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Gas Chromatography Analysis and Acute Toxicity study of Hydro-alcoholic extract of Oak Bark *(Quercus Pro Parte)* in Female Local Rabbits

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Abstract: The present study aimed to evaluate the phytochemical constituents of hydro-alcoholic oak bark extract using gas chromatography–mass spectrometry (GC–MS) analysis and to determine its acute toxicity (LD50) in female local rabbits. Oak bark was extracted using 70% ethanol, yielding 11.7% dark brown crystalloid extract. GC–MS analysis identified several biologically active compounds, with gallic acid representing the predominant constituent (49.06%), followed by linalool (7.74%), isobornyl acetate (6.12%), 3-deoxy-D-mannonic acid (4.02%), salicylic acid (3.76%), and α-bisabolol (3.22%). These compounds are known for their antioxidant, anti-inflammatory, antimicrobial, and tissue-protective activities. The acute toxicity study was conducted using the Up-and-Down method in female local rabbits. The estimated oral LD50 value of the oak bark extract was (1850.7) mg/kg body weight, indicating slight to moderate toxicity according to toxicity classification standards. Clinical signs observed in intoxicated animals were dose-dependent and included mild sedation, decreased locomotor activity, piloerection, hypothermia, bradycardia, salivation, muscular weakness, anorexia, tremors, convulsions, respiratory depression, and death at higher doses. Animals receiving lower doses recovered within 18–25 hours, whereas higher doses caused severe neurotoxic manifestations and mortality. The findings of the current study demonstrate that oak bark hydro-alcoholic extract contains numerous bioactive phytochemicals with potential therapeutic value. However, administration of high doses may induce significant toxic effects, particularly on the nervous and respiratory systems.

Keywords: Gas Chromatography,Acute Toxicity, LD50, Rabbits, Oak Bark.

Research Paper

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INTRODUCTION

Since the past, oak bark medicines have been utilized in European ethnomedicine. Leonhard Fuchs' 16th-century herbal compendium details the application of oak bark for its astringent and hemostatic properties in the treatment of wounds, ulcers, and gynecological ailments (Heinrich *et al*., 2012). Additionally, oak bark was utilized in traditional herbal medicine as a bath additive for the treatment of dermatological conditions, including weeping and pruritic dermatitis, excessive foot perspiration, burns, hemorrhoids, and internally for the management of oral thrush, pyrosis, and diarrhea (Alpaslan *et al*., 2023). The European Pharmacopoeia (Ph. Eur., 2014) states that oak bark can be sourced from three species: *Quercus robur L., Q. petrae* (MATT.) LIEBL., and Q. pubescens WILLD., with a preference for bark from 10–15-year-old trees. Due to its elevated tannin concentration (up to 20%) (Taib *et al*., 2020), oak bark possesses astringent, desiccating, emplastic, antimicrobial and anti-inflammatory activities, among others (Šukele *et al*., 2020). Currently, oak bark and its hydro-alcoholic extracts are predominantly utilized in phytotherapy and anthroposophic medicine (Banc *et al*., 2023), for the management of eczema, scrofula, menorrhagia, hemorrhoidal disorders, varices, and the development of fissures and rhagades in the skin and mucous membranes (Lorenz *et al*., 2016), as well as skin inflammations and allergic diseases (Burlacu *et al*., 2020). Bark is recognized for accumulating substantial quantities of polyphenolic chemicals that demonstrate a diverse array of biological effects, including antioxidants, antibacterial, and anti-inflammatory activities (Ferreira *et al*., 2015; Nisca and Tanase, 2025). Due to its excellent chemical constitution, oak bark has been utilized in traditional medicine for the treatment of wounds and dermatological conditions. Plentiful and cost-effective resources, such as byproducts of wood processing, constitute a promising supply of natural

antioxidant compounds (Bouras *et al*., 2015; Dróżdż and Pyrzynska, 2021). The aim of this study was to evaluate the extraction of major classes of polyphenolic compounds from bark oak (*Quercus robust L.*) with estimation of LD50 (acute toxicity) in female rabbits.

MATERIALS AND METHODS

Plant

Oak barks (*Quercus Pro Parte*) were procured from local marketplaces in Babylon, Iraq. The Oak was extracted in accordance with (Harbone, 1984; Al-Ameedi *et al*., 2023; Ayad *et al*., 2025).

GC-Mass Spectroscopy

The GC-MS analysis of Oak bark was performed using an Agilent 7820A GC system linked to a mass spectrometer (Agilent, USA), as seen in Fig. 3-1. The analytical column utilized was an Agilent HP-5 MS Ultra Inert (30 mm × 250 µm × 0.25 µm). A one microliter injection volume was utilized at a pressure of 11.933 psi. The temperatures for the GC inlet line, auxiliary heaters, and injector (splitless) were established at 250 °C, 300 °C, and 250 °C, respectively. The carrier gas employed was helium with a purity of 99.99%. The oven program temperature comprised four ramps: the initial ramp was maintained at 60 °C for 3 minutes, the second ramp ascended from 60 °C to 180 °C at a rate of 7 °C/min, the third ramp ascended from 180 °C to 280 °C at a rate of 8 °C/min, and the final ramp was sustained at 280 °C for 3 minutes.

Animals

This study utilized six female local rabbits (*Lepus cuniculus*) with an average body weight of 1800±125 g and an age of 8±1.4 months. The animals were accommodated in individual stainless-steel cages within the animal house at the College of Veterinary Medicine, Al-Qasim Green University, under standardized environmental conditions (temperature: 23±2 °C, humidity: 50±5%, 12-hour light/dark cycle). They were provided with unlimited access to food, including green leaves, hay, and water *ad libitum*. The rabbits utilized to ascertain the LD50 of Oak bark extract by the Up and Down technique (Dixon, 1980; Al-ameedi and Al-rekabi, 2015; Al-rekabi *et al*., 2021).

Ethics

The experimental design and procedures employed in this study received approval in compliance with animal welfare ethical standards from the Scientific Committee of the Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, Al-Qasim Green University, certificate number (qgec/16 /2025), as well as from the Ethics Committee of the College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq.

RESULTS

Plant Extraction

The Oak bark of (*Quercus Pro Parte)* were extracted by using 70% ethanol and the percentage of extract yield was 11.7%. The result of oak alcoholic extract was taken to full dryness, the color of extract was dark brown, and the extract was crystalloid in texture at room temperature as depicted in figure (1). These findings resembling results reported by many authors (Banc *et al*., 2023; Dnan *et al*., 2025).

Figure 1: Ethanolic extract of Oak bark

GC Mass Spectroscopy of Oak Bark Hydro-Alcoholic Extract

The phytochemical composition of the hydro-alcoholic extract of oak bark was analyzed using GC–mass spectrometry, as presented in Table (1). The analysis identified several bioactive compounds, with benzene dicarboxylic acid being the predominant constituent (49.06% area), followed by linalool (7.74%), isobornyl acetate (6.12%), and 3-deoxy-D-mannonic acid (4.02%).

Table 1: GC Mass analysis of Oak Bark hydro-alcoholic extract

No.	Compounds	R/T (min)	Chemical formula	Molecular weight	Area %	Biological activity
1	Gallic acid	19.822	C7H6O5	170.1	49.06	Osteoconductive Properties, antioxidant and inflammatory activities
2	Linalool	8.032	C10H18O	154	7.74	Strong anti-inflammatory and inhibition of osteoclast activity (reduce bone resorption)
3	Isobornyl acetate	9.406	C12H20O2	196	6.12	Antimicrobial, anti-inflammatory, sedative and Antibacterial Bone Cement Component
4	3-Deoxy-d-mannonic acid	2.153	C6H12O6	180	4.02	Increase cortical bone volume, and improve trabecular bone microarchitecture
5	Salicylic acid	18.980	C7H6O3	132	3.76	Anti-inflammatory and antibacterial agent
6	Alpha-Bisabolol	14.992	C15H26O	222	3.22	anti-inflammatory, antioxidant, and chondroprotective properties that contribute to bone health and potential tissue regeneration
7	aR-Turmerone	14.353	C15H20O	216	2.89	potent anti-inflammatory, antioxidant, and supporting a healthy microenvironment for bone metabolism
8	1H-Benzocyclohepten-9-ol,	15.300	C15H26O	222	2.48	promoting osteoblast proliferation and differentiation (bone matrix synthesis).
9	Caffeic acid	12.43	C9H8O4	180.1	1.54	stimulating the proliferation of bone-forming cells
10	1-Hexadecanol	17.480	C16H34O	242	1.26	Anti-inflammatory and antioxidant agent

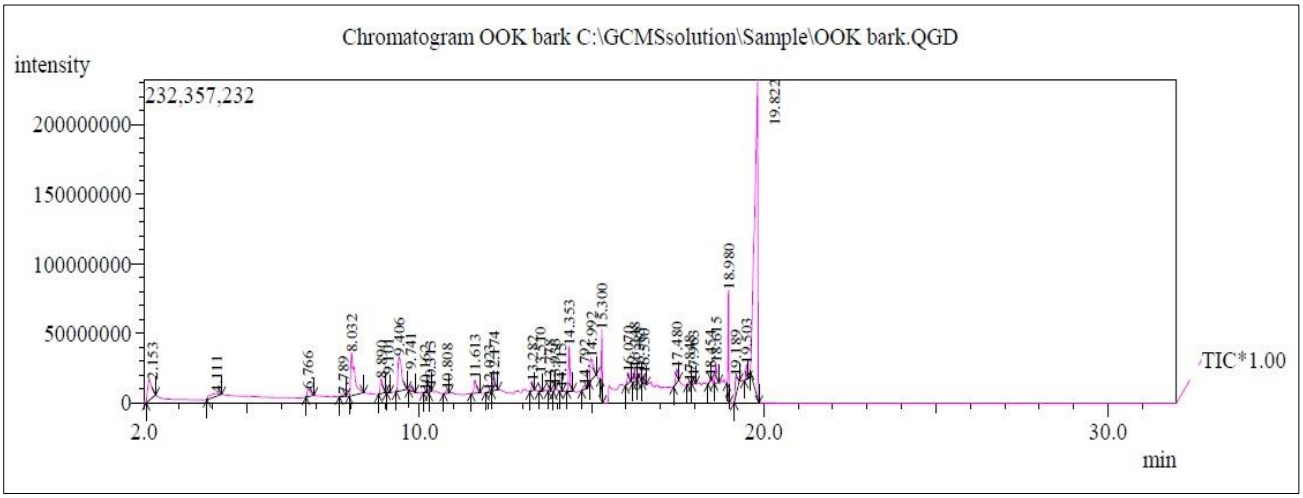

Figure 3: GC-mass analysis of Oak barks (*Quercus Pro Parte*) extracts

Acute Toxicity Study

The acute toxicity study found that the oral LD50 of Oak bark ethanolic extract as estimated by the Up and Down method is 1850.7 mg/kg BW orally in female local rabbit for 24 h (Table 2). The OB extract is considered slightly to moderately toxic with a class 4 according to the classification of toxicity rating which depends on LD50 value (Al-Rekabi *et al*., 2021).

Table 2: LD_{50} of Oak bark ethanolic extract by up and down method

Initial dose	Final dose	No. of animal	Results after 24hr.	Different Among doses	LD50 Mg/kg.bw orally
1500	1800	6	OOXOXX	300	1850.7

O=survival, X= dead

LD50 = xf + Kd

Xf = the last dose

K= factor from the table according to constant and Variable.

d= differences between doses used.

LD50=1800+(0.169*300) =1800+50.7

LD50=1850.7mg/kg.bw (orally in female local rabbit).

Clinical Signs

The clinical signs of acute oak bark toxicity observed in the experimental rabbits table (3) were dose-dependent and progressively intensified with increasing administered doses. Animals treated with 1500 and 1800 mg/kg BW exhibited early signs included mild sedation, reduced spontaneous movement, piloerection, decreased locomotor activity, and slight ataxia. As the intoxication progressed, hypothermia, bradycardia, salivation, and muscle weakness appeared within 60–120 minutes post-administration. Severe lethargy and inability to feed were observed after approximately 2.5 hours. However, the affected animals gradually recovered within 18–25 hours, and all animals in these groups survived. In contrast, rats administered higher doses (2100 mg/kg BW) developed more severe toxic manifestations. Early clinical signs included grooming behavior, piloerection, anorexia, and muscular tremors appearing within the first 10–60 minutes. Convulsions and marked depression were recorded after approximately 90 minutes to 2 hours, followed by arched back posture, crippling movement, and recumbency. The progression of toxicity culminated in respiratory arrest and death between 13–16 hours after dosing. Mortality was consistently observed in animals receiving 2100 and 1800mg/kg BW, indicating severe neurotoxic and respiratory depressive effects of amitraz at high doses.

Table 3: Clinical signs of the acute toxicity study (LD50) of oak bark

Dose mg/kg BW	Clinical signs during 24 hs	Time of Sings appeared	Results (O, X)
1500	-Mild sedation, reduced spontaneous movement, -piloerection, decreased locomotor activity, slight ataxia Hypothermia, bradycardia, salivation, muscle weakness Severe lethargy, inability to feed recovery	10 min 60 min 90 min 2 h 2.30 h 18 h 20h	O
1800	-Mild sedation, reduced spontaneous movement, -piloerection, decreased locomotor activity, slight ataxia Hypothermia, bradycardia, salivation, muscle weakness Severe lethargy, inability to feed recovery	10 min 60 min 90 min 2 h 2.30 h 11 h 25h	O
2100	grooming, piloerection anorexia, muscular tremors convulsions depression, arched back crippled recumbency respiratory arrest, death	10 min 60 min 90 min 2 h 2.30 h 3 h 14 h	X
1800	-Mild sedation, reduced spontaneous movement, -piloerection, decreased	10 min 60 min	O

Dose mg/kg BW	Clinical signs during 24 hs	Time of Sings appeared	Results (O, X)
	locomotor activity, slight ataxia Hypothermia, bradycardia, salivation, muscle weakness Severe lethargy, inability to feed recovery	90 min 2 h 2.30 h 10 h 17h	
2100	grooming, piloerection anorexia, muscular tremors convulsions depression, arched back crippled recumbency respiratory arrest, death	10 min 60 min 90 min 2 h 4 h 6 h 13 h	X
1800	grooming, piloerection anorexia, muscular tremors convulsions depression, arched back crippled recumbency respiratory arrest, death	10 min 60 min 90 min 2 h 7 h 8 h 16 h	X

X: Dead O: Live

DISCUSSION

The present study demonstrated that the hydro-alcoholic extract of oak bark (Quercus Pro Parte) contains several biologically active phytochemicals with potential pharmacological and toxicological significance. Gas chromatography–mass spectrometry (GC–MS) analysis revealed that gallic acid was the predominant compound identified in the extract, followed by linalool, isobornyl acetate, 3-deoxy-D-mannonic acid, salicylic acid, and α-bisabolol. These findings are consistent with previous reports indicating that oak bark is rich in polyphenols, tannins, flavonoids, and phenolic acids possessing antioxidants, antimicrobial, and anti-inflammatory activities (Banc *et al*., 2023; Burlacu *et al*., 2020). The high percentage of gallic acid observed in the current study may explain many of the biological effects of oak bark because gallic acid is recognized as a potent free radical scavenger and anti-inflammatory agent that protects tissues against oxidative damage (Nisca & Tănase, 2025; Aboktifa *et al*., 2025).

The presence of linalool and α-bisabolol in the extract further supports the therapeutic potential of oak bark. Linalool has been reported to exhibit significant anti-inflammatory and neuroprotective activities through suppression of pro-inflammatory cytokines and oxidative stress pathways (Peana *et al*., 2021). Similarly, α-bisabolol possesses antioxidant and cytoprotective properties and may contribute to tissue regeneration and cellular stabilization (Silva *et al.,* 2022; Al-Ameedi *et al*., 2026). Furthermore, salicylic acid identified in the extract is widely known for its anti-inflammatory and antimicrobial actions, which may synergistically enhance the medicinal value of oak bark preparations (Alpaslan *et al.*, 2023). Therefore, the diversity of phytochemicals detected by GC–MS confirms that oak bark extract is a rich source of bioactive compounds capable of exerting multiple pharmacological effects.

The extraction yield of 11.7% obtained in this study was relatively comparable with findings reported by other investigators using hydro-alcoholic solvents for extraction of oak bark phenolics (Banc *et al*., 2023). Ethanol-water mixtures are considered efficient solvents for extraction of tannins and polyphenolic compounds due to their polarity and ability to penetrate plant tissues effectively (Dróżdż & Pyrzynska, 2021). The dark brown crystalloid appearance of the extract may reflect the abundance of condensed tannins and phenolic constituents present in the bark.

The acute toxicity investigation demonstrated that the oral LD50 value of oak bark extract in female rabbits was 1850.7 mg/kg BW, indicating that the extract is slightly to moderately toxic according to toxicity classification systems. Similar observations were reported in previous toxicological studies of tannin-rich herbal extracts, where high doses produced neurological and gastrointestinal disturbances due to excessive phenolic intake (Ferreira *et al*., 2015). Although oak bark possesses beneficial medicinal properties, the current findings indicate that administration of high doses may induce toxic manifestations and should therefore be used cautiously.

The clinical signs observed during intoxication suggest that the oak bark extract exerted dose-dependent

neurotoxic and depressant effects on the central nervous system. Mild sedation, decreased locomotor activity, muscle weakness, and hypothermia observed at lower doses may be attributed to the depressant effects of certain terpenoids and phenolic compounds present in the extract. Linalool, for instance, has been shown to influence glutamatergic and cholinergic neurotransmission, producing sedative and anxiolytic effects (Peana *et al*., 2021). Additionally, the occurrence of bradycardia and salivation may indicate autonomic nervous system involvement associated with phytochemical-induced parasympathetic stimulation.

At higher doses, severe manifestations including tremors, convulsions, respiratory depression, and death were recorded. These effects may result from excessive oxidative imbalance and neuronal dysfunction induced by concentrated phytochemicals and tannins. Polyphenolic compounds at toxic concentrations may disrupt mitochondrial respiration and alter membrane permeability, ultimately impairing neuronal and respiratory functions (Silva *et al*., 2022;). Respiratory arrest observed before death may therefore represent the terminal consequence of progressive central nervous system depression.

The variation in survival between animals receiving 1800 and 2100 mg/kg BW confirms the narrow margin between therapeutic and toxic doses of the extract. This observation emphasizes the importance of dose standardization and safety evaluation before therapeutic application of oak bark products. Moreover, species variation in metabolism and sensitivity to phytochemicals should also be considered during toxicological assessment because rabbits may respond differently from rodents or humans.

CONCLUSION

Overall, the present findings support the traditional medicinal use of oak bark as a source of biologically active compounds with antioxidant and anti-inflammatory activities. However, the results also indicate that excessive administration may induce considerable toxicity. Therefore, additional studies are necessary to investigate chronic toxicity, histopathological alterations, biochemical changes, and the precise mechanisms underlying the toxic effects of oak bark extract.

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