Isolation and identification of fatty acids from berries of sea buckthorn (*Hippophae rhamnoides*)

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Five fatty acids, 2-hydroxydecanoic acid, nona-7-enoic acid, undec-9-en-7-ynoic acid, 13-phenyltridecanoic acid and 5, 9, 21-nonacosatrienoic acid have been isolated and characterized from the ethanol extract of the sea buckthorn berries (*Hippophae rhamnoides*). The structure of new fatty acid namely, undec-9-en-7-ynoic acid (*AS-3*) has been elucidated by the spectroscopic techniques.

Keywords: *Hippophae rhamnoides*, Sea buckthorn, Berries, Elaeagnaceae, Fatty acids

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Sea buckthorn (*Hippophae rhamnoides*) is a temperate, hardy, nitrogen fixing, deciduous, thorny bush of the Elaeagnaceae family and is native to Europe and Asia, growing widely on slopes and river sides in the dry temperate high altitude regions. In recent years, sea buckthorn has become an important raw material of health products and cosmetics especially in China and Russia. Its oil is gaining more and more popularity as special food supplement and ingredients in Japan, Europe and North America. The high content of fatty acids, carotenoids, tocopherols and phytosterols are special characteristic of the oil from pulp and peel of the berries. Although many compounds such as vitamin-C, sugars, fatty acid, flavanoids, sterols and other active ingredients from the berries of sea buckthorn have been extensively investigated, but still large number of compounds have not been identified so far. As a part of our ongoing research programme on bioresources, we have investigated the chemical composition of sea buckthorn berries, leaves and roots, and attempted to isolate and identify the bioactive compounds. To the best of our knowledge and literature search reveals that not much work has been reported with respect to the chemical constituents of this plant growing in cold desert of north-eastern Himalayas of India. In view of the above, the chemical studies of *Hippophae rhamnoides* plant have become important and essential to understand the mode of action of the bioactive component present. In the present study, we have described the isolation and identification of five fatty acids present in the ethanol extract of *Hippophae rhamnoides* fruit (berry).

Results and Discussion

The sea buckthorn fatty acids profile reveals that it contains nearly 90% unsaturated fats. The fruit extract (oil) has low indispensable fatty acid contents, but in the same way as seed oil, it showed also some therapeutic effects. The fruit extract also showed protective efficacy against sulfur mustard – a potential chemical warfare agent. Berries were extracted with 95% ethyl alcohol, extract so obtained was concentrated in vacuo, defatted with hexane and dissolved in minimum quantity of ethanol. The mixture was treated with diazomethane essentially to convert all the acids if present, into their corresponding methyl esters. Control TLC of the known methyl esters was also carried out which proved unequivocally the presence of methyl esters of the fatty acids. GC analysis was also carried out. GC profile showed the presence of large number of compounds; however, a few of the methyl esters of fatty acids could be separated by column chromatography and finally the structures of methyl esters of five fatty acids were confirmed by IR, $^1$H NMR, $^{13}$C NMR and GC-MS. The fatty acids had satisfactory IR, NMR and MS data and were compared with authentic samples. The spectral data for *AS-3* are given in detail, as it has not been reported earlier. The structures of all the methyl esters of fatty acids are shown in Figure 1.

The IR spectrum of *AS-3* displayed strong carbonyl absorption at 1731 cm$^{-1}$. Absorption at 3030 and 3300 cm$^{-1}$ are characteristic to olefinic (=C-H) and acetylenic (=C-H) stretching. Similarly, the
absorptions at 1600 and 2100 cm\(^{-1}\) correspond to C=C and C≡C stretching. The \(^1\)H NMR spectrum of AS-3 gave resonance corresponding to 18 protons. The spectrum showed singlet due to methyl group of ester at \(\delta\) 3.36. Similarly, vinylic protons appeared between \(\delta\) 5.5 and 6.10. The vinylic and acetylic linkage at C\(_9\) and C\(_7\) have been further confirmed by \(^{13}\)C NMR and also by mass spectral studies. The \(^{13}\)C NMR spectra of AS-3 showed carbonyl absorption at 172 ppm, while the absorption at 90 and 77 ppm as well as 115 and 139 ppm clearly indicated the presence of acetylenic group (C≡C) at C\(_7\) and vinylic group (C=C) at C\(_9\), respectively.

GC-MS of all the methyl esters (AS-1 to AS-5) showed the peaks at m/z 43 and 59 corresponding to CH\(_3\)-CO\(^+\) and CH\(_3\)-O-CO\(^+\) ions, respectively. Since all the methyl esters were unsubstituted at a-carbon atom, the peak at m/z 74 was also observed and this was due to the McLafferty rearrangement. The GC-MS of AS-3 gave an ion (M\(^+\)+H) at m/z 194, the peaks at m/z at 153 and 41 indicated the presence of CH\(_2\)-CO-(CH\(_2\))\(_2\)-C≡C\(^+\) and CH\(_2\)-CH\(_2\)=CH\(^+\) fragments. The formation of both the fragments clearly confirmed the presence of C≡C at C\(_7\) and C=C groups at C\(_9\) in AS-3.

**Experimental Section**

**General.** GC was performed on Chemito GC 1000 model with flame ionization detector (FID). A capillary column BP10 packed with 14% cyanopropylpolyphenyl polysiloxane and 86% dimethylpolysiloxane coated on fused silica were employed. The injection port and detector block both were maintained at 250ºC and the column oven was at programmed temperature profile started at 50ºC, ramped up to 280ºC at 10ºC/min. Dinitrogen was used as a carrier gas (at a flow rate of 30 mL/min). Air for FID was supplied at 300 mL/min and dihydrogen at 30 mL/min. In all analysis, 1\(\mu\)L samples were injected and peaks recorded on a computerized data acquisition station. IR was recorded as neat on Shimadzu-440 Infrared spectrometer (\(\nu\), cm\(^{-1}\)). The GC-MS analysis was performed on Varian 3400 GC coupled to a TSQ 7000 mass spectrometer (Finnigan Mat.). \(^1\)H NMR was recorded on a Bruker (400 MHz) spectrometer (chemical shifts in \(\delta\), ppm) against TMS as internal standard. TLC was carried out on silica gel 60 to 120 mesh size.

**Plant Material**

Plant material was collected in the month of Feb. 2003 from the cold desert of north eastern Himalayas (Leh, Ladakh).

**Extraction and Isolation**

Dried berries (1 kg, taken without crushing so that extraction was done only with pulp and not with seeds) were extracted with ethanol under reflux condition using Soxhlet apparatus. Complete extraction could be achieved in 8 hr. The extract was
concentrated on rotatory evaporator at 40ºC. After concentration, dark red-brown coloured sticky crude (oil) material was obtained. The extract (163.7 g) was defatted\textsuperscript{7-10} with hexane at room temperature (400 mL × 4). The defatted extract (20 g) was taken and dissolved in minimum amount of ethyl alcohol (35 mL), solvent was decanted. The residue was washed twice with a mixture of hexane-ethyl alcohol (3:1 and 1:1). The white crystalline solid obtained gave a positive test for acid on reaction with NaHCO$_3$ and also a white spot was observed on silica TLC plate indicating the presence of acids in the mixture. IR spectra also showed the presence of carboxylic group (3528 cm$^{-1}$). The presence of acidic group was confirmed by converting the acid into methyl ester by reacting it with diazomethane (prepared by the reaction of N-nitroso-N-methyl urea and KOH). After the complete work up, the mixture was subjected to column chromatography over silica gel. Five fatty acids AS-1-AS-5 were isolated with the following eluents mixture: benzene:methanol (99:1), (b) benzene:methanol (99:2), (c) benzene: methanol (95:5), (d) benzene:methanol (90:10) and (e) benzene:methanol (80:20), respectively. The structures of all esters were conformed by IR, $^1$H NMR and GC-MS.

**Spectral details of AS-3:** IR (KBr): 3030, 3300, 1731, 1600, 2100, 1224 cm$^{-1}$; $^1$H NMR (CDCl$_3$): $\delta$ 15.8 (CH$_3$), 17.7 (CH$_2$), 24.5 (CH$_2$), 28.1 (CH$_2$), 29 (CH$_2$), 35 (CH$_2$), 56 (CH$_3$-O-), 77, 90 (-C=C-), 114 and 138 (-C=C-), 172 (-C=O).

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**References**