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HPLC and GC-MS analysis of *Boswellia serrata* gum different extracts and evaluation of their antioxidant activity

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Abstract:-

Boswellia serrata is a tree that variant in size from moderate to large, this tree is native to Asian countries, consumed as a traditional food and play role in pharmaceutical cosmetics industries. The current study examined the anti-oxidant activity for the Gum of plant *Boswellia serrata*, extracted by employing the following: water, methanol, n-hexane and petroleum ether. The extraction process done using large size glass petri dishes followed by draying using oven at 70 ^{0}C on the first time but then soxhlet extractor has been employed at NLT 70 c^0 for 2 hours to avoid the loss in the materials and time which also followed by filtration using Gauze as pre filter then Whatman filter paper was employed. Qualitative chemical examinations were carried out for all of the extracts to recognize a variety of the main constituents for instance alkaloid, flavonoid, phenolic, glycoside, carbohydrate, tannin, phytosterol, fixed oil, protein, saponin, etc. Those extracts then went quantitative and qualitative apparatus used for further investigation such as TLC, HPLC and GC-Ms. The antioxidant activity of the extracts was determined using DPPH free radical savaging method by comparing the extracts to ascorbic acid as a standard using UV-VIS spectrophotometer at 517 nm. The highest IC50 value was observed for the extract of *methanol extract* (5882.35 µg/ ml) indicating the lowest DPPH radical scavenging activity. The lowest IC50 value was observed for the extract of *Petroleum ether* (458.30 µg/ ml) indicating the highest DPPH radical scavenging activity. IC50 values for *Hexane and Aqueous Extract* were (843.17 and 4000 µg/ ml) respectively. The IC50 values of all the extracts were greater as compared to that of ascorbic acid (7.27 µg / ml), indicating that extracts have low DPPH radical scavenging activity.

Keywords: *Boswellia serrata, TLC, HPLC and GC-Ms, antioxidant*

INTRODUCTION:-

Boswellia Serrata is a gum resin taken from a tree, which is sometimes scald (the entire species of Boswellia is usually known as Frankinsence) as aromatic its administered as alternative medicine. It was mostly used as a therapy in the Ayurvedic medicine (Asaad, and Alhomoud,2016). The antioxidant and anti-inflammatory effectiveness of *Boswellia*, unlike several anti-inflammatory chemical medicines, does not cause any kind of adverse effects on blood pressure, or side effect such as heart rate, respiration, beside other autonomic responses, and the resin possesses uncommonly low levels of toxicity (Pollastro, *et al.,* 2016). The gum resin of *Boswellia* was added to the list of safe compounds and its use is approved by US Food and Drug Administration (USFDA) as a food additive (Raja, *et al.,* 2011). *Boswellia* serrata is also classified as a phytopharmaceutical by (H15; Europe) which was revealed to be a significant anti-inflammatory, helpful against inflammation according to initiatory evidence for anti-inflammatory joint disorders (Woolley, *et al.,* 2012). Therapeutic potential of plants is mainly because of the presence of some bioactive mixes. between different kinds of bioactive compounds poly phenols are antioxidants responsible for the reducing or prevention of chronic diseases and health care (Yasuda, *et al.,* 2013). These Antioxidants are essential substances because they can shield the body from the free radicals that could cause some damage. Antioxidants exert their effect by scavenging the present of possible free radicals (i.e. reactive oxygen species (ROS) or reactive nitrogen species) generally present in organic frameworks (Devi, *et al.,* 2012). In order to investigate the importance of such non-steroidal future drugs, the researcher along with the guide decided to study the *Boswellia* serrata various crude extracts antioxidant activity using DPPH free radical savaging method. To achieve such a purpose, *Boswellia serrata* needs to undergo quantitative and qualitative tests to evaluate all of the extracts, then The antioxidant activity of the extracts was determined by employing DPPH free radical savaging method which compare *Boswellia Serrata* gum extracts to ascorbic acid as a standard using UV-VIS spectrophotometer at 517 nm.

MATERIALS AND METHODS:-

1- Extraction of Natural Plant Products:

Medicinal plants produce a variety of secondary metabolites each with a different functional group. Many approaches can be performed to extract the Boswellic acid which is plant material. Although water (Universal Solvent) is used as an extractant in many traditional protocols. Organic solvents of different polarities are selected in regular methods of extraction to achieve various solubility of phytochemicals. Solvent extraction procedures applied to plant natural products include maceration, percolation, soxhlet extraction, grinning using multifunctional house blender and draying oven.

2- Boswellia Serrata Gum Extracts

Procurement of material: The Gum of the plant *Boswellia serrata* were purchased from Herbal Store located at al saadoon street Baghdad, first extraction trail done manually using large size petri dishes but it consume a lot of time and materials this trial at plant biotechnology lab – department of biotechnology / university of Baghdad then process done at authors personal lab using soxhlet extractor.

3- Methodology:

The study includes phytochemical, TLC (qualitative, quantitative and HPLC) analysis of gum extracts and GC-MS studies (for identification of novel medicinal compounds) of *B. serrate*

4- Preliminary Phytochemical evaluation

All the extracts were screened using phytochemicals for the existence of different secondary metabolites such as Alkaloids, Tannins, flavonoids, Glycosides, Terpenoids, and proteins using regular phytochemical methods.

5- Qualitative chemical investigation of extracts

Qualitative compound examinations are carried out in favor of every extracts to recognize a variety of chief plant constituents approximating alkaloid, glycoside, carbohydrate, phenolic, tannin, phytosterol, fixed oil, fat, protein, amino acid, flavonoid, saponin, etc.

6- TLC Analysis

TLC of alkaloids Samples of the powdered gum extracted with all extraction method tested for alkaloid using a mixture of methanol and chloroform (1:15) .

TLC of flavonoids

Samples of the powdered gum extracted with all extraction method tested for flavonoid using a mixture of methanol and chloroform (1:19) .

TLC of phenols

Samples of the powdered gum extracted with all extraction method tested for flavonoid using a mixture of of methanol and chloroform (0.3:27) .

TLC of glycosides

Samples of the powdered gum extracted with all extraction method tested for glycoside using a mixture of water, methanol and Ethyl acetate (10:10:80) .

TLC of saponins

Samples of the powdered gum extracted with all extraction method tested for saponins using a mixture of water, methanol, glacial acetic acid and chloroform (8:12:34:64)

TLC of sterols

Samples of the powdered gum extracted with all extraction method tested for saponins using a mixture of water, methanol, glacial acetic acid and chloroform (8:12:34:64)

The color and Rf values of these spots were recorded under UV inspection light.

7- Estimation by High-Performance Liquid Chromatography (HPLC)

7.1 Preparation of stock solution of standard BSE:

Accurately weighed 50 mg standard BSE powder were dissolved in 25 mL of methanol to get a BSE stock solution.

7.2 Preparation of sample solution for analysis:

Accurately weighed 50 mg of BSE powder which were dissolved in 25 mL of solvent (depending on the type of extract) to get a BSE sample solution.

Chromatographic conditions:

The mobile phase consisted of acetonitrile–water (90:10, % v/v) adjusted to pH 4 with glacial acetic acid. Samples were analyzed using the following parameters: flow rate, 0.5 mL/min; injection volume, 20 µL; run time 18 min; temperature, 27 ± 2ºC; detection wavelength, 260 nm.

8- Identification of novel compounds in the gum extracts by Gas chromatography and Mass spectroscopy (GC-MS)

Analytical GC-MS system consisted of a GC-2010 (ST-EQ-052) gas chromatograph and a mass selective detector GC-MS-QP 2010 plus. Capillary column (50 miters, thickness - 0. 32 mm, diameter - 0.5µm).

GC oven temperature programmed at 80 C°for2 hrs. and increased to 250 C° at the rate of 3 C°/min. then increased to 290 C° at the rate of 10 C°/min. and maintain for 15 min.

9- Antioxidant Activity

- A quantity of 125 mg of DPPH powder was weighed.
- A volume of 200 ml of ethanol was added to dissolve the DPPH.
- Putted in the ultrasonic bath for 15 min.
- The DPPH solution was poured into 6 test tubes.
- A quantity of 500 µl of each extract was taken and put it in to marked test tube.
- The test tube no.5 is a positive control (ascorbic acid) while no.6 is negative control (ethanol).
- The speed of reaction was monitored from purple to yellow and the intensity of the color as compared with controls.
- Reading was done by using UV-VIS spectrophotometer at 517 nm .

Anti oxidant % = Abs of control – abs of test / Abs of control

10- Statistical analysis

Data of Antioxidant Activity, Paw volume and HRBC membrane protection testing was analyzed by using One-Way ANOVA Calculator. The results are revealed as Mean ±S.E.M (mean standard error). $p < 0.05$ was shown to be significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

RESULTS & DISCUSSION

1- Pharmacognostical Study

1.2 Phytochemical screening of *Boswellia serrata / gum resin*

A) Extraction of *Boswellia serrata / gum resin*

The course powder of *Boswellia serrata* was sequentially extracts through methanol, n-hexane and Petroleum Ether soxhlet apparatus. The result is described in Table No. 2. The *Boswellia serrata* gum extract with methanol extract was semi- solid, dark brownish and the yield was 14.18% w/w, Petroleum Ether semisolid, brown and the yield was 11.91% w/w, n-hexane extract was semi-solid, brown and the yield was 16.32% w/w and hot aqueous extract was oily, brown and the yield was 8.82% w/w.

Table No.1: Extraction of *Boswellia serrata* / gum resin

Sr.No	Extract (100gm)	Visual Color and consistency	Yield percentage
1	Methanol	Semi-solid Dark Brown	14.18%
2	Petroleum Ether	Semi-solid Brown	11.91%
3	n-Hexane	Semi-Solid yellow	16.32%
4	Aqueous	Oily brown	8.82%

Table No. 2: Phytochemical analysis of *Boswellia serrata / gum resin*					
Sr.No	Phytochemical Constitutes	Methanol extract	n-Hexane extract	Petroleum ether extract	Aqueous extract
1	Alkaloid	+	-	+	-
2	Flavonoids	+	-	-	-
3	Glycosides	+	-	-	-
4	Saponins	+	-	-	+
5	Steroids	-	-	+	-
6	Tannins	+	+	+	+
7	Anthroquinones	-	-	-	-
8	Phenolic compounds	+	+	-	-
Where: + = the presence of constitute, – = the absence of constitutes					

Table No. 3: TLC for various *Boswellia serrata* extracts					
Sr. No	**Phytochemical Constitutes**	**Rf value of Aqueous extract**	**Rf value of Petroleum ether extract**	**Rf value of n-Hexane extract**	**Rf value of Methanol extract**
1.	Alkaloid	0	0.31	0	0.34
2.	Flavonoids	0	0	0	0.15
3.	Glycosides	0	0	0.37	0.35
4.	Saponins	0.45	0	0	0.42
5.	Steroids	0.43	0.42	0	0
6.	Phenolic compounds	0	0.23	0.21	0.21

B) Preliminary phytochemical screening of *Boswellia serrata / gum resin*

Successive extract of oleogum resin of *Boswellia serrata* was screened in favor of various chemical investigations and the results are mentioned in Table No. 3.

The results in table (2) represents the presence or absence of secondary metabolites. Phytochemical analysis revealed more abundant glycosides, saponins and tannins. The phenolic compounds were present in a regular quantity and flavonoids were quite less. The alkaloids were absent in two extracts, while anthroquinones absent in all of them. Methanol extract found to contain the following Alkaloid, Tannins, Glycosides, Saponins, Flavanoids, and Phenolic compounds, while n-Hexane extract contains tannins and Phenolic compounds only. The Petroleum extract contains alkaloid, Steroid, and tannins. Aqueous extract contains Saponins and Tannins.

2- Qualitative estimation using thin layer chromatography:

The first aim of this study was to perform a comparative analysis on the composition of different methanol, n-hexane, petroleum ether and aqueous extracts of *Boswellia serrata* gum resin as a tool for the evaluation of the quality of the extracts.

Combination of TLC and HPLC analyses can be considered as a multidimensional analytical approach combining fast qualitative screening with an accurate and precise quantification of specific compounds. All of the extracts were tested using thin layer chromatography technique for qualitative examination and the results were as shown in the table No. 3

The Rf value for alkaloid was 0.34 in methanol and 0.31 in petroleum ether extract and negative in n-Hexane and aqueous extract, while Rf value for flavonoids 0.15 in methanol extract and negative n-Hexane and aqueous extract, glycoside 0.35 in methanol and 0.37 in n-Hexane extract and negative in petroleum ether and aqueous extract, saponins 0.42 in methanol and 0.45 in aqueous extract and negative in n-Hexane and petroleum ether, steroids 0.42 in n-Hexane and 0.43 in aqueous and negative in methanol and petroleum ether extract, phenolic compounds 0.21 in methanol, 0.21 n-Hexane and 0.23 in petroleum ether extract and negative in aqueous extract.

3- Estimation by High Performance Liquid Chromatography

One of the goals of this study was to develop a rapid and specific isocratic HPLC method for the estimation of boswellic acids (11-KBA and A-11-KBA) from all extracts. The proposed method utilizes an isocratic technique at room temperature without tedious sample preparation procedure and UV-Vis detection. It estimates both 11-KBA and A-11-KBA, the active acids among the boswellic acids. The mobile phase with pH 4.0 gives greater stability to the analytical column. The real advantage of the method is its low retention time: 11.495 and 15.612 min for 11-KBA and A-11-KBA, respectively. The chromatographic system was prewashed with a methanol and methanol–water (50:50, % v/v) mixture to remove any retained impurities in the column. The wavelength maxima of 11- KBA and A-11-KBA were found to be 260 nm; hence it was used for the detection of 11-KBA and A-11-KBA. The chromatogram of the standard *Boswellia serrata* extract solution shows two major peaks at 11.495 min and 15.612 min retention times. After injecting 20 µl of the standard , methanol extract, n-hexane extract, petroleum ether extract and aqueous extract using the conditions that mentioned in the previous chapter each of these extracts showed several peaks. The

peaks compared with the standard based on the retention time and area under the curve, retention time indicates the compound were as the area under the curve indicates the concentration of that compound. This means the qualitative and the quantitative estimation of each extract done respectively by such manner.

Table No. 4 represent the peaks which had been observed after the injection of standard to HPLC. Table No. 5 represent the peaks which had been observed after the injection of aqueous extract to HPLC. Table No. 6 represent the peaks which had been observed after the injection of methanol extract to HPLC. Table No. 7 represent the peaks which had been observed after the injection of n-hexane extract to HPLC.

Table No. 4: HPLC chromatogram for standard.

Peak	Retention time	Area under the curve	Identity
1	9.173	5566238	Unknown
2	9.797	4136109	Unknown
3	11.495	25919468	11-KBA
4	15.612	13883217	A-11-KBA
5	20.465	4060027	Unknown

Table No. 5: HPLC chromatogram for aqueous extract.

Peak	Retention time	Area under the curve	Identity
1	4.065	257691	Unknown
2	5.208	900647	Unknown
3	5.838	4868045	Unknown
4	6.073	1905474	Unknown
5	6.211	1428844	Unknown
6	6.371	4369197	Unknown
7	11.598	1083581	11-KBA
8	15.753	258783	A-11-KBA

Table No. 6: HPLC chromatogram for methanol extract.

Peak	Retention time	Area under the curve	Identity
1	9.192	49322713	Unknown
2	9.775	84617512	Unknown
3	10.917	67133943	Unknown
4	11.508	5183746	11-KBA
5	15.783	129550692	A-11-KBA

Table No. 7: HPLC chromatogram for n-hexane extract.

Peak	Retention time	Area under the curve	Identity
1	7.440	3485350	Unknown
2	7.789	5004797	Unknown
3	9.169	35425502	Unknown
4	9.849	43753378	Unknown
5	11.331	13656340	Unknown
6	11.608	74475589	11-KBA
7	16.539	148880601	A-11-KBA
8	21.311	67328671	Unknown

From the above results it's possible to say that the HPLC results depends on the type of solvent and its polarity which explains the high number of peaks observed. Based on the peaks appeared some degree of similarity in the retention time and area under the curve, this indicates that the results of the present study agrees with previous studies, despite some differences which could be caused by differences in the materials and devices source along with researcher handling during analysis along with the method of analysis that had been used.

4- Identification of novel compound by Gas chromatography–mass spectrometry (GC-MS):

Methanolic extract of *Boswellia serrata* was subjected to GC-MS study for identification of novel compounds. In the methanolic gum extract of *Boswellia serrata* , 14 compounds have been identified as shown in Table No. 8 by interpretation of mass spectrum (GC-MS).

Table No. 8: list of compounds in methanol extract

Sr.No.	Compound name	CAS	Formula	Mol. Weight
1	(1S,4R,5R)-4-Methyl-1-(propan-2-yl)bycyclo[3.0.1]hexane-3-one	546-80-5	C10H16	152.24
2	pentacyclic trierpenic acid	471-66-9	C30H48O3	456.70
3	1-Methyl-4-(1-Methyl ethenyl)-cyclohexane	138-86-3	C10H16	136.23
4	(1S-5S)-2,6,6-Trimethyl bi cyclo[3.1.1]hept-2-ene	778-70-8	C10H16	136.24
5	R-5-(1,2-dithiolan-3-yl)pentanoic acid	1200-22-2	C8H14O2S2	206.33
6	Cyclopentane, 1-ethyl-3-methyl-, cis- , cis-1-Ethyl-3-Methylcyclopentane , 1-Ethyl-3-methylcyclopentane ,	2613-66-3	C8H16	112
7	Butanoic acid, 3-methylbutyl ester , Butyric acid, isopentyl ester , Isoamyl butanoate , Isoamyl butylate ,Isoamyl butyrate , Isope	106-27-4	C9H18O2	158
8	Butanoic acid, 2-methylbutyl ester , 2-Methylbutyl butyrate ,	51115-64-1	C9H18O2	158
9	Hexane, 3-ethyl-4-methyl- , 3-Ethyl-4-methylhexane , 4-Ethyl-3-methylhexane , 3-Methyl-4-ethylhexane ,	3074-77-9	C9H20	128
10	Octane, 4,5-dimethyl- , 4,5-Dimethyloctane ,	15869-96-2	C10H22	142
11	Heptane, 3-ethyl-5-methylene	0-00-0	C10H20	140
12	Hexane, 2,3-dimethyl- , 2,3-Dimethylhexane ,	584-94-1	C8H18	114
13	Butanoic acid, 3-methylbutyl ester , Butyric acid, isopentyl ester , Isoamyl butanoate , Isoamyl butylate ,Isoamyl butyrate , Isope	106-27-4	C9H18O2	158
14	Butanoic acid, 2-methylbutyl ester , 2-Methylbutyl butyrate,	51115-64-1	C9H18O2	158

Table No. 9: list of compounds in aqueous extract				
Sr.No.	**Compound name**	**CAS**	**Formula**	**Mol. Weight**
1.	Pentacyclic trierpenic acid	471-66-9	C30H48O3	456.70
2.	1-Methyl-4-(1-Methyl ethenyl)-cyclohexane	138-86-3	C10H16	136.23
3.	(1S-5S)-2,6,6-Trimethyl bi cyclo[3.1.1]hept-2-ene	778-70-8	C10H16	136.24
4.	Cyclopentane, 1-ethyl-3-methyl-, cis- , cis-1-Ethyl-3-Methylcyclopentane , 1-Ethyl-3-methylcyclopentane ,	2613-66-3	C8H16	112
5.	Butanoic acid, 3-methylbutyl ester , Butyric acid, isopentyl ester , Isoamyl butanoate , Isoamyl butylate , Isoamyl butyrate , Isope	106-27-4	C9H18O2	158
6.	Butanoic acid, 2-methylbutyl ester , 2-Methylbutyl butyrate ,	51115-64-1	C9H18O2	158
7.	Hexane, 3-ethyl-4-methyl- , 3-Ethyl-4-methylhexane , 4-Ethyl-3-methylhexane , 3-Methyl-4-ethylhexane ,	3074-77-9	C9H20	128
8.	Octane, 4,5-dimethyl- , 4,5-Dimethyloctane ,	15869-96-2	C10H22	142
9.	Heptane, 3-ethyl-5-methylene	0-00-0	C10H20	140
10.	Hexane, 2,3-dimethyl- , 2,3-Dimethylhexane ,	584-94-1	C8H18	114
11.	Butanoic acid, 3-methylbutyl ester , Butyric acid, isopentyl ester , Isoamyl butanoate , Isoamyl butylate ,Isoamyl butyrate , Isope	106-27-4	C9H18O2	158
12.	Butanoic acid, 2-methylbutyl ester , 2-Methylbutyl butyrate ,	51115-64-1	C9H18O2	158

Table No. 10: list of compounds in n-hexane extract				
Sr.No.	**Compound name**	**CAS**	**Formula**	**Mol. Weight**
1	Pentacyclic trierpenic acid	471-66-9	C30H48O3	456.70
2	(1S-5S)-2,6,6-Trimethyl bi cyclo[3.1.1]hept-2-ene	778-70-8	C10H16	136.24
3	R-5-(1,2-dithiolan-3-yl)pentanoic acid	1200-22-2	C8H14O2S2	206.33
4	Cyclopentane, 1-ethyl-3-methyl-, cis- , cis-1-Ethyl-3-Methylcyclopentane , 1-Ethyl-3-methylcyclopentane ,	2613-66-3	C8H16	112
5	Butanoic acid, 3-methylbutyl ester , Butyric acid, isopentyl ester , Isoamyl butanoate , Isoamyl butylate , Isoamyl butyrate , Isope	106-27-4	C9H18O2	158
6	Butanoic acid, 2-methylbutyl ester , 2-Methylbutyl butyrate ,	51115-64-1	C9H18O2	158
7	Hexane, 3-ethyl-4-methyl- , 3-Ethyl-4-methylhexane , 4-Ethyl-3-methylhexane , 3-Methyl-4-ethylhexane ,	3074-77-9	C9H20	128
8	Octane, 4,5-dimethyl- , 4,5-Dimethyloctane ,	15869-96-2	C10H22	142
9	Hexane, 2,3-dimethyl- , 2,3-Dimethylhexane ,	584-94-1	C8H18	114
10	Butanoic acid, 3-methylbutyl ester , Butyric acid, isopentyl ester , Isoamyl butanoate , Isoamyl butylate , Isoamyl butyrate , Isope	106-27-4	C9H18O2	158
11	Butanoic acid, 2-methylbutyl ester , 2-Methylbutyl butyrate	51115-64-1	C9H18O2	158

Table No. 11: list of compounds in petroleum ether extract				
Sr.No.	**Compound name**	**CAS**	**Formula**	**Mol. Weight**
1	(1S,4R,5R)-4-Methyl-1-(propan-2-yl)bycyclo[3.0.1]hexane-3-one	546-80-5	C10H16	152.24
2	pentacyclic trierpenic acid	471-66-9	C30H48O3	456.70
3	1-Methyl-4-(1-Methyl ethenyl)-cyclohexane	138-86-3	C10H16	136.23
4	(1S-5S)-2,6,6-Trimethyl bi cyclo[3.1.1]hept-2-ene	778-70-8	C10H16	136.24
5	R-5-(1,2-dithiolan-3-yl)pentanoic acid	1200-22-2	C8H14O2S2	206.33
6	Cyclopentane, 1-ethyl-3-methyl-, cis- , cis-1-Ethyl-3-Methylcyclopentane , 1-Ethyl-3-methylcyclopentane ,	2613-66-3	C8H16	112
7	Butanoic acid, 3-methylbutyl ester , Butyric acid, isopentyl ester , Isoamyl butanoate , Isoamyl butylate , Isoamyl butyrate , Isope	106-27-4	C9H18O2	158
8	Butanoic acid, 2-methylbutyl ester , 2-Methylbutyl butyrate ,	51115-64-1	C9H18O2	158
9	Hexane, 3-ethyl-4-methyl- , 3-Ethyl-4-methylhexane , 4-Ethyl-3-methylhexane , 3-Methyl-4-ethylhexane ,	3074-77-9	C9H20	128
10	Octane, 4,5-dimethyl- , 4,5-Dimethyloctane ,	15869-96-2	C10H22	142
11	Heptane, 3-ethyl-5-methylene	0-00-0	C10H20	140
12	Butane, 2-methyl- , iso-Pentane , 1,1,2-Trimethylethane , 2-Methylbutane , iso-C5H12 ,Ethyldimethyl methane , Isoamyl hydrid	78-78-4	C5H12	72
13	Cyclopentane, 1-ethyl-3-methyl-, trans- , trans-1-Ethyl-3-Methylcyclopentane , 1-Ethyl-3-methylcyclopentane	2613-65-2	C8H16	112
14	Heptane, 3-ethyl-5-methylene-	0-00-0	C10H20	140
15	Butane, 2-methyl- , iso-Pentane , 1,1,2-Trimethylethane , 2-Methylbutane , iso-C5H12 ,Ethyldimethylmethane , Isoamylhydrid	78-78-4	C5H12	72

Aqueous extract of *Boswellia serrata* was subjected to GC-MS study for identification of novel compounds. In the aqueous gum extract of *Boswellia serrata* , 12 compounds have been identified as shown in Table No. 9 by interpretation of mass spectrum (GC-MS).

The n-Hexane extract of *Boswellia serrata* was subjected to GC-MS study for identification of novel compounds. In the n-Hexane gum extract of *Boswellia serrata* , 11 compounds have been identified as shown in Table No. 10 by interpretation of mass spectrum (GC-MS).

Petroleum ether extract of *Boswellia serrata* was subjected to GC-MS study for identification of novel compounds. In the Petroleum ether gum extract of *Boswellia serrata* , 15 compounds have been identified as shown in Table No. 11 by interpretation of mass spectrum (GC-MS).

Table No. 12: DPPH radical scavenging activity of extracts of *B. serrata*, and the IC50 values

Extracts/Sample	Concentration in µg/ml	DPPH radical scavenging activity(%)	IC50 µg/ml
Ascorbic acid	3	27.36±1.79	7.27
	6	40.20±1.63	
	9	71.90±3.92	
	12	89.30±0.25	
	15	90.76±0.39	
Methanol Extract	200	2.80±0.08	5882.35
	400	4.00±0.15	
	600	5.28±0.38	
	800	6.33±0.50	
	1000	8.28±0.14	
Hexane Extract	200	12.24±3.51	843.17
	400	35.87±3.19	
	600	40.77±3.02	
	800	47.97±0.36	
	1000	50.80±0.87	
Petroleum ether Extract	200	24.68±0.80	458.30
	400	51.88±0.75	
	600	76.13±1.16	
	800	91.06±0.72	
	1000	95.89±0.09	
Aqueous Extract	200	3.28±1.36	4000
	400	6.52±0.71	
	600	8.33±0.42	
	800	9.38±0.84	
	1000	11.66±0.54	

5- Antioxidant Activity:

The DPPH radical scavenging activity of extracts of *B. serrata* Methanol n-Hexane Petroleum ether Aqueous extracts, and the IC50 values have been shown in Table No. 12.

From table 12, it is clear that as the concentration of extracts increases, there is an increase in the DPPH radical scavenging activity. The highest IC50 value was observed for the extract of *methanol extract* (5882.35 µg/ ml) indicating the lowest DPPH radical scavenging activity. The lowest IC50 value was observed for the extract of *Petroleum ether* (458.30 µg/ ml) indicating the highest DPPH radical scavenging activity. IC50 values for *Hexane and Aqueous Extract* were (843.17 and 4000 µg/ ml) respectively. The IC50 values of all the extracts were greater as compared to that of ascorbic acid (7.27 µg / ml), indicating that extracts have low DPPH radical scavenging activity.

REFERENCES:-

1) Asaad, M.; Alhomoud, M. (2016) Proulcerogenic effect of water extract of Boswellia sacra oleo gum resin in rats. Pharm. Biol., 54, 225–230.
2) Pollastro, F.; Golin, S.; Chinese, G.; Putra, M.Y.; Moriello, A.S.; de Petrocellis, L.; Garcia, V.; Munoz, E.; Taglialatela-Scafati, O.; Appendino, G. Neuractive and anti-inflammatory Frankincense cembranes: A structure-activity study. J. Nat. Prod. 2016, 79, 1762–1768.
3) Raja AF, Ali F, Khan IA, Shawl AS, Arora DS, Shah BA and Taneja SC (2011). Antistaphylococcal and biofilm inhibitory activities of acetyl-11-keto- β-boswellic acid from Boswellia, BMC Microbiol., 11; 54.
4) Woolley CL, et al. Chemical differentiation of Boswellia sacra and Boswellia carterii essential oils by gas chromatography and chiral gas chromatography-mass spectrometry. J Chromatogr A. (2012)
5) Yasuda GT, et al. (2013). Rheumatoid arthritis. In JE Pizzorno, MT Murray, eds., Textbook of Natural Medicine, 4th ed., pp. 1769-1784. St. Louis: Elsevier.
6) Devi PR, et al. (2012) Safety Evaluation of Alcoholic Extract of Boswellia ovalifoliolata Stem-bark in Rats. Toxicol Int.
7) Pawar RK, Sharma S, Singh KC, Sharma RK. Physico-chemical standardization and development of HPTLC method for the determination of β-boswellic acid from Boswellia serrata Roxb.(exudate) Int J App Pharm. 2011;3:8–13.
8) Roy A., Mould D. R., Wang X. F., Tay L., Raymond R., Pfister M. (2009). Modeling and simulation of abatacept exposure and interleukin-6 response in support of recommended doses for rheumatoid arthritis. J. Clin. Pharmacol.
9) Singh JA, et al. (2012). 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthritis Care and Research, 64(5): 625-639.
10) Song Z, et al. Determination of alpha-pinene and octyl acetate contents in Boswellia serrata. Zhongguo Zhong Yao Za Zhi. (2012)