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ISOLATION AND IDENTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM THE WILD PLANT, SUADEA VERMICULATA AGAINST APHIS CRACCIVORA KOCH

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ABSTRACT

Biological and phytochemical studies were conducted on *Suadea vermiculata* to investigate the biologically active compounds of this plant against *Aphis caraccivora* Koch. Results showed that, fraction C with LC_{50} 0.081 mg/cm^2 and LC_{95} 0.226 mg/cm^2 was the most effective compared with the crude extract LC_{50} 0.104 mg/cm^2 and LC95 1.338 mg/cm^2 followed by fraction A (LC_{50} 0.103 mg/cm^2 and LC_{95} 0.352 mg/cm^2) and then fraction B which had the lowest LC_{95} value (0.709) than those of crude extract (1.338). Data also indicated that these fractions composed mainly of fatty acids, fatty acids methyl esters in addition to hydrocarbons. The main active component of fraction A, fatty acids methyl esters with percent area 70.698 % followed by 22.452 % monoterpenes, lenoleic acid with percent area 2.692 and low amount of hydrocabons with area percent 0.745. As regarding fraction B hydrocarbons represented the main components with percent area 56.425 %. The other compounds identified in this fraction were fatty acids 14.066 %, methyl anthranilate 15.706 % and methyl salicylate 7.650 %. Fraction C was the highest toxic fraction against *A. craccivora*, its chemical constituents were fatty acids methyl esters with 15.717 %, fatty acids 10.063 % and monoterpens 7.991 %. Methyl salicylate identified in this fraction with area percent 4.539 in addition to 15.470 % hydrocarbons.

Keywords: Identification, Biological activity, *Suadea vermiculata, Aphis caraccivora*.

INTRODUCTION

Use of botanical pesticides (natural plant products) in an agro-ecosystem is now emerging as one of the primes means to product crop production free environment from pesticidal pollution, which is a global problem. Pervious screening tests were carried out in order to evaluate the toxicity of different plant extracts using different solvents against *Aphis craccivora* (Abdallah et al., 2004). Comparison among the toxicity of the 29 plant extracts using different solvents, showed that the ethyl acetate extracts of *Atriplex Semibaccata*, *Suadea vermiculata* and *Halocenemon strobilacium* were the most toxic against *A. craccivora* . Barakat et al., (2005) isolated and identified some of biological active compounds of *Atriplex Semibaccata* against *Aphis craccivora*. *Suadea vermiculata* belongs to family Chenopodiaceae that contains a large number of plants considered as having high biological activity. Some of them were used in the traditional medicine in

some countries (Mahasneh *et al*., 1996). Therefore, the present study was conducted to investigate:

1-Fractionation of the ethyl acetate crude extract of *S. vermiculata*.
2-Biological activity of the promising fractions against *A. craccivora*.
3-Isolation and identification of insecticidal components of the crude extract and their fractions by using GC/MS.

MATERIALS AND METHODS

1-Plant material

The wild plant *Suadea vermiculata* (fam:Chenopodiaceae) was collected from different areas in Sinai. Sample of the collected plant was left to dry under laboratory conditions. Dried parts of plant were ground using an electric mill, sieved and kept for extraction.

The chemical constituents of S. *vermiculata* were extracted with ethyl acetate and steam distillation. The ethyl acetate extract was further fractionated by column chromatography using solvent mixtures with different polarity.

2-Preparation of the essential oil and crude extract

Samples of the plant were hydrodistilled for 3 hours using a Clevenger type apparatus. The oils were separated and dried over anhydrous sodium sulphate and kept in freezer at – 80 ^{0}C until analysis. Crude extract was prepared according to the method described by Freedman et al., (1979).

3-Fractionation of the ethyl acetate crude extracts

Fractionation of the crude extract, *S.vermiculata* was performed on 50 cm length X 2.5cm diameter column packed with a slurry of silica gel 60 (particle size 0.063-0.2 mm, 70-230 mesh ASTM), purified in pentane/diethyl ether (95/5 v/v). The ethyl acetate crude extract was applied directly on the top of the column then eluted with the following solvents:

No. of phase	Phase	Ratio	Amounts	Fractions
١	Benzene	١٠٠	٢٠٠ ml	1-20
٢	Benzene / Diethyl ether	80 : 20	200 ml	21-40
٣	Benzene / Diethyl ether	60 : 40	200 ml	41-60
٤	Benzene / Diethyl ether	50 : 50	200 ml	61-80 A
٥	Benzene / Diethyl ether	20 : 80	200 ml	81-100 B
٦	Diethyl ether	100	200 ml	10 l-120 C
٧	Acetone	100	200 ml	121-140
٨	Ethanol	100	200 ml	141-160

These fractions were collected and the solvent was evaporated till dryness. Then they were examined for biological activity against *A. craccivora* using residual film technique. All percent mortalities were corrected for the natural mortality according to Abbott's formula (1925) Mortality curves were drawn on probit logarithmic graph paper according to the method developed by Finney (1971).

4-Identification and determination of essential oil and ethyl acetate crude extract and their fractions by GC/MS analysis

The obtained essential oil, ethyl acetate crude extracts and their fractions of the wild plant, *Suadea vermiculata* were analyzed using GC-MS apparatus. GC-MS Finnigan mat SSQ 7000 Digital DEC 3000. Work station: Digital DEC 3000. Ionization mode Eleven 70. Column: DB-5 capillary column 30-m length, 0.32-mm i.d and 0.25 µm thicknesses. Carrier gas: Helium at 13 psi. Temperature-programming initial column temperature was set at 50°C for 3 min then the temp. was increased by 7 °C/min to reach 250°C, and held for 10 min. at 250 °C. Injector temperature was 200°C and the injected volume was 1 µL. Transition-line and ion source temperatures were 250°C and 150 °C, respectively. The mass spectrometer had a delay of 3 min to avoid the solvent peak and then scanned from m/z 40 to m/z 350. Ionization energy was set at 70 eV. Identification was based on the comparison with the MS computer library (NIST -Software Package, Finnigan) and on the respective retention indices. The separated components were identified by matching them with the National Institute of Standards and Tech (NIST) mass spectral library data.

RESULTS AND DISCUSSION

1- Biological studies

Ethyl acetate extracts of *S.vermiculata* showed superior toxicity with LC_{50} 0.104 in comparisons with petroleum ether, chloroform and ethanol extracts (Abdallah et al.,2004). So , ethyl acetate crude extract of *S.vermiculata* was further fractionated by column chromatography using different solvent mixtures possess different polarity. Fractions which eluted with benzene/diethyl ether (1:1) (fraction A), Benzene/diethyl ether (1:4) (fraction B) and diethyl ether (Fraction C) were the most effective against *Aphis craccivora*.

The results in Table (1) showed that fraction C with LC_{50} 0.081 mg/cm2 and LC_{95} 0.226 mg/cm^2 was the most effective against *Aphis craccivora* compared with the crude extract LC_{50} 0.104 mg/cm^2 and LC_{95} 1.338 mg/cm2 followed by fraction A LC_{50} 0.103 mg/cm^2 and LC_{95} 0.352 mg/cm^2. In addition to the previous fractions another one fraction namely B had the lowest LC_{95} value (0.709 mg/cm^2) than those of crude extract (1.338 mg/cm^2).

Table (1): LC_{50}, LC_{95} and Slope values of *Suadea vermiculata* crude extract and its different fractions against *Aphis craccivora*.

Treatments	LC_{50} mg/cm2	LC_{95} mg/cm^2	Slope
Crude extract	0.104	1.338	2.42
Fraction A	0.103	0.352	3.09
Fraction B	0.245	0.709	3.57
Fraction C	0.081	0.266	3.17

2-Identification of essential oil constituents

The chemical name and area % for the nineteen identified compounds in the essential oil are shown in Table (2). The oil is rich in monoterpenes (68.23 %) and sesquiterpene (8.59 %). The main components in this class being α-Terpinene (21.12 %), 2- Carene (17.10 %) followed by α -Phellandrene (8.26 %), α -Pinene (7.02 %), Myrcene (6.02 %) and Camphene (4.64 %), in addition to small amounts of lower and higher homologes. The oil is poor in hydrocarbons, which constitute only 4.86 % of the oil, comprising mainly Pentadecane (2.49 %), Tridecane (0.91 %), Tetradecane (0.73 %) and Hexadecane (0.72 %). The oil contains also 4.82 % of Tetradecanoic acid, the only fatty acid identified, in addition to Lumiflavine 4.67 % and others.

Table (2): Chemical constituents of the main compounds of the essential oil extracted from the wild plant *Suadea vermiculata*.

Peak no.	Name of compounds	Area %
1	α -pinene	7.023
2	Camphene	4.641
3	β -pinene	0.662
4	2-Carene	17.009
5	Myrcene	6.017
6	α -Phellandrene	8.263
7	α -Terpinene	21.122
8	D-Limonene	2.462
9	β -Phellandrene	0.940
10	Propionic acid	1.226
11	Tridecane	0.913
12	Tetradecane	0.732
13	Cis-alpha-copaene-8-ol	8.589
14	Pentadecane	2.493
15	Hexadecane	0.719
16	Agarospirol	5.647
17	α -amyrin	1.923
18	Tetradecanoic acid	4.822
19	Lumiflavine	4.667

3- Identification of the crude extract constituents

The components detected, their area % and formula are compiled in Table (3) Twenty-nine compounds were identified. Fatty acids and their esters constituted 72.70 % of the crude extract, with Hexadecanoic acid (39.65 %), Octadecanoic acid methyl ester- (14.21 %) and Tetradecanoic acid (6.49 %) as the main components. Other members in the same class were identified but in a lower quantities; the methyl ester of Pentadecanoic acid (5.63 %), Hexadecanoic acid, methyl ester (4.08 %) and Dodecanoic acid (2.64 %). Long-chain hydrocarbons amounted to 19.912 %. The main hydrocarbons identified were Heptadecane (6.04 %), Hexadecane (5.62 %) and higher homologes Pentacosane 2.301 %, Hexacosane 1.010 % and Heptacosane 1.121 %. The percentage of ketonic compounds was 10.81 %; trimethyl Pentadecanone (4.30 %) was the main components. The crude extract contained also, Phytol as diterpene alcohol (2.60 %).

Table (3): Chemical constituents of the main compounds of the ethyl acetate crude extract for the wild plant *Suadea vermiculata*.

Peak no.	Name of compounds	Area %
1	Acetic acid, 2-methyl-propyl ester	1.320
2	2,2-Dimethyl valeric acid	0.319
3	2-Pentanone 4-Hydroxy-4- methyl	2.710
4	Carbamic acid, acetyl-, ethylester	0.575
5	Acetic acid, octyl ester	0.473
6	Benzene, 1, 3, 5 - trimethyl	1.460
7	1,5-Heptadiene, 3, 3, 5- trimethyl	1.112
8	1, 2, 3- Propanetriol, monoacetate	1.636
9	Decane, 3, 6- dimethyl	0.690
10	Tetradecane	1.562
11	5, 9-Undecadien-2-one,6, 10- dimethyl-(Z)-	1.498
12	2, 6,10-Dodecatrien-1-ol, 3, 7, 11- trimethyl- (Z,E)	1.086
13	2H-Pyran-2-carboxylic acid,5-ethyltetrahydro-2,3- dimethyl-6-oxo-	0.473
14	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4, 7a- trimethyl	1.383
15	Dodecanoic acid	2.639
16	3,5-Heptanedione, 2,2,6,6-tetramethyl	0.920
17	Hexadecane	5.620
18	Heptadecane	6.042
19	Tetradecanoic acid	6.492
20	Octadecane	3.818
21	Phytol	2.601
22	2-Pentadecane, 6,10,14-trimethyl	4.300
23	2- Pentadecanoic acid, 13- methyl-, methyl ester	5.626
24	Hexadecanoic acid	39.655
25	Hexadecanoic acid, 2- methyl-, methyl ester	4.077
26	8-Octadecanoic acid, methyl ester, (E)	14.210
27	Pentacosane	2.301
28	Hexacosane	1.010
29	Heptacosane	1.121

4- Identification of fraction A constituents

This fraction showed high toxic effect against *Aphis craccivora* especially at LC_{95} level 0.352 mg/cm^2 with the toxicity of crude extract (Table 1).

The GC - MS chromatogram of fraction A showed the presence of 16 identified compounds (Table 4). The main component in this fraction identified as 8-Octadecanoic acid, methyl ester with area percent 70.70 followed by Phytol with percent area 11.31. d-Limonene and Linoleic acid, ethyl ester with percent area 4.80 and 2.69 were detected. The fraction includes the following classes: monoterpene (11.14 %), sesquiterpene (1.36 %), hydrocarbon (0.75 %), alcohols (12.35%) and fatty acid esters (73.39 %). Among the major components of the fraction, d-Limonene has a pleasant orange -like odour and it is classified as one of the most widely distributed terpenes, occurring in many plant extracts and essential oils.

Table (4): Chemical constituents of the main components in fraction A from the ethyl acetate crude extract of *Suadea vermiculata*.

Peak no.	Name of compounds	Area %
1	α -pinene	0.736
2	Camphene	0.540
3	β -pinene	0.851
4	2-Carene	0.516
5	Myrcene	0.818
6	α -Phellandrene	0.859
7	α -Terpinene	0.671
8	d-Limonene	4.795
9	β -Phellandrene	1.358
10	2(4H)-Benzofuranone, 5, 6, 7a-tetrahydro-4, 4, 7a-trimethyl	0.998
11	Tridecanol	1.047
12	Tridecane	0.745
13	α -Bisabolol	1.367
14	Phytol	11.308
15	8-Octadecanoic acid, methyl ester, (E)	70.698
16	Linoleic acid ethyl ester.	2.692

5- Identification of fraction B constituents.

This Fraction showed higher LC_{50} value (0.245 mg/cm^2) than those of crude extract (0.104 mg/cm^2) (Table1), and LC_{95} (0.709 mg/cm^2) lower than those of the crude extract (1.338 mg/cm^2).

The components of the Fraction B and the area percentages are compiled in Table (5). Fourteen components were separated on a 30-m DB-5 fused silica capillary column. The fraction contains 56.435 % hydrocarbon, the main of which are Heptacosane (9.89 %), Pentacosane (13.55 %) and Octadecane (8.01 %). It contains also about 39.50 % of carbonyl compounds, mainly Benzoic acid, 2-(methylamino)-, Methyl ester (15.706 %) and Methyl salicylate (7.650 %). Alcohols (4.09 %) and fatty acid: Hexadecanoic acid (14.07 %).

Table (5): Chemical constituents of the main components in fraction B from the ethyl acetate crude extract of *Suadea vermiculata*.

Peak no.	Name of compounds	Area %
1	2-Pentanone, 4-Hydroxy-4- methyl	2.080
2	Methyl salicylate	7.650
3	Tridecane	4.801
4	Tetradecane	6.640
5	Benzoic acid, 2-(methylamino)-, methyl ester	15.706
6	Hexadecane	7.238
7	Heptadecane, 3- methyl	6.297
8	Octadecane	8.011
9	Hexadecanoic acid	14.066
10	Pentacosane	13.549
11	carissanol	4.089
12	Heptacosane	9.889

6- Identification of fraction C constituents.

In comparison with the toxicity of crude extract and the other two fractions A and B. This fraction showed the highest toxic effect at LC_{50} and LC_{95} (LC_{50} 0.081 mg/cm^2 and LC_{95} 0.226 mg/cm^2) (Table 1).

The components found in fraction C altogether with their percentages are shown in Table (6). Twenty-three compounds were identified. Fatty acid and their esters amounted to 29.50 % of the fraction. The main fatty acids were Hexadecanoic acid (13.05 %) and the methyl ester and Octadecanoic acid (8.00 %). The percentage of alcoholic compounds was 28.13 %; Phytol (6.28 %), Patchoul alcohol (4.97 %) and Stigmasterol (5.73 %) being the main compounds. In addition, the fraction contained 6.70 % ketone, 4.54 % Methyl salicylate and 5.16 % α -Amyrin. Hydrocarbons constituted 15.47 % of the fraction with Heptadecane (5.65 %) and Hexadecane (4.12 %) as the main component.

Table (6): Chemical constituents of the main components in fraction C from the ethyl acetate crude extract of *Suadea vermiculata.*

Peak no.	Name of compounds	Area %
1	2-Pentanone 4-hydroxy-4- methyl	3.034
2	R-(-)-2,2-dimethyl -1,3-dixolane-4-methanol	1.211
3	Butanedionic acid, methyl-, dimethyl ester	3.602
4	1, 5-Heptadiene, 3,3,6- trimethyl	2.232
5	1,4-Anhydro-d-mannitol	1.437
6	2-Cyclopentene-1-one-3,4,4- trimethyl	1.867
7	Mannitol	2.098
8	Methyl salicylate	4.539
9	D- mannitol, 1,2:5,6-bis-O-(1- methyl ethylidene)-	2.054
10	Pentadecane	3.471
11	Hexadecane	4.117
12	Heptadecane	5.648
13	Tetradecanoic acid	4.042
14	3-Eicosyne	3.561
15	2-Pentadecanone, 6,10,14- trimethyl	2.665
16	Pentadecanoic acid – 13- methyl-, methylester	4.415
17	Hexadecanoic acid	13.052
18	8-Octadecanoic acid, methyl ester	7.991
19	Phytol	6.276
20	Patchoul alcohol	4.967
21	Stigmasta-7,16-dien-3-ol, (3-beta,5-alpha,)	5.730
22	1-Dotriacontanol	6.886
23	α -Amyrin	5.160

Table (7) summarizes the active components in the ethyl acetate crude extract and their fractions of *S. vermiculata.* Crude extract composed mainly Fatty acids with area percent 46.147 %, Fatty acids methyl esters with percent area 23.913%, in addition to Hydrocarbons with total percent area 22.164%. The main active component of fraction A, Fatty acids methyl esters with percent area 70.698% followed by 22.452 % Monoterpenes, Lenoleic

acid with percent area 2.692% and low amount of Hydrocabons with area percent 0.745%. As regarding fraction B Hydrocarbons represented the main components with percent area 56.425%. The other compounds identified in this fraction were Fatty acids (14.066 %), Methyl anthranilate 15.706 % and Methyl salicylate 7.650 %. Fraction C was the highest toxic fraction against *A. craccivora*, its chemical constituents were Fatty acids methyl esters with 15.717 %, Fatty acids 10.063 % and Monoterpens 7.991 %. Methyl salicylate identified in this fraction with area percent 4.539% in addition to 15.470 % Hydrocarbons.

Table (7): percent area of active components of the ethyl acetate crude extract and their fractions of *S. vermiculata*.

Compounds	% Area of the compounds in fractions:			
	Crude extract	Fraction A	Fraction B	Fraction C
*-Monoterpenes				
-Monocyclic	-	8.501	-	-
-Bicyclic α -pinene	-	2.643	-	-
*- Diterpenes				
- Phytol		11.308		7.991
Total	-	22.452	-	7.991
**-Fatty acids				
Tetradecanoic acid	6.492	-		5.648
Hexadecanoic acid	39.655	-	14.066	4.415
Total	46.147	-	14.066	10.063
*-Fatty acids esters				
*- Methyl esters				
-Pentadecanoic acid	5.626	-	-	2.665
-Octadecanoic acid	14.210	70.698	-	13.052
-Others	4.077			
Total	23.913	70.698		15.717
*-Ethyl esters				
-Linoleic acid	-	2.692	-	-
Total	-	2.692	-	-
*-Hydpocarbons	22.164	0.745	56.425	15.470
*- Methyl anthranilate	-	-	15.706	-
*- Methyl salicylate	-		7.650	4.539
LC50 0.081 mg/cm2	0.104	0.103	0.245	0.081

Obviously, crude extract and its biologically active fractions contain active constituents e.g. the presence of Methyl salicylate is in close agreement with the data obtained by Mahasneh *et al.* (1996). They reported that, the petroleum ether extract of *Suadea vermiculata* used in the traditional medicine of Bahrain have an antimicrobial activity, however they were not discussed the reasons, but obviously through the GC - MS analyses, the antimicrobial effect of such extract is due to the presence of Methyl salicylate.

Methyl salicylate is a repellent for black bean aphid (Hardie, et al., 1994). It stimulates specific olfactory cells in the primary rhinaria on the sixth and fifth antenna segments, respectively, of the black bean aphid, *Aphis fabae*. It also inhibited attraction to volatiles from its host, broad bean (*Vicia faba*). Methyl salicylate is widely distributed as a component of certain plants,

e.g., in the Rosaceae and Salicaceae. It is also a volatile metabolite of salicylic acid, a plant phenolic that is a known allelopathic agent (Balke et al., 1987) and a compound capable of inducing various secondary metabolite - based defense mechanisms, such as production of phenolics by the inducible phenylalanine ammonia - lyase (PAL) system Ward *et al.,* (1991).

Sauvion et al. (1996)) reported that mannose - binding lectins assayed in artificial diets for their toxic and growth - inhibitory effects on nymphal development of the peach - potato aphid *Myzus persicae*.

More than 20 compounds were identified in benzene/ether (1:1) (Fraction A). Monoterpenes and unsaturated fatty acid esters characterize this fraction in comparison to the others (Tables 4, 5 and 6).

Hardie *et al.* (1994) showed the repellent effect of Monoterpenoid myrtenal, which is metabolically, related to one of the identified component in fraction A, α -Pinene.

α -Pinene is an abundant component of defensive resins produced by gymnosperms. Ment, *Mentha virdis* L. and peppermint, *Mentha pipertia* L. was reported to have deterrent and toxicity effects on *Tetranychus urticae*. Qualitative and quantitative analysis of mint and peppermint essential oils showed that both oils were mainly characterized by high concentration of total Terpene compounds 88.53 and 92.67% respectively (Momen *et al*. 2001).

In addition to Methyl salicylate, long - chain hydrocarbons constitute 56.43 % of the fraction B, 22.16 % in the crude and 15.47 % in fraction C. These hydrocarbons e.g. Hexadecane, Heptadecane and Octadecane in addition to Hexadecanoic acid may explain the biological activity of this fraction. In a similar experiment performed by Peterson *et al.,* (1989), hexane extract of *Chenopodium ambroioides* contain mainly long-chain Hydrocarbons and Fatty acid esters, that was toxic against *Tribolium castaneum* and *Sitophilus granarius*.

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فصل وتعريف المكونات النشطة بيولوجياً من نبات السويده البرى *Suadea vermiculata* ضد حشرة من البقوليات *Aphis craccivora*

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٢*- قسم الآفات ووقاية النبات- المركز القومى للبحوث - القاهرة.

أجريت دراسات بيولوجية وكيميائية لفصل وتعريف المكونات الفعالة بيولوجياً لنبات السويده البرى ضد حشرة من البقوليات.

أظهرت النتائج أن الجزء المفصول C، كان الأكثر فاعلية ضد الحشرة المختبرة، وكانت قيم LC_{50} ، LC_{95} كالآتى : ٠,٠٨ ، ٠,٢٢٦ ، ملليجرام/سم٢ مقارنة مع ٠,١٠٤ ، ١,٣٣٨ ملليجرام/سم٢ للمستخلص الخام، يليه الجزء المفصول A (٠,١٠٣، ٠,٣٥٢ ملليجرام/سم٢)، لنفس القيم المذكورة ، ثم الجزء B الذى أعطى قيمة LC_{95} ٠,٧٠٩ الأقل من مثيلتها للمستخلص الخام (١,٣٣٨) وقد بينت النتائج أيضا أن المكونات الرئيسية الفعالة لهذه الأجزاء المفصولة هى أحماض دهنية، أحماض دهنية وإستراتها بالإضافة إلى الهيدروكربونات. ووجد أن المركبات النشطة للجزء A هى الأحماض الدهنية وإستراتها بنسبة ٧٠,٦٩٨ % يليها التربينات الأحادية ٢٢,٤٥٢%، حمض اللينوليك بنسبة ٢,٦٩٢ مع وجود نسبة ضئيلة من الهيدروكربونات (٠,٧٤٥%). وبالنسبة للجزء B تمثل الهيدروكربونات المكونات الرئيسية بنسبة ٥٦,٤٢٥ %، الأحماض الدهنية ١٤,٠٦٦% ، ميثيل انثرانيلات ١٥,٧٠٦ %، ميثيل سلسلات ٧,٦٥٠ % . الجزء الثالث C وهو أعلى الأجزاء سميه ضد الحشرة موضع الإختبار فهو يحتوى على إسترات أحماض دهنية بنسبة ١٥,٧١٧ % ، أحماض دهنية ١٠,٠٦٣ % ثم التربينات الأحادية ٧,٩٩١ % ، ميثيل سلسلات ٤,٥٣٩ % بالإضافة إلى هيدروكربونات ١٥,٤٧٠ %.