

Phytochemical profile and acute toxicity study of an aqueous extract of *Petroselinum crispum* (Apiaceae) leaves

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World Journal of Advanced Research and Reviews, 2025, 27(03), 1677-1682

Publication history: Received on 18 August 2025; revised on 23 September 2025; accepted on 26 September 2025

Article DOI: <https://doi.org/10.30574/wjarr.2025.27.3.3332>

Abstract

This work aims to promote African pharmacopoeia. To this end, the composition and acute toxicity of the aqueous extract of *Petroselinum crispum* (Apiaceae) leaves, a plant used in various traditional medicine treatments, were determined. This study began with the preparation of the aqueous extract of *Petroselinum crispum* leaves by maceration in distilled water and filtration. The resulting extract was then used for phytochemical analysis using known methods. Qualitative phytochemical tests performed with this extract revealed the presence of sterols, polyterpenes, polyphenols, catechin tannins, flavonoids, alkaloids, quinones, and saponins. Subsequently, an acute toxicity study was conducted on female Wistar mice by force-feeding them with single doses of 2000 or 5000 mg/kg body weight, following the OECD 423 guideline. It revealed that the extract was not toxic orally at the doses studied.

Keywords: Aqueous Extract; *Petroselinum crispum*; Phytochemical; Acute Toxicity; OECD 423

1. Introduction

Since ancient times, humans have used plants to meet their basic needs, including food, clothing, and medical needs. Currently, over 80% of the world's population uses medicinal plants for treatment, due in part to their effectiveness and, in part, to the lack of access to medications prescribed by modern medicine [1]. This is the case with parsley (*Petroselinum crispum* (Mill.) is a biennial aromatic plant of the Apiaceae family [2]. All parts and the essential oil of parsley have been shown to be diuretic, spasmolytic, and have anti-urolithiasis activity. Preparations based on parsley leaves have demonstrated hypoglycemic, antioxidant, anti-inflammatory, antibacterial, and hypoglycemic properties [3]. Furthermore, these modern medications, although effective, are not without adverse effects [4]. This research aims to evaluate the phytochemical properties and assess the acute toxicity of *Petroselinum crispum* leaves, which are necessary to better rationalize their use.

2. Material and methods

2.1. Material

2.1.1. Plant Material

Petroselinum crispum (Apiaceae) leaves purchased at the Gouro market in Adjamé (Abidjan, Côte d'Ivoire) were used as plant material. This plant was identified at the FÉLIX HOUPHOUËT-BOIGNY University at the National Floristic Center (CNF).

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2.1.2. Animal Material

The animal material consisted of Swiss strain *Mus musculus* (Muridae) mice, weighing between 18 g and 25 g, from the animal facility of the Higher Normal School (ENS) at the Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire).

2.2. Methods

2.2.1. Preparation of the total aqueous extract of *Petroselinum crispum* (Apiaceae) leaves

The *Petroselinum crispum* leaves were dried away from sunlight at room temperature (30 +/- 2°C) for 21 days and then ground using an electric grinder to obtain a powder. One hundred (100) grams of the *Petroselinum crispum* powder were ground three times in a blender for 3 minutes in 1.5 L of distilled water. The resulting ground product was filtered through absorbent cotton and then through Whatman No. 1 filter paper. The collected filtrate was oven-dried at 68.5°C for 72 hours. After drying, the aqueous extract of *Petroselinum crispum* leaves (EAPc) was in powder form.

2.2.2. Phytochemical study of the total aqueous extract of *Petroselinum crispum* (Apiaceae) leaves

Secondary metabolites were detected using the analytical techniques described in the work of [5]. For these tests, a solution of the aqueous extract was prepared by dissolving 5 g of the extract in 50 ml of distilled water.

2.2.3. Detection of sterols and polyterpenes

To detect sterols and polyterpenes, 5 ml of EAPc solution was evaporated to dryness, without carbonizing the residue, in a dish over a sand bath. The residue was then dissolved while hot in 1 ml of acetic anhydride and carefully added to 0.5 ml of concentrated sulfuric acid along the wall of the test tube containing the solution. The appearance of a purple or violet ring, turning blue and then green, indicates a positive reaction. Polyphenol Detection

One drop of an alcoholic solution of ferric chloride (FeCl₃ 2%) was added to 2 ml of the solution. Ferric chloride, in the presence of polyphenol derivatives, causes a more or less dark blue-black or green color, indicating the presence of polyphenol derivatives.

2.2.4. Flavonoid detection

In a capsule, 2 ml of the total aqueous extract of *Petroselinum crispum* were evaporated to dryness, and the residue was taken up in 5 ml of hydrochloric alcohol. The solution was poured into a test tube, and 2 to 3 magnesium shavings were added. The resulting orange-pink or purplish color indicates a positive reaction. The color was compared to that of standard quercetol (0.05 mg/ml) treated with the same amount of reagent to demonstrate the presence of flavonoids.

2.2.5. Alkaloid detection

The characterization of alkaloids begins with the evaporation to dryness of 6 ml of the total aqueous extract of *Petroselinum crispum* in a dish. The residue is taken up in 6 ml of 60°C alcohol. The resulting alcoholic solution is divided into two test tubes. Two drops of Dragendorff's reagent are added to the first tube. The appearance of a reddish-brown precipitate indicates a positive reaction. Two drops of Bouchardat's reagent are added to the second tube. The appearance of a reddish-brown precipitate or color indicates a positive reaction.

2.2.6. Saponin detection

This investigation is based on the property of aqueous solutions containing saponins to foam after shaking. 15 ml of the aqueous solution is placed in a test tube 16 mm in diameter and 16 cm high. The tube is capped before vigorously shaking vertically for 10 seconds, then allowed to stand for 10 minutes. If, after this standing time, the foam height is greater than 1 cm, then saponins are present.

2.2.7. Tannin detection

Tannins are divided into two groups:

- Catechol tannins, non-hydrolyzable in nature, formed from catechol polymers in condensed form.
- Gallic tannins, derived from gallic acid and combined in the form of hydrolyzable glycosides.

2.2.8. Catechol Tannin Detection

To the remaining 5 ml of the evaporated EA solution, 15 ml of Stiasny's reagent (30% formalin, concentrated HCl) were added. The mixture was kept in a water bath at 80°C for 30 minutes, then cooled. The observation of a large flake precipitate indicates the presence of catechol tannins.

2.2.9. Detection of gallic tannins

Another 5 ml of the previous solution of the total aqueous extract of *Petroselinum crispum* is filtered and saturated with sodium acetate. The addition of three drops of ferric chloride (2% FeCl₃) causes the appearance of an intense blue-black color, indicating the presence of gallic tannins in the medium. A control test is performed with gallic acid.

2.2.10. Detection of quinone compounds

Free or combined quinone compounds are detected using the Borntraeger reaction. 2 ml of the EAPc solution are evaporated to dryness in a dish, and the residue is taken up with 5 ml of hydrochloric acid diluted 1/5. The resulting solution is poured into a test tube and kept in a boiling water bath for 30 minutes. After cooling completely, 20 ml of chloroform are added. The chloroform phase is then recovered, to which 0.5 ml of half-strength ammonia (Borntraeger's reagent) is added. The appearance of a color ranging from red to purple is proof of the presence of quinone compounds.

Detection of sterols and polyterpenes

To detect sterols and polyterpenes, 5 ml of the aqueous extract solution is evaporated to dryness, without carbonizing the residue, in a dish over a sand bath. The residue is then dissolved while hot in 1 ml of acetic anhydride and carefully added to 0.5 ml of concentrated sulfuric acid along the wall of the test tube containing the solution. The appearance of a purple or purple ring, turning blue and then green, indicates a positive reaction. A control test is performed with a chloroform cholesterol solution.

2.2.11. Acute toxicity study of the extract in mice

The acute toxicity class method is a study that allows us to assess the dose levels that can cause the death of a laboratory animal, to determine the symptoms of acute poisoning, and the circumstances of death. It was conducted in accordance with the acute oral toxicity method described in OECD Guideline 423 [6]. This acute oral toxicity study was conducted on mice weighing between 18 g and 25 g. The limit test method was adopted in this study. Three groups of three mice were created. The first group (control) was treated with distilled water, and the second group received a dose of 2000 mg/kg body weight in a volume of 1 ml per 100 g of body weight of cPAE. Administration was carried out using a suitable gastric tube, and the animals were fasted the day before with free access to water.

The animals were observed individually at least once during the first 30 minutes and regularly during the first 24 hours after treatment. Special attention was paid to them during the first 4 hours after the observation session, when they were given food again. Observations continued daily for 14 days. The parameters observed were: tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. The skin, hair, eyes, and mucous membranes, as well as the respiratory system, were examined.

- In case of death, the lower dose of 300 mg/kg of body weight was used.
- In the absence of death in the treated group, the test was repeated at a dose of 5000 mg/kg of body weight, administered to another group of 3 mice.

3. Results

3.1. Phytochemical study of the total aqueous extract of *Petroselinum crispum* leaves

Phytochemical analysis of the total aqueous extract of *Petroselinum crispum* leaves revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, and saponins. It also revealed the presence of alkaloids and catechol tannins. However, gallic tannins and quinone compounds were not present in the aqueous extract of *Petroselinum crispum* (Table 1).

Table 1 Results of the phytochemical study of the aqueous extract of *Petroselinum crispum* leaves

Researched Compounds		Test or Reagents	Result
Sterols and Polyterpenes		Liebermann	+
Polyphenols		Ferric Chloride	+
Flavonoids		Cyanidine	+
Saponins		Vigorous Shaking	+
Quinonic Compounds		Borntraeger	-
Alkaloids		Dragendorff	+
		Bouchardat	+
Tannins	Catechol	Stiasny	+
	Gallic	Hydrochloric Acid	-

(+): Presence of the compound (-): Absence of the compound

3.2. Acute toxicity study of the aqueous extract of *Petroselinum crispum* leaves

Gavage of the aqueous extract of *Petroselinum crispum* leaves at a dose of 2000 mg/kg body weight did not alter the behavior of the mice. During the 14-day observation period, the 2000 mg/kg body weight dose did not cause any mouse deaths. Thus, the maximum dose of 5000 mg/kg body weight was administered. It resulted in decreased motor skills and grouping in the corners of the cage immediately after administration. During the 14-day observation period, neither the 2000 nor the 5000 mg/kg body weight doses caused any mouse deaths. The 50% lethal dose (LD50) is therefore greater than 5000 mg/kg of body weight (Table 2).

Table 2 Number of mice and percentage of mortality of mice treated with aqueous extract of *Petroselinum crispum* leaves (EAPc)

	Doses of EAPc (mg/kg BW)	Number of mice tested	Number of deaths
1	Distilled water	3	0
2	2000	3	0
3	5000	3	0

4. Discussion

Qualitative phytochemical tests performed with the aqueous extract of *Petroselinum crispum* (Apiaceae) leaves revealed the presence of sterols, polyterpenes, polyphenols, catechol tannins, flavonoids, alkaloids, and saponins. However, the absence of gallic tannins and quinone compounds was noted in this extract. A study by Akhtar *et al.* [7] on the hydroalcoholic extract of *Petroselinum crispum* leaves revealed the same compounds observed in EAPc, with the exception of sterols and polyterpenes. In the literature, the chemical composition of EAPc varies according to the authors Kouar *et al.* [8], during a comparative study of the phytochemical composition of the leaves of *Petroselinum crispum*, *Tymus satureioides*, and *Spirulina platensis* carried out in Morocco, had confirmed the presence of tannins, flavonoids, sterols, and triterpenes. However, our results are consistent with those of Fejes *et al.* [9] and Francis et Isaksen [10], who obtained the same results.

The study of the acute toxicity of the aqueous extract of *Petroselinum crispum* leaves in female mice provided an estimate, according to the OECD Guidelines and Recommendations 423 [6], of the 50% lethal dose (LD 50). The results showed that the administration of EAPc at doses of 2000 and then 5000 mg/Kg of body weight did not cause any animal deaths in 24 hours and during 14 days of observation. According to the OECD Globally Harmonized System of Classification (GHS), EAPc is classified in category 5 or not classified as non-toxic products by oral route [6]. The LD50 is therefore greater than 5000 mg/kg of body weight by oral route. This lack of toxicity has also been observed with other plants of the traditional African pharmacopoeia such as the leaves of *Moringa oleifera* (Moringaceae), *Mitragyna inennis* (rubiacaceae) by this same route on mice. This is confirmed by the studies carried out by Asiedu-Gyekye *et al.* [11]

and those of Ossou *et al.* [12] who obtained the same results. A study by Kablan *et al.* [13] showed similar results with EAPc at 5000 mg/kg body weight, confirming its non-toxic potential even at high single doses.

5. Conclusion

In the context of plant resource development, the Apiaceae family stands out as one of the most studied due to the richness of its bioactive compounds with notable phytotherapeutic properties. Qualitative analysis of an aqueous extract of *Petroselinum crispum* leaves, carried out using phytochemical screening, revealed the presence of various secondary metabolites, the presence of which in the extract is believed to be responsible for many of the therapeutic properties attributed to this plant. Furthermore, toxicity assessment showed that this extract is non-toxic orally, suggesting that this administration method is a preferred approach for potential pharmacological use.

Compliance with ethical standards

Acknowledgments

The authors express their gratitude to the members of the teaching and research Unit for their valuable contribution to this study, as well as to the administration of the Higher Normal School which made the animal facility available for carrying out the various manipulations.

Disclosure of conflict of interest

No conflict of interest in our document.

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