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Estimation of free fatty acids, iodine value and saponification value of sea oil and its FTIR study

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Abstract

Sea buckthorn oil is red-orange oil derived from sea buckthorn plants. Oil content in seeds of sea buckthorn is on average 7–11 % while oil content of the fruit pulp is around 1.5–3%. Seed oil is characterized by high contents of polyunsaturated fatty acids while pulp oil contains monounsaturated fatty acids and carotenoids. Both oils also contain dense amounts of tocopherols, tocotrienols and plant sterols. Present Paper deals with Estimation of Free Fatty acids, Iodine value and Saponification value of Sea Buckthorn Oil and FTIR study of Sea Buckthorn Oil. Fat or oil contains small quantity of free fatty acids. On storing, the free fatty acid contents of the fat or oil increases. The free fatty acid contents are determined by direct titration of fat or oil with standard KOH solution. The acid value is defined as the numbers of milligrams of KOH required to neutralize the free fatty acid present in 1 g of the fat or oil. The iodine value of oil is the number of grams of iodine taken up by 100 g of the oil. It is determined by reacting a known volume of excess solution of iodine monochloride in acetic acid (Wij's solution) with oil and then back titrating unreacted iodine with Sodium thiosulphate solution. Saponification value of an oil or fat is defined as the number of milligrams of Potassium Hydroxide required to hydrolyse (saponify) one gram of oil completely. A known amount of oil is refluxed with excess amount of standard alcoholic potash solution and the unused alkali is titrated against standard acid solution using phenolphthalein as an indicator.

Keywords: Sea Buckthorn Oil, Estimation of Free Fatty acids, Estimation of Iodine value, Estimation of Saponification value, FTIR study.

Introduction

Sea buckthorn oil is red-orange oil derived from sea buckthorn plants. The most commonly used species for this purpose is Hippophaerhamnoides. Species belonging to this genus accumulate lipids in the mesocarp (the fruit pulp), so the oil can be extracted from either the seeds or the pulp. The resulting oils (seed oil and pulp oil, also called fruit or berry oil) are used in dietary supplements, nutraceuticals, cosmetics and skin care products.

Oil content in seeds of sea buckthorn is on average 7–11 % while oil content of the fruit pulp is around 1.5–3% (per fresh weight). Seed oil is characterized by high contents of polyunsaturated fatty acids while pulp oil contains monounsaturated fatty acids[1] and carotenoids[2]. Both oils also contain dense amounts of tocopherols, tocotrienols[3] and plant sterols[4].

Oils from sea buckthorn seeds and pulp differ considerably in fatty acid composition. While linoleic acid and α-linolenic acid are the major fatty acids in seed oil, sea buckthorn pulp oil contains approximately 65% combined of the monounsaturated fatty acid, palmitoleic acid, and the saturated fatty acid, palmitic acid[1]. This results in a major difference between the sea buckthorn oil extracted from seeds and the sea buckthorn oil extracted from the fleshy part of the fruit, in term of appearance and consistency. Sea buckthorn fruit oil is dark orange in color and has a thick consistency (it is liquid at room temperature, but becomes much thicker if refrigerated), whereas the seed oil is pale yellow and does not solidify under refrigeration.

Few other vegetable oils contain a similar quantity of these fatty acids. The high proportion of unsaturated fatty acids are also responsible for the relatively poor shelf life, as they cause sea buckthorn oil to turn rancid quickly[5]. α-Tocopherol is the major vitamin E compound in sea buckthorn[6]. Seed oil also contains considerable amounts of gamma-tocopherol. The total amount of tocopherols and tocotrienols is roughly 64–300mg/100g in seed oil and 100–481mg/100g in pulp oil[3].

As carotenoids are the pigments that give sea buckthorn berry its distinctive orange-red color, these compounds are present in considerable amounts both in pulp oil and in seed oil; the average carotenoid content of pulp oil is 350 mg per 100 grams, as compared to 67.5 mg per 100 grams in seed oil. The total content of carotenoids in pulp oil varies (300–2000 mg/100 g) greatly between different growth locations and subspecies[7], and between components, where total carotenoids were up to 85 mg/100 g in seed oil, and up to 1000 mg/100 g in pulp oil. In general, the main carotenoids present in pulp oil are beta-carotene, zeaxanthin and lycopene[2].

Both seed and pulp oil also contain considerable amounts of plant sterols (12–23 g/kg and 10–29 g/kg of oil, respectively). Beta-sitosterol is the major sterol compound throughout the berry which constitutes 57–83% of total sterols[4]. Sea buckthorn oil is frequently used in cosmetic products, especially topical products marketed for aging skin and dry skin[5]. Sea buckthorn oil is used in some shampoos and other hair care products. It has sometimes been used as a type of topical medication for skin disorders[8] although there is no high-quality research proving effectiveness or safety of any such uses. Taking sea buckthorn oil as a dietary supplement does not help with eczema[9]. In a few East Asian countries, the fruit pulp oil is put on skin burns[5].

Present Paper deals with Estimation of Free Fatty acids, Iodine value and Saponification value of Sea Buckthorn Oil and FTIR study of Sea Buckthorn Oil.

Methodology

Estimation of Free Fatty acids of Sea Buckthorn Oil: Fat or oil contain small quantity of free fatty acids. On storing, the free fatty acid contents of the fat or oil increases. The free fatty acid contents are determined by direct titration of fat or oil with standard KOH solution. The acid value is defined as the numbers of milligrams of KOH required to neutralize the free fatty acid present in 1 g of the fat or oil.

$RCOOH + KOH \rightarrow RCOOK + H_2O$

Preparation of 0.1 N Oxalic Acid: 0.63 g of oxalic acid was dissolved in 100ml of distilled water.

Preparation of 0.1 N Potassium Hydroxide: 0.56 g of KOH was dissolved in 100 ml of distilled water.

Standardization of 0.1 N KOH: 10 ml of oxalic acid was taken in 100ml of volumetric flask and 2-3 drops of phenolphthalein indicator was added. Then titrated with 0.1 N KOH. At end point it turns colourless to pink.

Titration of Sea Buckthorn Oil: 1gm of Sea Buckthorn Oil was taken in 100 ml of conical flask, 5ml alcohol and 2-3 drops of phenolphthalein indicator was added. Then titrated with 0.1 N KOH. At end point it turns colourless to pink.

Estimation of Iodine value of Sea Buckthorn Oil: The iodine value of oil is the number of grams of iodine taken up by 100 g of the oil. It is determined by reacting a known volume of excess solution of iodine monochloride in acetic acid (Wij's solution) with oil and then back titrating unreacted iodine with Sodium thiosulphate solution.

Preparation of 0.1 N Sodium thiosulphate: 2.48 g of sodium thiosulphate was dissolved in 100 ml of distilled water.

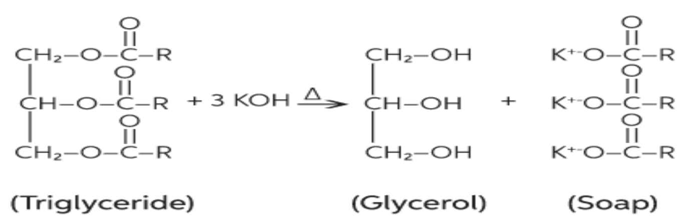
Preparation of 0.1 N $CuSO_4$: In 100 ml volumetric flask 2.49 g of $CuSO_4$was taken and dissolved in distilled water and to it 3-4 drops of concentrated $H_2SO_4$was added and volume was made up to 100 ml with distilled water.

Standardization of Sodium thiosulphate: 10ml Standard $CuSO_4$ was taken in 100ml of conical flask and then NH_4OH was added till turbidity appeared. Then dilute acetic acid was added till the turbidity vanished. And 10 ml 10% of KI was added. Then it was titrated with sodium thiosulphate till pale yellow colour appeared. Then 1 ml of starch solution was added and titration was continued with sodium thiosulphate, till blue colour converted into white, then pinch of ammonium thiocyanate was added, blue colour was appeared then again titrated with Sodium thiosulphate till the blue colour turned into white.

Titration of Sea Buckthorn Oil: 1g oil, 20ml of Carbon tetrachloride and 30ml Wij's solution was taken in 250ml of conical flask and stoppered the flask. It was then shaken and allowed it to stand in the dark for 1 hour with occasional shaking.10ml of 10% KI, 60ml of distilled and 2-3 ml of freshly prepared starch solution was added and then titrated with Sodium thiosulphate till the colour disappeared. Noted the end point.

Blank Titration: Blank titration was performed without taking oil. Noted the end point.

Estimation of Saponification value of Sea Buckthorn Oil: Saponification value of an oil or fat is defined as the number of milligrams of Potassium Hydroxide required to hydrolyse (saponify) one gram of an oil completely. A known amount of oil is refluxed with excess amount of standard alcoholic potash solution and the unused alkali is titrated against standard acid solution using phenolphthalein as an indicator. The reaction is as follows:



$$2KOH + H_2C_2O_4 \rightarrow K_2C_2O_4 + 2H_2O$$

Preparation of 0.5 N Oxalic acid: 3.15 g of the Oxalic acid was dissolved in 100 Distilled water.

Saponification: 1g of given Sea Buckthorn Oil was taken in a 250 ml conical flask. To it 10 ml of 0.5 N KOH solution was added. Air condenser was fitted to the conical flask. The reaction mixture was refluxed on a boiling water bath for half an hour. The resulting solution was cooled and titrated it against standard oxalic acid solution. Noted the end point.

Blank Titration: Titration was performed using blank solution (without oil) and noted the end point.

FTIR Analysis: FTIR can be routinely used to identify the functional groups. FTIR spectra of Sea Buckthorn Oil was obtained at room temperature by using an FTIR Spectrophotometer - Shimadzu - IR Affinity – 1. The spectra is collected in range from 350 - 4700 cm-1.

SEM Analysis: The Electron Microscope is an essential component for scientific analysis of a variety of materials. Scanning Electron Microscope (SEM) comprises a powerful tool in studying (cell and molecular biology, anatomy, microbiology, pathology and forensic science) biological specimens, food stuffs and several other areas of material sciences (electronics, metallurgy, polymer and surface science). Morphological graphs of the Sea Buckthorn Oils provided by scanning electron microscopy (Digital Scanning Electron Microscope - JSM 6100 - JEOL) with a Link analytical system operating at 10 KV (acceleration voltage).

Results and Discussion

Estimation of Free Fatty acids: is as follows:

Table-1: Standardization of KOH.

Volume of oxalic acid taken	Volume of 0.1N KOH required	Constant reading V_1
10ml	10 ml	10 ml
10ml	10ml	
10ml	10 ml	

Table-2: Titration of Sea Buckthorn Oil.

Volume of alcohol	Volume of 0.1N KOH	Constant reading V_2
5 ml	1 ml	1 ml
5 ml	1 ml	
5 ml	1 ml	

Normality of oxalic acid (N_1) = $\frac{Weight\ of\ Oxalic\ Acid \times 10}{63} = \frac{0.63 \times 10}{63} = 0.1$

Normality of potassium hydroxide (N_2) = $\frac{N1 \times 10}{V1} = \frac{0.1 \times 10}{10} = 0.1$

1litre 1N KOH ≡ 56g KOH

V_2 ml N_2 NKOH ≡ $\frac{V2 \times 56}{1000} \times \frac{N2}{1}$gKOH

≡ $V_2 \times 56 \times N_2$ mg KOH

≡$1 \times 56 \times 0.1$ mg KOH

≡5.6 mg KOH

5.6 mg KOH is required to Neutralize the free fatty acid present in 1g of oil. Hence the acid value of Sea Buckthorn Oil is 5.6.

Estimation of Iodine value: As follows:

Table-3: Standardization of Sodium thiosulphate.

Volume of $CuSO_4$	Vol. of Sodium thiosulphate	Constant reading V_1
10 ml	10 ml	10 ml
10 ml	10 ml	
10 ml	10 ml	

Table-4: Titration of Sea Buckthorn Oil.

Sea Buckthorn Oil	Vol. of Sodium thiosulphate V_2
1 g	12.0 ml

Table-5: Blank Titration.

Sea Buckthorn Oil	Vol. of Sodium thiosulphateV_3
0 g	12.3 ml

Normality of $CuSO_4$ Solution (N1) $= \frac{2.49 \times 10}{249.5} = 0.1$

Normality of Sodium Thiosulphate Solution (N_2) = $\frac{N1 \times 10}{V1} = \frac{0.1 \times 10}{10} = 0.1$

Iodine Value = $\frac{(V3-V2) \times N2 \times 127 \times 100}{Wt\ of\ Sample \times 1000} = \frac{(12.3-12) \times 0.1 \times 127 \times 100}{1 \times 1000} = 0.381$

Hence the Iodine value of Sea Buckthorn Oil was found to be 0.381.

Estimation of Saponification value: Volume of 0.5 N Oxalic acid used for blank Titration (V_1) = 9 ml, Volume of 0.5 N Oxalic acid used for Sea Buckthorn Oil Titration (V_2) = 7 ml.

Normality of Oxalic Acid = $\frac{Wt\ of\ Oxalic\ acid\ X10}{63} = \frac{3.15\ X\ 10}{63} = 0.5$

Volume of Oxalic Acid used = (V_1-V_2) = (9-7) = 2 ml

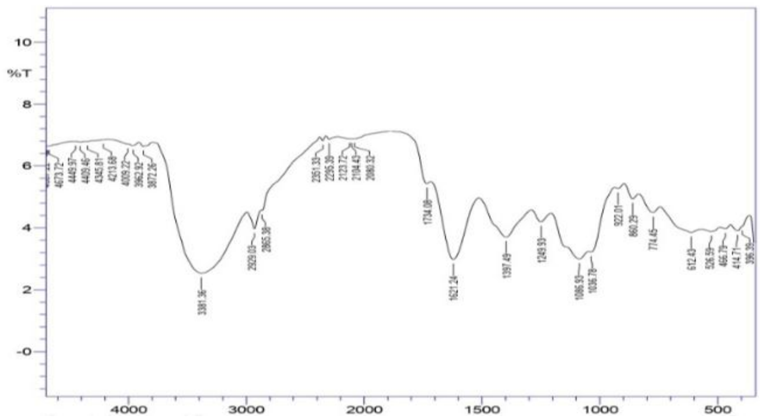
1liter of 1 N Oxalic Acid≡ 56g KOH

Hence (V_1-V_2) ml of 0.5N Oxalic acid≡ $\frac{56\ (V1-V2) X\ 0.5}{1000\ X\ 1}$

≡ $\frac{56 \times (9-7)\ X\ 0.5}{1000 \times 1} =$ 0.056 g KOH = 56 mg KOH

56 mg KOH is required to saponify 1 g Sea Buckthorn Oil. Therefore, Saponification value of Sea Buckthorn Oil was found to be 56.

SEM Analysis: Scanning Electron Microscope image of Sea Buckthorn Oil shows that the material mainly consisted of spherical particles with 1-10 µm in diameter, and has a smaller aggregated particle size.



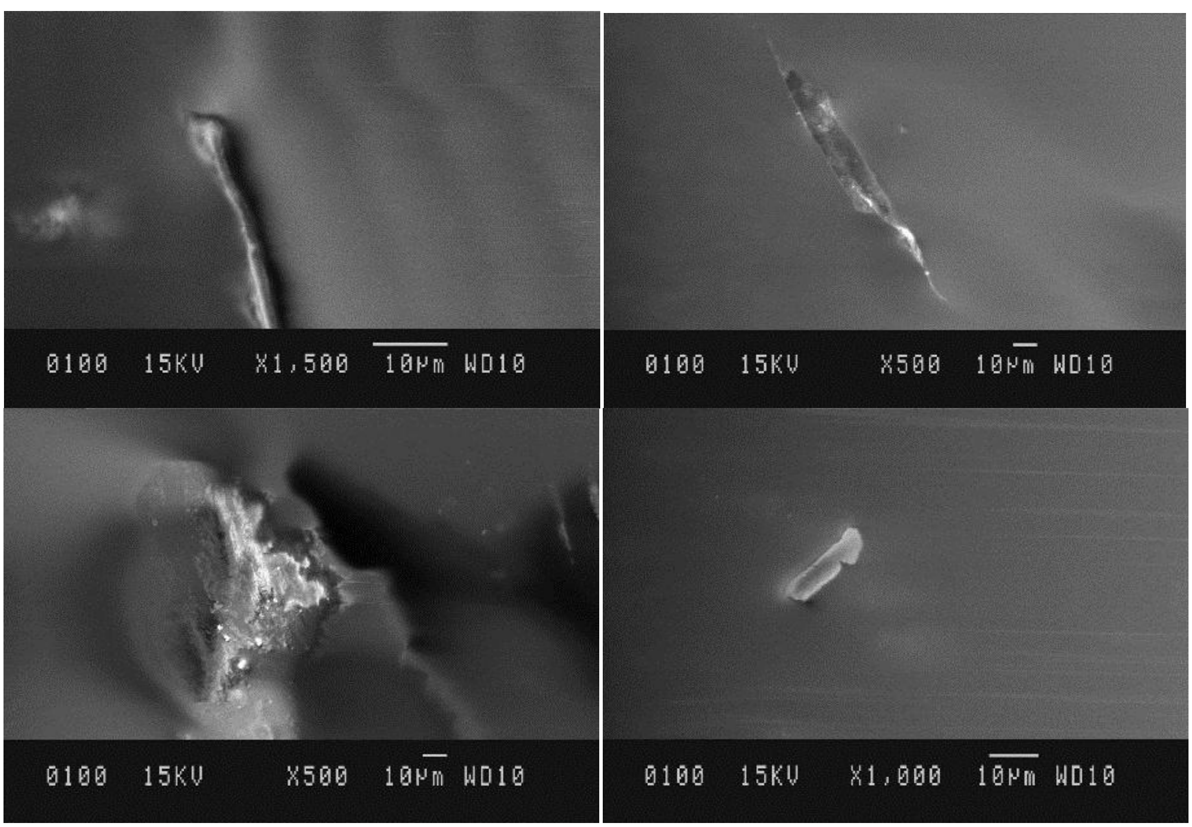
No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	396.39	4.0448	0.0119	397.35	367.46	41.0719	0.0317
2	414.71	3.9072	0.1607	444.61	397.35	66.1633	0.4825
3	466.79	3.9729	0.0914	488.01	445.58	59.2617	0.2362
4	526.59	3.8828	0.1077	569.03	488.98	112.5131	0.5111
5	612.43	3.8526	0.2797	743.59	569.99	240.9002	3.6843
6	774.45	4.4942	0.3215	835.21	744.55	120.3354	1.4259
7	860.29	4.9376	0.2886	895.97	836.18	77.2515	0.7414
8	922.01	5.2731	0.077	936.48	896.94	50.3581	0.1321
9	1036.78	3.2213	0.2132	1047.39	937.44	151.4443	0.9938
10	1086.93	2.9913	0.5457	1206.53	1048.36	231.8163	7.2173
11	1249.93	4.1933	0.334	1290.43	1207.49	112.8532	1.3476
12	1397.49	3.7005	1.0641	1512.26	1291.4	304.0061	12.095
13	1621.24	2.9763	2.2672	1716.72	1513.22	283.4848	22.5271
14	1734.08	5.4302	0.2151	1888.39	1717.68	201.9114	0.3748
15	2080.32	6.8804	0.0078	2086.1	1889.36	227.2271	0.0986
16	2104.43	6.8764	0.0028	2117.93	2087.07	35.8785	0.0028
17	2123.72	6.8774	0.0033	2225.95	2118.9	124.2485	0.0251
18	2295.39	6.8613	0.0994	2323.36	2226.91	111.9179	0.2713
19	2351.33	6.8197	0.128	2375.44	2324.32	59.4018	0.2056
20	2865.38	4.5664	0.0312	2871.17	2376.4	607.557	0.0444
21	2929.03	3.9684	0.5716	2992.69	2872.13	164.8822	2.9629
22	3381.36	2.5329	3.0662	3794.14	2993.65	1145.670	138.7752
23	3872.26	6.6311	0.1316	3909.88	3795.11	134.8033	0.5365
24	3962.92	6.6573	0.0747	4003.43	3910.84	108.7218	0.2447
25	4009.22	6.7076	0.0048	4173.17	4004.39	197.0884	0.0124
26	4213.68	6.8461	0.0004	4214.64	4174.14	47.1531	0.0024
27	4345.81	6.791	0.0109	4368.96	4215.61	178.8773	0.044
28	4409.46	6.7755	0.0185	4447.08	4369.92	90.1494	0.0405
29	4449.97	6.7937	0.0003	4463.47	4448.04	18.0209	0.0002
30	4673.72	6.6411	0.0076	4683.37	4464.44	256.6155	0.0122
31	4687.22	6.6419	0.0001	4690.12	4684.33	6.815	0
32	4695.9	6.6418	0.0003	4700.73	4691.08	11.3584	0.0001

Figure-1: FTIR spectra of Sea Buckthorn Oil.

Table-6: Interpretation of IR Spectra of Sea Buckthorn oil.

Spectra region wave number cm^{-1}	Intensity and Pattern of peak	Bond causing Absorption	Compound Class
4695.9	Weak and Broad	-	-
4687.22	Weak and Broad	-	-
4673.72	Weak and Broad	-	-
4449.97	Weak and Broad	-	-
4409.46	Weak and Broad	-	-
4345.81	Weak and Broad	-	-
4213.68	Weak and Broad	-	-
4009.22	Weak and Broad	-	-
3962.92	Weak and Broad	-	-
3872.26	Weak and Broad	-	-
3381.36	Strong and Broad	O-H Stretching	Alcohol
2929.03	Medium and Sharp	C-H Stretching	Alkane
2865.38	Weak and Broad	C-H Stretching	Alkane
2351.33	Weak and Broad	-	-
2295.39	Weak and Broad	-	-
2123.72	Weak and Broad	C≡C Stretching	Alkyne
2104.43	Weak and Broad	C≡C Stretching	Alkyne
2080.32	Weak and Broad	-	-

1734.08	Medium and Broad	-	-
1621.24	Strong and Broad	C=C Stretching	α, β – unsaturated ketone
1397.49	Medium and Broad	O-H Bending	Carboxylic acid, Alcohol
1249.93	Weak and Broad	-	-
1086.93	Strong and Broad	C-O Stretching	Primary alcohol, Secondary alcohol
1036.78	Strong and Broad	S=O Stretching	Sulfoxide
922.01	Weak and Broad	-	-
860.29	Weak and Broad	-	-
774.45	Weak and Broad	-	-
612.43	Weak and Broad	-	-
526.59	Weak and Broad	-	-
466.79	Weak and Broad	-	-
414.71	Weak and Broad	-	-
396.39	Weak and Broad	-	-



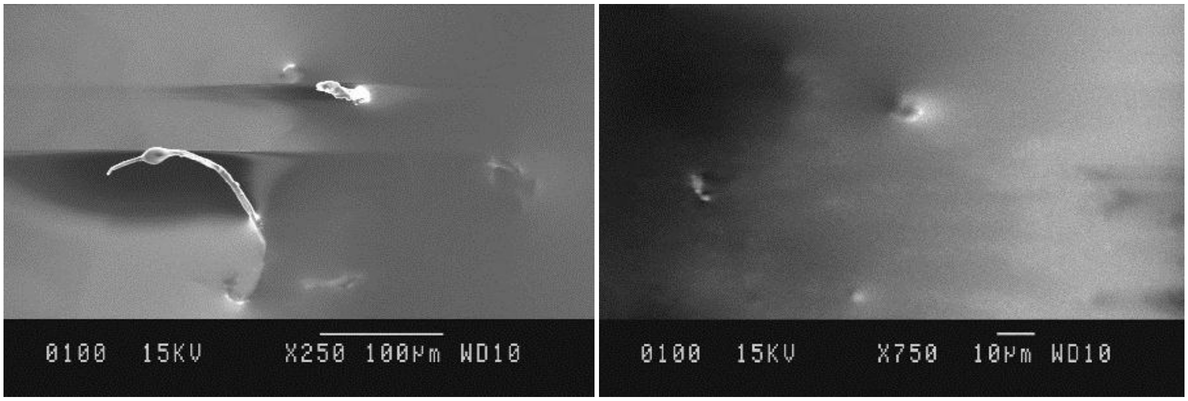


Figure-2: Scanning Electron Microscope images of Sea Buckthorn Oil.

Conclusion

The acid value of Sea Buckthorn Oil is 5.6.The Iodine value of Sea Buckthorn Oil was found to be 0.381 and Saponification value of Sea Buckthorn Oil was found to be 56.

Interpretation of FTIR Spectra of Omega-3 Salmon Oil shows presence of various functional groups such O-H Stretching – Alcohol; C-H Stretching – Alkane; C≡C Stretching – Alkyne; C=C Stretching - α, β – unsaturated ketone; O-H Bending - Carboxylic acid, Alcohol; C-O Stretching - Primary alcohol, Secondary alcohol; S=O Stretching – Sulfoxide.

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