati M, Singh R, Biotechnological interventions in sea buckthorn (*Hippophae* L.). Curr Status Future Prospects, 2011; 25: 559-75.
2. Lai, B., Ahuja, P.S. and Gupta, A.K. Application of Seabuckthorn in Amchi system of medicine. *In:* Proceedings of Intemational Workshop on Seabuckthorn, w.e.f. 18*"-21^' Feb., New Delhi, India, 2001.
3. Dwivedi SK., Parimelazhagan T., Singh SB., Ahmed Z. Seabuckthorn (*Hippophae* spp.): The golden bush. SSPH publication, 2009; 5-12.
4. Kaur T., Gurpreet Singh G., Kapoor D.N. A review on pharmacognostic, phytochemical and pharmacological data of various species of *Hippophae* (Sea buckthorn). International Journal of Green Pharmacy, 2017; 62-75.
5. Xiao Z. Chemical study on the flavonoids of *Hippophae rhamnoides*. *Acad J Sichuan Med Univ (Chinese),* 1980; 11: 174-176.

6. Ranjith A, Kumar SK, Venugopalan VV, Arumughan C, Sawhney RC, Fatty acids, tocols and caratenoides in pulp oil of three seabuchthorn species (*H. rhamnoides, H. salicifolia and H. tibetana*) grown in the Indian himalayas. *JAOCS*, 2006; 83: 359-384.
7. Soxhlet F. Die gewichtsanalytische bestimmung des michfettes. Dingler' poly technisches Journal, 1879; 232-461.
8. Indian Standard methods of sampling and test for oils and fats. IS: 548(Part1) 1964 Reaffirmed, 2010.
9. Harborne, J.B. Phytochemical Methods. Chapman and Hall Ltd., London, 1973; 49-188.
10. Sadashivam, S. and Theymoli Balasubramanian In: Practical Manual in Biochemistry Tamil Nadu Agriculture University, Coimbatore, 1987; 14.
11. Alam Zeb. Chemical and Nutritional constituents of Seabuckthorn juice. Pakistan Journal of Nutrition, 2004; 3(2): 99-106.
12. Lu, R. The chemical composition of *Hippophae* fruits in China. In: Proceedings of International Symposium on Seabuckthorn (*Hippophae rhamnoides* L.). Barnaul, Russia, 1993.
13. Mironov, V.A. Chemical composition of *Hippophae rhamnoides* of different populations of the USSR. In: Proc. International Symposium on Seabuckthorn (*H.rhamnoides* L.). Xian, China, 1989.
14. Nath K, Solanky KU, Mahatma MK, Madhubala, Swami RM Role of Total Soluble Sugar, Phenols and Defense Related Enzymes in Relation to Banana Fruit Rot by *Lasiodiplodia theobromae* [(Path.) Griff. and Maubl.] During Ripening. J Plant Pathol Microb, 2015; 6: 299.
15. Leung DT, Sacks SL. Docosanol: a topical antiviral for herpes labialis, Expert Opinion on Pharmacotherapy, 2004; 5(12): 2567-2571.
16. Catherine E. Ulbricht An Evidence-Based Systematic Review of Beta-Sitosterol, Sitosterol (22,23- dihydrostigmasterol, 24-ethylcholesterol) by the Natural Standard Research Collaboration, Journal of Dietary Supplements, 2016; 13(1): 35-92.
17. Rainer L., Heiss CJ. Conjugated linoleic acid: health implications and effects on body composition. Journal of the American Dietetic Association, 2004; 104(6): 963-968.