



Phytochemical Screening, and Acute Toxicity Detection of The Ethanolic Tuberos Root Extracts of *Euphorbia condylocarpa* in Albino Rats

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ABSTRACT

The tuberos root of spurge *Euphorbia condylocarpa* M. Bieb, that is pertain related to family (Euphorbiaceae) are ordinarily used as a laxative activity in folk medicine. The present study was intended to explore the phytochemical screening as well as lethal dose of ethanol root tubers extract of spurge in constipated rats. For acute toxicity assessment, several doses (500, 1000, 3000, 5000 mg/kg) of the extract were administrated orally to albino rats. Symptoms of toxicity and possible death of animals were monitored for 24 hrs, to ascertain the Median lethal dose (LD 50) of extract. The results displayed that three compounds were found in ethanolic root tubers in the content determination with LC-, MS/MS: Galic acid in a concentration (440µg/g), Protocatechuic acid in a concentration (71 µg/g), Quercitrin (82µg/g). Qualitative phytochemical test indicated the existence of terpenoids, alkaloids, phenols, flavonoids, steroids, tannin, and coumarins of root tuber ethanolic extracts. The maximum antioxidant activity of tuberos root extract was at concentration of 5mg/ml (58.69%), while minimum antioxidant activity was at 0.625 mg/ml (17.18%). Treating albino rats with 5000 mg/kg resulted in noticeable signs of acute toxicity and mortality in the tested animals. The LD50 of this plant was 3600 mg/kg.

Keywords: *Euphorbia condylocarpa*, Antioxidant activity, Acute toxicity, Rats, Ethanol extract.

INTRODUCTION

The Euphorbiaceae is a diverse family of flowering plants, with about 300 genera and 800 species. The *Euphorbia* genus is notable within this family for comprising over 2000 species. These species may manifest as herbs, shrubs, trees, and occasionally as succulents and cacti (Kgosiemang *et al.*, 2025). Aromatic and medicinal plants, corresponding to their exploit, can be retailed as fresh and dried yields. Medicinal plants can be commercialized as either fresh or dried products depending on their application. Medicinal plants are used as raw materials for the production of pharmaceuticals produced by the industry (Parvin *et al.*, 2023). Phytochemicals are bioactive molecules that supply originated from the plant for their defense. They can be derived from diverse sources including fruits, vegetables, herbs, whole grains, and nuts. Notable phytochemicals involved saponin, phytosterols, polyphenols, isoprenoids, carotenoids, dietary fibers, and certain polysaccharides (Dev Sharma *et al.*, 2023). Humans depend on plants for most of the nutrients and calories (Usman *et al.*, 2025, Agwan *et al.*, 2025). The root of *Euphorbia condylocarpa* M. Bieb (spurge) is utilized in folk medicine for the treating cancer, costiveness, and migraines, and as well serves an emollient and for the extraction of various phenolics Fig. (1) (Nasrollahzadeh *et al.*, 2014). Phytochemicals, the bioactive natural chemical molecules that provide medicinal properties to plants, are widely categorized in to two types; primary and secondary metabolites (Asha and Thirunavukkarasu, 2015). Constipation is a gastrointestinal motility disease characterized by rare bowel motions. An estimated 14% (3.2 million) of the world's population is constipated (Rdg *et al.*, 2023). Spurge plant in folk medicine is used a potent drug to treat constipation and intestinal infections (Mohammadi *et al.*, 2017a). The acute toxicity test is a mode for identifying harmful influences that manifest shortly after the administration of a chemical, either in a single dose or in multiple doses within a 24-hour period. Clinical symptoms arising from the specified dosage include tumors, cardiac irregularities, behavioral, activity disorders, gastrointestinal issues, weakness and respiratory depression. Furthermore, the acute toxicity of a substance can be assessed by calculating the LD₅₀. Lethal dose within 24 hrs after administration of toxicant, LD₅₀ is a dose that statistically can kill 50% of experimental animals (Rinanda *et al.*, 2025).

Hematological and biochemical parameters are crucial as they indicate the health state of animals upon administration of plant extracts, at the same time toxicological investigations assessed the safety of these extracts and their potential effects on users (Oloninefa *et al.*, 2023).

This study was aimed to investigate the phytochemical screening and acute toxicity of tuberous root ethanolic extract, and the assessing the potentiality of the extract as laxative agent in animals model constipation on rats.

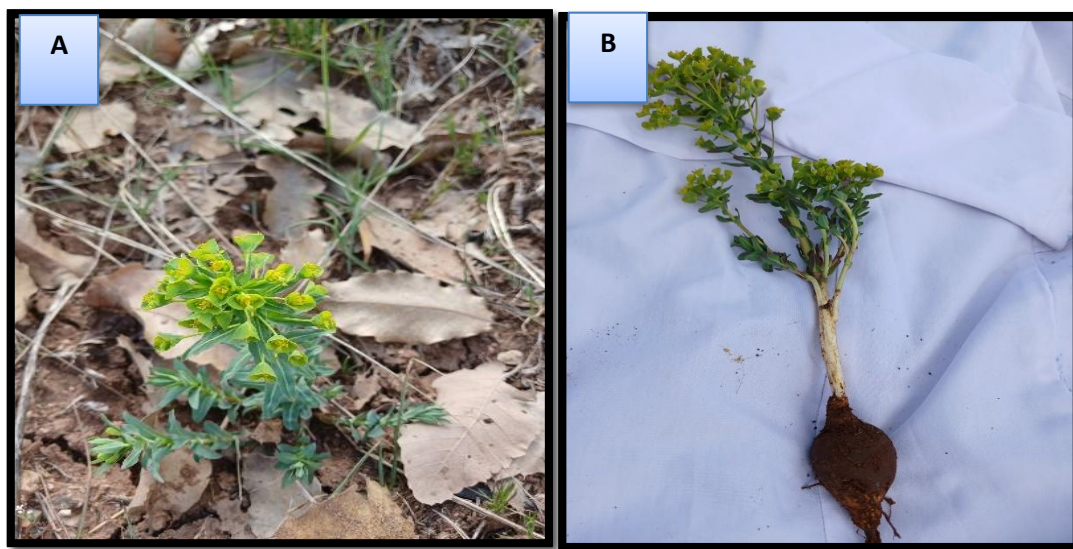


Fig. 1: A. The whole plant of spurge B. Tuberous root of Spurge in Hiran region

MATERIALS AND METHODS

Preparing the ethanolic extraction

Tuberous root of spurge plants was collected from Hiran hills in Iraq and washed with distilled water, then dried in shade under sterile conditions. After that the root tubers were ground by an electric mill, and the powder was kept in plastic cans and stored in the refrigerator at 4°C until used. 50 grams of dried powdered root tubers were subjected extraction with 500ml of absolute ethanol (ETH.) by exploiting a Soxhlet apparatus at 60 °C for 8 hours Fig. (2). The extracts were filtered and concentrated by rotary evaporator under diminished pressure at 40°C. The extracts were frozen till utilized (Mohammadi and Arabhosseini, 2024). Ingredient concentration was prepared from powdered ethanolic extract as prepared different doses (500, 1000, 3000, 5000 mg/g body weight), by dissolving plant crude extract in Dimethyl sulfoxide (DMSO).

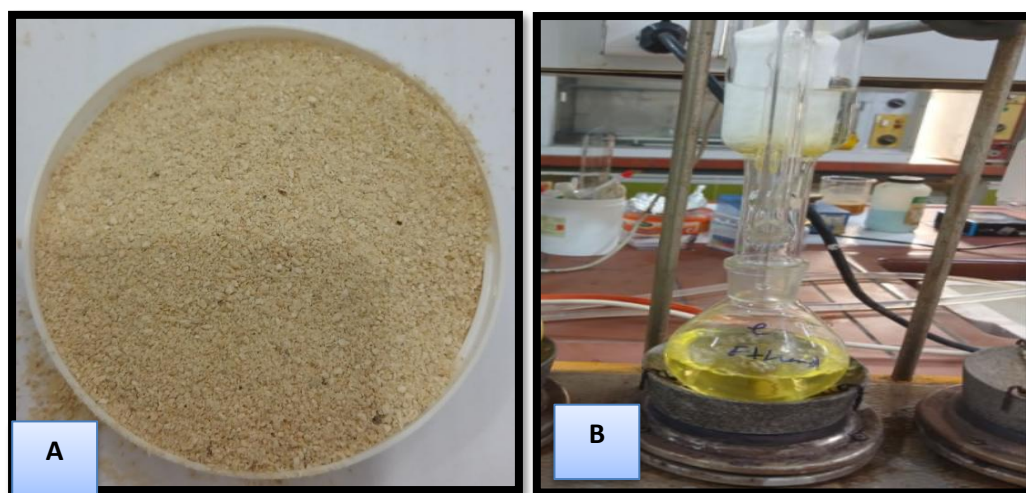


Fig.2: A. Tuberous root powder

B. Soxhlet extraction process of tuberous root of spurge

Qualitative analysis of phytochemicals in tuberous root of spurge plants

To make phytochemical screening of *Euphorbia condylocarpa* tuberous root, the methods adopted by (Savithramma *et al.*, 2011) were applied. The plant ethanolic extract was subjected to the following tests:

Terpenoids detection

By mixing 0.5 ml of the plant extract with 2 ml of concentrated sulphuric acid and chloroform V/V, the existence of terpenoids is verified by the emergence of a reddish or brown interface.

Steroid detection

The existence of steroids is proved by adding 2 ml of chloroform and 1 ml of sulphuric acid to 0.5 ml of plant extract. The formation of reddish-brown interface rings confirmed that steroids are present.

Saponins detection

To identify saponins, 2ml of plant extract and 2ml of distilled water were stirred in a graduated cylinder for 15 minutes. Saponins were detected if 1-centimeter foam was formed.

Flavonoid detection

2 ml of plant extract were added to 1 ml of 2N of sodium hydroxide. Appearance of yellow color indicating the presence of flavonoids.

Phenols detection

1ml of plant extract was mixed with 2 ml of distilled water and a few drops of 10% ferric chloride. Phenols are present if blue/ green color development took place.

Tannins detection

1 ml of 5% ferric chloride was added to 2 ml of the plant extract. Greenish-black or dark blue coloring is an indicator of tannins presence.

Coumarins detection

1ml of 10% of sodium hydroxide was combined with 1 ml of plant extract to recognize the coumarins. The yellow color emergence is a sign of coumarins presence in the extract.

Alkaloids detection

Two drops of Wagner reagent with to 2 ml of plant extract mixed decently. The emergence of a reddish color indicates the existence of alkaloids (Dahanayake *et al.*, 2019)

Antioxidant activity (DPPH assay)

The antioxidant activity (%A) of the sample, and reference was assayed with slight modifications according to the methods described by (Dessalegn, 2025). Various concentrations ranged from (0.625, 1.25, 2.5, 5 mg/ml) of the extract, and ascorbic acid (as commercial antioxidants) were held in disparate test tubes. 0.06% W/V DPPH solution (2,2-dophenyl-1-picrylhydrazyl) prepared by dissolving 0.06 gm of DPPH in 100ml of absolute ethanol (0.0%w/v) 1 ml of the extract mixed with 1 ml of DPPH solution, and so 1ml of standard ascorbic acid mixed with 1ml DPPH. Each mixture left at room temperature for 30 min in the dark. Then, the absorbance of each prepared solution was measured spectroscopically at 517 nm. Absolute ethanol considered as blank. Scavenging capacity of the DPPH radical was calculated as follows:

$$\text{DPPH scavenging (\%)} = (\text{A control} - \text{A sample} / \text{A control}) \times 100$$

Detection toxicity and Lethal dose 50 (LD₅₀) of the tuberous root ethanolic extraction vivo:

This test was operated at the College of Pharmacy, the Howler Medical University, the test procedure was executed corresponding to (Epa, 2002) test guideline. The female albino rats (*rattus norvegicus*) that were left fasting for overnight, weighting (200-235g), were maintained under standards conditions (12hr. light, 12hr. dark) photoperiod, at a humidity of 5±60 %, temperature 22±3 °C, provided with standard diets and unrestricted access to tap water. In standard plastic cages (38×18×30) cm, all animals were housed and bedded with wood chips sterilized with UV light about 1.5 hr. and informally divided in to distinct groups (n=5). The normal control group were supplied only with DMSO, while the other groups received a single dose ethanolic root tuber extract of spurge orally at dosages of 500, 1000, 3000, and 5000 mg/kg. The volume of the extract solution administered orally contingent upon on the size of the animals not exceeding 1ml/100 g of their body weight. Following the administration of the solution extract, eating may be withheld for 1-2 hr. During the acute toxicity study symptoms of toxicity were seen the first 24 hr., together the rat body weight was measured weekly from the first 7 days and again at 14 days post treatments. The LD₅₀ value was determined based on the death of the rats reported after 24hours.

Determination of Median lethal dose (LD₅₀):

After 24 hr. of the treating the animals with ingredient doses of the tuberous root extract; the rate of mortality was recorded and the LD₅₀ was calculated by using methods employment by (Ahmed, 2015) as follows:

$$\text{LD}_{50} = \text{LD}_{100} - (a \cdot b) / n$$

LD₁₀ lethal dose causing 100% of all animal death.

a= the difference between two successive doses of administrative extract.

b= the average number of dead animals in two successive doses.

n= sum of animal's total number within a group.

Measurement of body weight:

The animal's weight was recorded individually weekly in grams, at the beginning of the experiment and end of each week.

Phytochemical analysis:

The mass spectroscopy analysis was meticulously conducted by employing a state-of-the-art Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer, which was equipped with an Electrospheric Ionization (ESI) source capable of functioning in both positive and negative ionization modes. The data obtained from LC-MS/MS analyses were systematically collected and processed using the Lab Solutions software developed by Shimadzu, based in Kyoto, Japan, as referenced in the works of (Ertas *et al.*, 2015). To ensure precision and accuracy in quantification, the analyses were executed in a multitude of response monitoring (MRM) modes, encompassing both quantitative and confirmation measures. This approach aimed to enhance the reliability of the results by incorporating a dual assessment strategy, reinforcing the robustness of the analytical procedures employed in the Mass Spectrometry analysis. This utilization of advanced technology and comprehensive data processing software underscored the commitment to a thorough and meticulous investigation of the molecular components under scrutiny.

Liquid chromatography- Tandem Mass Spectrometry Analysis:

Fifty-six organic acids, both phenolic and non-phenolic, commonly found in plant resources, were identified and measured. Linear regression equations and the linear scopes of the standards compound under investigation are illustrated in Fig. (3) correlation coefficients consistently exceeded 0.99. (Table 5) presents the results demonstrating the limit of detection (LOD) and quantitation (LOQ) for proposed analytical way. The LOD for analyzed compounds varied from 0.05 to 25.8 g/L, while the LOQ ranged from 0.17 to 85.9 g/l. furthermore, the recovery rates for the phenolic compounds fell within the range of 96.9% to 106.2%.

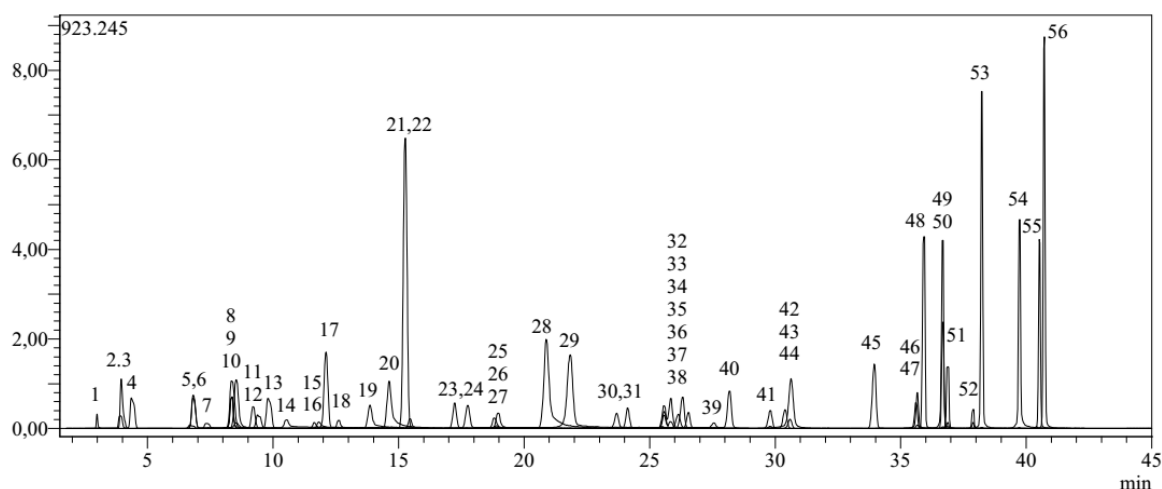


Fig. 3: LC-MS/MS chromatogram for reference phytochemical compound.

Analysis of data

The data were statistically analyzed using one-way analysis of variance (ANOVA) with statistical package for the social sciences (SPSS) version 27. The means were analyzed using Duncan's Multiple Range test at probability of 0.05 of significance (Wellington *et al.*, 2020). The data were presented as the means.

RESULTS AND DISCUSSION

Qualitative phytochemicals determination in leaves and tuber roots of *E. condylocarpa* M. Bieb qualitative phytochemicals were analyzed by using ethanol solvent of root tubers of *E. condylocarpa*.

The data revealed that the contiguity of major phytochemicals groups such as terpenoids, alkaloids, phenols, flavonoids, tannins, coumarins and the results assigned as (+) for the occurrence together with (-) for lack of phytochemicals (Table 1) and Fig. (4). The outcomes exhibited that the presence of different phytochemicals in root tubers of *E. condylocarpa* (Table 3). It is attainable that

these phytochemicals are responsible for therapeutic potentials. Terpenoids display myriad pharmacological activity operating as quick agent against inflammation, cancer, viruses in addition to bacteria. Flavonoids are possessed to have antioxidant outcomes impeding as well as succession of tumors. Tannins possess antibacterial, antitumor, besides antiviral schemes. Alkaloids are strong antibacterial performs (Adil *et al.*, 2024).

Table (1): Qualitative Phytochemical screening of tuberous root ethanolic extract.

Compound extract	Plant parts	Terpenoid	steroid	Saponin	Flavonoids	phenol	Tannin	Coumarin	alkaloid
Ethanol	Root tuber	+	+	-	+	+	+	+	+

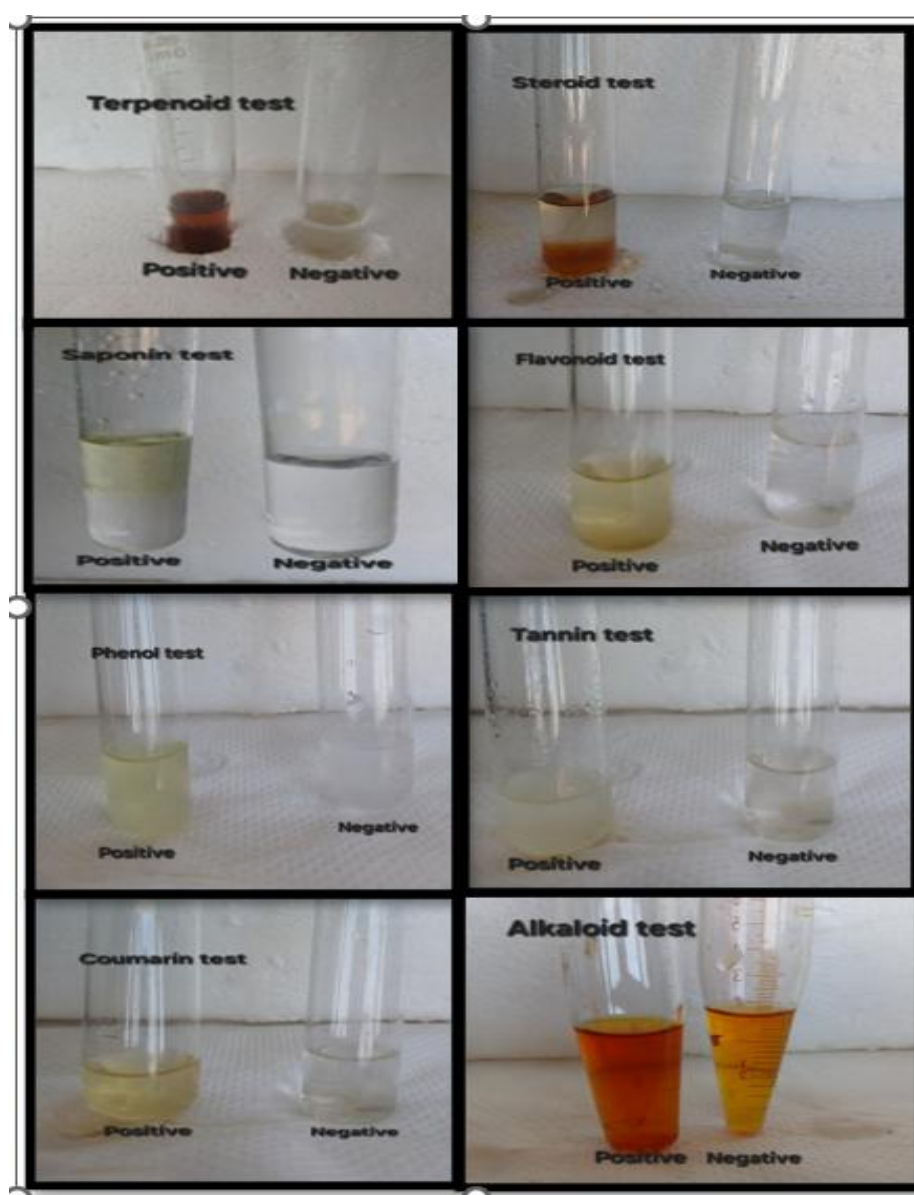


Fig. 4: Color changing during Phytochemical test for ethanolic tuberous root extract of *E. condylocarpa*.

Phytochemical screening and quantitative of the root tubers of spurge plant:

Quantitative phytochemical screening of ethanolic extract of root tuber of spurge which is determined with LC-, MS/MS Fig. (5), showed the presence of Galic acid (440 μ g/g), in peak number (4), Protocatechuic acid (71 μ g/g) in peak number (6), Quercitrin (82 μ g/g) in peak number (41), (Table 1). The extreme of the biological active chemicals are soluble in ethanol more than any other solvents, which active against organism (Abdul-Ghany *et al.*, 2011).

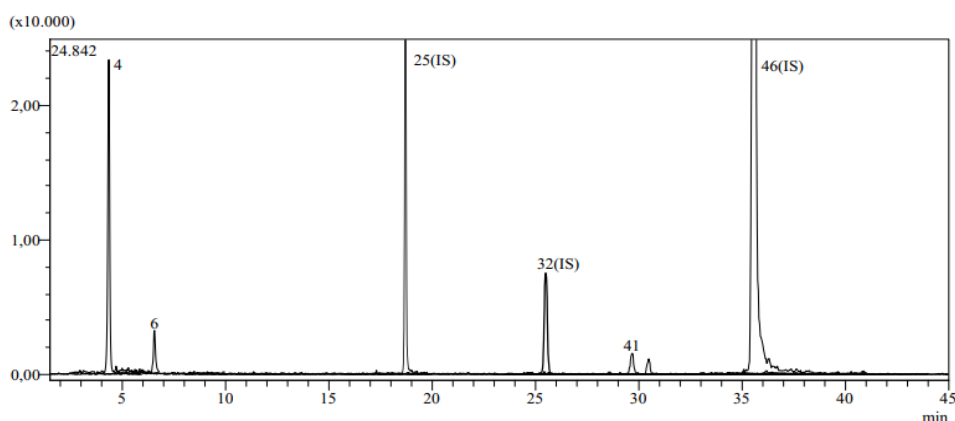


Fig 5: LC-MS/MS chromatogram for analytical parameters and results of phytochemical compounds.

Antioxidant activity

Root tuber spurge extract exhibits a DPPH radical scavenging activity. The maximum antioxidant activity of the ethanolic extract at 5mg/ml was (58.69%). While minimum antioxidant activity was at 0.625 mg/ml (17.18%). The results are illustrated in (Table 2) and Fig. (6). The standard antioxidant compound ascorbic acid displayed the highest DPPH scavenging activity of (80.59%) at 5mg/ml. The result of Mohammadi and Arabhosseini (2024), showed that the antioxidant activity of the *E. condylocarpa* tuberous root extract contain various antioxidant compounds, variability of phenolic compound rate with the free radical scavenging free radical capacity of DPPH may be to the presence of other antioxidant compounds exclude for the polyphenol compounds.

Table (2): Antioxidant activity of plant extract, ascorbic acid measured by DPPH assay

Plant part	Concentration (mg/ml)	DPPH scavenging activity %
Root tubers	0.625	17.18 f
	1.25	30.37 e
	2.5	35.64 de
	5	58.69 c
Ascorbic acid	0.625	43.65 d
	1.25	69.33 b
	2.5	73.27 ab
	5	80.59 a

*Different letters refer to significant different between values at $P \leq 0.05$.

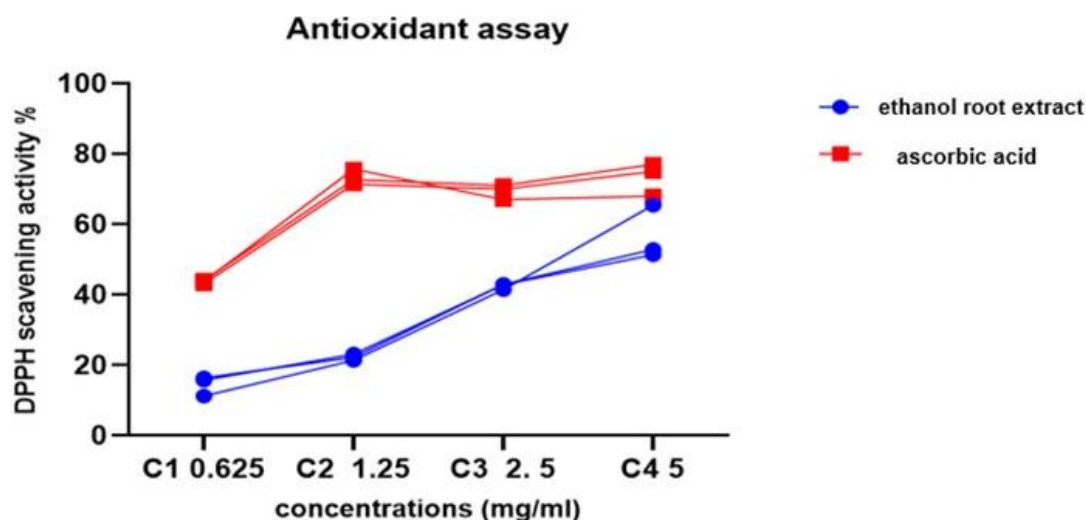


Fig. 6: Antioxidant activity of spurge plant ethanolic extract compared with ascorbic acid

Ethanol root extract

Studying toxicity signs and LD₅₀ of *E. condylocarpa* ethanolic extract in albino rats. The lethal dose or toxicity assessment were reported through the treatment duration in the treatment groups given a single dose orally of 500, 1000, 3000, 5000 mg/kg of root tubers of spurge during 14 days of the experiment, the treated rat showed some signs of toxicity such as hypoactivity, few lacrimation, diarrhea, and bloat, in a dose- dependent manner. The number of rats mortality during 24hr recorded in the groups that received 1000,3000,5000 mg/kg respectively, and the LD₅₀value was determined to be 3600mg/kg Fig. (7) and (Table 4) body weight, which *E. condylocarpa* pertains to Euphorbiaceae family which is possessed to be poisonous. This aligns with the research conducted by (Mohammadi *et al.*, 2017b) which examined cytotoxic effect of the root tubers of the *E. condylocarpa*, their findings indicated that ethanolic crude extracts exhibited significant cytotoxic activity. In this inspection *E. condylocarpa* ethanolic extract at dose 5000mg/kg has detrimental effect on the tested rats. Their body weights dramatically diminished following the orally administration of single dose of ethanolic extracts of root tuber of spurge after 7 and 14 days of administrating 500, 1000 mg/kg of the ethanolic extract, which indicative of the laxative activity of this extract. This extract induced symptoms of toxicity including hypoactivity which may correlate with diminished mobility activity regulated by the central neural system. Lacrimation might indicate a muscarinic effect associated with cholinergic poisoning while bloating could stem from physical obstruction or esophageal damage, impacting respiration and potentially leading to death (Rahman *et al.*, 2020). Toxicity signs of the plant ethanolic extract were depending on: Hypoactivity, lacrimation, diarrhea, bloat.

Table (4): Observed toxicity symptoms in albino rats according to different concentrations of *Euphorbia condylocarpa* ethanolic extract:

The extract Concentrations (mg/kg b.w.)	Number of Treated rats	Number of Dead rats	Symptoms
500	5	0	Non*
1000	5	2	Hypoactive, few lacrimation, diarrhea, bloat
3000	5	3	Hypoactive, lacrimation, diarrhea, bloat
5000	5	5	Hypoactive, lacrimation, diarrhea, bloat
LD ₅₀	3600 mg/kg		

*non: no toxicity symptoms



Fig. 7: Rats died within 24hrs after administrating 5000 mg/kg BW. of *E. condylocarpa* tuberous root ethanolic extract.

Effect of *E. condylocarpa* tuberous root extract on body weight

As illustrated in (Table 5); there were significant difference in body weight at ($P < 0.05$) in rats treated with different concentration of *E. condylocarpa* tuberous root ethanolic extract compared with control group, the decrement in rat weight were higher after 14 days of treatment.

Table (5): Body weights of rats (g) after treatment with different concentrations of *E. condylocarpa* tuberous root ethanolic extract:

Dose: mg/Kg body weight	Change in body weight (g)		
	0 time	After 7 days	After 14 days
Control	205.200 a	216.400 a	225.600 a
500	209.200 a	181.66 b	197.66 b
1000	204.600 a	188.666 b	193.333 b
3000	211.600 a	D*	D*
5000	201.600 b	D	D

D referred to that: rats were died during 24 h after treatments, D*= only 2 rats survive and excluded from the statistical analysis. According to Duncan's Multiple Range test, different letters referred to significantly different at $p < 0.05$.

CONCLUSIONS

This investigation disclosed the qualitative phytochemical screening of the tuberous root ethanolic extract consists of terpenoids, steroids, alkaloids, tannins, phenols, flavonoids and coumarins, while LC MS/MS showed the presence of Galic acid, Protocatechuic acid, Quercitrin when administration of tuberous root ethanolic extracts of spurge plant orally as acute toxicity in rats.

The employment of herbal materials is always beneficial for health with low side effects. The ethanolic extracts of root tubers of spurge plants contained secondary metabolites of pharmacological

importance which may be responsible for the acute toxicity effects at high dosed and may be responsible for intestinal irritation and movement. These finding support that *Euphorbia condylocarpa* useful as a laxative in Kurdistan folk medicine.

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الفحص الكيميائي النباتي، والتحرى عن السمية الحادة للمستخلص الايثانولي لجذر درنات نبات *Euphorbia condylocarpa* لدى الجرذان

هلاله رحمان قادر

قسم العلوم البيئية والصحة/ كلية العلوم/ جامعة صلاح الدين/ أربيل/ العراق

عبد الغني عمر اسماعيل السارممي

قسم علوم الحياة/ كلية العلوم/ جامعة صلاح الدين/ أربيل/ العراق

الملخص

تعتبر درنات جذور نبات الفرييون (*Euphorbia condylocarpa* M. Bieb) التابع لفصيلة Euphorbiaceae عادة كملين في الطب الشعبي.

هدفت هذه الدراسة الى استكشاف الفحص الكيميائي النباتي، بالإضافة الى الجرعة المميتة، لمستخلص جذر درنات نبات الفرييون الايثانولي في الجرذان. لتقييم السمية الحادة، اعطيت جرعات متعددة (500، 1000، 3000، 5000 ملغ/كغم) من المستخلص عن طريق الفم. رصدت اعراض السمية واحتمالية نفوق الحيوانات لمدة 24 ساعة لتحديد المتوسطة الجرعة المميتة (LD50) من المستخلص. اظهرت النتائج وجود عشرة مركبات في درنات الجذور الايثانولية عند تحديد محتواها باستخدام LCMS/MS. وقد تم الحصول على كميات اقل من حمض (440µg/g) Galic acid، (71µg/g) Protocatechuic acid، Quercitrin(82µg/g). اظهر اختبار الكيميائي النباتي النوعي وجود في المستخلصات الايثانولية لدرنات الجزر. كان اقصى نشأة مضاد للأكسدة عند تركيز 5ملغم/مل (58.69%)، بينما كان الحد الأدنى للكمية عند 0.625ملغم/مل (17.18%). اظهر اختبار السمية الحادة ان مستخلص جذر درنات نبات بجرعة واحدة مقدارها 5000 ملغم /كغم، قد احدثت اثارا جانبية مرتبطة بالعلاج، من حيث السمية والوفيات لدى الحيوانات المختبرة. بلغت الجرعة المميتة (LD 50) لهذا النبات 3600 ملغم/كغم، اي اقل من 5000 ملغ /غم من زيادة وزن الجسم.

الكلمات الدالة: *Euphorbia condylocarpa*، النشاط المضادة للاكسدة، السمية الحادة، الجرذان، مستخلص ايثانولي