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THE CHEMICAL COMPOSITION OF THE FIXED AND VOLATILE OILS OF *NIGELLA SATIVA* L. AND ITS PHYSICO-CHEMICAL PROPERTIES

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ABSTRACT

Nigella sativa name as black seed or Kalonji seed belongs to family of rananculacea. It is widely grown in different part of world in Europe, Middle East, and Western Asia, Phytochemically; it contains fixed oil, essential oil, protein and alkaloids saponin. The chemical composition of the extracted fixed oil (total fatty acid composition) and volatile oil of *Nigella sativa* L. seeds were determined by GC. The compounds Thymoquinone was found in aqueous extract 5.53 % and 0.33 % in chloroform extract and very small amount in n-hexane. There are many fatty acids found in fixed oil of *Nigella sativa* and the major compounds were stearic acid (28.59%) and palmitic (7.3%) and linoleic (6.27%).and oleic acid (2.75%). Various physical constants of fixed oil like refractive index, specific gravity and viscosity were also determined.

KEYWORDS*: Nigella sativa* L., Fixed Oil, Volatile Oil Composition, physico-chemical properties

INTRODUCTION

The seeds of *N. sativa (The black seed)* are the source of the active ingredient of this plant. The actual importance of *N. sativa* to the Muslims came from the holy saying of the Prophet Mohammed "Prayers and peace be upon him" in the black seed is the medicine for every disease except death.[1]

N. sativa oil has been shown to possess 67 constituents, many of which are capable of inducing beneficial pharmacological effects in human.[2] By HPLC analysis of *N. sativa* oil, thymoquinone (TQ), dithymquinone (DTQ), thymohydroquinone, and thymol are considered the main active ingredients. *N.sativa* seeds contain other ingredients, including nutritional components such as carbohydrates, fats, vitamins, minerals and proteins, including eight of essential amino acids.[3] Carbohydrates like monosaccharides in the form of glucose, rhamnose, xylose, and arabinose, are found. *N. sativa* seeds are rich in the unsaturated and essential fatty acids. Chemical characteristics, as well as fatty acid profile of the total lipids, revealed that the major unsaturated fatty acid is linoleic acid, followed by oleic acid.[3] The major phospholipid is phosphatidylcholine, followed by phosphatidylethanolamine, phosphatidylserine, and phosphatidylinisitol respectively. The seeds contain carotene which is converted by the liver to vitamin A.[4] The *N. sativa* seeds are also a source of calcium, iron, and potassium.[5]

Amin *et al*.[6] found that the Black cumin oil contained about thirty-two fatty acids (99.9%) in the fixed oil. The major fatty acids were linoleic acid (50.2%), oleic acid (19.9%), margaric acid (10.3%), cis-11, 14-eicosadienoic acid (7.7%) and stearic acid (2.5%).

The objective of this study was carried out to extract of volatile and fixed oil from *Nigella sativa* L. by different methods.

MATERIALS AND METHODS

Plant material

The dried seeds of *Nagilla Sative* were collected from local market in Baghdad during November / 2014 and identified by biolgy department in the college of sciences / Baghdad University. The seeds have been grinded by a coffee grinder to a fine powder to be used directly after grinding.

Isolation of essential oil from *Nagilla sativa* seed

There are two methods using to extracted and isolated essential oil.

First: Isolation of essential oil by Steam Distillation method

The essential oil extracted according to Koedam,[7] One-hundred gram of the powdered *Nagilla sativa* seeds have been placed in a special flask connected to a steam generation source, the flask was connected to a condenser which in turn connected to a collecting flask

immersed in ice or cold water, the steam was passed from the steam generator to the flask. the temperature of the water– *Nagilla* seeds mixture have been raised to about 102 C˚(avoiding high pressure), the water steam which carried the essential oil molecules passed through the condenser where its temperature had been decreased below the boiling point of water and was collected in the collecting flask (both the water and essential oil), the collected mixture has been transferred to a separator funnel and kept in cold place over night, then the oil layer could be collected, dried over anhydrous sodium sulfate, weighted, and stored in a sealed vial dark colored at 4 C˚.

Second : Isolation of essential oil from *Nagilla* seeds using Clevenger

The hydro distillation method had been used for the extraction of essential oil from (NS). By using clavenger apparatus. The distillation flask of 1000 ml contained water about 1/2 of its volume and 100 gm of the powdered plant material .The operation have been proceeded by heating the flask at 100 C˚, heat was applied to the flask and the volatile oil was carried with the steam to a cold condenser, where the volatile oil and steam returned to their liquid states. The process continued for about 6 hrs till getting largest amount of the oil. Generally, the lighter oil rises to the top of the separator and can be drawn off from time to time as the layer built up. The water, that contains traces of slightly soluble fractions of the oil is drawn off continuously from the bottom of the separator and was returned to the flask containing the plant material. The essential oil collected was dried over anhydrous sodium sulphate, weighed and stored in a sealed dark colored vial at 4 C˚.

The yield percentage of essential oil was determined using the formula described by Rao *et al.*[8] where the amount of essential oil recovered (g)

$$\text{Yield (\%)} = \frac{\text{Amount of essential oil recovered (g)}}{\text{Amount of plant material distilled}} \times 100$$

Preparation of the *Nigella* Methanol Extract

The extract has been prepared according to the method used by Ozaki *et al.*, [9] with some modification. 100 gram of the crude powder of seeds was refluxed with 350 ml of 70% methanol (1:7) in soxhlet apparatus for 8 hours, the solution have been filtered through a filter paper and evaporated to dryness under vacuum at 30 C˚, the dried extract have been weighed and stored at 4 C˚.

Preparation of the *Nigella* Chloroform Extract

The extract has been prepared according to the method used by Ozaki et al,[9] with some modification. 100 gram of the crude powder of seeds was refluxed with 350 ml of 70% methanol (1:7) in soxhlet apparatus as shown for 8 hours, the solution have been filtered through a filter paper and evaporated to dryness under vacuum at 30 C˚, the dried extract have been weighed and stored at 4˚C.

Preparation of the *Nigella* Hexane Extract

The extract has been prepared according to the method used by Ozaki et al[9] with some modification. 100 gram of the crude powder of seeds was refluxed with 350 ml of 70% methanol (1:7) in soxhlet apparatus as shown for 8 hours, the solution have been filtered through a filter paper and evaporated to dryness under vacuum at 30˚C, the dried extract have been weighed and stored at 4˚C.

Evaluation of some physical characteristics of *Nagilla* seeds fixed oil

The physical futures were evaluated according to the methods mentioned by,[10] and.[11] as follows:

1. odor, color and taste : The odor, color and taste of the essential oil have been evaluated by sensory testes through smelling, tasting and the degree of color.
2. The solubility of the essential oil have been evaluated by using different solvents.
3. Density : The value of density of the essential oil of *Nagilla Sativa* seeds determined by weighting 1 ml of the oil using sensitive balance then dividing the weight of the oil on the volume of the same oil.
4. Viscosity: The value of viscosity have been evaluated by putting 15ml of the essential oil of *Nagilla Sativa* seeds in the viscometer at 25 C˚ and read the result.

Gas Chromatography analysis

Analysis of the oil was carried out by *ibn sina center* in Baghdad university, Gas Chromatography analysis was carried out on Shimadzu GC-14A gas chromatograph with FID detector and SE-30 column (length and inner diameter). The operating conditions was as the follow carrier gas was He (30 ml/min constant flow) , the oven temperature for first 2 min was 100 C˚ and then increased at a rate of 10 C˚ / min until 300 C˚ hold for 2 min , injector and detector temperature were set at 325 C˚ and 350 C˚ respectively.

RESULTS AND DISCUSSION

Table (1) shown that the volatile oil for black seed (*N. sativa*) was 0.5 gm /100 gm when extracted by steam distillation method , while we obtained 0.8-1.0 gm/100gm when used Clevenger method. Other studies found that volatile oil (0.4-1.5%) [12,13,14]. And also Hajhashemi *et al*., (2004) found that the volatile oil was 0.4 to 2.5% . Because the little amount gave by steam distillation and Clevenger method we could not be studies the physical chemical properties.

Table (1): The volatile oil quantity (g/100g) extracted by different methods.

Type of extraction methods	Weight (g/100gm)
Steam distillation	0.5
Clevenger	0.8-1.0

From the results table (2) shown that the fixed oil for black seed was 35g/100gm when extracted by methanol solvent compared with chloroform solvent when extracted with it gave higher amount 42 g/100gm and 38 m/100gm when extracted with n-hexane. Hajhashemi *et al*.[15] found that the *N. sativa* seeds contain 36 to 28% fixed oil. Another study by Gharby, et al.[16] Nigella seeds from Morocco afforded 37% and 27% of oil after hexane- or cold press-extraction, respectively.

Table (2): The fixed oil quantities of extraction by soxhelt with various solvents.

Type solvent	Weight (g/100gm)
Methanol extract	35
Chloroform extract	42
n-hexane	38

The Physico- chemical properties of *Nagilla* seeds essential oil and fixed oil extracted by different solvents have been shown in Table (3). The odor of the essential oil was strong like spicy odor and have been characterized by a colorless to pale yellow color. The essential oils of *Nagilla* seeds was insoluble in water but soluble in some organic solvents like ethanol, ether and chloroform and petroleum ether.

Specific gravity is the ratio of the density of a respective substance to the density of water at 4°C.Specific gravity values of oils are less than 1 for most of the oils except few containing oxygenated aromatic compounds,[17] Table (3) also shown that specific gravity for the fixed oil which extracts by methanol, chloroform and n-hexane were 0.9194, 0.9227 and 0.9473

(gm/ml) at 25 ^{o}C respectively. Another study by [18] found that the specific gravities estimated with the seed oils for Jordanian *N. sativa* 0.9071 at 25 ^{o}C.

The range of refractive index (RI) for the fixed oil was 1.341-1.466 at 20^{o}C and that depends upon extracted methods and the temperature. The results for this study higher than found by Al-Bahtiti, [18] that refractive index of the oils were found to be 1.4683 for Jordanian *N. sativa* at 30^{o}C and the study by Alvl *et al.*[19] found that refractive index for menthol crystal was 1.44 at 20^{o}C in 70% alcohol solution.

The viscosity (cSt) for the fixed oil for *Nagilla* seeds which extracts by different solvents, methanol, chloroform and n-hexane was 40.65 , 54.38 and 42.85 respectively.

Table(3): The physico-chemical properties of the fixed oil for the different extracts solvent.

	Specific gravity at 25^{o}C (g/ml)	Viscosity (cst)	Refractive index at 20^{o}C
Methanol extract	0.9194	40.65	1.341
Chloroform extract	0.9227	54.38	1.466
n-hexane extract	0.9473	42. 85	1.462

Table (4), The constituents were identified by matching their Gas chromatography and by comparison of their retention indices with literature values[20]. Retention indices were determined using retention times of (standard fatty acids) that have been injected to the same instrument and under the same chromatographic conditions. There are many fatty acids found in Nigella sativa L. seeds. One of them is stearic acid which we found 28.59% when extracted by n-hexane solvent but gave 15.32% when extracted by chloroform ad we don't found any amount when extracted by methanol solvent and aqueous extracts. The linoleic acid found in this study 6.27% n chloroform extract and 0.022%, 0.245% in n-hexane and methanol extract respectively. The oleic acid concentration found 0.644% and 2.748% in chloroform and n-hexane extract respectively but also no found any amount in methanol and aqueous extracts. Finally, the linoleic acid concentration 6.27% when extracted by chloroform, but methanol gave 0.245% and n-hexane solvent gave 0.022%.Nickavara et al.[21] found that Nigella sativa seeds form Iran contained form main fatty acids linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%).

No significant differences were observed between the fatty acids compositions of methanol extract, chloroform and n-hexane solvent-extracted oils, but the thymoquinone compound

obtained by Aqueous extract method which gave higher mount 5.531%when extracted the *Nigella* seeds compared to another methods (Table 4) (Figure 1 and 2). Fatty acid composition of Nigella seed oil from Morocco was also found very similar to that of Nigella seed oils from different geographical origin with the major exceptions of Nigella seed oils from Egypt[22] and Tunisia[23].

Table (4): The fatty acids concentration and thymoquinone compound obtained by GC-chromatography for *Nigella sativa* L. seed extracted by different solvent.

					Fatty acids					
	Thymoquinone		Oleic acid		Stearic acid		Palmitic acid		Linoleic acid	
Methanol extract	----		-----		-----		Time	13.246	Time	14.732
							Conc.	0.0159	Conc.	0.245
Chloroform extract	Time	5.814	Time	16.469	Time	15.319	Time	13.772	Time	14.771
	Conc.	0.3316	Conc	0.6442	Conc.	2.546	Conc.	0.3318	Conc.	6.2718
n-hexane extract	Time	6.629	Time	16.434	Time	15.101	Time	13.153	Time	14.307
	Conc.	0.0208	Conc.	2.7483	Conc.	28.5951	Conc.	7.2614	Conc.	0..0224
Aqueous extract	Time	6.437	Time	------	Time	-----	Time	13.88	Time	----
	Conc.	5.5315	Conc.	-------	Conc.	------	Conc.	1.2677	Conc.	-----

Table (5): The time (min.) appearance and fatty acids standard concentration assay by GC chromatography.

	Time (min.)	Conc.
Thymoquinone	5.749	1.533
Oleic acid	16.06	27.10
Stearic acid	15.80	0.488
Palmatic acid	13.856	1.889
Linoleic acid	14.456	2.540

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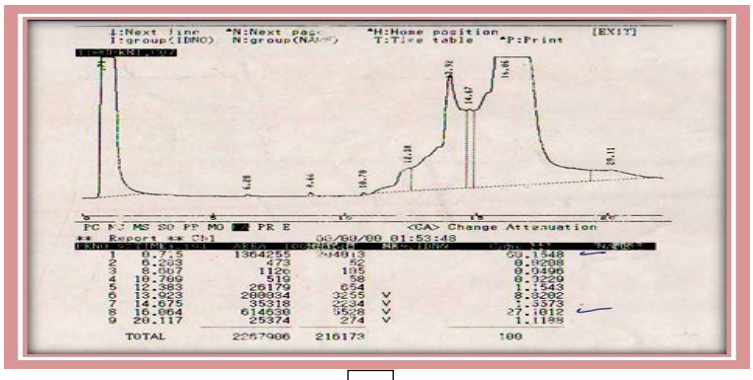

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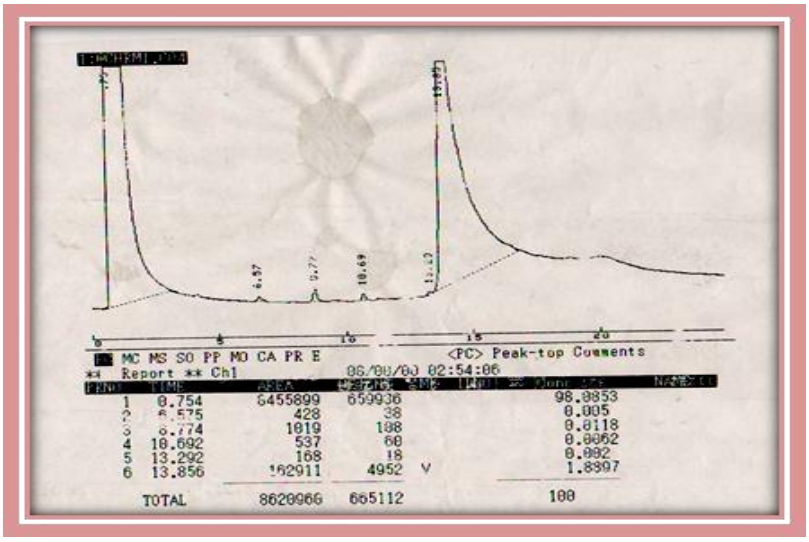
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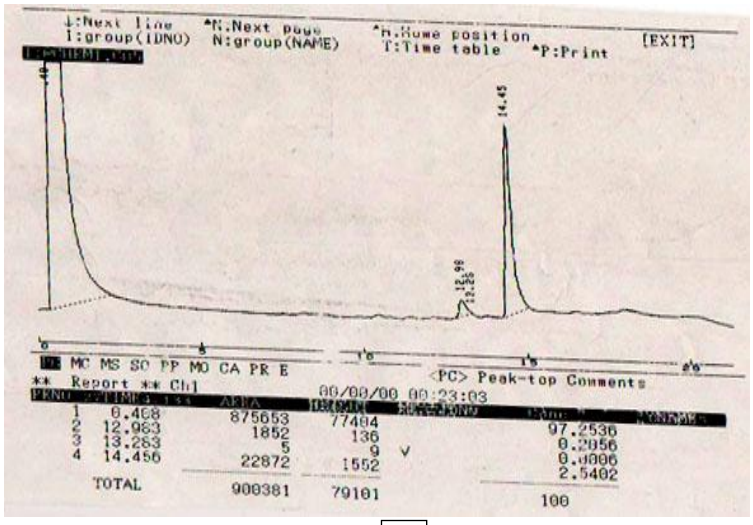
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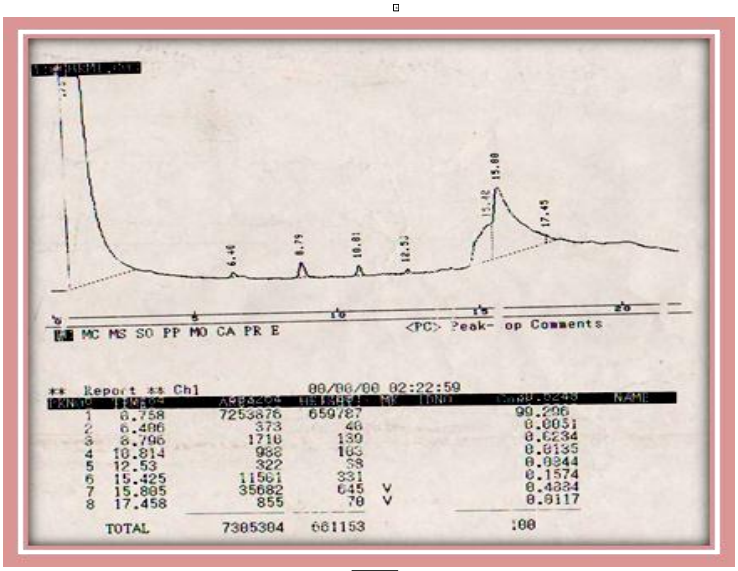
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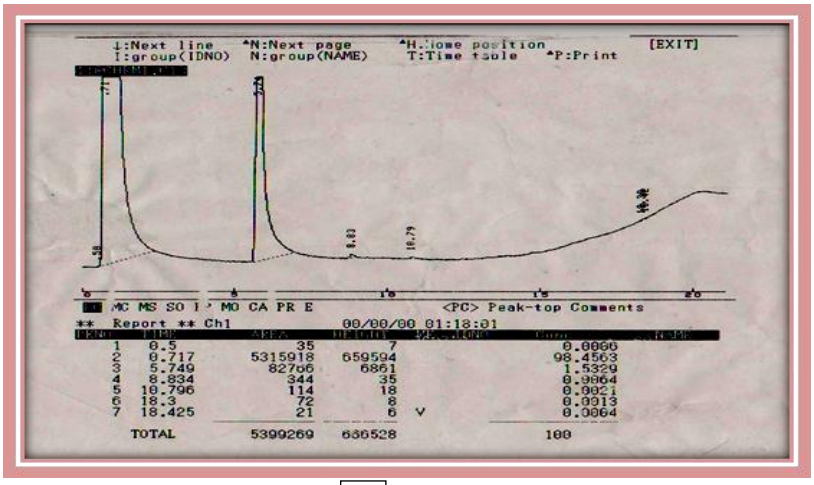
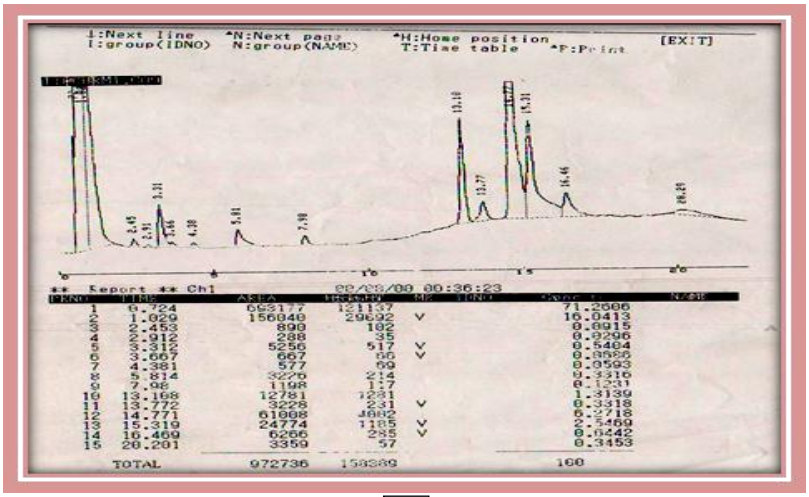


E

Figure (1): The Gas Chromatography pattern for Fatty acids standard. A. oleic acid, B. palmitic acid, C. Linoleic acid, D. Stearic acid, E. Thymoquinone



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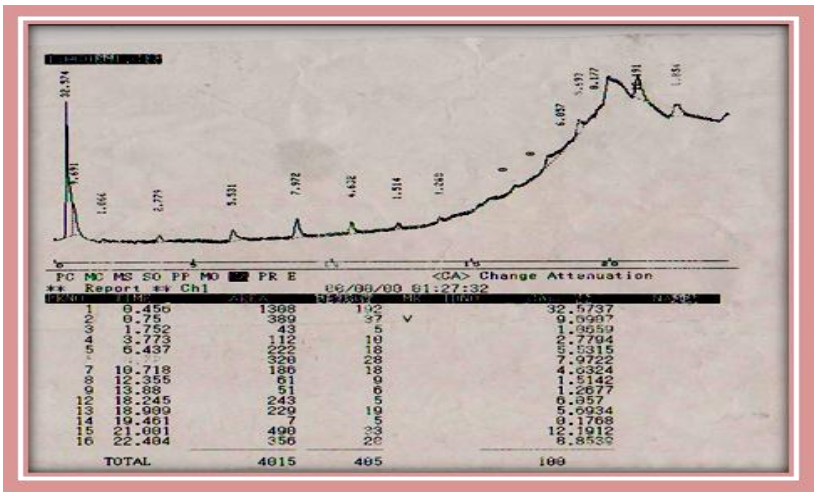

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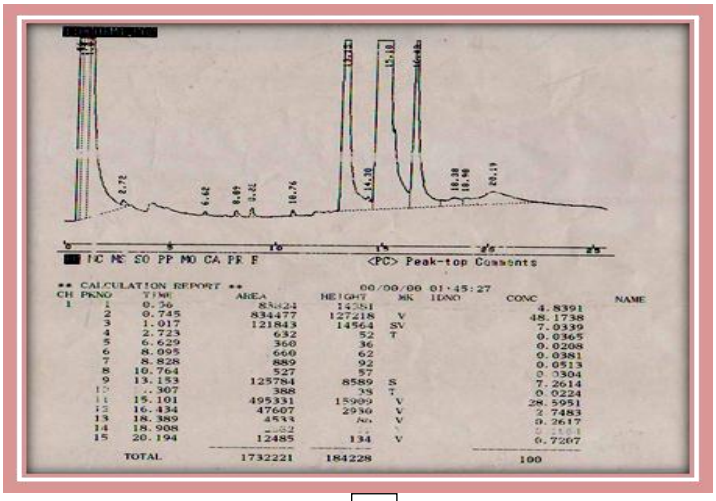

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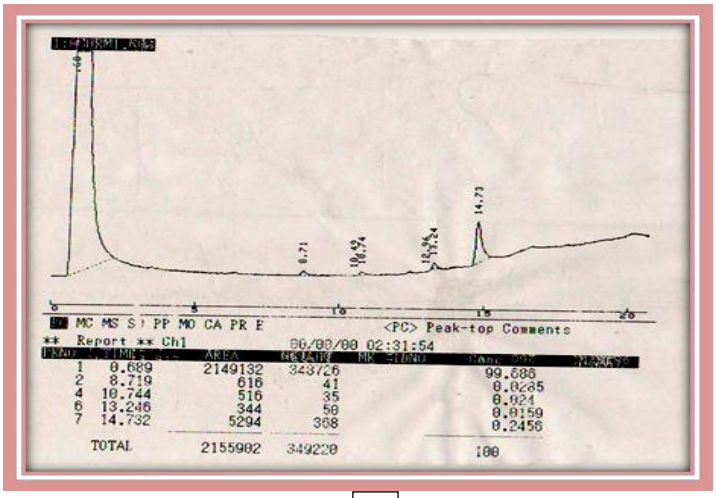

D

Figure (2): The Gas Chromatography for oil seeds extracted by different solvents. A. Aqueous extraction, B. Methanol extraction, C. Chloroform extraction, D. n- exane extraction.

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