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EFFECT OF SPRAYING WITH BIO STIMULATORS "AGROSIGN AND LIQUID HUMUS" IN THE CHEMICAL CONTENT AND PRODUCTION OF BIOFUEL VEHICLES FOR OIL OF LOCAL MINT PLANT (*MENTHA SPICATA* L.)

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Abstract

The experiment was conducted in the agricultural season 2016/2017 in one of the private orchards in Al-Haritha area, 10 km away from the center of Basrah governorate. The effect of spraying with the vital agrosign biomass at 0, 0.75 and 1.5 ml-1 and liquid humus at 0, 0.5 and 1.0 ml in the chemical content and production of Biofuel Vehicles for oil of local mint plant (*Mentha spicata* L.). The biochemical constituents of extracts obtained from the treatments showed that the ethanol extract was analyzed by Gas chromatography- mass spectroscopy (GC-MS) technique. The results showed 51 peaks indicate the presence of 51 compounds (phytochemical constituents). Most of the chemical components found in all treatment Cyclohexasiloxane dodecamethyl higher percentage in 8 and 9 treatment (23.11 and 26.95% respectively), All treatment associated with the main constituents of the extracts were Cyclohexasiloxane dodecamethyl higher percentage in 8 and 9 (26.95%), Cyclooctasiloxane tetradecamethyl higher percentage in 5 treatment (27.41%), Epinephrine (beta), 3TMS derivative found in 1,2,3,5,6 treatment. As well as, the extract of treatment 7 useful algal oil for biofuel production and study the GC-MS characteristics of biofuel extracted from it. Results showed that the presence of dodecane, hexadecane, pentadecane and heptadecane, 7- methyl. In addition, octadecane, 3-methyl, and tetradecane in 2 and 3 treatment. which confirmed the biodiesel.

Key words : biomass, cyclohexasiloxane, mint extract, GC-MS technique.

Introduction

Mint leaves have been used in medicine for thousands of years and the Babylonians, they used it to treat indigestion and Chinese to colic, and Romans to ease poisoning and poisoning (Briggs, 1993; Evans, 1999). Mint is *Mentha* sp. Of perennial herbaceous plants that grow wildly in different parts of the world and native to the Mediterranean basin and back to the oral family Lamiaceae, spread its cultivation in most countries include America, India, Algeria, Egypt, Syria, Turkey, Iraq and Iran. The Mint plant *Mentha* sp. has an aromatic scent, it is fresh or dried leaves are used as spices to improve the taste of the food. Peppermint oil is colorless or yellow, containing 60% carvon and other substances, mainly lemons, flanders, menthol and tanning materials as well as volatile oils and medical substances pain relievers and convulsions and enter the aromatic oil in the composition of several medicines and treatment of gastric ulcer and the food industry and cosmetics(Sukhmal *et al.,* 2004 ; Znini *et al.,* 2011). Peppermint oil is produced in the world and the United States by approximately 8000 tons / year Mint oil is the most widely used volatile oil used in the treatment of asthma, sinusitis, relieve cough and inflammation throat, It is used as a disinfectant and its detergent in mouth lotions, gums and pastes teeth, since it leads to muscle relaxation may help to relieve painful convulsions and treatment of colds and flu (Mohammed *et al.,* 2015). Marine plant extracts are natural extracts of herbs, plants and seaweeds, it is used widely in many areas as a source of natural growth and nutrient growth regulators, and uses soil fertilizers and enhancers As they contain not only the major and minor nutrients, amino acids and vitamins B1, B2, C, E, but also contain cytokines, oxins and gebrilins (Crouch, 1990). Agrosign organic fertilizer extracted from marine grass *Ascophyllum nodosum,* it is a fertilizer solution, consists of 40% organic

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matter, free amino acids of 12%, organic nitrogen of 8.5%, sulfur of 1.1% and calcium of 0.4%. Rasha is used on the total vegetation of all crops, It is derived from amino acids of plant origin and contains organic nitrogen and a high percentage of free amino acids and thus helps the plant to overcome stress conditions (Matroad and AL-Ali, 2017).

Humic acid an organic biocomponent that has a significant effect on plant growth, development and increase and improve physical, chemical and biochemical properties and the elements are ready for absorption by the plant It stimulates enzymes and plant hormones (Nardi *et al.,* 2004; Mikkelsen, 2005; Rajpar *et al.,* 2011).

According to the World Energy Outlook of the International Energy Agency, the energy demand is anticipated to increase until 2035 and this emission would rise by 20% UNEP. (2009). Biofuel derived from vegetable oils or animal fats. It can be played a significant role in the production of biofuel such as *Camelina sativa*, *Cola nitida, Brassica napus*, *Cocos nucifera*. As well as seaweed has also been used for biodiesel (Sharmila *et al.,* 2016; Mebude *et al.,* 2017).

Biofuel comprised of monoalkyl esters derived from vegetable oils or animal fats. Spent cooking oil plays a significant role in the production of biofuel. The other feed stocks of biodiesel include *Camelina sativa*, *Brassica napus*, *Cocos nucifera*, *Zea mays*, *Jatropha curcas* and *Helianthus annuus*. Now a day's seaweed has also been used as a feed stock for biodiesel (Sharmila *et al.,* 2016).

Because there was no previous study in Iraq to produce biofuel, Therefore, the aim of this study to produce these compounds of oil local mint plant (*Mentha spicata* L.) Using bio stimulators "agrosign and liquid humus" Produced from seaweed and seaweed by GC-MS analysis.

Materials and Methods

The experiment was conducted in the agricultural season 2016/2017 in one of the private orchards in the area of Haritha, which is 10 Km from the center of Basrah Governorate in a green clay soil with a pH of 7.8 and an electrical conductivity (E.C) 1.3 dsi-sammens $^{-1}$ and Organic Article 2.15%, To determine the effect of spraying with bio stimulators "agrosign and liquid humus" In the Chemical content and production of Biofuel Vehicles for oil of local mint plant (*Mentha spicata* L.).

Divide the test field into 27 panels with an area of 1 m ^{2} for each panel and prepare each experimental unit board. After two weeks of planting, the plants were sprayed with bio stimulators Agrosign with a concentration of 0, 0.75 and 1.5 ml^{-1} and liquid Humus With a concentration of 0, 0.5 and 1.0 ml^{-1} With a comma of one week between the Sprayed and the other. All service operations were performed and the need for plants to fertilize, as the plants gave the nitrogen fertilizer with urea at a rate of 150 kg. hectare^{-1} with the first two payments after one month of planting and the second one month after the first addition. Experimental measurements of five plants were taken in each experimental unit at the end of the growth seasons and included.

Preparation of oily mint extract

The steam distillation method was used to extract the pilot oil from leaves of mint plant, using the Clevenger apparatus and described in the constitution (British Herbal pharmacopoeia, 1996) the extraction was 10: 1 (weight / volume) (mint leaves / water) water vapor carries volatile oils towards the condensate And then to the oil trap as the oil is collected in it and float on The surface of the water collects oil and moisturizes and keeps the bottles clean, dry and nurtured to be Small, dark color, tight cover and 4C^{0} Until use.

Chemical components of oil

The quantitative and qualitative components of oil were analyzed using Gas chromatograph device connected to mass spectrometer type Shimadzu GC MS –QP2010 Ultra. Dilute with 1 ml of hexane after prepare mathel aster for fat samples Injection 0.1 Microliter of the formed solution in a device Gas Chromatographs GC MS (Mebude and Adeniyi, 2017). The experiment was silent Randomized Complete Block Design With a global experience (Al Rawy and Kalf, 1980).

Results and Discussion

The GC-MS chromatogram of *Mentha spicata* leaves extract treated with Humus and Agrasen (Table 1 and Fig. 1-15) showed 51peaks indicate the presence of 51 compounds (phytochemical constituents). Most of the chemical components found in all treatment Cyclohexasiloxane dodecamethyl higher percentage in 8 and 9 treatment (23.11and 26.95% respectively),

The biochemical constituents of extracts obtained from the treatments showed that the extract was analyzed by Gas chromatography- mass spectroscopy (GC-MS) technique. All treatment associated with the main constituents of the extracts were Cyclohexasiloxane dodecamethyl higher percentage in 8 and 9 (23.11 and 26.95%), Cyclooctasiloxane tetradecamethyl higher percentage in 5 treatment (27.14%), Epinephrine (beta),

Table 1: Compounds present in Biofuel extracted from *Mentha spicata* leaves treated with Agrosing and Humus.

S.N.	Compound	1Mint oil	2(0.5 ml/L Humus	3(1 ml/L Humus	40.75 ml/L Agro.	50.75 Agro.+ 0.5 Hu	60.75 Agro. + 1 Hu	71.5 Agro.	81.5 agro._ 0.5 Hu	91.5 Agro. + 1 Hu.
1	Hepasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	1.46	2.11	-	-	-	-	-	-	-
2	Hepasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	0.55	0.55	-	-	-	-	-	-	-
3	Octadecanoic acid, 2,3-dihydroxypropyl ester	8.66	8.66	-	-	-	-	-	-	-
4	Oxalic acid, disohexyl ester	3.76	3.76	-	-	-	-	-	-	-
5	1-Deoxy-d-mannitol	1.33	1.43	-	-	-	-	-	-	-
6	Eicosane, 2-methyl	17.51	17.55	-	-	-	-	-	-	-
7	Ethanol, 2-(octadecyloxy)-	19.09	19.09	-	-	-	-	-	-	-
8	2,4-Di-tert-butylphenol	2.13	2.13	-	-	-	-	-	-	-
9	2,4-Di-tert-Butyl-m-cresol	1.55	1.55	-	-	-	-	-	-	-
10	1-Hexadecanol, 2-methyl	3.20	3.03	-	-	-	-	-	-	-
11	Heptadecane	2.32	2.44	-	-	-	-	5.13	-	-
12	Pentasiloxane,dodecamethyl	-	-	-	-	25.03	27.14	1.26	23.40	23.40
13	Tridecanedioic acid	17.78	16.76	-	-	-	-	-	-	-
14	Docosane,1-Iodo, Octacosane	0.67	0.67	-	-	-	-	-	-	-
15	Octadecane	1.48	1.48	-	-	-	-	-	-	-
16	Dodecane, 1,2-dibromo-	0.55	0.54	-	-	-	-	-	-	-
17	Cyclononasiloxane, octadecamethyl-	0.79	1.17	-	-	-	-	-	-	-
18	Cyclodecasiloxane, eicosamethyl-	0.95	1.25	-	-	-	-	-	-	-
19	Glycidyl palmitate	17.78	-	-	-	-	-	-	-	-
20	Glycidol stearate	16.50	-	-	-	-	-	-	-	-
21	Decane, 5,6-dimethyl-and Octane, 4,5-diethyl-	4.93	2.31	-	-	-	-	-	-	-
22	Decane, 4-ethyl	-	-	-	-	-	-	-	-	-
23	Heptane, 4- ethyl	3.13	3.13	-	-	-	-	-	-	-
24	Octane, 4- methyl-	4.65	4.56	-	-	-	-	-	-	-
25	Octadecane, 6-methyl	-	1.33	-	-	-	-	-	-	-
26	Heptacosane	-	3.22	-	-	-	-	-	-	-
27	Camphene	-	9.37	-	-	-	-	2.06	-	-
28	Benzenethiol	-	-	-	-	-	-	6.51	-	-
29	Pentasiloxane, dodecamethyl-	-	-	-	-	25.03	27.14	1.26	23.40	23.40
30	Pulegone	-	-	-	-	-	-	7.06	-	-
31	Octadecane	1.48	-	-	-	-	-	2.36	-	-
32	Hentriacontane	0.72	-	-	-	-	-	1.56	-	-
33	Cyclohexasiloxane, dodecamethyl-	20.72	23.11+	23.11	7.65	25.81	10.80	1.74	23.11	26.95
34	Cyclopentasiloxane, decamethyl-	-	-		4.72	6.76	6.70	-	-	-
35	Epinephrine, (.beta.)-, 3TMS deriv ative	-	-	5.27	-	6.67	6.70	-	-	-
36	1,5-Diphenyl-2H-1,2,4-triazoline-3 -thione	-	-	-	-	24.51	31.04	-	-	-
37	Cycloheptasiloxane, tetradecamethy-	19.60	19.60	19.60	15.41	25.03	27.14	-	-	-
38	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13, 15,15-hexadecamethyl-	9.22	1.46	-	-	-	-	-	-	-
39	Tetrasiloxane, decamethyl-	-	-	-	-	-	8.40	-	-	-
40	Aspidospermidine-1-ethanol, 17-hyd	-	-	5.25	-	-	-	-	-	-
41	Benzimidazol-5-amine, 1-(4-ethoxyp	-	-	38.06	-	-	-	-	-	-

Table 1 contd.....

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S.N.	Compound	1Mint oil	2(0.5 ml/L Humus	3(1 ml/L Humus	40.75 ml/L Agro.	50.75 Agro.+ 0.5 Hu	60.75 Agro. + 1 Hu	71.5 Agro.	81.5 agro._ 0.5 Hu	91.5 Agro. + 1 Hu.
42	Melamine, 3TMS derivative	-	-	13.96	15.92		15.92	-	-	-
43	Decane	0.59	0.59	-	-	-	-	-	-	-
44	9-Octadecenoic acid (Z)-, phenylmethyl ester	17.62	17.30	-	-	-	-	-	-	-
45	Pentadecane	-	2.50	-	-	-	-	4.64	-	-
46	Dodecane	-	-	-	-	-	-	2.10	-	-
47	Hexadecane,7,9-dimethyl	0.55	-	-	-	-	-	10.22	-	-
48	Eicosane	0.72	0.57	-	-	-	-	18.76	-	-
49	Nonane	0.90	1.12	-	-	-	-	-	-	-
50	Tetradecane	0.74	0.74	-	-	-	-	-	-	-
51	Hexadecane	19.48	4.23	-	-	-	-	8.76	-	-

Agro. = Agrosing Hu.= Humus

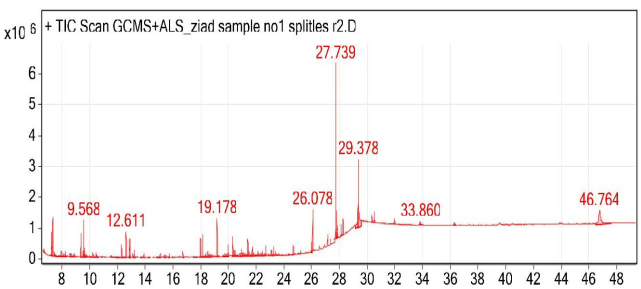

Fig. 1: GC-MS chromatogram of the leaves of *Mentha spicata* control treatment.

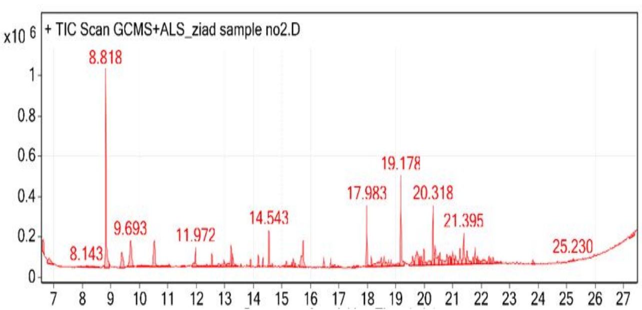

Fig. 2: GC-MS chromatogram of the leaves of *Mentha spicata* treated with 0.5 m1/L of Humus.

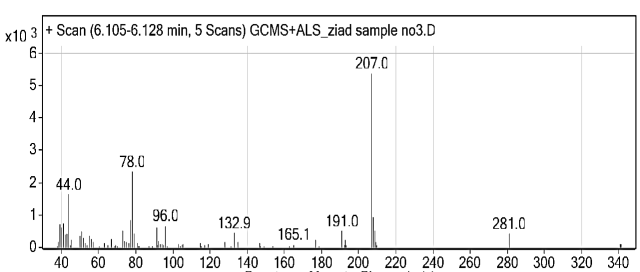

Fig. 3: GC-MS chromatogram of the leaves of *Mentha spicata* treated with 1 m1/L of Humus.

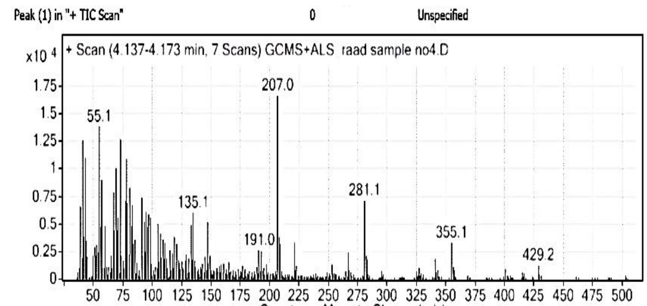

Fig. 4: GC-MS chromatogram of the leaves of *Mentha spicata* treated with 0.75 m1/L of Agrosan.

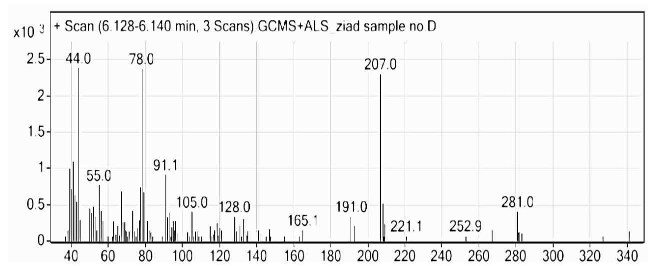

Fig. 5: GC-MS chromatogram of the leaves of *Mentha spicata* treated with Agrosan 0.75 m1/L of and 0.5 m1/L Humus.

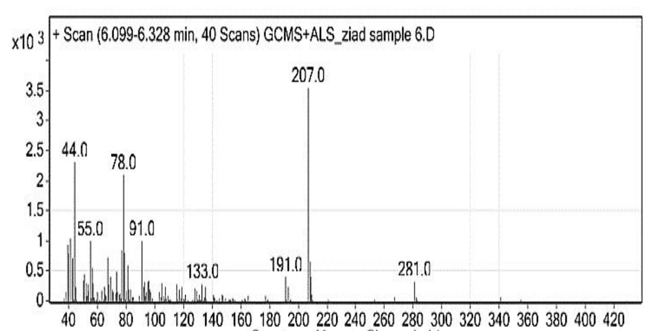

Fig. 6: GC-MS chromatogram of the leaves of *Mentha spicata* treated with 0.75 m1/L of Agrosan and 1 m1/L of Humus.

3TMS derive active found in 1, 2, 3, 5, 6 treatment. As well as, the extract of treatment 7 useful algal oil for biofuel production and study the GC-MS characteristics of biofuel extracted from it. Results showed that the presence of dodecane, hexadecane, pentadecane and heptadecane, 7- methyl. In addition, octadecane, 3-methyl, and tetradecane in 2 and 3 treatment. which confirmed

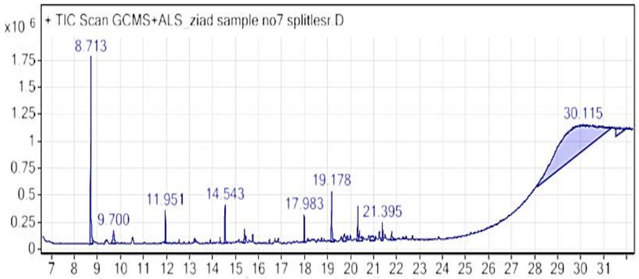

Fig. 7: GC-MS chromatogram of the leaves of *Mentha spicata* treated with 1.5 m1/L of Agrosan and Humus.

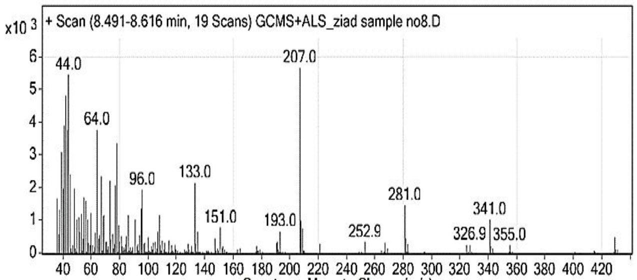

Fig. 8: GC-MS chromatogram of the leaves of *Mentha spicata* treated with 1.5 m1/L of Agrosan and 0.5 m1/L Humus.

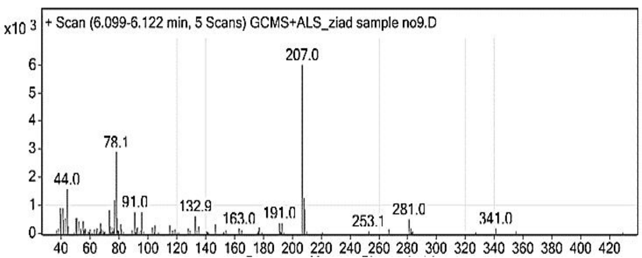

Fig. 9: GC-MS chromatogram of the leaves of *Mentha spicata* treated with 1.5 m1/L of Agrosan and 1 m1/L Humus.

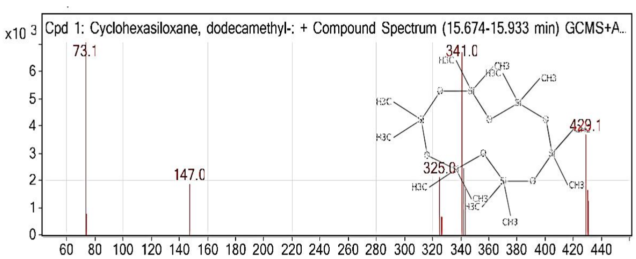

Fig. 10: Fig. 2 Mass spectrum of Cyclohexasiloxane, dodecamethyl-

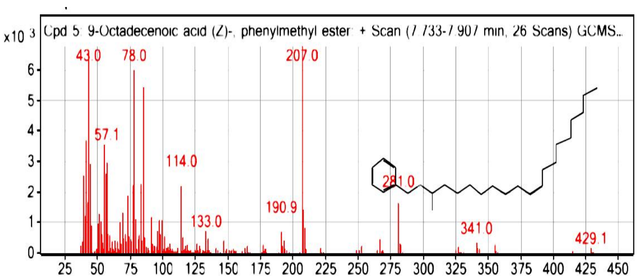

Fig. 11: Fig. 2 Mass spectrum of 9-Octadecenoic acid (Z)-, phenylmethyl ester.

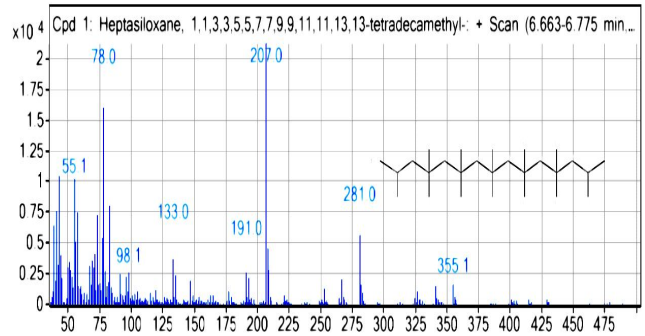

Fig. 12: Fig.2 Mass spectrum of Hepasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11,13,13- tetradecamethyl.

MS Spectrum

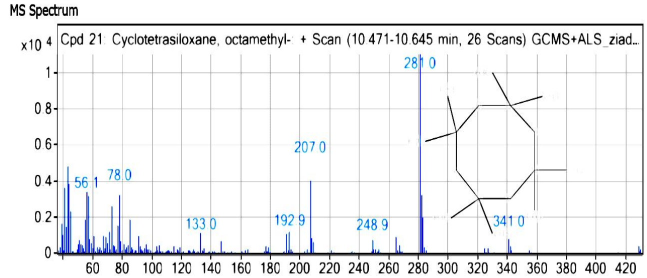

Fig. 13: Mass spectrum of Cyclononasiloxane, octadecamethyl-

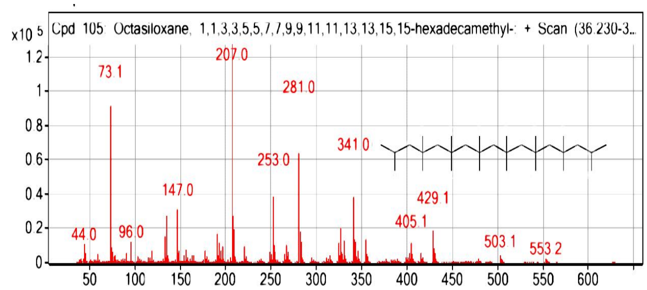

Fig. 14: Mass spectrum of Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-

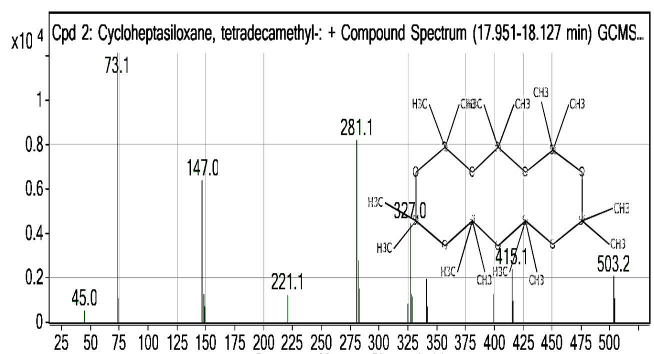

Fig. 15: Mass spectrum of Cycloheptasiloxane, tetradecamethy-

the biodiesel? The chemical compounds identified in the extract of *Mentha spicata* are presented in table 1. The results agree with literatures, by presence camphene (9.37 and 2.06%) in treatment 2 and 7 respectively, and Pulegone (7.06%) (De Sousa Barros *et al*, 2015).

The result of the present investigation reveals that the extract of Mint possessed significant anti-inflammatory, antimicrobial, antitumor, and anti-oxidant properties (Znini *et al.,* 2011; Falé, *et al.,* 2013). Presence of Cyclohexasiloxane, dodecamethyl which is used in personal care products, anti-perspirant and antifungals alluded to the report of (7).

In this work, marine algae were used for extracting biofuel and its compounds were analyzed to confirm the presence biodiesel. A treatment of mint extract can balance the characteristics of a biofuel. It contains Cyclohexasiloxane and dodecamethyl ester (Fig. 1-15; Table 1) which indicated amount of Cyclohexasiloxane and dodecamethyl ester was found in the extract. Next to that maximum amount of 21.18% Cycloheptasiloxane, tetradecamethyl (ester of myristic) 27.14 in treatment 6, octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13, 15,15-hexadecamethyl (24.33) in treatment 2 were found. dodecamethyl and tetra decamethyl table 1 were found to be present in maximum amount in the extracted biofuel. These compounds are the main constituents of biodiesel (Sharmila *et al.,* 2016). According to Singh and Singh (2010) reported that oils found as biofuels, some essential oil found in this study such as Pulegone and Camphene.

Results showed that the bio-oil consists of alkanes (Heptadecane, tetradecane, dodecane, hexadecane, pentadecane, tridecane and octadecane.), alkenes (1-hexadecane), carboxylic acids (decanoic acid,) and cyclic hydrocarbons. As well as, some compositions were such as ketones, alcohols, aldehydes. The products of bio-oil were matching with the literature (Erdogdu *et al.,* 2019). Many of researchers soured that it is possible to use the obtained bio-oil (phenolic compounds; resin; alkane content and additive for naphthalene) as an alternative fuel after application of the purifying and purification processes (Johansson *et al.,* 2016; Erdogdu *et al.,* 2019). The major chemical compounds identified alcohols, organic acids, ketones, in the bio-oil yield were alkanes, alkenes, phenols, aromatics and nitrogenated compounds esters, aldehydes, nitrogen compounds, aromatic hydrocarbons and furans (Heo *et al.,* 2010; Butt 2006; Liaw *et al.,* 2012; Johansson *et al.,* 2016; Erdogdu *et al.,* 2019). The bio-oil potential as a replacement of a petroleum based fuel should be considered seriously especially in the current energy scenario of high petroleum fuel cost as well as the projected fuel shortage in the year 2020.

Conclusion

In conclusion, this study has shown that the extract of *Mentha spicata* leaves have various compounds of interest microbiologically and pharmaceutically. It is an important characteristic of biofuel.

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