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Toxicological, histological and field application of *Ricinus communis* methanol extract controlling the black rat *Rattus rattus* (Rodentia: Muridae) compared to bromodialone

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Abstract

One of the rodents that cause severe economic problems on all fronts is the black rat *Rattus rattus* (L.) (Rodentia: Muridae) (*R. rattus*). The current work was conducted to investigate the effect of the methanolic extract of castor seeds, *Ricinus communis* (*R. communis*), used as natural materials of plant origin in the control process against *R. rattus* compared to the recommended rodenticide, Bromadiolone. Toxicity analysis showed LD_{50} values of 1.44 and 484.29 mg/kg.b.w; for bromadiolone and *Ricinus* extract, respectively. Body weight and average bait consumption were well assessed using free-choice and non-choice feeding methods. Histopathological examination of the liver and kidney sections treated with *Ricinus* extract showed some alteration, such as congestion and cellular infiltration around the central vein, a congested and lobulated glomerular capsule, and vacuolar degeneration of some convoluted tubules. GC/MS analysis revealed the presence of 14 compounds in the methanol extract. A field experiment was also conducted, where the reduction was 66.33% of the extract while it was 80 % for the recommended rodenticide supercaid.

Introduction

Rodents are one of the major pests that affect food security by impacting food availability and safety. They are problematic to agriculture and public health since they can inflict considerable economic damage associated with their diversity, generalist feeding habits and high reproductive rates (Swanepoel *et al.*, 2017). Rodents are considered one of the most important pests in Egypt and other countries causing great economic losses to grow crops, stored crops, poultry and animal farms (Neena and Babbar, 2010 and Singla and Babbar, 2012).

The black rat *Rattus rattus* (L.) (Rodentia: Muridae) (*R. rattus*) is one of the most commonly encountered and economically important rodents; it does not only inflict heavy damage to stored food but also have nuisance value being a disease carrier or vector. It is purely an indoor pest (Brooks *et al.*, 1987). Conventional pesticides possess inherent toxicities that endanger the health of farm operators, consumers, and the environment. Such negative effects of conventional pesticides on human health led to a resurgence in interest in the botanical pesticides because of their minimal costs and fewer ecological side effects.

The chemistry of separating and identifying, and the application of new compounds, especially natural products of plant origin, is in continuous and widespread progress, and the use of new compounds and their evaluation on pests in general and on rats ,in particular, is not an exception. Due to their environmental friendliness, natural compounds have been utilized and are still employed to control rodents without the use of pesticides or synthetic compounds (Isman, 1997 and Tripathi *et al*., 2008). *Ricinus communis* L. (Euphorbiaceae) (*R. communis*) commonly known as the castor oil seed plant, the seeds are poisonous to people, animals, and insects. Castor oil has the ability to heal wounds and cure ailments; bactericide, insecticide and larvicidal (Gibbs *et al*., 2002).

Therefore, the present study aims to assess the effect of *R. communis* methanol extract as a potential rodenticide compared to bromadiolone against the black rat *R. rattus,* under laboratory and field conditions.

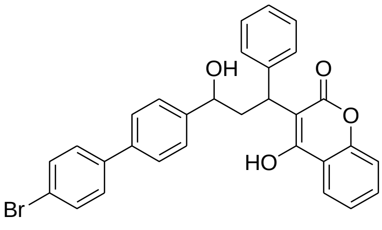
Materials and methods

1. Tested compounds:

1.1. Methanol extract of *Ricinus communis*.

1.2. Bromadiolone**:**3-[3-(4′-bromo[1,1′-biphenyl]-4-yl)-3-hydroxy-phenylpropyl]4-hydroxycoumarin.

Molecular formula $C_{30}H_{23}BrO_4$ which were obtained from Glaxowellcome U.K. Besiers, France and ICI companies.



Bromadiolone

1.3. Supercaid 0.005% anticoagulant, readymade bait was obtained from KZ Co. Egypt.

2. Experimental animals:

The black rat *R. rattus,* was captured from a field in Egypt's Mansoura region. Animals were transported to the lab of the Plant Protection Research Institute, where they were individually caged in laboratory cages (50×30×30) with sawdust for two weeks to acclimate. They ate a typical diet of (65% crushed maize + 25% ground wheat + 5% sugar + 5% corn oil) and water *ad libitum*. The mature, healthy animals were divided into three groups (each of five rats). Rats typically weighed 150–200 g.

3. Collection of *R. communis* seeds and extraction:

R. communis seeds were collected from shrubs distributed in Aga district, Mansoura governorate, Egypt. The plant's authenticity was established in the Herbarium, Faculty of Science, Mansoura University. Seeds were cleaned from dirt and immersed in tap water for washing, then well dried. The outer coverings of the seeds were removed by hand; the pulp seeds were then pressed mechanically through hydraulic pressure to remove all of the oil in the pulp. The cake was dried using a desiccator, and the final residue was well dried. Two kilos of dry powder Castor seeds were soaked in methanol and extracted with 3.5 L of methanol for 24 hours by using a soxhlet extractor. The solvent was evaporated by a rotary vacuum evaporator to obtain the viscous extract. This crude extract was subjected to GC-MS chromatographic analysis.

4. Laboratory Experiments:

4.1. Evaluation of toxicity and LD values:

R. rattus was administered oral doses of *Ricinus* extract (250, 500, 1000, and 2000 mg/kg body weight) via stomach tubes. Bromadiolone dosages of 1, 1.5, 2, and 4 mg/kg body weight were also administered orally. The rats received these dosages after a 12-hour fast, followed by two hours of food and water, while the control animals just received distilled water orally. For up to 7 days, mortality percentages were recorded. Using the statistical technique of probit analysis, the LD_{50} and LD_{90} values were determined **(Abbott, 1925).**

4.2. Non-choice feeding method:

Three groups of five rats each were caged individually for each treatment. The first group was offered 50 grams of crushed maize containing 1% *Ricinus* extract, and the second group was offered 1% supercaid for 4 successive days. As a check group, they received normal crushed maize. Daily, the amount of bait consumed was calculated. The treated bait was taken out, and the animals that survived were fed the normal diet and monitored for 28 days (Shefte *et al*., 1982). Rat mortality and body weight were measured throughout this period.

4.3. Free-choice feeding method:

By comparing its consumption with that of a standard challenge diet, the free choice feeding method was utilized to assess the acceptability of *Ricinus extract* bait (Palmateer, 1974). Five rats were caged separately to consume 1% *Ricinus* extract or 1% supercaid, with one serving as a control. In small, separate dishes, each rat received 50 g of crushed maize with either 1%, one of the mentioned compounds and 50 g of the standard diet. To prevent feeding preferences for a specific position, the placement of the two dishes was changed every day. Daily consumption of the poisoned bait and standard diet was recorded for four successive days, after which the poisoned bait was removed and the survivors were fed the standard diet. For up to 28 days, dead animals were recorded daily. According to data on bait acceptance (Mason *et al*., 1989):

$$\textbf{Bait acceptance \%} = \frac{\textbf{Consumed amount of treated bait}}{\textbf{Consumed amount of treated bait + standard diet}} \times 100$$

5. Histological investigation:

Histological examination of the liver and kidney tissues to determine the impact of the lethal dose, LD_{50} of the two test compounds. Animals were intubated orally for 24 hrs. Animals were sacrificed and dissected three days after starting the treatment to obtain liver and kidney samples from the treated and control groups. The specimens were fixed in 10% formalin. Following that, the samples were taken to the Histopathology Laboratory at the Mansoura University Faculty of Medicine for examination. Hematoxylin and eosin were used to stain tissue sections of a thickness of 5µ to illustrate the histological examination **(Creasy *et al*., 2021).**

6. Gas chromatography–mass spectrometry (GC-MS) analysis:

The chemical composition the performed sample using GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 60°C and then increased by 6°C /min to 250°C withhold 1 min then increased to 300 at 30 C/min. The injector temperature was kept at 270°C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and transfer line were set at 200 °C and 280 °C respectively. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST14 mass spectral database (Abd El-Kareem *et al*., 2016).

7. Field experiment:

In the fields of the Aga district, Dakahlia Governorate, a field evaluation of crushed maize bait containing 1% of *Ricinus* extract and super caid treated groups was carried out. The entire region is infected with the black rat, *R. rattus*. For each compound, a feddan-sized portion received treatment, whereas a comparable portion served as the control. The rat population density was determined both before and after treatment using the food consumption method (Dubock, 1982). Each plastic bag containing 100g of the suggested bait, which weighed 2 kg, was placed in the chosen plot for five days, and the amount of tested bait consumed was recorded. The following calculations were made to determine the population reduction:

$$\textbf{Population reduction \%} = \frac{\textbf{Pre-treatment consumed - Post-treatment consumed}}{\textbf{Pre-treatment consumed}} \times 100$$

8. Statistical analysis:

LD_{50} values were expressed as mg/kg body weight. One-way analysis of variance (ANOVA) was performed on all the data, which were all presented as mean ± SE (St and Wold, 1989). Using Tukey's method, confidence intervals with a 95% simultaneous confidence level were created. Significant was considered as 0.05 probability. All statistical analysis was performed using Cohort Software (Cho *et al*., 2004).

Results and discussion

1. Acute toxicity test (LD_{50}):

The rodenticidal activities of *Ricinus extract* and bromadiolone were investigated against the black rat, *R. rattus*; results are summarized in Table (1). Bromadiolone shows a higher mortality percentage followed by *Ricinus extract; the* LD_{50} and LD_{90} values were (1.44 and 2.67 mg/kg.b.w) and (484.29 and 1491.83 mg/kg.b.w) for bromadiolone and *Ricinus* extract, respectively. Al-Khafaji *et al.* (2017) observed that the toxin's oral LD_{50} of *R. communis* in rabbits was 352.58 mg**.** The LD_{50} in mice with various behavioral abnormalities following oral administration of the extract was 1100 mg/kg body weight in the acute toxicity test of the ethanolic extract of castor seed (AL-Jborrey *et al.,* 2018**).** According to Asran *et al.* (2016), the LD_{50} values for male, female, and juvenile *R. norvegicus* with bromadiolone were 0.81, 0.91, and 0.78 mg/kg b.w., respectively.

Table (1): The acute toxicity of *Ricinus extract* and bromadiolone against *Rattus rattus.*

Treatment	LD_{50} (mg/kg. b. w.)	LD_{90} (mg/kg. b. w.)	Slope ± S.E.
Ricinus extract	484.29	1491.83	2.62 ± 0.22
Bromadiolone	1.44	2.67	4.76 ± 0.48

S.E. = Standard Error.

2. Effect of *Ricinus extract* and supercaid baits against *Rattus rattus*:

2.1. Using the non- choice feeding method:

The results of the non-choice feeding test of *Ricinus* extract and supercaid baits against *R. rattus* were shown in Table (2). The average bait consumption was 31.7 and 25.5 gm. which gave mortality rates 70 and 90% and mean days to death of 9.3 and 5.3 days for *Ricinus* extract and supercaid, respectively, when compared to the control group. Also, the data in Table (2) showed that the *Ricinus* extract and supercaid baits significantly changed the body weight of the tested animals, with the mean body weight of treated rats falling from 201.3 and 213.8 gm. to 147.5 and 136.3 gm., respectively, with a body weight reduction% of -26.73 and -36.25 gm. for each treatment compared to the control group.

2.2. Using the free-choice feeding method:

The results of the free-choice feeding method test of *Ricinus* extract and super caid baits against *R. rattus* were indicated in Table (3). The acceptance percents were 41.26% and 47.44%, with mortality of 65% and 80% and mean days to death of 12 and 6.5 days for *Ricinus* extract and supercaid, respectively. There was a significant decrease in the body weight of the tested rats, with mean body weight losses from (187.5 and 200.0 gm.) to (158.9 and 145.0 gm.), and body weight reduction% was (- 15.25 and - 27.5 gm.) for *Ricinus* extract and supercaid respectively, when compared to the control group. Olayioye *et al*. (2014) showed that rats fed castor oil seeds lost body weight and showed negative impacts on their liver and spleen weights compared to control rats given a normal diet. Haile *et al*. (2019) investigated the mice's decreased food intake over time, which likely had an impact on the functioning of numerous organ systems, including the central nervous system, maybe the cause of their decreased activity and behavioral changes after receiving *R. communis* leaf extract. According to Asran *et al*. (2016), male, female, and juvenile albino Norway rats (*R. norvegicus*) treated with LD_{50} values of Bromadiolone showed body weight reduction percentages of (13.9, 21.8 and 10.2%). Moreover, the mean day to death for individuals who were treated was (6.0, 6.0, and 4.6 days).

3. Histological changes:

3.1. Liver:

The classic hepatic lobule, as observed in histological sections of the liver of the control *R. rattus*, was formed of a central vein and masses of liver cells (Hepatocytes) arranged in the shape of liver cords radiating from the vein. The hepatocytes had vesicular and central nuclei and were polygonal or rounded in shape. Endothelial and Von Kupffer cells lined the narrow blood sinusoids that separated the hepatic cords from one another (Figure 1A). Rat liver sections from those given *Ricinus* extract treatment showed congestion in the central vein, some areas of degeneration, and cellular infiltration around the central vein upon histological investigation (Figure 1B). Bromadiolone-treated rats' histological liver assessment revealed haemorrhage in the central vein and blood vessels, necrosis in some hepatic regions, and pyknotic nuclei were also observed (Figure 1C).

3.2. Kidney:

Malpighian renal corpuscles, proximal convoluted tubules, and distal convoluted tubules were observable in the renal cortex, which was the kidney's normal histological structure. A normal glomerulus with a thin glomerular basement membrane and normal Bowman's capsules formed the malpighian renal corpuscles (Figure 2A). Following treatment with *Ricinus* extract, the kidney's histological changes included a congested, atrophied, and lobulated glomerular capsule, vacuolar degeneration of some convoluted tubules, haemorrhage in the glomerulus, and the interstitial blood vessels (Figure 2B). Following treatment with Bromadiolone, the glomerular capsule atrophies with rupture in Bowman's capsules, some glomeruli disappear, some areas become necrotic, the proximal and distal convoluted tubules are disorganized, and there is severe haemorrhage in the glomeruli and tubules (Figure 2C).

Table (2): Effect of *Ricinus extract* and supercaid against *Rattus rattus* using the non- choice feeding method.

Treatment	Av. Body weight (g)			Av. bait consumption/day (g)	Mortality %	Day to death	
	Before	After	Reduction%			Range	Mean
Ricinus extract	201.3 ±18.3^{a}	147.5 ±22.1^{b}	- 26.73	31.7 ± 2.01^{ab}	70	7-12	9.3
Supercaid	213.8 ±12.5^{a}	136.3 ±17.7^{b}	- 36.25	25. 5 ± 3.58^{b}	90	4-7	5.3
Control	227.5 ±6.61^{a}	261.3 ±7.17^{a}	+ 14.86	46.8 ± 6.55^{a}	0.0	0.0	0.0
P-Value	0.416	0.001	—	0.022			

Values is the mean ± SE. of the same column that do not share the same letter (a, b, c) are significantly different.

Table (3): Effect of *Ricinus extract* and Super caid against *Rattus rattus* for using the free-choice feeding method.

Treatment	Av. Body weight (g)			Av. bait consumption / day (g)		Acceptance %	Mortality %	Day to death	
	Before	After	Reduction %	Untreated	Treated			Range	Mean
Ricinus extract	187.5± 14.9^{a}	158.9± 12.8^{b}	- 15.25	22.35± 2.67	15.7± 1.85	41.26	65	9-15	12
Super caid	200.0± 16.8^{a}	145.0± 19.4^{b}	- 27.5	24.43± 7.84	22.05±7.64	47.44	80	5-8	6.5
Control	227.5± 6.61^{a}	261.3± 7.17^{a}	+ 14.86	46.8 ± 6.55		100	0.0	0.0	0.0
P-Value	0.15	0.00	—	—	—				

Values is the mean ± SE. of the same column that do not share the same letter (a, b, c) are significantly different.

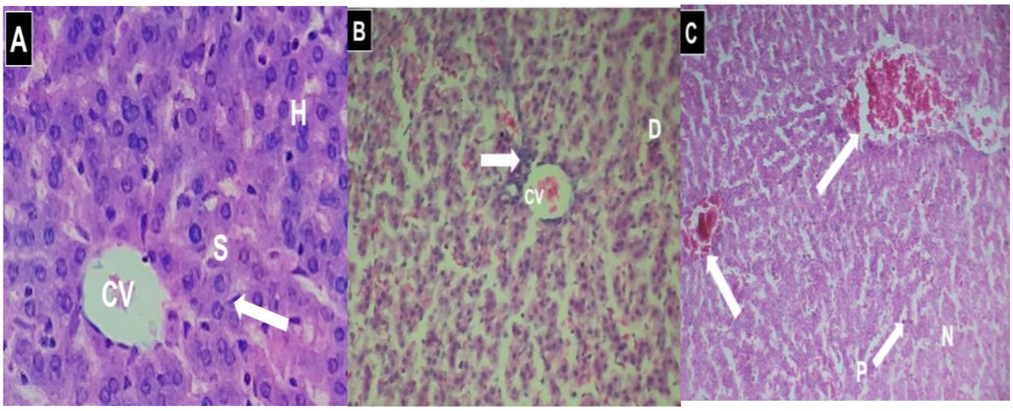


Figure (1): Photomicrographs of sections in livers of *Rattus rattus* [A]: Control, showing a central vein (CV), radiating cords of liver cells separated by blood sinusoids (S) and Kupffer cells (Arrow) Heapatocytes (H). [B]: After treatment with *Ricinus* extract, displaying congested central vein (CV), degeneration (D) cellular infiltration (Arrow). [C]: After treatment with Bromadiolone, demonstrating heamorrahge in central vein and blood vessels (Arrows), necrosis (N) and pyknotic nuclei (P). (H & E X400).

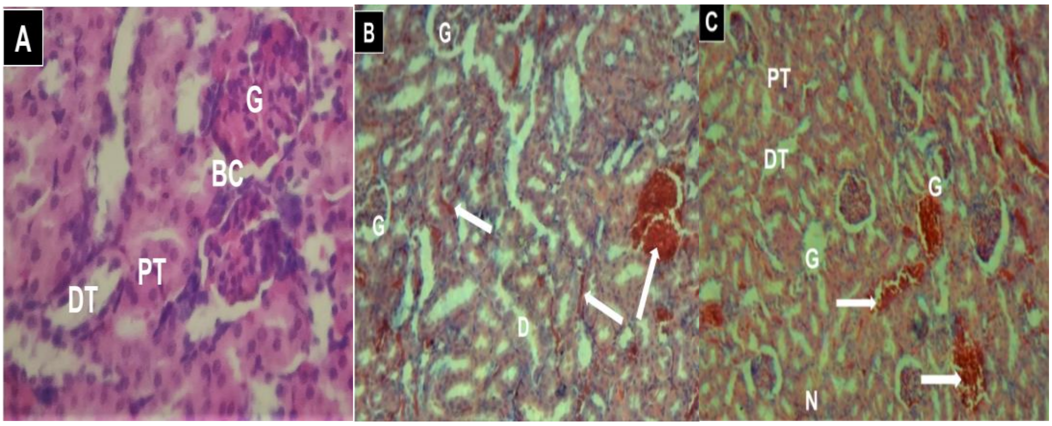


Figure (2): Photomicrographs of sections in kidney (renal cortex) of *Rattus rattus* [A]: Control, showing Malpighian renal corpuscles with normal glomerulus (G) and Bowman's capsules (BC), normal proximal convoluted tubules (PT) and distal convoluted tubules (DT). [B]: After treated with *Ricinus* extract, displaying congested and lobulated glomerular capsule (G), vacuolar degeneration (D) and heamorrahge in (G) and the interstitial blood vessels (Arrows). [C]: After treated with Bromadiolone, showing atrophied, disappearance of some (G), necrosis (N), disorganized (PT) and (DT) convoluted tubules and sever heamorrahge in (G) and tubules (Arrows) (H & E X400).

The liver is responsible for a variety of processes, including the transfer and storage of metabolites, aiding in food digestion, storing glucose, and creating blood-clotting factors and removing harmful substances from the body. The kidney's main tasks include eliminating waste products from metabolism, foreign substances and maintaining homeostasis (Effendy *et al*. 2006). According to Haile *et al*. (2019), mice treated with *R. communis* leaf extract exhibited histological changes in their livers in both male and female mice. These changes include cytoplasmic vacuolation of hepatocytes, dilation and congestion of central and portal veins, activation of kupffer cells, pyknotic nuclei, and slight sinusoidal congestion. AL-Jborrey *et al*. (2018) observed that when *R. communis* seed ethanol extract was administered to albino mice, a histopathological examination of their livers revealed congestion of blood vessels that may have been caused by the extract's activity in the biotransformation of xenobiotics. At 50 and 100 mg/kg body weight, there is an infiltration of inflammatory cells, primarily monocytes and congested renal tissues in the kidney. Studies on the histology of albino rats treated with ¼ and ⅒LD_{50} values of bromadiolone or chlorophacinone revealed dilated central and portal veins, congestion with lymphocytic infiltration, and necrosis in the liver, while degenerative changes were seen in the kidney with necrobiosis in the lining epithelial cells of renal tubules, congestion in the glomerular tuft of the hypertrophied glomeruli (Rady *et al.* 2013). Atrophy of the glumeruli, damage to the flattened epithelial tissue of the renal tubules, which results in haemorrhage, and rupture of the tubular basement membrane, which results in inflammation (Samia *et al.* 2009). Degeneration may result from a disruption in the cell's metabolism, which causes morphologic abnormalities (Abdin, 1981). Cell necrosis and vacuolation can be caused by an acute injury that elicits an inflammatory response (Alberts *et al.*, 2001).

4. Chemical constituents of *R. communis* seed: 4.

Castor seed methanol extract was greenish-red color after 72 hours of solvent extraction; the LD_{50} value of the methanol extract of seeds was 484.29 mg/kg. b. w. GC-MS analysis revealed the presence of many compounds with wide molecular weight range as shown in Table (4) and Figure (3) . Fourteen compounds were identified depending on their retention times, as follows: 1,2,3-Propanetriol, Erythritol, (2-Mercaptoethyl)guanidine, Shikmic acid, Hexadecanoic Acid, Methyl Ester, Pentadecanoic Acid, 14-Methyl-, Methyl Ester, n-Hexadecanoic acid, 9-Octadecenoic Acid (Z), Methyl 9-cis,11-trans octadecadienoate, Cyclopropanebutanoic acid,2-2-2-(2 pentylcyclopropyl)methyl]cyclopropyl]methyl] cyclopropyl]methyl], methyl ester, 9,12-Octadecadienoic acid (Z,Z), Methyl 12-hydroxy-9-octadecenoate, 9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)], 9,12-Octadecadienoyl chloride, (Z,Z). The methanol extract of *R. communis* seeds and leaves is lower toxicity than the petroleum ether extract, this is maybe according to that methanol removes some of the toxic substances in *R. communis* like ricin (Daood and Abdullah Salih, 2021).

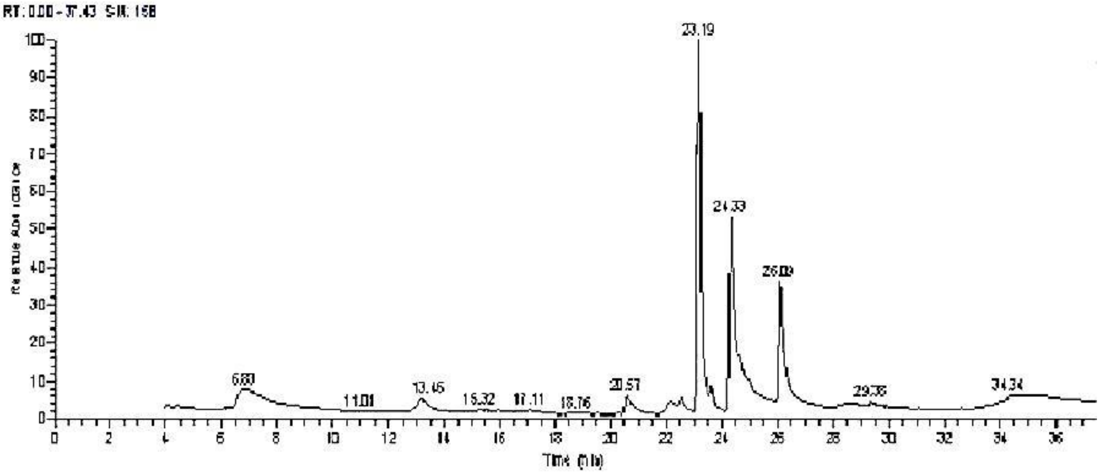
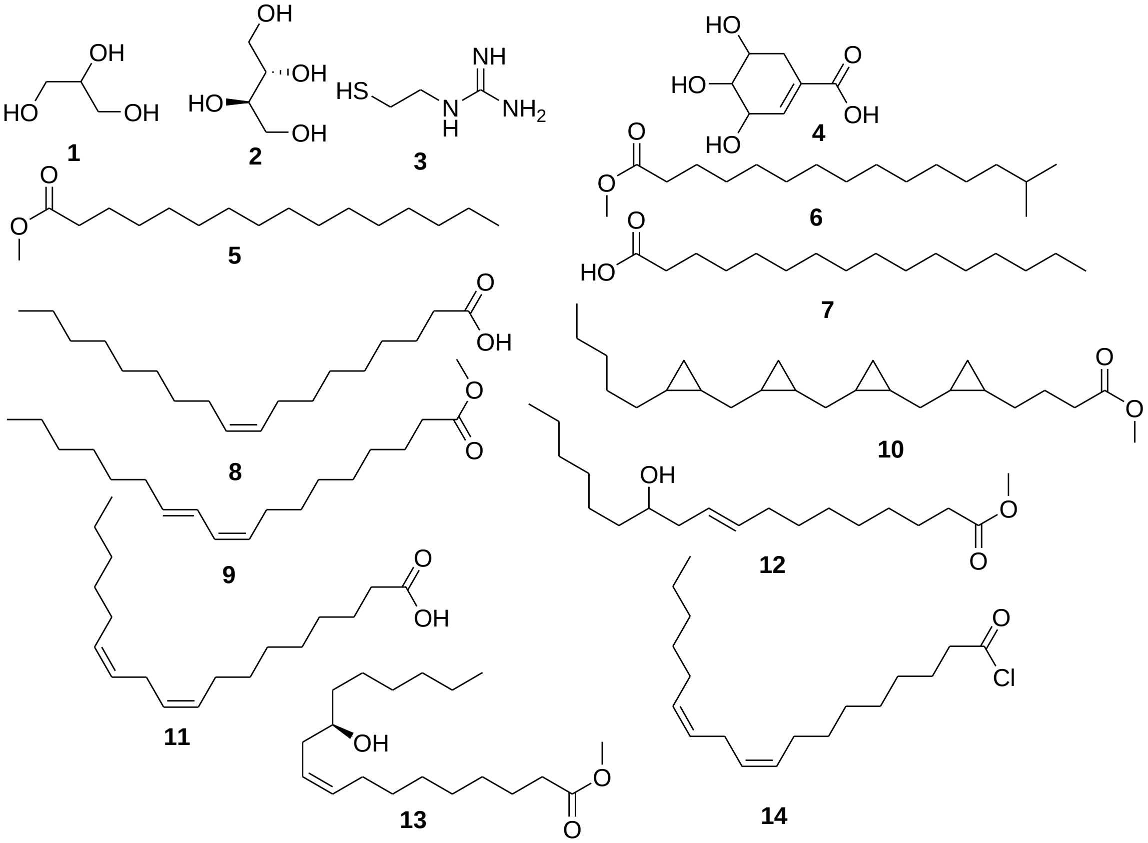


Figure (3): GC/MS analysis of *Ricinus communis* seed

Table (4): Main components of *Ricinus communis* seed identified by GC/MS.

No	Retention time (min.)	Compound name	Molecular formula	Peak Area %
1	6.73	1,2,3-Propanetriol	$C_3H_8O_3$	3.08
2	6.81	Erythritol	$C_4H_{10}O_4$	3.53
3	6.83	(2-Mercaptoethyl) guanidine	$C_3H_9N_3S$	4.12
4	13.46	Shikmic acid	$C_7H_{10}O_5$	2.25
5	20.57	Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	2.02
6	22.11	Pentadecanoic Acid, 14-Methyl-, Methyl Ester	$C_{17}H_{34}O_2$	1.12
7	22.17	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.66
8	22.54	9-Octadecenoic Acid (Z)-	$C_{18}H_{34}O_2$	0.85
9	23.1	Methyl 9-cis,11-trans octadecadienoate	$C_{19}H_{34}O_2$	18.45
10	23.19	Cyclopropanebutanoic acid, 2- [[2- [[2- [(2 pentylcyclopropyl) methyl] cyclopropyl] methyl] cyclopropyl] methyl]-, methyl ester	$C_{25}H_{42}O_2$	23.17
11	23.6	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	1.6
12	24.33	Methyl 12-hydroxy-9-octadecenoate	$C_{19}H_{36}O_3$	19.38
13	26.09	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	$C_{19}H_{36}O_3$	12.14
14	34.34	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	3.15

The chemical structures of *Ricinus communis* seed compounds using the GC-MS technique.



5. Field application:

Data in Table (5) indicated the relative efficiency of *Ricinus* extract and the recommended anticoagulant rodenticide supercaid against the black rat, *R. rattus*, under field conditions using the bait consumption technique. Pre-treatment average consumption of untreated crushed maize for *Ricinus* extract and Super caid was 180 and 200 g, respectively, while post-treatment consumption was 60.6 and 40 g for both. The 80.00 population % reduction that supercaid bait achieved was followed by the 66.33 reduction % by *Ricinus* extract. Ahmed *et al.* (2017)

used the food consumption method to evaluate supercaid 0.005% against *Rattus norvegicus Berk.* (Rodentia: Muridae) in a poultry farm and found population reductions of 79.98% and 46.04% inside and outside the building, respectively. The field and laboratory results agree, revealing that the *Ricinus* seed extracts effectively reduced and controlled the rat population.

Table (5): Field performance of *Ricinus extract* as a bait against the black rat *Rattus rattus* compared with supercaid rodenticide at Dakahlia Governorate.

Applied Compound	Bait consumption (gram /feddan)			
	Pre-treatment Mean ± SE	Treatment Mean ± SE	Post-treatment Mean ± SE	Reduction %
Ricinus extract	**180 ± 30.2^a**	**165 ± 13.5^a**	**60.6 ± 6.42^b**	**66.33**
Supercaid	**200 ± 42.5^a**	**137.2 ± 11.2^a**	**40 ± 6.12^b**	**80.00**
Control	**208 ± 41.1^a**	—	**184 ± 39.6^a**	—
P-Value	**0.86**	**0.15**	**0.002**	—

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