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RESEARCH ARTICLE

Chemical Constituents of the Goat Margarine and Antibacterial Activity against Bacterial Pathogens in Sudan

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Abstract

The margarine constituents were examined by Gas Chromatography-Mass Spectrometry (GC-MS), it contained 41 compounds, fifteen of them were identified as the major compound, Hexadecanoic acid, methyl ester (22.39%), Methyl stearate (14.92%), methyl elaidate (13.80%), Methyl tetradecanoate (10.74%), Capric acid, methyl ester (8.34%) Lauric acid (4.52%) methyl octanoate (2.70%), linoleic acid methyl ester (2.57%), Methyl 11-octadecenoate (1.90%), methyl caproate (1.77%), Methyl pentadecanoate (1.72%), Methyl (8E,11E)-8,11-octadecadienoate (1.70%), Heptadecanoic acid, methyl ester, (1.46%),trans-13-Octadecenoic acid, methyl ester (Oleic acid) (1.20%), Methyl palmioleate (1.00%). The effect of goat margarine, against four different pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhimurium*, and *Bacillus cereus*, were carried out by using a disc diffusion technique, the highest antibacterial activity was detected against *Salmonella typhimurium* and the lowest one against *Escherichia coli*. The antibacterial activity of the antibiotics, (Ciprofloxacin, Tetracycline Ceftriaxone, Chloramphenicol and Gentamycin), were tested by the disc diffusion technique, and by measuring zones of inhibition, shows that there were differences, among all antibiotic the highest activity of antibiotic against bacteria was due to the action of ciprofloxacin. Ceftriaxone and Tetracycline antibiotic give lowest activity. Among the bacteria the highest inhibition zone by antibiotic against *Salmonella typhimurium*, and the lowest one against *Escherichia coli*

Keywords: Goat margarine; GC-MS; antibiotics; pathogenic bacteria; disc diffusion method.

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INTRODUCTION

Traditional goat margarine can be made by separation the fat from butter. The structure of margarine contain of carbon, hydrogen and oxygen, those atoms were difference bonded together (saturated and unsaturated). Goat milk contain mostly unsaturated fatty acids (monounsaturated (MUFA), polyunsaturated fatty acids (PUFA)), which all are known to be beneficial for human health, especially for cardiovascular conditions[1,2,3], essential fatty acids have nutritional importance it is a source of omega-3 and omega-6[4,5,6,7] the goat margarine have less amount of trans- acids. The extracts of goat margarine are mainly, oleic acid have activity against bacterial[8] Linoleic acid methyl ester prevent body against microorganism[9,] trans-13-Octadecenoic acid, methyl extract against inflammation, prevent against cancer, act as cosmetic, prevent skin from many decrease, anemia and act as insects ide[10]. Hexadecanoic acid, methyl ester act as Antibacterial and antifungal, methyl octanoate and n-hexa-decanoic acid have higher activity against bacteria[11,12,13,14]. Margarine come from goat milk lipid have best activity against allergic, inflammation, it have high nutritive value[15.] Caproic and caprylic acids, they have possess antimicrobial activity, total fat content have higher activity for prevention bacteria.

Objective

The present study was to know the goat margarine chemical Compound and the activity of it against pathogenic bacteria attacked human, compare it with the antibiotics.

MATERIALS

Goat milk collected from domesticated goat, piece of cloth, yogurt, refrigerator and milk pudding machine, Gas Chromatography-Mass Spectrometry (GC-MS).

Microorganism

All the microorganisms used in this work were obtained from the National Research Centre (Khartoum) Sudan. The identification of bacterial was carried out by conventional biochemical methods according to the standard micro-biological techniques These microbes were, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhimurium* and *Bacillus cereus.*

METHODS

Manufacture of margarine

Traditional method

Milk filtered from any impurities by piece of cloth, coagulation the milk by yogurt After the milk reaches the level of good coagulation, put in the refrigerator for at least 4 hours to cool down The coagulated milk is placed in a special machine or can be placed in a tightly sealed container and is dipped for about an hour with the hands, but in the machine for milk pudding it takes about 45 minutes then collect the butter from above the milk and washed with cold water then heat and collect margarine.

Sample Preparation

2g of the goat margarine was mixed thoroughly with 7ml of alcoholic sodium hydroxide (Noah). The mixture was then shake for 5 minutes. The content of the test tube was left to stand overnight.1 ml of Super saturated sodium chloride (NaCl) was added and shaken, added 2ml of normal hexane and then the contents were shake thoroughly for three minutes. Then the n-hexane layer (the upper layer of the test tube) was formed, 5 µl from the n-hexane extract was diluted with 5 ml of diethyl ether, filtered the mixture and dried with 1g of anhydrous sodium sulphate, and 1µl of the diluted sample was injected in the GC/MS instrument.

Method of analysis

Gas Chromatography-Mass Spectrometry (GC/MS) Conditions

The identification of goat margarine constituents were carried out by using Gas chromatography with mass spectrometry, model (GC/MS-QP2010-Ultra)' Shimadzu Company, Japans with serial number 020525101565SA and capillary column (Rtx-5ms-30m x 0.25mm x 0.25 µm). Injection of the sample by using split mode, the carrier gas was helium passed with flow rate 1.61 ml/min, the rate of temperature was began from 6°C with flow rate 10c/min to 30°C as end temperature degree with 3 minutes hold time, the injection port temperature was 30°C, the temperature of the ion was 20°C and the interface temperature was 25°C. The goat margarine sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 27 minutes. The identification of the

Table 1. Chemical constituents of goat margarine identified by GC-MS

ID#	Name	Ret.Time	Area	Area%
1.	Hexanoic acid, methyl ester	3.300	16420096	1.77
2.	Octanoic acid, methyl ester	5.957	25031032	2.70
3.	4-Decenoic acid, methyl ester, Z-	8.753	1973471	0.21
4.	Decanoic acid, methyl ester	8.871	77338694	8.34
5.	Dodecanoic acid, methyl ester	11.566	41934099	4.52
6.	Tridecanoic acid, 12-methyl-, methyl ester	13.570	1724913	0.19
7.	Methyl Z-11-tetradecenoate	13.881	2696851	0.29
8.	Methyl tetradecanoate	14.031	99602220	10.74
9.	Pentadecanoic acid, methyl ester	14.727	3919991	0.42
10.	Tetradecanoic acid, 12-methyl-, methyl ester	14.825	7091336	0.76
11.	Pentadecanoic acid, 14-methyl-, methyl ester	15.144	15978254	1.72
12.	7,10-Hexadecadienoic acid, methyl ester	15.919	291271	0.03
13.	7-Hexadecenoic acid, methyl ester, (Z)-	15.982	4681016	0.50
14.	9-Hexadecenoic acid, methyl ester, (Z)-	16.030	9266601	1.00
15.	11-Hexadecenoic acid, methyl ester	16.069	2138576	0.23
16.	Hexadecanoic acid, methyl ester	16.289	207249077	22.39
17.	Hexadecanoic acid, 15-methyl-, methyl ester	16.881	7748042	0.84
18.	Hexadecanoic acid, 14-methyl-, methyl ester	16.977	8482084	0.91
19.	cis-10-Heptadecenoic acid, methyl ester	17.046	5044403	0.54
20.	Heptadecanoic acid, methyl ester	17.261	13576564	1.46
21.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.995	23805616	2.57
22.	9-Octadecenoic acid (Z)-, methyl ester	18.081	127920245	13.80
23.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.105	4085216	0.44
24.	11-Octadecenoic acid, methyl ester	18.135	17578257	1.90
25.	trans-13-Octadecenoic acid, methyl ester	18.172	11153676	1.20
26.	Methyl stearate	18.290	138367840	14.92
27.	8,11-Octadecadienoic acid, methyl ester	18.468	15794339	1.70
28.	9,11-Octadecadienoic acid, methyl ester, (E,E)-	18.590	775753	0.08
29.	10-Nonadecenoic acid, methyl ester	18.966	2042381	0.22
30.	Nonadecanoic acid, methyl ester	19.195	2051960	0.22
31.	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	19.577	1780403	0.19
32.	11-Eicosenoic acid, methyl ester	19.887	1881376	0.20
33.	Eicosanoic acid, methyl ester	20.104	3864392	0.42
34.	Heneicosanoic acid, methyl ester	20.974	1297791	0.14
35.	Methyl 6,9,12,15,18-heneicosapentaenoate	21.406	1940477	0.21
36.	Docosanoic acid, methyl ester	21.812	1495112	0.16
37.	Tricosanoic acid, methyl ester	22.618	873501	0.09
38.	Vinyl decanoate	23.314	2191100	0.24
39.	Tetracosanoic acid, methyl ester	23.393	317580	0.03
40.	Sulfurous acid, pentadecyl pentyl ester	23.693	8958125	0.97
41.	Octanoic acid, 4-pentadecyl ester	23.831	6844877	0.74

compound by comparing their retention index and mass fragmentation patents with those are found in the library, the National Institute of Standards and Technology (NIST).

Disc diffusion method

For microbial sensitivity test using the paper disc diffusion method, to screen the antibacterial activity of goat margarine and performed by using Muller Hinton agar. Sterile 5 mm diameter paper disc soaked with the margarine was placed gently on the media, which had been freshly inoculated with each of the

Table 2. Inhibition zoon (in mm) for different concentrations of goat margarine

Microorganism	Concentration of the margarine (µg/disc)			
	12.5	25	50	100
Salmonella typhimurium	0	12	13	15
Pseudomonas aeruginosa	0	11	13	14
Escherichia coli	0	10	11	11
Bacillus cereus	0	12	14	14

Table 3. Inhibition zoon (in mm) for different antibiotics

Bacterial test strains (No. tested)	Antibiotics concentration in (µg/disc)m				
	Ciprofloxacin	Gentamycin	Chloramphenicol	Tetracycline	Ceftriaxone
Salmonella typhimurium	25	15	18	9	12
Pseudomonas aeruginosa	*26*	*10*	*12*	*12*	14
Escherichia coli	14.9	11	13	10	10
Bacillus cereus	21	13	10	11	6

Ciprofloxacin (5µg), Gentamycin (10µg), Chloramphenicol (10µg), Tetracycline (25µg) and Ceftriaxone (30µg),

organisms. The plates were incubated for 24 hours at 37°C. The results were recorded by measuring the zone inhibition by goat margarine, also used the same method for microbial sensitivity test of the antibiotic[17].

RESULTS AND DISCUSSION

Table 1 and Fig. 1 shows that the chemical constituents of the goat margarine are given in the order of the retention times 41 constituents were identified, 15 of them constituted as major compound such as Hexadecanoic acid, methyl ester (22.39%), Fig. 2. Methyl stearate (14.92%), Fig. 3. 9-Octadecenoic acid (Z)-, methyl ester (13.80%), Fig. 4. Methyl tetradecanoate (10.74%), Fig. 5. Capric acid, methyl ester (8.34%). Fig. 6. Lauric acid, methyl ester (4.52%), Fig. 7. Methyl octanoate (2.70%), Fig. 8. Linoleic acid methyl ester (2.57%), Fig. 9, Methyl 11-octadecenoate (1.90%), Fig. 10. Hexanoic acid, methyl ester (1.77%), Fig. 11. Methyl pentadecanoate (1.72%), Fig. 12. Methyl(8E,11E)-8, 11-octadecadienoate (1.70%), Fig. 13. Heptadecanoic acid, methyl

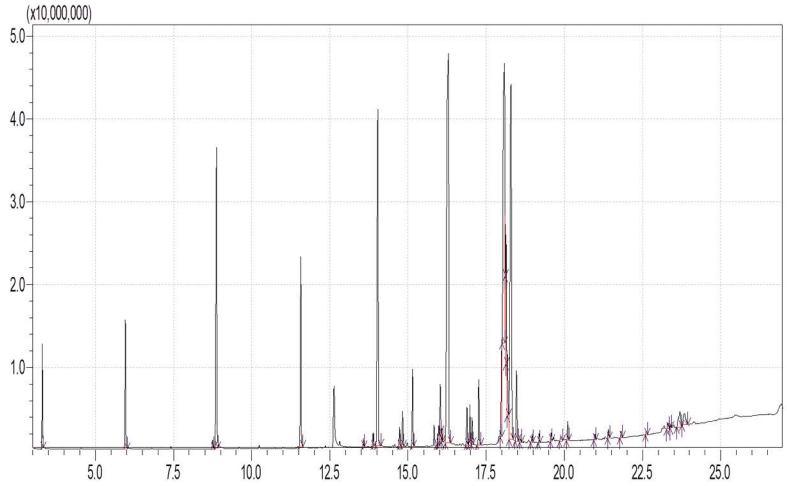

Fig. 1. GC/MC of goat margarine

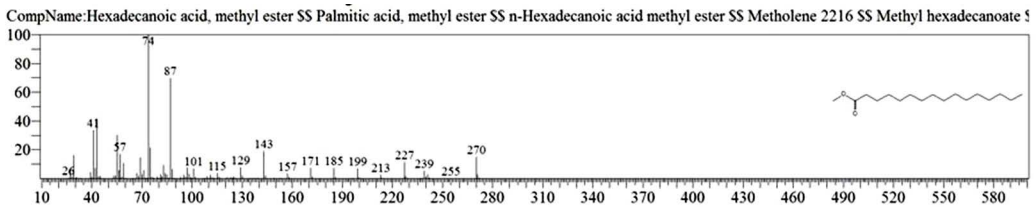

Fig. 2. Hexadecanoic acid methyl ester

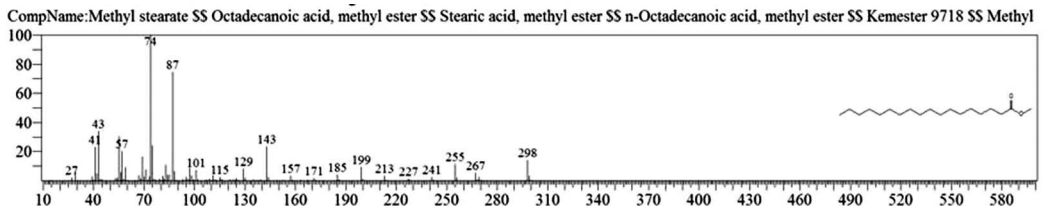

Fig. 3. Methyl streate

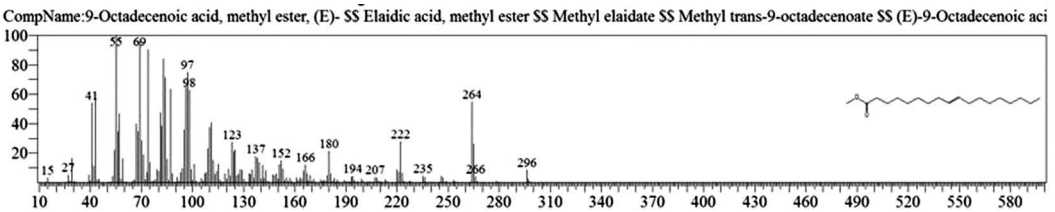

Fig. 4. 9-Octadecenoic acid methyl ester

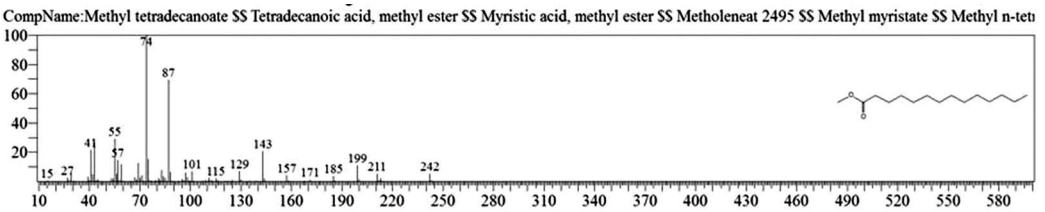

Fig. 5. Methyl tetradecanoate

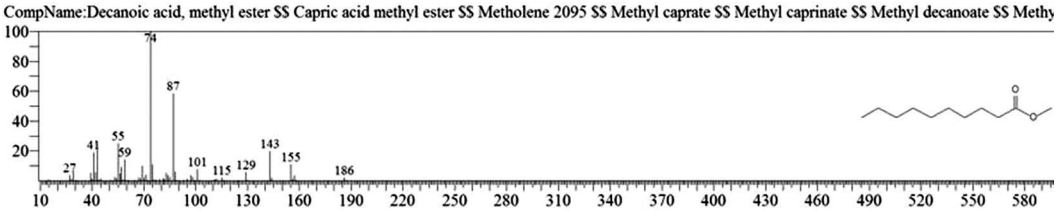

Fig. 6. Decanoic acid methyl ester

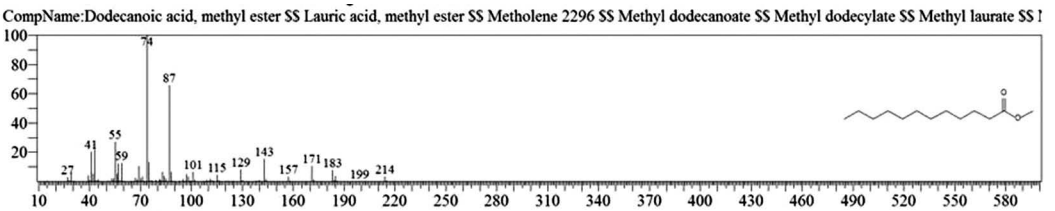

Fig. 7. Dodecanoic acid methyl ester

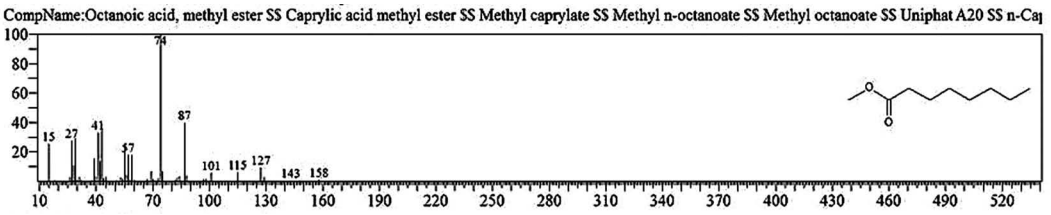

Fig. 8. Octanoic acid, methyl ester

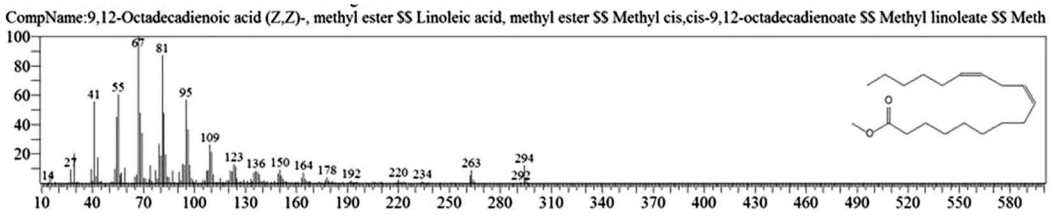

Fig. 9. 9,12-Octadecadienoic acid (z,z).,methyl ester

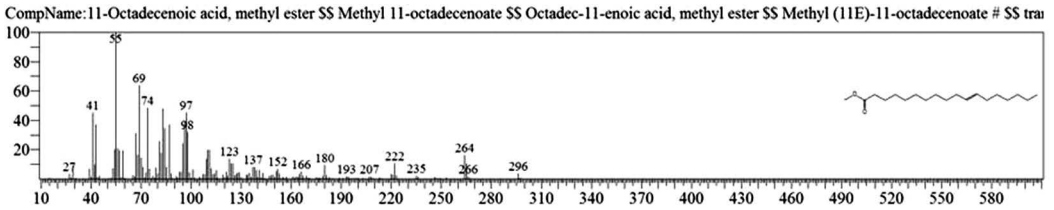

Fig. 10. 11,Octadecenoic acid, methyl ester

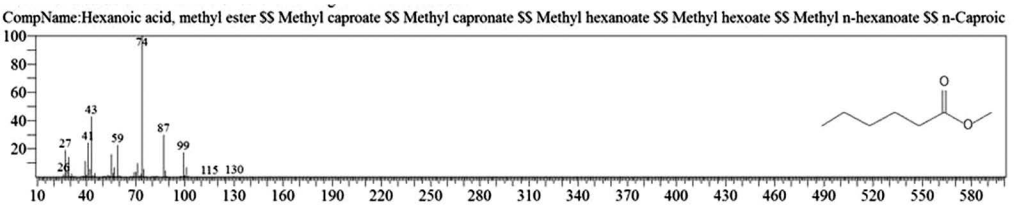

Fig. 11. Hexanoic acid, methyl ester

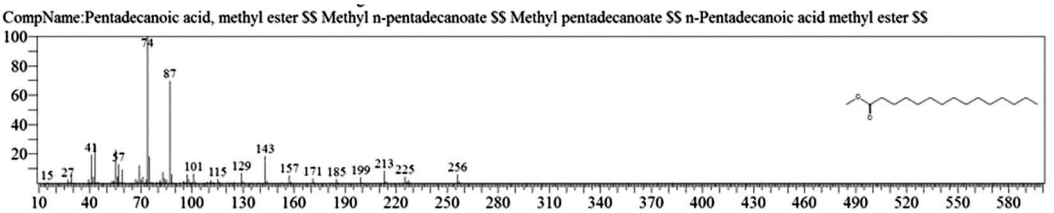

Fig. 12. Pentadecanoic acid, methyl ester

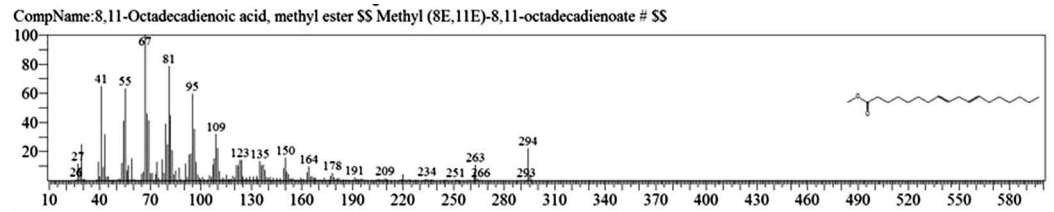

Fig. 13. 8,11,-Octadecadienoic acid methyl ester

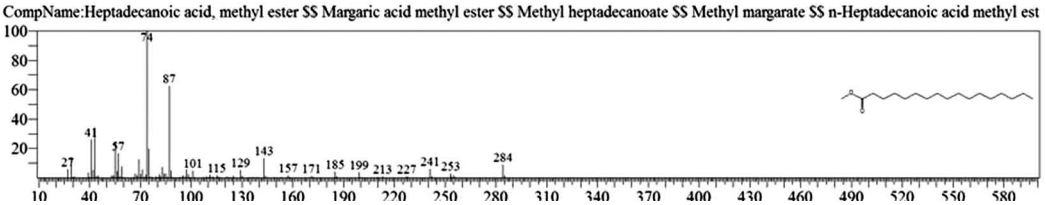

Fig. 14. Heptadecanoic acid, methyl ester

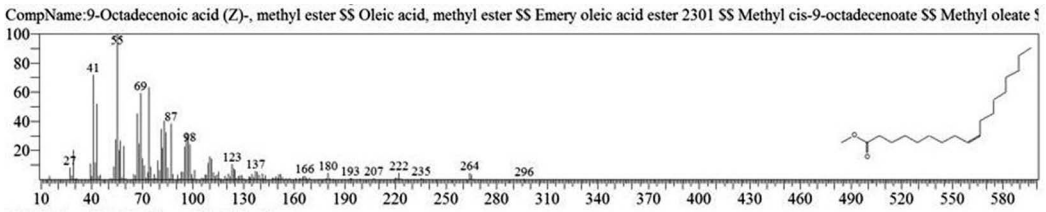

Fig. 15. Oleic acid

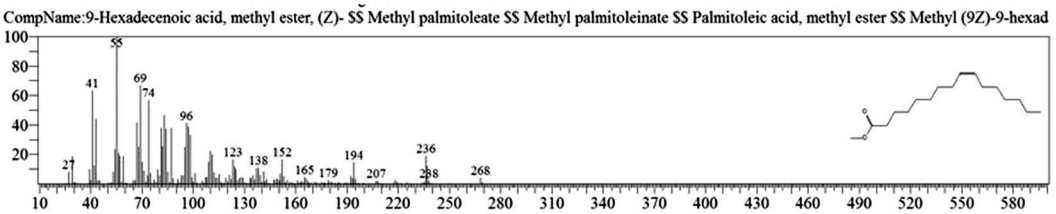

Fig. 16. 9- Hexadecenoic acid, methyl ester

ester (1.46%) Fig. 14. Oleic acid (1.20%), Fig. 15. 9-Hexadecenoic acid, methyl ester, (Z)- (1.00%), Fig. 16, these compound were found active during infla-mmation, act during oxidationas antioxidant, hypocholesterolemic highly active against pest, insect and nematode[8,18,19]. Table 2 shows that the antibacterial activity of the goat margarine against four different pathogenic organisms *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhimurium* and *Bacillus cereus*, (the highest concentration of the goat margarine is (100 mg/ml) and the lowest one is (12.5 mg/ml) there was differences effect among bacteria, the highest inhibition zone by goat margarine was detected against *Salmonella typhimurium* and the lowest inhibition zone against *Escherichia coli*, in lower concentration (12.5) there was no result. these results conformity with those obtained in previous studies[16,19,20] Table 3. Shows that the inhibition zones (in mm) for the antibiotic there was differences among them, highest activity of antibiotic against bacteria was due to the action of ciprofloxacin among all antibiotic and the lowest inhibition detected by Ceftriaxone and Tetracycline antibiotic, the highest inhibition zone among the bacteria by antibiotic against *Salmonella typhimurium* and the lower inhibition zone is *Escherichia coli*, there was no high different between antibiotic, (Gentamycin, Chloramphenicol, Ceftriaxone and Tetracycline) and goat margarine against bacteria.

CONCLUSION

This work comes to conclude that the goat margarine contain non-polar components had potent antibacterial activity against pathogen bacteria, and have no high different between it and synthetic antibiotic in the activity against microorganism,

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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