

Full Length Research Paper

Determination of alkaloid compounds of *Ricinus communis* by using gas chromatography- mass spectroscopy (GC-MS)

Ameera Omran Hussein¹, Imad Hadi Hameed^{1*}, Huda Jasim¹ and Muhanned Abdulhasan Kareem²

¹Department of Molecular Biology, Babylon University, Hilla City, Iraq.

²Centre of Environmental Research, Babylon University, Hilla City, Iraq.

Received 29 January, 2015; Accepted 3 March, 2015

In this study, the alkaloid compounds of *Ricinus communis* have been evaluated. The chemical compositions of the leaf ethanol extract of *R. communis* were investigated using gas chromatography-mass spectroscopy (GC-MS). GC-MS analysis of *R. communis* alkaloid leaf ethanol extract revealed the existence of the n-haxadecanoic acid, octadecanoic acid, 1-hexadecanol. 2-Methyl, gibb-3-ene-1. 10decarboxylic acid, 2,4a. 7trihydroxy-1-methyl-8-methylene, 1.4a-lactone. 10-methyl, L-valine, ethyl ester, hexadecamethyl, tetradecamethyl, octadecamethyl, butanedioic acid, hydroxyl. diethyl ester, 1.1.3.3.5.5.7.7.9.9.11.11.13.13.15.15 hexadecamethyl, triethyl citrate, diethyl phthalate, and 3-octadecene.

Key words: Alkaloids, ethanol, gas chromatography-mass spectroscopy (GC-MS) analysis, *Ricinus communis*.

INTRODUCTION

The castor oil plant *Ricinus communis*, also known as *Palma(e) Christi* or wonder tree (Figure 1), is a perennial scrub of the spurge family Euphorbiaceae. *R. communis* probably originates from Africa and was used in ancient Egypt and by the Romans and Greeks (Waller and Skursky, 1972). Apart from the highly toxic ricin and the less toxic *R. communis* agglutinin, the plant contains another toxic compound, the low molecular weight alkaloid ricinine (MW = 164.2 g/mol). Ricinine or 3-cyano-4-methoxy-N-methyl-2-pyridone (CAS 524-40-3) belongs to the group of piperidine alkaloids. It was first discovered and named by Tuson in the seeds of *R. communis*, while searching for its medically active compounds even before ricin was known. Subsequently, its chemical structure

was identified and its biosynthesis and metabolism was studied (Waller and Skursky, 1972; Mann and Byerrum, 1974).

Ricinine can be found in all parts of the plant and it is a quite strong insecticide. The castor seeds contain approximately 0.2% of the alkaloid. *R. communis* contains a complex cocktail of toxic substances including the type II ribosome-inactivating protein (RIP) ricin, the haemagglutinin RCA120 and the alkaloid ricinine. Furthermore, other compounds like fatty acids, flavonoids and saponins have been found to exhibit deleterious effects on bacteria, virus, fungi, invertebrates and higher animals, seemingly giving the plant some sort of protection in a hostile environment (Sitton and West,

*Corresponding author. E-mail: imad_dna@yahoo.com. Tel: 009647716150716.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)



Figure 1. Plant of *Ricinus communis*.

1975; Taylor et al., 1994; Upasani et al., 2003; Bigi et al., 2004; De Assis et al., 2011) Furthermore, allergenic reactions against *R. communis*, in particular the seed dust, were realized. Low molecular proteins, 2S albumins, have been identified as the main allergenic compounds (Thorpe et al., 1988; Bashir et al., 1998; Deus-de-Oliveira et al., 2011). Experimental intoxication studies underline the major contribution of ricin as compared to other hazardous compounds found in the seeds (He, 2010). The oil and seed have been used as folk remedies for warts, cold tumors, indurations of the abdominal organs, whitlows, lacteal tumors, indurations of the mammary gland, corns, and moles, etc. Castor-oil is a cathartic and has labor-inducing properties. Ricinoleic acid has served in contraceptive jellies (Allardice, 1993). Ricin, a toxic protein in the seeds, acts as a blood coagulant. The oil is used externally for dermatitis and eye ailments. The seeds, which yield 45 to 50% of a fixed oil, also contain the alkaloids ricinine and toxalbumin ricin, and is considered purgative, counter-irritant in scorpion-sting and fish poison. The leaves, applied to the head, is used to relieve headache and as a poultice for boil (Foster, 1990).

Phytochemical interactions of poisons lead to injury or death of living tissues. Toxicology is like science and an art like medicine. It includes observational data gathering

and data utilization to predict outcome of exposure in human and animals. The ancient humans categorized some plants as harmful and some as safe. The aim of the present work is to study the toxic nature of the powder of *R. communis* leaves.

MATERIALS AND METHODS

Collection and preparation of plant

In this research, the leaves were dried at room temperature for 10 days and when properly dried the leaves were powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature.

Extraction and identification of alkaloids

The powdered leaves (2 g) were boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml

of dilute sulphuric acid. The various extracts were then used for the following test.

Mayer's test

To the filtrate in test tube I, 1 ml of Mayer's reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids (Evans, 2002).

Dragendoff's test

To the filtrate in test tube II, 1 ml of Dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Wagner's test

To the filtrate in tube III, 1 ml of Wagner's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS analysis of the methanol extract of *R. communis* was carried out using a Clarus 500 Perkin Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold-Perkin Elmer Turbo mass 5.1 spectrometer with an Elite - 1 (100% dimethyl poly siloxane), 30 m × 0.25 mm ID × 1 µm of capillary column. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization system which was operated in electron impact mode with ionization energy of 70 eV (Imad et al., 2014, 2015a; Muhanned et al., 2015). The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C at the rate of an increase of 5°Cmin⁻¹, and maintained for 9 min. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 ml was employed (split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed at 110°C (isothermal for 2 min), with an increase of 100°C min⁻¹ to 200°C, then 5°C min⁻¹ to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min and the total GC-MS running time was 36 min. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45 to 450 (m/z). The mass detector used in this analysis was Turbo Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo Mass ver 5.2 (Imad et al., 2015b; Mohammed et al., 2015).

RESULTS

GC-MS analysis of alkaloid compound clearly showed the presence of nine compounds. The alkaloid compound, formula, molecular weight and exact mass are as shown in Table 1. The GC-MS chromatogram of the nine peaks of the compounds detected are as shown in Figure 2-4. Chromatogram GC-MS analysis of the methanol extract of *R. communis* showed the presence of nine major peaks and the components corresponding to the peaks

were determined as follows. The first set up peaks were determined to be n-haxadecanoic acid. The second peaks were indicated to be octadecanoic acid. The next peaks were considered to be 1-hexadecanol. 2-methyl, Gibb-3-ene-1. 10decarboxylic acid, 2,4a. 7trihydroxy-1-methyl—8-methylene, 1.4a-lactone. 10-methyl, L-Valine, ethyl ester, hexadecamethyl, tetradecamethyl, octadecamethyl, Butanedioic acid hydroxyl. Diethyl ester, 1.1.3.3.5.5.7.7.9.9.11.11.13.13.15.15 hexadecamethyl, Triethyl citrate, Diethyl Phthalate, and 3-Octadecene (Figures 5 to 11). The identified phytocompounds have the property of antioxidant and antimicrobial activities (He, 2010; Deus-de-Oliveira, 2011).

DISCUSSION

Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects. Continued further exploration of plant derived antimicrobials is needed today.

Castor bean may become a weed in neglected crop land and pasture. It is not difficult to control through cultivation and mowing. Of greater concern than its weedy potential is the high toxicity of its seeds, which contain ricin, a water-soluble protein. Even a small amount of masticated seed is likely to cause death. Humans and horses are especially vulnerable. Fatal doses are from 2.5 to 6 seeds for humans and about 6 seeds for horses (CISR, 1972). The symptoms are stomach irritation, diarrhea, abdominal pain, increased heart rate, profuse sweating, collapse, and convulsions. Broken seeds can cause skin irritation. The foliage is only slightly toxic (Anonymous, 2000). It is advisable to completely eliminate castor bean from pastures, especially horse pastures, and pinch off flowers of ornamental plants to prevent possible poisoning of children. When assessing the numerous reports on intoxications with ricin, *R. communis* seeds or *R. communis*-containing feed and fertilizer, some general aspects have to be considered. The term ricin in any toxicological publication suggests a degree of homogeneity or a lack of variability that might be expected for pure chemicals (Despeyroux et al., 2000; Thullier and Griffiths, 2009).

Furthermore, other compounds like fatty acids, flavonoids and saponins have been found to exhibit deleterious effects on bacteria, virus, fungi, invertebrates and higher animals, seemingly giving the plant some sort of protection in a hostile environment (Upasani et al., 2003; De Assis et al., 2011). Furthermore, allergenic reactions against *R. communis*, in particular the seed dust, were realized.

Conclusion

R. communis is a native plant of Iraq. It contains chemical

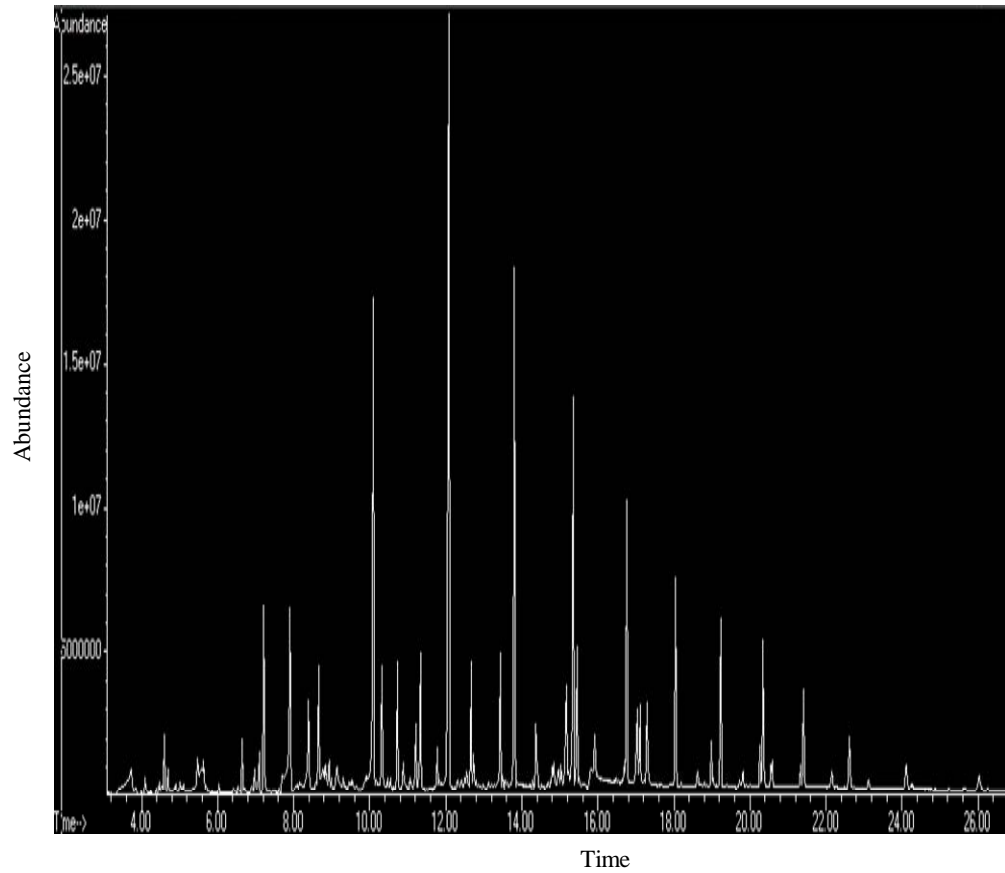


Figure 2. GC-MS Profile of leaves extract of *Ricinus communis*.

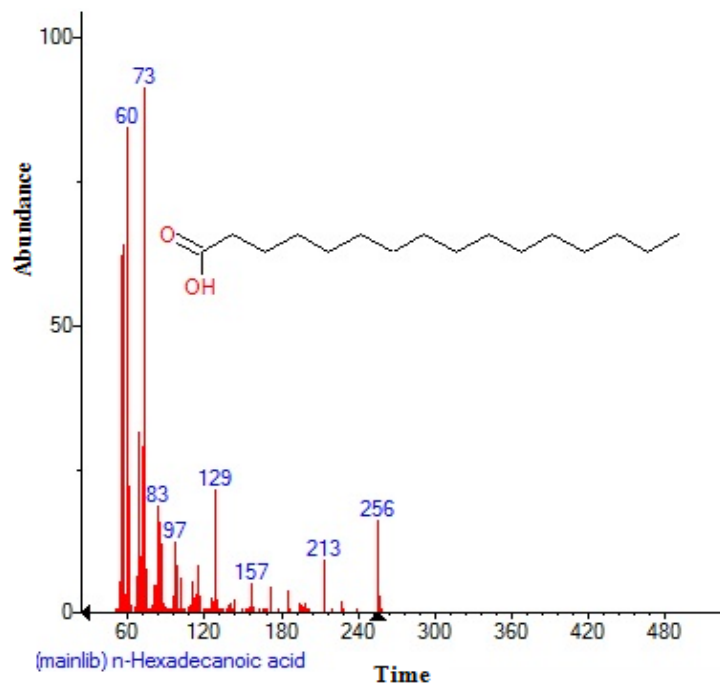


Figure 3. Structure of n-haxadecanoic acid present in the leaves extract of *Ricinus communis* using GC-MS analysis.

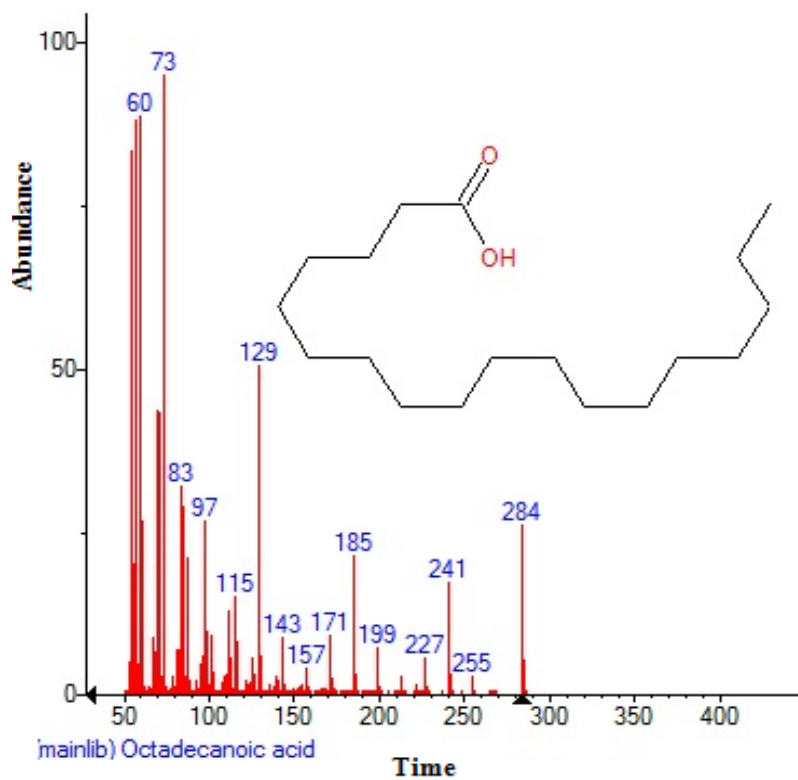


Figure 4. Structure of Octadecanoic acid present in the leaves extract of *Ricinus communis* using GC-MS analysis.

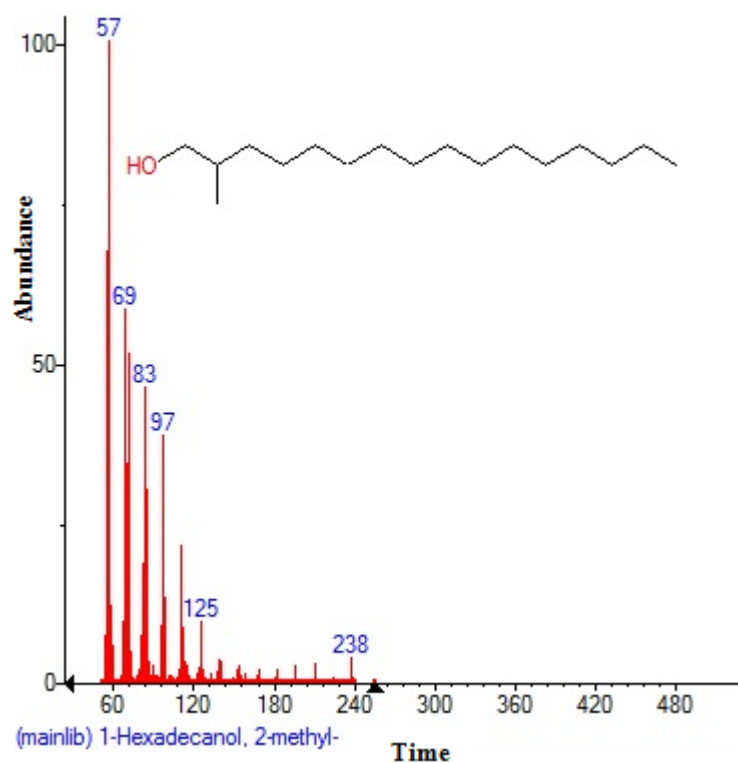


Figure 5. Structure of 1-hexadecanol, 2-methyl present in the leaves extract of *Ricinus communis* using GC-MS analysis.

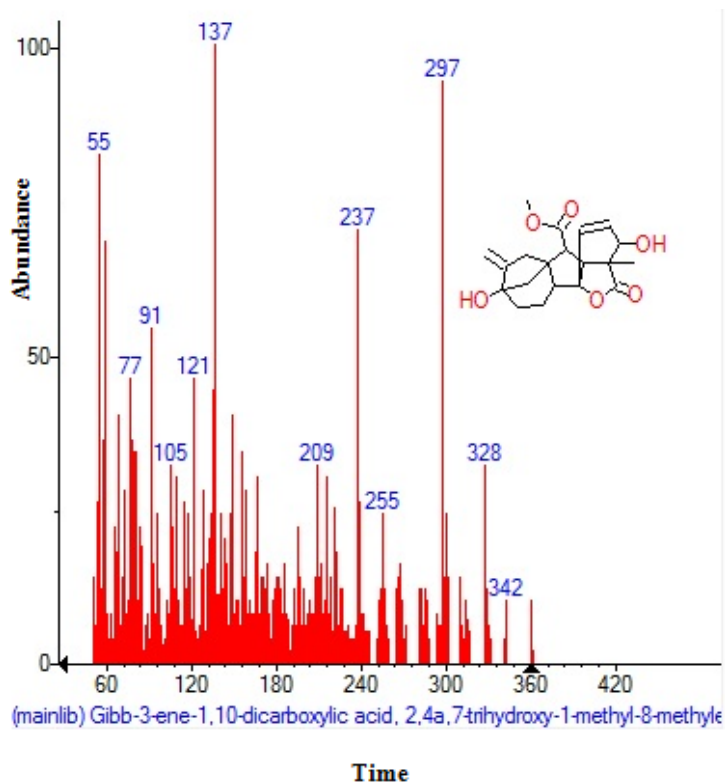


Figure 6. Structure of Gibb-3-ene-1,10-dicarboxylic acid, 2,4a,7-trihydroxy-1-methyl-8-methyl-1,4a-lactone, 10-methyl present in the leaves extract of *Ricinus communis* using GC-MS analysis.

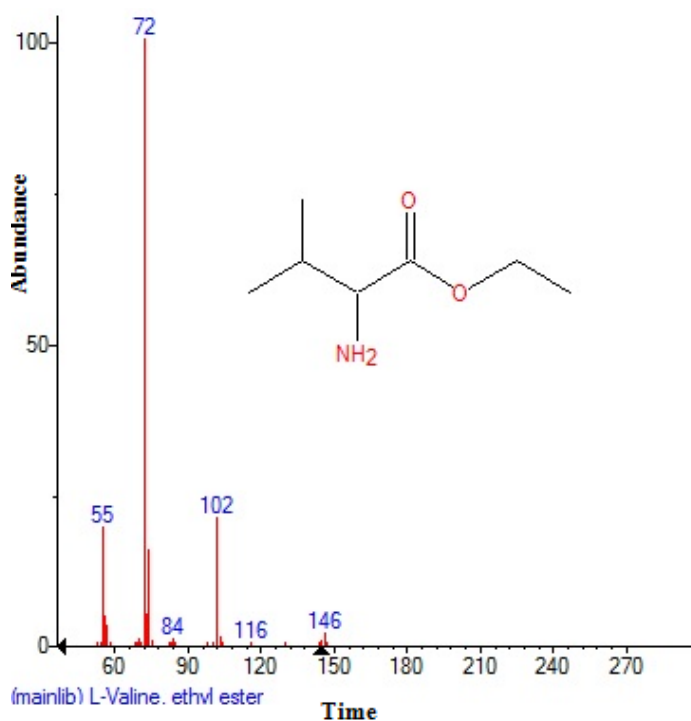


Figure 7. Structure of L-Valine, ethyl ester present in the leaves extract of *Ricinus communis* using GC-MS analysis.

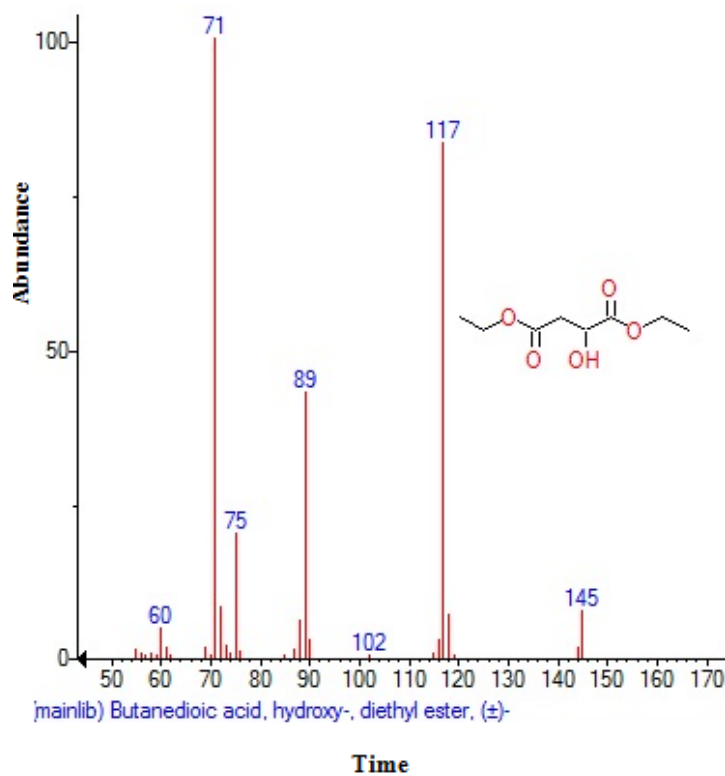


Figure 8. Structure of Butanedioic acid hydroxyl diethyl ester present in the leaves extract of *Ricinus communis* using GC-MS analysis.

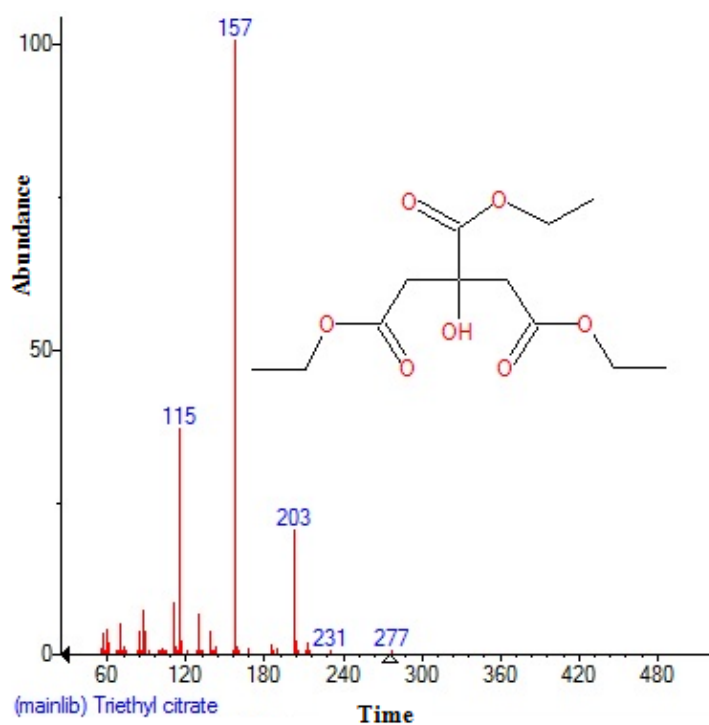


Figure 9. Structure of Triethyl citrate present in the leaves extract of *Ricinus communis* using GC-MS analysis.

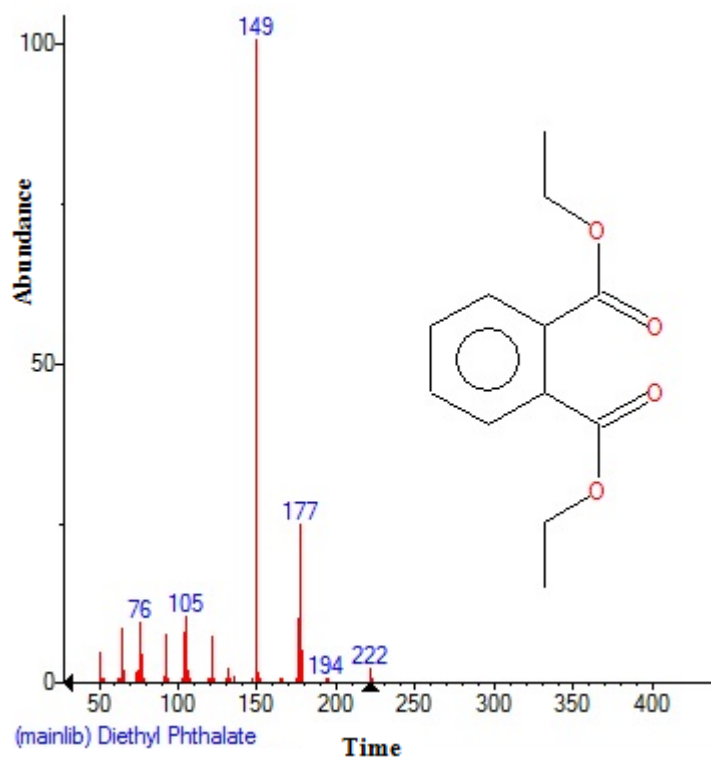


Figure 10. Structure of diethyl phthalate present in the leaves extract of *Ricinus communis* using GC-MS analysis.

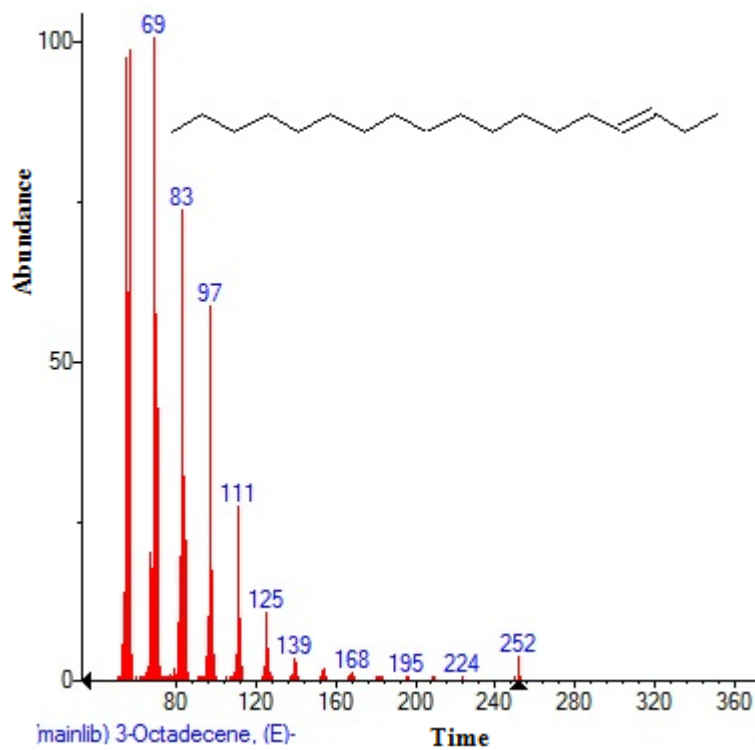


Figure 11. Structure of 3-octadecene present in the leaves extract of *Ricinus communis* using GC-MS analysis.

Table 1. Compounds present in the leaves extract of *Ricinus communis* using GC-MS analysis.

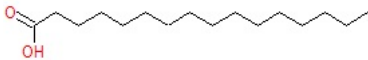
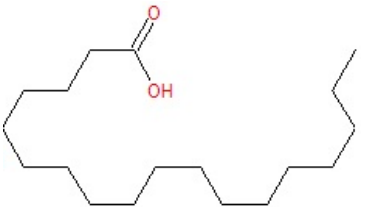
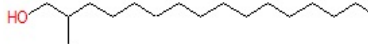
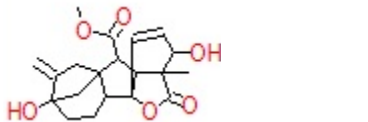
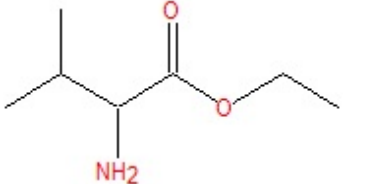
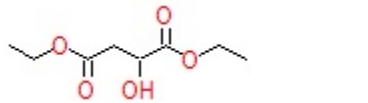
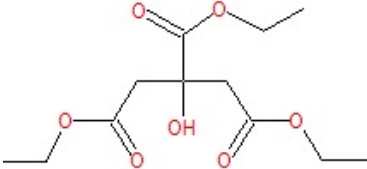
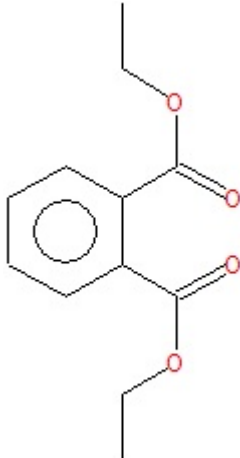
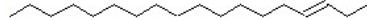
Serial No.	Phytochemical compound	RT (min)	Formula	Molecular weight	Exact mass	Chemical structure
1	n-haxadecanoic acid	15.177	C ₁₆ H ₂₃ O ₂	256	256.24023	
2	Octadecanoic acid	17.043	C ₁₈ H ₃₆ O ₂	284	284.27153	
3	1-hexadecanol. 2-methyl	17.300	C ₁₇ H ₃₆ O	256	256.276615	
4	Gibb-3-ene-1. 10decarboxylic acid ,2,4a. 7trihydroxy-1-methyl —8-methylene, 1.4a-lactone. 10-methyl	18.628	C ₂₀ H ₂₄ O ₆	360	360.157288	
5	L-Valine, ethyl ester	4.088	C ₇ H ₁₅ NO ₂	145	145.110279	
6	Butanedioic acid hydroxyl. Diethyl ester	7.207	C ₈ H ₁₄ O ₅	190	190.084124	

Table 1. Contd.

7	Triethyl citrate	11.778	C ₁₂ H ₂₀ O ₇	276	276.120903	
8	Diethyl Phthalate	11.344	C ₁₂ H ₁₄ O ₄	222	222.089209	
9	3-Octadecene	13.348	C ₁₈ H ₃₆	252	252.281701	

constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthmatic. So, it may be concluded that *R. communis* is a very important indigenous medicinal plant which requires more exploration to utilize its medicinal property.

ACKNOWLEDGEMENT

The authors thank Dr. Abdul-Kareem Al-Bermani,

Lecturer, Department of Biology, for valuable suggestions and encouragement.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

REFERENCES

Allardice P (1993). A-Z of Companion Planting. Cassell Publishers Ltd. ISBN 0-304-34324-2.

Bashir MEH, Hubatsch I, Leinenbach HP, Zeppezauer M, Panzani RC, Hussein IH (1998). Ric c 1 and Ric c 3, the allergenic 2S albumin storage proteins of *Ricinus communis*: Complete primary structures and phylogenetic relationships. Int. Arch. Allergy Immunol. 115:73-82.

Bigi MF, Torkomian VL, de Groot ST, Hebling MJ, Bueno OC, Pagnocca FC, Fernandes JB (2004). Activity of *Ricinus communis* (Euphorbiaceae) and ricinine against the leaf-cutting ant *Atta sexdens rubropilosa* (hymenoptera: Formicidae) and the symbiotic fungus *Leucoagaricus gongylophorus*. Pest Manag. Sci. 60:933-938.

De Assis EM, Fernandes IM, Santos CS, Mesquita LX, Pereira RA, Maracajá PB, Soto-Blanco B (2011). Toxicity of castor bean (*Ricinus communis*) pollen to honeybees. Agric. Ecosyst. Environ. 141:221-223.

- Despeyroux D, Walker N, Pearce M, Fisher M, McDonnell M, Bailey S, Griffiths G, Watts P (2000). Characterization of ricin heterogeneity by electrospray mass spectrometry, capillary electrophoresis, and resonant mirror. *Anal. Biochem.* 279:23-36.
- Deus-de-Oliveira N, Felix SP, Carrielo-Gama C, Fernandes KV, Damatta RA, Machado OL (2011). Identification of critical amino acids in the IgE epitopes of Ric c 1 and Ric c 3 and the application of glutamic acid as an IgE blocker. *PLoS One* 6:2011.
- Evans WC (2002). *Trease and Evans Pharmacognosy*, 15th edition. WB Saunders Company Ltd, London. pp. 137-139,230-240.
- Foster S, Duke JA (1990). *A Field Guide to Medicinal Plants*. Eastern and Central N. America. Houghton Mifflin Co.
- He X, McMahon S, Henderson TD, Griffey SM, Cheng LW (2010). Ricin toxicokinetics and its sensitive detection in mouse sera or feces using immuno-PCR. *PLoS One* 5(9), e12858.
- Imad H, Muhanned A, Aamera J, Cheah Y (2014). Analysis of eleven Y-chromosomal STR markers in middle and south of Iraq. *Afr. J. Biotechnol.* 13(38):3860-3871.
- Imad HH, Mohammed AJ, Muhanned AK (2015a). Forensic analysis of mitochondrial DNA hypervariable region HVII (encompassing nucleotide positions 37 to 340) and HVIII (encompassing nucleotide positions 438-574) and evaluate the importance of these variable positions for forensic genetic purposes. *Afr. J. Biotechnol.* 14(5):365-375.
- Imad HH, Muhanned AK, Rafid HH (2015b). X-chromosome short tandem repeat, advantages and typing technology review. *Afr. J. Biotechnol.* 14(7):535-541.
- Mann DF, Byerrum RU (1974). Activation of the de novo pathway for pyridine nucleotide biosynthesis prior to ricinine biosynthesis in castor beans. *Plant Physiol.* 53:603-609.
- Mohammed AJ, Imad HH, Muhanned AK (2015). Detection of New Variant "Off-ladder" at the (D12S391, D19S433 and D1S1656 loci) and Tri-allelic Pattern at the D16S539 Locus in a 21 Locus Autosomal Short Tandem Repeat Database of 400 Iraqi Individuals. *Afr. J. Biotechnol.* 14(5):375-399.
- Muhanned AK, Ameer IA, Imad HH, Mohammed AJ (2015). A New Polymorphic Positions Discovered in Mitochondrial DNA Hypervariable Region HVIII From Central and North-Central of Iraq. *Mitochondrial DNA* 23:1-5.
- Sitton D, West CA (1975). Casbene: An anti-fungal diterpene produced in cell-free extracts of *Ricinus communis* seedlings. *Phytochemistry* 14:1921-1925.
- Taylor S, Massiah A, Lomonosoff G, Roberts LM, Lord JM, Hartley M (1994). Correlation between the activities of five ribosome-inactivating proteins in depurination of tobacco ribosomes and inhibition of tobacco mosaic virus infection. *Plant J.* 5:827-835.
- Thorpe SC, Kemeny DM, Panzani RC, McGurl B, Lord M (1988). Allergy to castor bean. II. Identification of the major allergens in castor bean seeds. *J. Allergy Clin. Immunol.* 82:67-72.
- Thullier P, Griffiths G (2009). Broad recognition of ricin toxins prepared from a range of *Ricinus* cultivars using immunochromatographic tests. *Clin. Toxicol. (Phila.)*. 47:643-650.
- Upasani SM, Kotkar HM, Mendki PS, Maheshwari VL (2003). Partial characterization and insecticidal properties of *Ricinus communis* L. foliage flavonoids. *Pest Manag. Sci.* 59:1349-1354.
- Waller GR, Skursky L (1972). Translocation and metabolism of ricinine in the castor bean plant, *Ricinus communis* L. *Plant Physiol.* 50:622-626.