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Research Article

ANTIOXIDANT POTENTIALS AND FATTY ACID COMPOSITION OF EXTRACTS *STERCULIA TRAGACANTHA* LINDL. LEAVES FROM RAJA AMPAT WEST PAPUA PROVINCE INDONESIA

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ABSTRACT

Sterculia tragacantha Lindl. plants are endemic plants existing in Papua and have the potential of secondary metabolite compounds. This research aimed to determine the antioxidant activity and fatty acid composition of *S. tragacantha* Lindl. leaf extract. The extract was obtained by maceration using methanol and n-hexane solvents. The antioxidant activity testing was carried out using DPPH (1,1-diphenyl 2-picrylhydrazyl) free radicals while the identification of fatty acid compounds was done using Gas Chromatography-Mass Spectrometry (GCMS). The results showed that the methanol and n-hexane extracts of *S. tragacantha* Lindl. leaves had a strong antioxidant activity of 2.25 ±0,09 ppm and 27,24 ± 1,25 ppm respectively. Meanwhile, the fatty acid main contents of the methanol and n-hexane extracts of *S. tragacantha* Lindl. leaves were Methyl palmitate, Methyl linolenate, Linolelaidic acid methyl ester, trans-9-elaidic acid methyl ester, and Methyl palmitoleate.

Keywords: Antioxidants, Fatty Acid, FTIR, Sterculia, GCMS

INTRODUCTION

Antioxidant compounds have a highly important role in health. Various scientific evidence has shown that antioxidant compounds can reduce the risk of chronic diseases such as cancer and coronary heart disease. Additionally, antioxidants can inhibit or delay the oxidation of oxidated substrates in chain reactions, thus antioxidant compounds are very important for disease prevention[1-7]. The main character of antioxidant compounds is its ability to capture free radicals[8]. Free radicals can oxidize nucleic acids, proteins, fats, and even DNA as well as initiate the onset of degenerative diseases[9]. Antioxidant compounds resulted from plants, including vitamin C, vitamin E, carotene, phenol groups especially polyphenols, and flavonoids, are known to potentially reduce the risk of degenerative diseases[8,10]. Use of antioxidants was known to prevent damage to body cells and food components[11]. The compounds that had potential as antioxidants were generally the compounds of flavonoids, phenolics, and alkaloids[12].

Fatty acids are straight-chain monocarboxylic acids found in nature as esters in fat molecules or triglycerides. Triglyceride hydrolysis results in saturated and unsaturated fatty acids based on the presence of double bonds of carbon chains in the molecule. Unsaturated fatty acids (having double bonds) contained in the oil can be in two forms namely cis and trans isomers. Natural unsaturated fatty acids are mostly cis fatty acids, only a few are trans fatty acids. The number of Trans Fatty Acids (TFA) can increase in fatty foods, especially margarine due to the applied processing such as hydrogenation and heating at high temperatures[13]. The basic components of fats are fatty acids and glycerols obtained from the hydrolysis of fats, oils and other lipid compounds. Fatty acids forming fats can be distinguished based on the number of C (carbon) atoms, the presence of double bonds, the number of double bonds, and the location of double bonds. Based on the chemical structure, fatty acids are divided into Saturated Fatty Acids (SFA) and Unsaturated Fatty Acids (UFA). Saturated fatty acids do not have double bonds while unsaturated fatty acids have double bonds. Saturated fatty acids are further categorized into Mono Unsaturated Fatty Acid (MUFA) with 1 (one) double bond, and Poly Unsaturated Fatty Acids (PUFA) with 1 or more double bonds[14]. The bioactive which is often used at most by compounds from oil or fat groups in functional food formations is Omega-3 fatty acids, classified as Poly Unsaturated Fatty Acids (PUFAs). PUFA can reduce the level of VLDL and LDL in the blood because the liver does not convert it to VLDL[15]. WHO recommends that fat consumption for adults is a minimum of 20% of total energy (around 60 grams/ day)[16]. Meanwhile, according to the American Heart Association (AHA), fat consumption for adults should be < 10% of total energy consumption[16].

One of the plants that have the potential as a natural antioxidant and fatty acids is *S. tragacantha* Lindl. Plants of *S. tragacantha* Lindl. are one of the endemic plants existing in Papua[17]. *S. tragacantha* Lindl. leaves contain flavonoids, tannins, phenols, and polyphenols[18]. This study aimed to determine the antioxidant activity, fatty acid content, and functional group of the extract of *S. tragacantha* Lindl. leaves.

MATERIALS AND METHODS

Sample Handling: Samples of *S. tragacantha* Lindl. leaves were taken from Gag Island, Raja Ampat, West Papua, Indonesia in July, 2017. They were authenticated by Genetika Science Indonesia for taxonomic test and showed as *S. tragacantha* Lindl. They were suitable with Accession/Voucher (herbarium) FTG, FGX.12-17(FTG) and database accession AF022126[19,20]. The

specimens were deposited in their herbarium for reference purposes. The samples were then cleaned and dried naturally by being aerated for 7 days. The dried samples were mashed/ grinded using a machine and filtered with a mesh size of 65, and thereafter stored for further test.

Extraction: In this research, there were two different types of solvents, namely methanol (polar) and n-hexane (non-polar). The use of the two types of solvents was intended to extract both polar and nonpolar chemical components and to know the antioxidant properties of *S. tragacantha* Lindl. leaves in each solvent. Extraction method used in this research was maceration extraction. The extraction was done with a ratio between the sample and the solvent of 1:3 for 48 hours at room temperature[21]. The extraction results were then filtered with filter paper using a vacuum filter tool. The filtrate resulted from the filtration was here after evaporated at 40° C to obtain the solid extract to be collected and stored at 0° C for further tests.

Antioxidant Activity Test with DPPH: In this section, the first step was making a 0.2 mM DPPH solution in pro analyst ethanol. The next step was making a sample stock solution with 1000 ppm concentration of pro analyst ethanol, which was then diluted to obtain the sample solution with a series of concentration of 5, 10, 15, 20, and 25 ppm for extraction results using methanol solvent, the sample solution with a series of concentration of 50, 100, 150, 200, and 250 ppm for extraction results using n-hexane solvent, and the sample solution with a series of concentration of 2, 3, 4, 5, and 6 ppm for pure vitamin C. After that, 4 ml sample solution was taken at each concentration, which was then reacted with 1 ml of the 0.2 mM DPHH solution[22]. The subsequent step was measuring its absorbance at 517 nm wavelength. It was thereafter made a blank solution containing no sample (4ml pro analyst ethanol with 1ml DPPH). After that, the damping percentage (% *inhibition*) was calculated with the following formula:

$$Inhibition\ (\%) = \frac{\text{Abs. Blank} - \text{Abs. Sample}}{\text{Abs. Blank}} \times 100\ \%$$

Regression between % inhibition and concentration of the solution obtained this following equation:

$$Y = a\,(x) + b$$

Description:

- Y states the sought value of IC (inhibitor concentration), which is 50; and
- X states the value of IC_{50}. The value of IC_{50} states the concentration of sample solution needed to reduce DPPH by 50%.

Amino Acid Test with GC-MS : GC-MS characteristics used were Shimadzu with QP2010S type, 280° C injector temperature, split mode injector, 1 minute sampling time, 40-270° C column temperature with the initial temperature setting of 40° C hold on for 5 minutes, which then took 10 minutes for reaching the highest temperature of 270° C (23° C/ minute) hold on for 60 minutes so that it was needed a total time of 88 minutes to reach 270° C temperature, detector temperature of 280° C, interval temperature of 250° C, He-carrier gas, main pressure of 500-900, Flow control mode pressure, pressure of 10.9 Kpa, total flow of 58.8 ml/ m, column flow of 0.55 ml/m, linear acceleration of 26.0 cm/ s, cleaning flow of 3.0 ml/ m, split ratio of 99.8, Rtx-5MS column type, length column of 30.00 m, thickness of 0.25 µm, diameter of 0.25, and EI (Electron Impact)-ionizer type of 70 eV.

Functional Group Analysis with Fourier Transform Infrared Spectrophotometer (FTIR)

The samples tested in this research were liquid. In the sample preparation, a teflon gasket was inserted into the holder, so did with Window 1. Henceforth, sequentially, the spacer, Window 2, and the range were also inserted into the holder, continued with closing the holder with the cover. The samples were injected fully into the holder and then the injection hole was covered. The samples were ready to be scanned with FTIR. The FTIR system contained in Fourier Transform Infrared Spectrophotometer is FTS 1000 Schimitar (Model Name) made by Agilent Technologies, and specifications of the hand press tool for making pellets is Pike Technologies (Brand) with a capacity of 9 tons and pressing time of 1-3 minutes.

RESULTS AND DISCUSSION

Antioxidant Activity

The methanol and n-hexane solid extracts of *S. tragacantha* Lindl. leaves were tested using DPPH IC_{50} method. The antioxidant activity of each extract and vitamin C are presented in Table 1.

Table 1 shows that *S. tragacantha* Lindl. leaves extracted with methanol solvent had a very strong antioxidant activity, indicated by a lower antioxidant activity value than pure vitamin C. Almost similarly, *S. tragacantha* Lindl. leaves extracted with n-hexane solvent also had a strong antioxidant activity even though its value was higher than the antioxidant activity value of pure vitamin C. The higher the antioxidant activity is, the lower the IC_{50} value will be[22].

The DPPH antioxidant activities of Kepuh leaves (*S. foetida*) in ascorbic acid extract, n-hexane extract, ethyl acetate extract and methanol extract reached 2,61±0,007 µg/ml, 300,932±1,13 µg/ml, 57,24±0,11 µg/ml dan 4,56±0,03 µg/ml respectively. Meanwhile, hantap leaves (*Sterculia coccinea* Jack.) had an IC_{50} value of 6.48 ppm[23]. As for klika faloak leaves (*S. quadrifida* R.Br), the IC_{50} value reached 4.8101 ppm, indicating a strong antioxidant activity[24]. The faloak (*S. quadrifida* R.Br) concentration of 16.00 mg/ mL can effectively reduce the free radical content in the liver organ of tilapia[25].

Fatty Acid Contents of *S. tragacantha* Lindl. Leaves Extracted with Methanol

The methanol and n-hexane solid extracts of *S. tragacantha* Lindl. were also examined for determining its fatty acid contents. The GC-MS test result chromatogram and fatty acid compounds of the methanol extract of *S. tragacantha* Lindl. leaves are presented in Figure 1 and Table 2.

The main fatty acid contents of the methanol extract of *S. tragacantha* Lindl. leaves were Methyl palmitate with % relative of 33.42%; Methyl linolenate with % relative of 32.63%; Linolelaidic acid methyl ester with % relative of 9.19%; trans-9-elaidic acid methyl ester with % relative of 5.81%; Methyl palmitoleate with % relative of 4.06%; Methyl octadecanoate with % relative of 2.49%. The major fatty acids of the total lipid from *Sterculia urens* seed were stearic acid (31.72%), linoleic acid (28.83%) and palmitic acid (26.79%). Eicosadienoic acid (4.98%) and eicosatrienoic acid (2.96%)[26].

Fatty Acid Contents of *S. tragacantha* Lindl. Leaves Extracted with n-hexane

The GC MS test result chromatogram and fatty acid compounds of the n-hexane extract of *S. tragacantha* Lindl. leaves are shown in Figure 2 and Table 3.

The main fatty acid contents of the n-hexane extract of *S. tragacantha* Lindl. leaves were Methyl palmitate (32.03%) ; Methyl linoleate (23.3%); Linolelaidic acid methyl ester (10.04%), Methyl palmitoleate (6.7%), trans-9-elaidic acid

methyl ester (6.45%), gamma-linolenic acid methyl ester (3.62 %), Methyl octadecanoate (2.62%), and cis-10-pentadecenoic acid methyl ester (2.35 %).

Based on Table 2 and 3, *S. tragacantha* Lindl. leaves extraction both with methanol and n-hexane solvents was dominated by SAF (Saturated Fatty Acids) and PUFA (Poly Unsaturated Fatty Acids) as well as a little MUFA (Mono Unsaturated Fatty Acids). Saturated and Unsaturated Fatty Acids have the antifungal potential[27]. Hydrophobic groups in fatty acids have a role in bioactivity. Increased hydrophobicity with long chains can reduce solubility in water and hydrophobic groups can inhibit fatty acid interaction with phospholipid membranes[28]. The action target of fatty acids is the cell membrane. Here, fatty acids will disrupt electron transport chains and oxidative phosphorylation. Besides interfering with cellular energy production, the action of fatty acid may also result from the inhibition of enzyme activity, impairment of nutrient uptake, generation of toxic peroxidation, and auto-oxidation degradation of products or direct lysis of bacterial cells[29].

Unsaturated fatty acids have better antimicrobial activities than saturated fatty acids. This is because unsaturated fatty acids contain C=C bonds which can help fatty acids enter membranes[30]. Besides, unsaturated fatty acids can increase oxidative stress on fungal membranes[31]. Palmitate acid (C:16) is another antifungal fatty acid[32].

Palmitoleic acid has the ability to inhibit the attachment of *Candida albicans* to the skin[33]. Meanwhile, linoleic acid (18:2) is able to inhibit fungal growth and is effective as an antifungal[34]. Moreover, linoleic acid and its derivatives have the ability to damage the structure of the fungal cell wall that inhibits the glucan enzyme synthase[35].

Table 1: IC_{50} Antioxidant Activity of *S. tragacantha* Lindl. Leaves

Extraction Solvent	IC_{50} (ppm) ± SD
Methanol	2.25 ±0.09
n-hexane	27.24±1.25
Pure Vitamin C	3.59 ± 0.04

Table 2: Fatty Acid Composition of Methanol Extract *S. tragacantha* Lindl. Leaves

No.	Compound Name	Retention Time min	Area counts*min	Height counts	Relative Area (%)	Amount ug/mL
1	Methyl laurate	13.897	895404	17979357	0.57	711.3318
2	Methyl tridecanoate	17.233	289378	5228768	0.18	263.1911
3	Methyl tetradecanoate	19.233	1668455	31972182	1.05	1476.2446
4	Myristoleic acid methyl ester	19.92	1717393	24407794	1.08	1721.8516
5	Methyl pentadecanoate	21.726	275852	5069332	0.17	268.14
6	Cis-10-pentadecenoic acid methyl ester	23.532	2334874	40575853	1.47	2457.7508
7	Methyl palmitate	24.114	52939744	1008921204	33.42	49793.8576
8	Methyl palmitoleate	24.461	6432776	107323952	4.06	6814.19
9	Methyl heptadecanoate	26.539	1323273	16051117	0.84	1795.306
10	Cis-10-Heptadecenoic acid methyl ester	27.018	611723	9080166	0.39	688.8309
11	Methyl octadecanoate	29.664	3947121	42061995	2.49	4140.1044
12	Trans-9-elaidic acid methyl ester	30.304	9199555	84501618	5.81	12220.5739
13	Cis-9-oleic methyl ester	30.589	643225	6048386	0.41	653.8969
14	Linolelaidic acid methyl ester	32.164	14554133	125312524	9.19	22668.3533
15	Gamma-linolenic acid methyl ester	33.715	3020009	23273321	1.91	5086.1339
16	Methyl linolenate	35.249	51701318	417538218	32.63	88044.8306
17	Methyl arachidate	38.347	410547	4400513	0.26	573.1258
18	Cis-11,14,17-Eicosatrienoic acid methyl ester	43.071	302751	3956001	0.19	614.5966
19	Methyl cis-5,8,11,14,17-Eicosapentanoate	43.265	431934	6190411	0.27	964.3233
20	Methyl cis-5,8,11,14-eicosatetraenoic	43.384	253333	3534909	0.16	522.8016
21	Methyl docosanoate	43.752	596584	7611878	0.38	866.7045
22	Methyl erucate	44.296	1223496	10614656	0.77	2038.3093
23	Cis-13,16-Docosadienoic acid methyl ester	45.075	865277	12936391	0.55	1540.3438
24	Methyl tricosanoate	46.704	1536134	8768787	0.97	2596.0573
25	Methyl lignocerate	47.581	753335	10048120	0.48	1061.195
26	All cis-4,7,10,13,16,19-Docosahexaenoate	47.867	181145	2840037	0.11	552.7579
27	Methyl nervonate	48.67	314255	3618521	0.2	414.7221
	Total		**158423025.1**	**2039866012**	**100**	

Table 3: Fatty Acid Composition of n-hexane Extract *S. tragacantha* Lindl. Leaves

No.	Compound Name	Retention Time min	Area counts *min	Height counts	Relative Area (%)	Amount ug/mL
1	Methyl laurate	13.904	584734	11702626	0.56	464.5275
2	Methyl tridecanoate	17.237	366285	6579268	0.35	333.1389
3	Methyl tetradecanoate	19.236	1641377	30839904	1.58	1452.2853
4	Myristoleic acid methyl ester	19.917	1627625	23703238	1.57	1631.8507
5	Methyl pentadecanoate	21.726	297494	5212106	0.29	289.1762

6	Cis-10-pentadecenoic acid methyl ester	23.535	2440403	43219732	2.35	2568.8336
7	Methyl palmitate	24.117	33303862	628714891	32.03	31324.8166
8	Methyl palmitoleate	24.461	6962479	120505184	6.7	7375.3004
9	Methyl heptadecanoate	26.542	921702	11029228	0.89	1250.4873
10	Cis-10-Heptadecenoic acid methyl ester	27.025	681423	8464656	0.66	767.3164
11	Methyl octadecanoate	29.674	2723929	29247239	2.62	2857.1076
12	Trans-9-elaidic acid methyl ester	30.314	6707945	65667666	6.45	8910.7505
13	Cis-9-oleic methyl ester	30.596	487127	4363794	0.47	495.2089
14	Linolelaidic acid methyl ester	32.161	10438050	89804402	10.04	16257.4707
15	Gamma-linolenic acid methyl ester	33.708	3781521	27732798	3.64	6368.6306
16	Methyl linoleate	35.252	24230190	188809788	23.3	41262.8349
17	Methyl arachidate	38.351	560101	5688876	0.54	781.9044
18	Cis-11,14,17-Eicosatrienoic acid methyl ester	43.092	227442	2946337	0.22	461.7158
19	Methyl cis-5,8,11,14,17- Eicosapentanoate	43.279	305656	4495156	0.29	682.398
20	Methyl docosanoate	43.762	489252	7027664	0.47	710.776
21	Methyl erucate	44.306	676198	4796164	0.65	1126.5272
22	Cis-13,16-Docosadienoic acid methyl ester	45.081	547164	7856147	0.53	974.0465
23	Methyl tricosanoate	46.707	1421489	8099206	1.37	2402.3078
24	Methyl lignocerate	47.581	623821	8384345	0.6	878.7536
25	All cis-4,7,10,13,16,19- Docosahexaenoate	47.887	698767	8790425	0.67	2132.2644
26	Methyl nervonate	48.683	1224356	12144138	1.18	1615.7797
Total			**103970390**	**1365824978**	**100.02**	

Table 4: Infrared Spectrum Vibration Types of *S. tragacantha* Lindl. Leaves

No.	Vibration Types	***S.tragacantha* Leaves Extract**	
		Methanol	**n-hexane**
1	OH stretching dan N-H stretching (4000-3200 cm^{-1})	3451.031	3449.291
2	N=O stretching (1680 - 1610 cm^{-1})	1648.71	1646.831
3	-NO2 Asymmetric stretching (1600-1510 cm^{-1})	1542.44	-
4	C=C stretching (1600-1450 cm^{-1})	1542.44	-
5	H-C-H bending (1490-1150 cm^{-1})	1384.957	1384.82
6	S=O stretching (1420-990 cm^{-1})	1036.482	-
7	C-O-C stretching (1310-1020 cm^{-1})	1036.482	-
8	C=S stretching (1225-1045 cm^{-1})	-	1022.165
9	C=C-H stretching deformation (1000-780 cm^{-1})	-	856.72
10	C-H deformation (900-670 cm^{-1})	670.187	687.67
11	C-X stretching (850-500 cm^{-1})	588.476	599.77

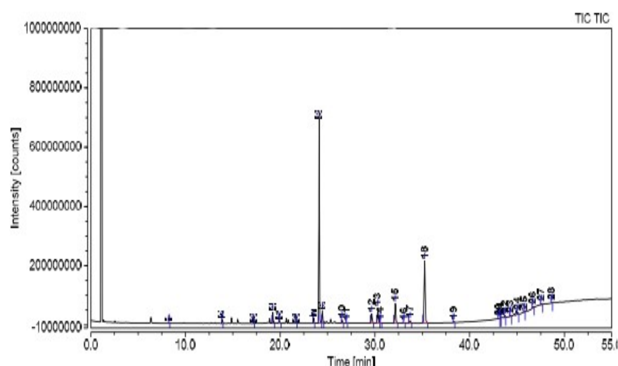

Figure 1: Fatty Acids GC-MS Chromatogram of Methanol Extract *S. tragacantha* Lindl. Leaves

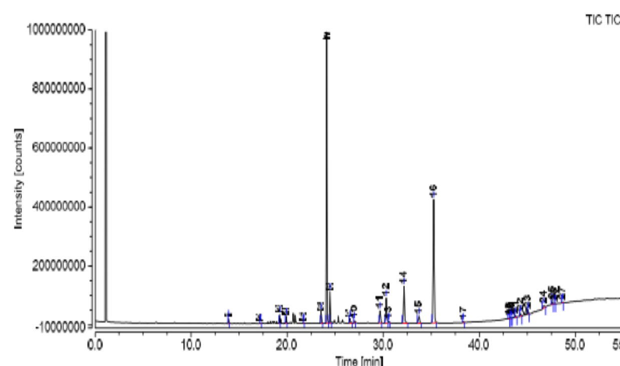

Figure 2: Fatty Acids GC-MS Chromatogram of n-hexane Extract *S. tragacantha* Lindl. Leaves

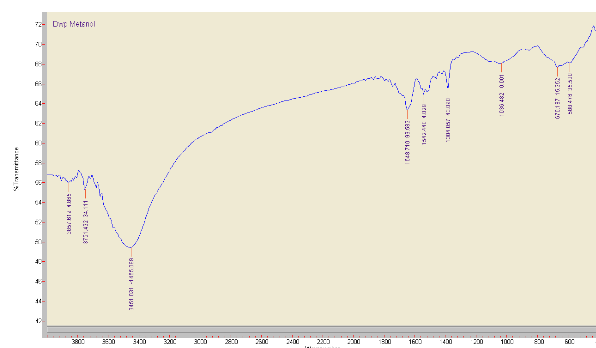

Figure 3: Infrared Spectrum of Methanol Extract *S. tragacantha* Lindl. Leaves

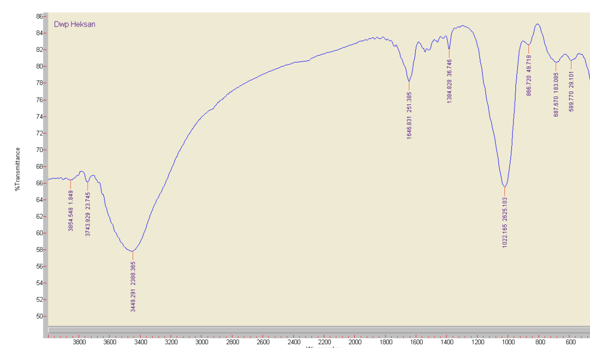

Figure 4: Infrared Spectrum of n-hexane Extract *S. tragacantha* Lindl. Leaves

Functional Group of *S. tragacantha* Lindl. Leaves Extract

The results of *S. tragacantha* Lindl. leaves extraction both using methanol and n-hexane solvents were analyzed with FTIR (Fourier Transform Infra Red) for determining the functional groups. The FTIR infrared spectrums of methanol and n-hexane extracts of *S. tragacantha* Lindl. leaves and the types of spectrum vibration can be seen in Figure 3, 4, and Table 4.

The FTIR spectroscopy analysis was based on the characteristics of the functional groups found in each extract. Data presented in Table 4 and 5 show that both methanol and n-hexane extracts of *S. tragacantha* Lindl. leaves contained phenols which were indicated by an active OH group. Furthermore, it can be seen that the methanol and n-hexane extracts of *S. tragacantha* Lindl. leaves had a wave number of respectively 3451.031 cm^{-1} and 3449.291 cm^{-1}, indicating a hydroxyl (OH) stretching vibration of the phenol component[36]. In addition, the OH group in the wave number showed the presence of free fatty acids. An uptake with a wave number between 3600-2500 cm^{-1} indicates an OH group presenting free fatty acids[37].

There was also OH bound to the side chain or ether function group (R-O-R) at a wavelength of 1036.482 cm^{-1} and 1022.165 cm^{-1}, constituting OH bound to each ring of glucose. As reported [38], C-O-C bonds of hexane rings are detected in the wave number of 1160 cm^{-1}, and C-OH is located on the side chain in the wave number of 1078 cm^{-1}.

CONCLUSION

Extracts of *S. tragacantha* Lindl. leaves showed a strong antioxidant activity. Based on the characteristics of the functional groups extracts of *S. tragacantha* Lindl. leaves contained important compounds such as phenols, free fatty acids. Extracts of *S. tragacantha* Lindl. leaves were dominated by SAF (Saturated Fatty Acids) and PUFA (Poly Unsaturated Fatty Acids) as well as a little MUFA (Mono Unsaturated Fatty Acids).

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