



# Chemical composition, anti-diabetic, antioxidant, and anti-inflammatory potentials of *Cotula cinerea* growing in Algerian Sahara

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## Abstract

Our study aimed to examine the phytochemical composition and biological features of *Cotula cinerea*, an indigenous plant found in the Algerian Sahara. The aqueous maceration process was used to get the extract of *Cotula cinerea*, which showed a high concentration of polyphenols ( $34.01 \pm 1.08$  mg GAE/g) and flavonoids ( $24.10 \pm 0.82$  mg EQ/g). The toxicity studies on rats demonstrated no detrimental effects, confirming the extract's safety. Notably, the use of *C. cinerea* extract showed significant results in lowering blood sugar levels. The diabetic rats that received the extract showed significantly reduced glucose levels ( $D500 = 1.04 \pm 0.13$  g/l) compared to those without treatment ( $2.92 \pm 0.23$  g/l). In addition, all other biochemical parameters in the diabetic rats who received treatment were comparable to or superior to those of the control group. The extract's antioxidant capacity was verified through in vitro testing utilizing DPPH ( $IC_{50} = 0.54 \pm 0.023$  mg/ml) and FRAP ( $IC_{50} = 0.51 \pm 0.012$  mg/ml) tests. Ascorbic acid was used as a reference, with concentrations of  $0.06 \pm 0.005$  mg/ml and  $0.11 \pm 0.014$  mg/ml for the respective assays. Moreover, the plant's anti-inflammatory properties were assessed both in vitro and in vivo. Its effectiveness was ranked in the following order: The order of effectiveness, from highest to lowest, is as follows: Aspirin, Diclofenac, Extract (500 mg/kg b.w.), Extract (250 mg/kg b.w.). The findings emphasize the potential of *C. cinerea* as a promising reservoir of bioactive chemicals with notable hypoglycemic, antioxidant, and anti-inflammatory activities.

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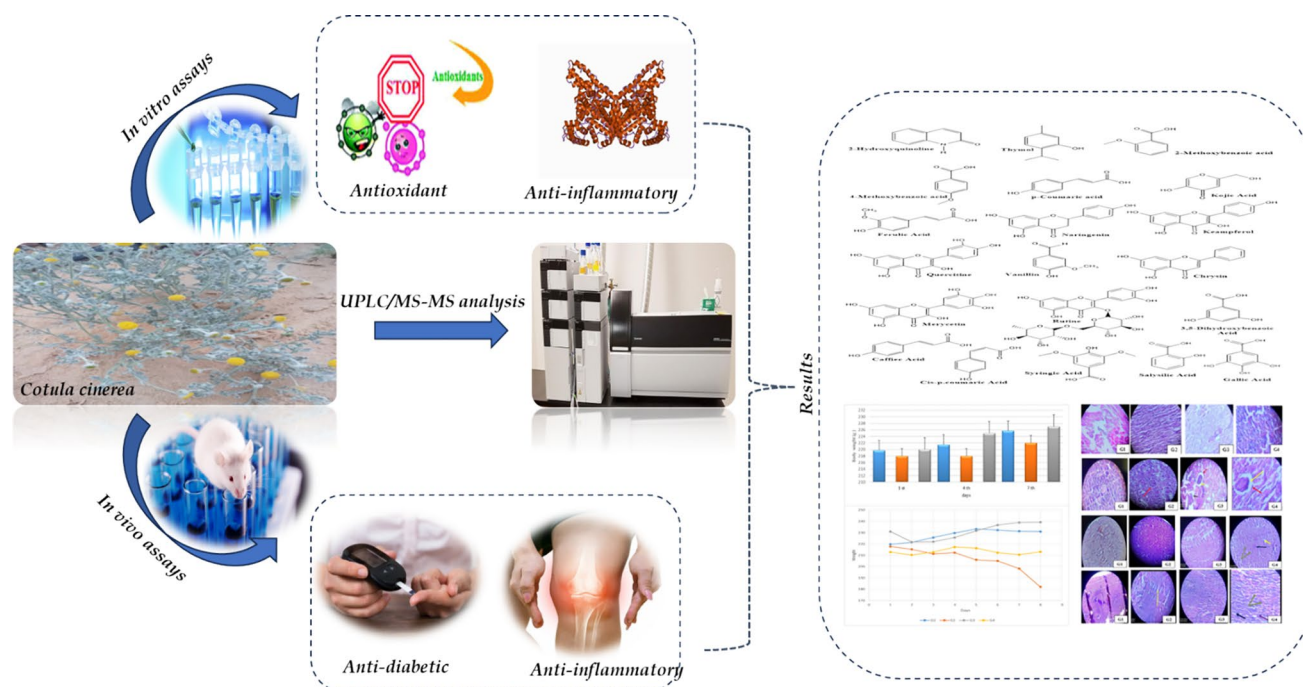
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## Graphical abstract



**Keywords** *Cotula cinerea* · Aqueous extract · LC–MS/MS · Anti-diabetic · Antioxidant · Anti-inflammatory

## Introduction

Synthetic antioxidants that are now in use may harm health or perhaps encourage them (Branen 1975; Barlow 1990); as a result, their use is now subject to stricter regulations, and replacing them with naturally occurring antioxidants is becoming more popular. Together with the well-known and conventionally utilized natural antioxidants found in tea, wine, fruits, vegetables, and spices (Kanner et al. 1994; Madsen and Bertelsen 1995; Wang et al. 1996; Velioglu et al. 1998; Fogliano et al. 1999), numerous other plant species have been studied to find new antioxidants. Certain naturally occurring antioxidants, like sage and rosemary, are currently used in the food and pharmaceutical industries as dietary supplements or antioxidant additives (Koleva et al. 2002).

Nowadays, the phytochemical study of medicinal plants has become a significant focus of most scientific research in medicine to treat incurable diseases. In recent years, the incidence of inflammatory diseases has risen sharply and is becoming a global scourge. Under normal conditions, inflammation is a self-limiting physiological process; however, in some pathological cases, inflammation may persist and become uncontrollable, leading to various chronic inflammatory diseases (Ricklin and Lambris 2013; Bardaa et al. 2020). Oxidative stress is mainly responsible for the

onset of several chronic diseases, such as inflammatory diseases, hypertension, cancer, diabetes, aging and neurodegenerative disorders (Nardi et al. 2016). Presently, there is a great deal of interest in using medicinal plants for their therapeutic effects in both developed and emerging countries (Agisho et al. 2014). The World Health Organization (mondiale de la Santé 2013) indicates that 80% of the world's population uses these plants to treat several diseases. Therapeutic herbs are an essential source of bioactive molecules such as polyphenols. The latter are a group of natural compounds with significant antioxidant and anti-inflammatory properties (Saxena et al. 2013; Ansari et al. 2020).

Diabetes is one of the most prevalent diseases in the world (Jayakumar et al. 2010). According to the International Diabetes Federation (IDF), diabetes is a highly prevalent condition worldwide, impacting more than 463 million persons as of 2019. The projected global prevalence of this condition is expected to reach 4.5 million by 2030, according to the Federation (2017). Moreover, assuming current trends persist, this figure will increase to 700 million by 2045. Recent studies emphasize the substantial rise in type 2 diabetes, mainly influenced by lifestyle factors such as unhealthy eating habits and insufficient exercise. Research highlights the significance of timely diagnosis and intervention to mitigate problems such as cardiovascular disease, renal failure, and visual impairment. The advancements in

diabetes management, such as sophisticated glucose monitoring devices and novel medication categories, are enhancing patient outcomes and elevating their quality of life (Mohajan and Mohajan 2023). In Algeria, it was estimated at 1.3 million diabetics; this number could reach nearly 4.2 million in 2025 (Boudjelthia et al. 2018). On the other hand, the bioactive compounds contained in medicinal plants are responsible for their activity.

Therapeutic herbs are an essential antidiabetic source of bioactive molecules such as polyphenols. The latter are a group of natural compounds with significant antioxidant and anti-inflammatory properties (Saxena et al. 2013; Ansari et al. 2020). The phenolic compounds, flavonoids, terpenoids, alkaloids, and other phytochemicals exert antidiabetic effects, such as lowering blood sugar, lipid peroxidation and insulin resistance, increasing insulin levels, inhibiting hexokinase enzymatic activity, and acting as antioxidant and anti-inflammatory agents (Modak et al. 2007; Mukherjee et al. 2013). Algeria is rich in herbal remedies and has excellent traditional medicine expertise (Bouzabata 2017). *Cotula cinerea* belongs to the Asteraceae family and is among the plants used in Algerian folk medicine.

The objective of the present study is to evaluate the biological activities of this spontaneous medicinal plant, *Cotula cinerea*, in vitro and in vivo, including antioxidant, anti-inflammatory, and antidiabetic activity.

## Materials and methods

### Plant material

*Cotula cinerea* Del. (*Brocchia cinerea* Vis.) is a Saharo-Arabic species common throughout the Sahara in slightly sandy soils (Quezel and Santa 1963). It is a small annual plant with a woolly appearance, 5–15 cm, entirely tomentose (Fig. 1). The whitish-green leaves and stems are covered with tiny, dense hairs forming velvet coats. The flowers are small golden yellow half-pompoms at the end of a short flower (Quezel and Santa 1963, Ould El Hadj et al. 2003). *Cotula* species are traditionally used as anti-inflammatory, antipyretic, antiprotozoal, analgesic, bacteriostatic, or antiseptic agents, as well as in the treatment of digestive disorders (Jana et al. 1992; Larhsini et al. 2002; Refaey et al. 2022; Mekhadmi et al. 2023).

### Preparation of the aqueous extract by maceration

The most popular technique in conventional medicine now in use is water extraction since it is easy to use and comfortable. With minor adjustments, this kind of extraction was carried out using the techniques published by Majhenič et al. (2007) and Bougandoura and Bendimerad (2012). Mix 10 g



Fig. 1 *Cotula cinerea*

of *C. cinerea* powder with 100 ml of distilled water, shake, and let it sit for 21 h. After that, the extract was macerated for 3 h and filtered through filter paper. It was then dried in an oven at no more than 50 °C. The extract was obtained as a thin, solid film that was scraped off with a flat spatula and kept in closed glass vials wrapped with aluminum foil in the refrigerator at 4 °C until needed. Qualitative phytochemical assays were performed to identify the several families of secondary metabolites using the aqueous extract of *C. cinerea*, such as reducing sugars, alkaloids, saponins, and polyphenols (flavonoids, tannins).

### High-performance liquid chromatography analysis (HPLC)

The HPLC–Uv analysis of the *C. cinerea* extract was employed by a Shimadzu LC 20 AL High-Performance Liquid Chromatography (HPLC) system with a Hamilton 25 µL universal injector. The analytical column utilized was a Shim-pack VP-ODS C18 (4.6 mm × 250 mm, 5 µm), type Shimadzu. The UV–VIS SPD 20A (Shimadzu) detector was employed. Acetonitrile and 0.1% acetic acid were combined to create the mobile phase. Before use, the mobile phase's contents were passed through a 0.45 µm membrane filter and then pumped at a flow rate of 1 mL/min from the solvent reservoir to the column. Chouikh et al. (2018) introduced a solution of plant extracts containing 20 µL into the mobile phase flow. The high pressure that powers the mobile phase was modified using a pump. After the mobile phase in the effluent was detected at  $\lambda = 268$  nm, the separated compounds will be determined using the column for 50 min. The results will be recorded as chromatographic curves on the computer. As discussed below, the calibration of standards was used in this work to compare the quantification of a few peaks.

## Ultra-performance liquid chromatography-mass spectrometry (UPLC/MS–MS) analysis

In the UPLC/MS–MS analysis of *C. cinerea* extract, direct injection was utilized with 5  $\mu$ L without a column (Restek Ultra C18 3  $\mu$ m 150  $\times$  4.6 mm) on a SHIMADZU 8040 Ultra-High sensitivity with UFMS technology, which was outfitted with a binary bump Nexera XR LC-20AD, to optimize the standards for polyphenols. With a total flow rate of 0.4 ml/min, gradient elution was employed with a mobile phase comprised of solvent A (water), solvent B (methanol), and 0.1% formic acid. The ESI parameters were as follows:

- DL temperature, 250 C;
- Nebulizing gas flow, 3.00 L/min;
- Heat block, 400 C;
- Drying gas flow, 15.00 L/min;
- CID gas, 230 KPs;
- Conversion dynode, 6.00 kV.

Table 1 presents an overview of MS/MS detection parameters.

### Animal material

Five female Wistar Albino strain white rats weighing between 180 and 250 g were used in the current investigation. The rats were aged between 9 and 11 weeks. These animals were reared in an animal facility at the University of El-Oued's Faculty of Natural and Life Sciences after being brought from the Pasteur Institute in Algiers. The rats were grown and housed in plastic cages containing sawdust, which was changed three times a week until the experiment was over, during the adaption phase, which lasted for two weeks before the experiment under controlled settings (12 h of lighting and 24 °C temperature). The guidelines outlined in the manuals for the care and

management of experimental animals (CCAC, 1984) are followed while handling and treating any rat. Using an electronic scale, measurements were obtained twice a week to track the female rats' weight changes.

### Study of the toxicity of *C. cinerea* extract on rats

After a 16-h fast, save for water, 15 male Wistar rats were randomly assigned to three groups of five rats each, with the following treatments: G1: untreated; G2: healthy; G3: 500 mg/kg of body weight (b.w.); G4: 1000 mg/kg b.w. The aqueous extract of *C. cinerea* was administered orally to each group once daily using a gavage syringe, which was maintained in identical circumstances. Throughout the seven-day trial, the rats' weight and any toxicity indicators were noted using a modified version of the Pissang et al. (2022) methodology (Pissang et al. 2022).

### Evaluation of the antidiabetic activity of *C. cinerea* in vivo

Following a 12-h fast, 16 adult Wistar Albino rats were given an intraperitoneal injection of alloxan (Sigma, UK). The injection was made fresh and administered at 150 mg/kg b.w. 5 ml of physiological water solution was used (Sabu et al. 2002). The water bottles were switched out with ones containing 5% glucose solution for 24 h following the injection to prevent any potential normal or deadly hypoglycemia. The enormous release of insulin and the death of pancreatic  $\beta$  cells generated by alloxan (Chahlia 2009). After 48 h, fasting blood sugar levels were used to demonstrate that all the rats under study had induced diabetes (Owoyele et al. 2005). Treatment with *C. cinerea* extract was initiated 48 h after diabetes induction El Kabbaoui (2019) reports that the *C. cinerea* extract treatment was started 48 h after diabetes induction and continued orally (gastric gavage) for 21 days (El Kabbaoui 2019). There were four groups of five rats each from all the rats. Groups 1 and 2 were the usual group on a standard diet; group 3 was the diabetic group on a standard diet and daily treatment with 500 mg/Kg b.w. of *C. cinerea* extract; and group 4 was the diabetic group on a standard diet plus daily treatment with 250 mg/Kg b.w. of *C. cinerea* extract. Blood sugar and weight were the two indicators that were regularly checked during the treatment. Biochemical markers such as glucose, triglycerides, total cholesterol, serum urea, creatinine, and aspartate aminotransferases (ASAT or SGOT) and alanine aminotransferases (ALT or SGPT) were also taken into account. Additionally, histological sections of the kidneys, spleen, liver, and heart were created using the method outlined (Houlot 1984).

**Table 1** Phytochemical test of aqueous extract of *C. cinerea*

Secondary metabolites	Reactions	Observation
Polyphenol	+++	Greenish blue
Flavonoids	++	Yellow
Tannins	–	No foam
Alkaloids	++	White precipitation (Mayer's reagent)
	++	Reddish-brown precipitation (Drajesdorf's reagent)
Saponins	–	No foam
Reducing sugars	+	Brick red precipitation
Terpenes	+	Appearance of a red–purple ring

(+): Weakly positive test, (++) : Positive test, (+++): Strongly positive test, (–): Negative test

## Evaluation of the in vitro antioxidant activity of *C. cinerea*

The capacity of an extract or a plant compound to eliminate free radicals was estimated in several ways; in our study, we evaluate the two following methods:

### Free radical scavenging test (DPPH)

This method, a crucial tool in our research, involves completely dissolving 4 mg of DPPH in methanol. Take 12 mg of the extract for the plant and dissolve it in 2 ml of methanol. Take 200  $\mu$ l of the extract dissolved in methanol and add 800  $\mu$ l of the DPPH solution (3 repetitions), homogenize the solution, incubate the tubes in the dark for 30 min, then measure the absorbance of the prepared solutions at 517 nm by a spectrophotometer, Ascorbic acid was used as a standard (Dziri et al. 2012; Azouaou et al. 2020).

### Ferric reducing-antioxidant power (FRAP) iron reduction test

The activity of the reducing power was meticulously determined according to the method of Oyaizu (1986) and Benzie and Strain (1996). Using this procedure, 0.25 ml of the plant's aqueous extract at various concentrations (between 0.0625 and 1 mg/ml) was added to 0.625 ml of a buffer solution (pH = 6.6, potassium phosphate: 0.2 M), which was followed by 0.625 ml of a 1% potassium ferricyanide [ $K_3Fe(CN)_6$ ] solution in distilled water. For 20 min, each test tube was kept in a water bath at 50 °C. 2.5 ml of 10% trichloroacetic acid (TCA) was added to stop the reaction. For ten minutes, each tube was centrifuged at 3000 rpm. After transferring 0.625 ml of the supernatant to a new tube, 0.125 ml of the freshly made ferric chloride  $FeCl_3$  0.1% in distilled water and 0.625 ml of distilled water were added. The reaction medium's absorbance was measured at 700 nm compared to an identically prepared blank. Ascorbic acid served as the representative of the positive control.

### Evaluation of anti-inflammatory activity in vitro

With slight modifications, the egg white was employed to assess the anti-inflammatory activity following the protein denaturation method outlined by Chandra et al. (2012). 200  $\mu$ l of fresh egg albumin was combined with 2.8 ml of aqueous extract at several doses (0.0625 mg/ml) and 2.8 ml of phosphate buffer saline (PBS, pH 6.4). Instead of the aqueous extract, 2 ml of distilled water was used as the control. The reference medication utilized was acetylsalicylic acid. Following 15 min at room temperature, the combinations were heated for 5 min at 70 °C in a water bath. At 660 nm, the absorbance was measured following cooling.

The percentage inhibition (PI%) of protein denaturation was calculated according to the following formula:

$$\text{Inhibition percentage (PI\%)} = \left[ \frac{(\text{Control Abs} - \text{Sample Abs})}{\text{Control Abs}} \right] \times 100$$

PI%: Inhibition percentage.

Control Abs: Control absorbance.

Sample Abs: Sample absorbance.

### Evaluation of anti-inflammatory activity in vivo

This activity was evaluated according to the method of Amezouar et al. (2013) and Harchaoui (2019) with slight modifications. The procedure entails giving the rat a 16-h fast before giving it the product intragastrical. Five groups of five rats were created. According to Belguidoum et al. (2023), the first group was treated with 500 mg/Kg b.w., the second was treated with 250 mg/Kg b.w., the third and fourth were treated with aspirin and diclofenac (positive control), and the final group was not treated (negative control) (Belguidoum et al. 2023). The percentage of inhibition of edema was calculated according to the following formula:

$$\% \text{ I edema} = \left[ \frac{(\text{Dn}-\text{D0}) \text{ control} - (\text{Dn}-\text{D0}) \text{ treated}}{(\text{Dn}-\text{D0}) \text{ control}} \right] \times 100.$$

Dn: the diameter of the leg at time t after the injection of carrageenan.

D0: the initial diameter of the paw before causing edema.

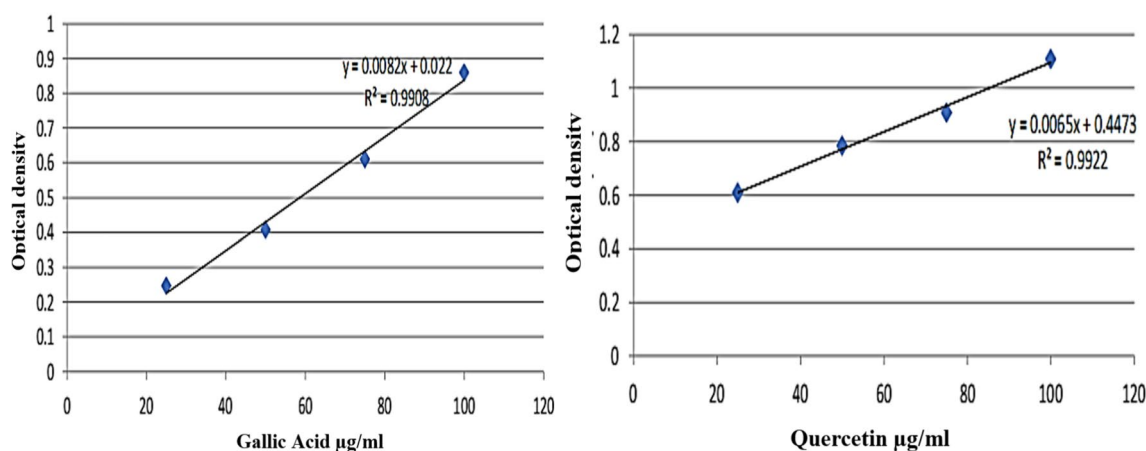
### Statistical analyzes

Results are reported as the mean of three replicates ( $n=3$ ), with calculations performed using EXCEL 2016 and R 4.2.3., including mean and standard deviation ( $\pm$  SD) for each studied parameter. Statistical analysis was performed using the statistical software R 4.2.3. The obtained data were carried out using principal component analysis (PCA), ANOVA, and the correlation between the groups treated with the *Cotula cinerea* aqueous extract and the control depending on the biochemical parameters.

## Results

### Phytochemical test

The present study allowed us to obtain a dry extract weighing 9.11% of the yield. Phytochemical analyzes of *C. cinerea* showed that it is very rich in polyphenols and flavonoids;



**Fig. 2** Calibration curve for gallic acid (total phenols) and quercetin (flavonoids)

**Table 2** Concentration ( $\mu\text{g}/\text{mg}$  extract) of major phenolic compounds identified by HPLC in the extract of *C. cinerea*

Compounds ( $\mu\text{g}/\text{ml}$ )	RT	Extract
Gallic Acid	5.29	3.269
Chlorogenic Acid	13.392	13.011
Vanilic Acid	15.531	0.866
Caffeic Acid	16.277	0.306
Rutin	28.37	13.120
Naringin	34.788	0.357
Quercetin	45.047	1.481

RT retention time

however, it is poor in alkaloids, terpenes, and tannins. While saponins and reducing sugars were absent (Table 1).

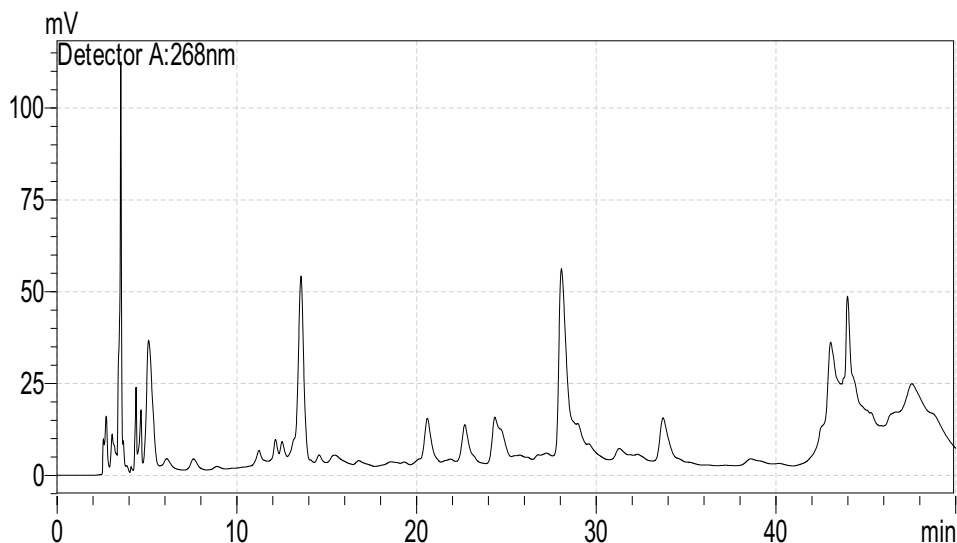
### Qualitative results

Based on the qualitative results showing the significant presence of polyphenols and flavonoids, we selected these last two metabolites for quantitative tests. According to the results, our extract contains polyphenols at a rate of  $34.01 \pm 1.08$  mg GAE/g and flavonoids estimated at  $24.10 \pm 0.82$  mg EQ/g (Fig. 2).

### High-performance liquid chromatography (HPLC)

Phenolic compounds detected in the *C. cinerea* extract by HPLC–Uv analysis are listed in Table 2.

**Fig. 3** HPLC profile of aqueous extract of *C. cinerea*



The HPLC–Uv analyzes have identified seven phenolic compounds in the aqueous extract of *C. cinerea*: gallic acid, chlorogenic acid, vanillic acid, caffeic acid, rutin, naringin and quercetin (Table 2, Fig. 3).

### Liquid chromatography mass spectrometry (LC–MS)

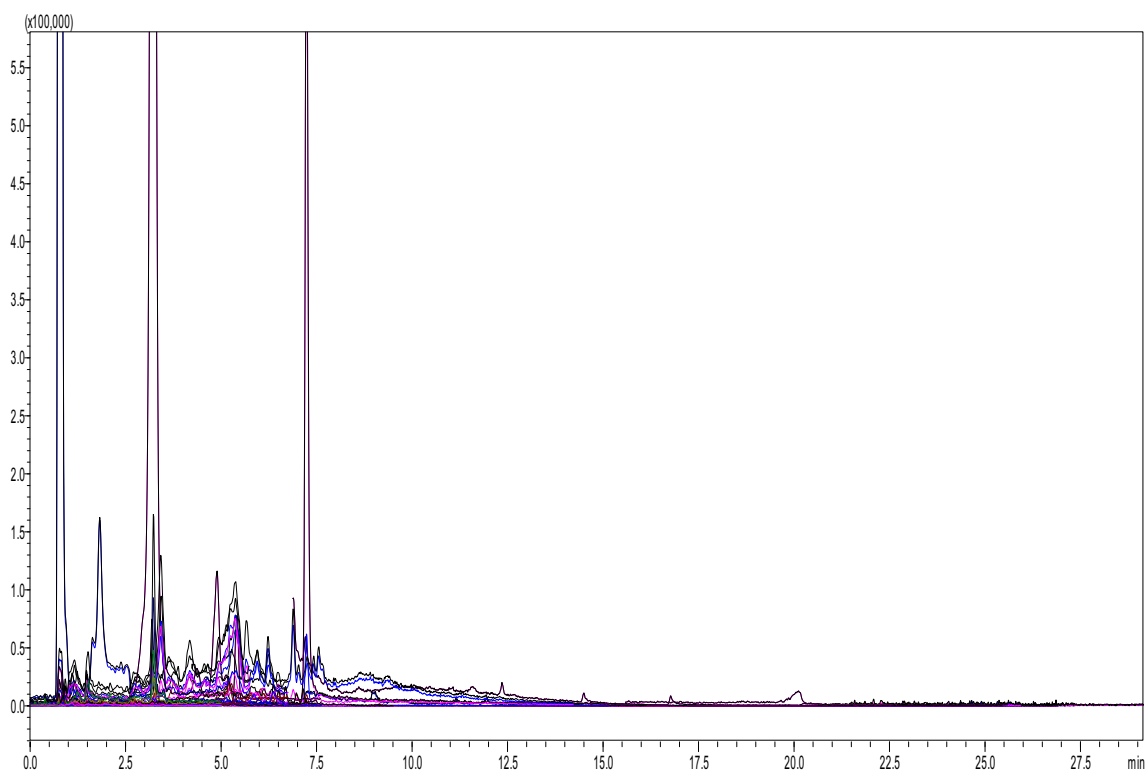
The LC–MS/MS analysis of the aqueous extract of *C. cinerea* led to the tentative identification and characterization of 24 phytochemical compounds by comparing their retention times with those of standards (Table 3, Figs. 4, and 5). The outcome concerning the LC–MS/MS analysis of our extracts confirms the richness of this extract by a different type of phenolic compounds, including phenolic acids and their derivatives (2-methoxybenzoic acid, 4-methoxybenzoic acid, p-coumaric acid, kojic acid, ferulic acid, vanillin, folic acid, catechin hydrate, sinapic acid, 4-hydroxy coumarin, 3,5-dihydroxybenzoic

acid, caffeic acid, cis-p coumaric acid, syringic acid, salicylic acid, gallic acid chlorogenic acid). These compounds can interfere with inflammatory, antioxidant and antidiabetic functions and are highly recommended to prevent inflammatory diseases. Among the compounds detected in our extract by LC–MS/MS analysis, eight flavonoids (naringenin, kaempferol, quercetin, chrysin, myricetin, rutin utensil and hesperetin) were characterized. The immune system interaction and antioxidant properties of flavonoid compounds are thought to contribute to their therapeutic potential (Hosseinzade et al. 2019). An expanding corpus of studies has examined the efficacy of different flavonoids in treating various types of epilepsy. Furthermore, the structure of flavonoids is essential and gives rise to their anti-inflammatory properties. Since the hydroxyl groups have a planar ring structure with unsaturation at C2–C3, their locations are crucial for imparting this characteristic. For flavonoids

**Table 3** Detected molecules from the aqueous extract of *C. cinerea* according to the LC/MS–MS results

ID#	Compound	Molecular Formula	Molecular Weight	ESI Charge (±)	m/z	Ret. Time
1	2-Hydroxyquinoline	C <sub>9</sub> H <sub>7</sub> NO	145.16	(+)	146.0500 > 101.0000	5.101
2	Thymol	C <sub>10</sub> H <sub>14</sub> O	150.22	(+)	151.7500 > 88.1000	0.768
3	2-Methoxybenzoic Acid	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	152.15	(+)	153.0500 > 135.0500	5.37
4	4-Methoxybenzoic Acid	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	152.15	(+)	153.0500 > 70.7500	3.423
5	p-Coumaric acid	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	164.16	(+)	165.1000 > 101.2000	0.779
6	Kojic Acid	C <sub>6</sub> H <sub>6</sub> O <sub>4</sub>	142.11	(+)	143.0000 > 69.0500	3.231
7	Ferulic Acid	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	194.18	(+)	194.9000 > 177.1500	7.206
8	Naringenin	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	272.25	(+)	272.9500 > 177.0000	5.321
9	Beta carotene	C <sub>40</sub> H <sub>56</sub>	536.87	(+)	537.2000 > 23.1000	23.551
10	Keampferol	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	286.24	(+)	287.1000 > 255.2500	1.121
11	Quercitine	C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>	302.23	(+)	303.1000 > 262.2000	9.032
12	Vanillin	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	152.15	(+)	153.1000 > 65.1500	5.672
13	Chrysin	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	254.24	(+)	255.0500 > 153.0500	22.275
14	Merycetin	C <sub>15</sub> H <sub>10</sub> O <sub>8</sub>	318.23	(+)	336.2500 > 46.1500	22.37
15	Folic Acid	C <sub>19</sub> H <sub>19</sub> N <sub>7</sub> O <sub>6</sub>	441.14	(+)	442.9000 > 323.4500	ND
16	Rutine	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	610.5	(+)	611.2000 > 73.2000	6.689
17	Catechin Hydrate	C <sub>15</sub> H <sub>16</sub> O <sub>7</sub>	308.28	(–)	306.9500 > 169.1000	ND
18	Sinapic Acid	C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	224.21	(–)	223.0000 > 208.1500	ND
19	4-hydroxy coumarin	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	162.14	(–)	160.8000 > 117.1000	ND
20	3,5-Dihydroxybenzoic Acid	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	154.12	(–)	153.1000 > 109.1000	3.232
21	Caffeic Acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	180.16	(–)	179.1500 > 135.0500	5.415
22	Cis-p.coumaric Acid	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	164.16	(–)	163.1500 > 119.1500	20.118
23	Syringic Acid	C <sub>9</sub> H <sub>10</sub> O <sub>5</sub>	198.17	(–)	196.9500 > 182.0000	6.616
24	Salysilic Acid	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	138.12	(–)	137.1000 > 93.1500	7.238
25	Gallic Acid	C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>	170.12	(–)	169.1000 > 125.0500	21.07
26	Luteonil	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	286.24	(–)	284.9500 > 133.0000	21.886
27	Hesperitin	C <sub>16</sub> H <sub>14</sub> O <sub>6</sub>	302.28	(–)	300.9000 > 255.2500	16.77
28	Chlorogenic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	354.31	(–)	352.9000 > 177.1500	0.87

ND not detected



**Fig. 4** The LC–MS/MS chromatogram profile of the extract of *C. cinerea*

to continue having an anti-inflammatory action, the hydroxyl groups located at the B ring's 3' and 4' positions are essential (Al-Khayri et al. 2022). Therefore, it is conceivable to discuss flavonoids' anti-inflammatory effect on epilepsy treatment. A review published in 2023 by Shyam et al., have examined the anti-inflammatory activity of naringenin, kaempferol, quercetin, chrysin, myricetin, rutin luteonil and hesperitin by employing different methods (Rabidas et al. 2023). Other publications have also documented the antidiabetic activity of flavonoids. It has been reported the antidiabetic effects of hesperidin and quercetin have been applied in various ways (Lu and Yip 2023).

### Toxicity of *C. cinerea*

Oral administration of the aqueous extract of *C. cinerea* at doses of 500 and 1000 mg/kg b.w. to rats did not induce any sign of acute toxicity during the 14 days of observation, either on the skin, hair, eyes, behavior, somatomotor activity, sleep, or mortality. A typical course of body growth was observed (Fig. 6).

Oral administration gavage of the *C. cinerea* extract presented a natural increase in the growth rate for the

two treated batches compared to the control. Thus, no sign of toxicity was observed. In addition, we noticed binge eating in treated mice. This means the non-toxicity of these doses allowed us to study their antidiabetic and anti-inflammatory activity in vivo.

### Effect of diabetes induction in rats

In this study, rats made diabetic by intraperitoneal injection with 150 mg/kg of Alloxan (Sabu et al. 2002) showed hyperglycemia after 48 h after the injection compared to control rats (Fig. 7). This was consistent with the results of other previous studies. This effect was explained by the cytotoxicity of Alloxan to pancreatic Langerhans cells, which can cause severe necrosis of the latter and thus causes a significant drop in insulinemia, which induces type 1 diabetes (Lenzen and Munday 1991; Szkudelski 2001).

### Analysis of biochemical parameters

The results illustrated in Table 4 below showed the antidiabetic potential of the aqueous extract of *C. cinerea* on rats treated with it (250–500 mg/kg bw).

The application of *C. cinerea* extract on hypoglycemic individuals showed the existence of a very significant



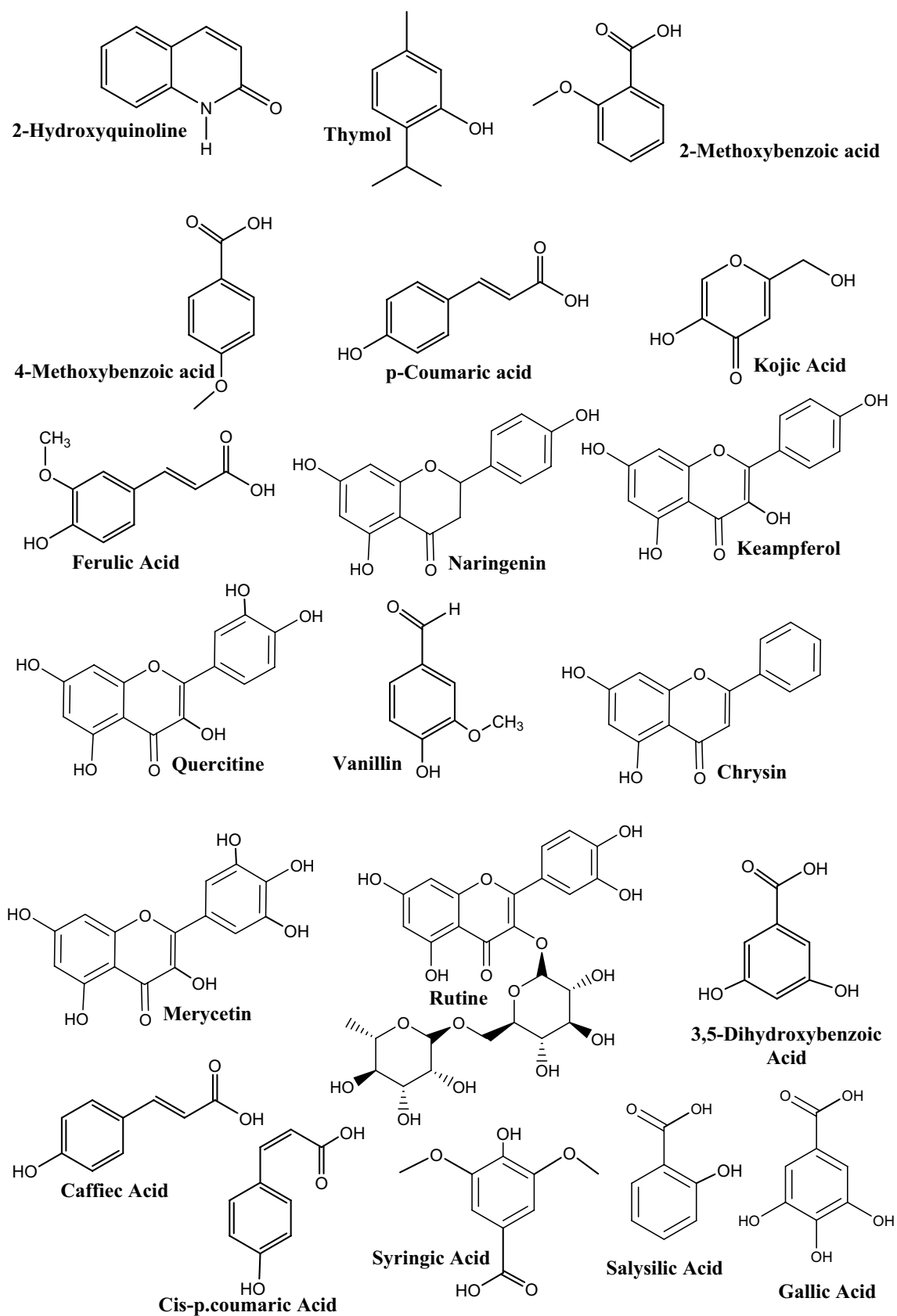


Fig. 5 Chemical structures of molecules detected in the extract of *C. cinerea*

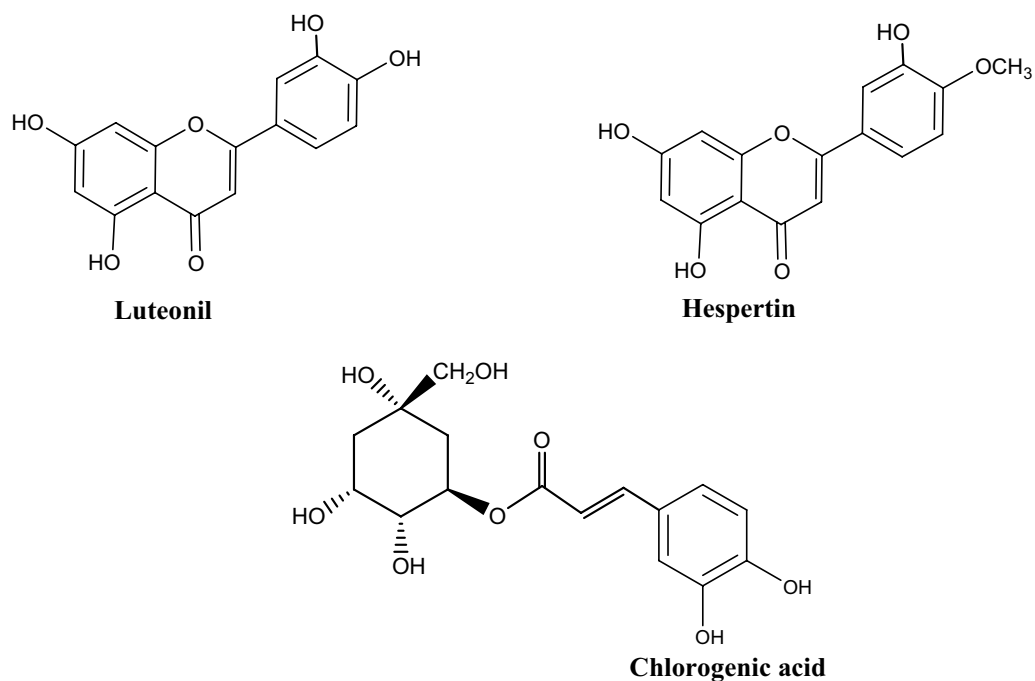
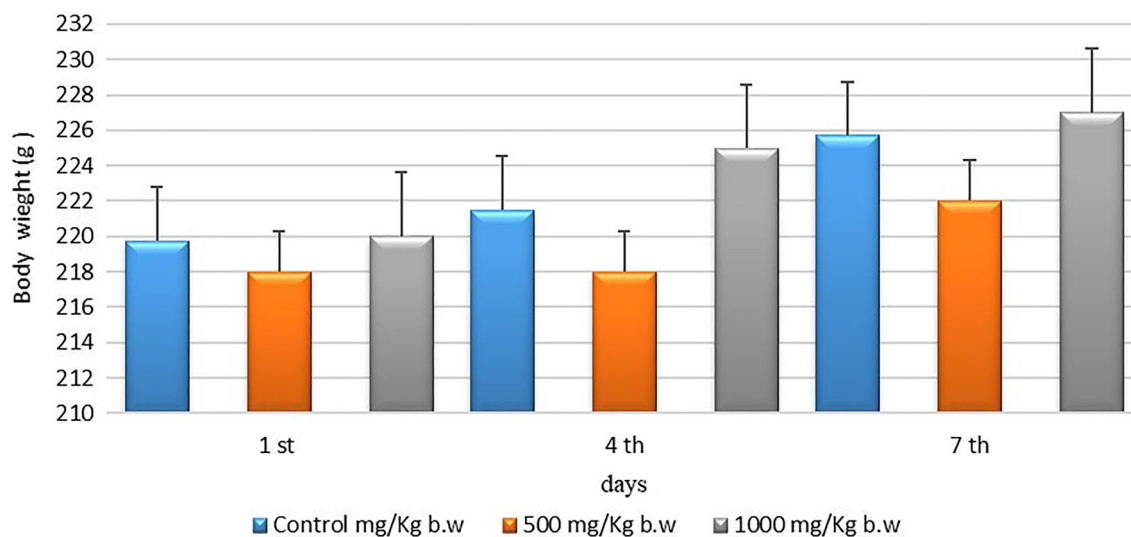


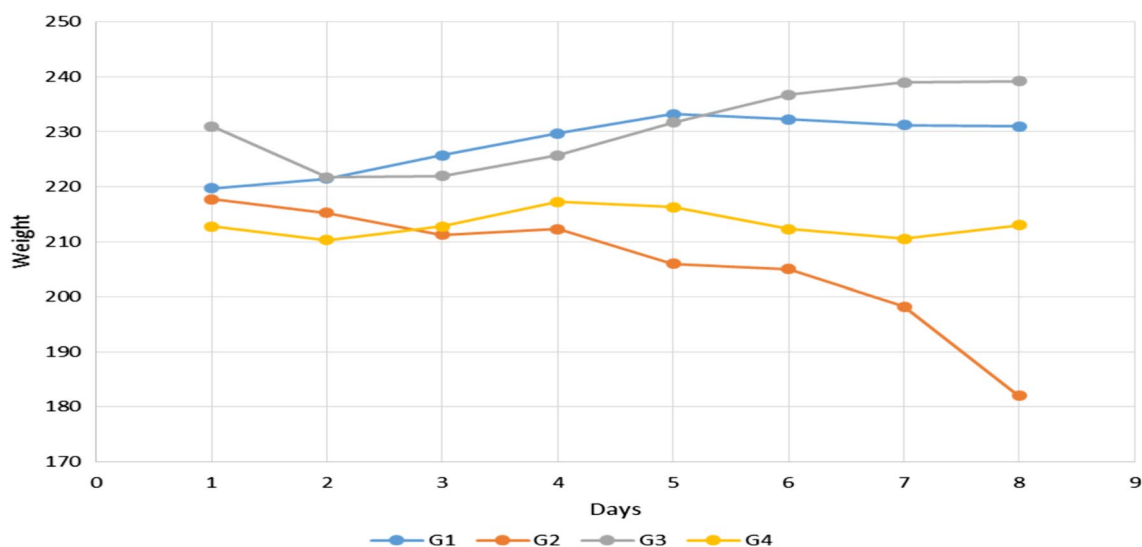
Fig. 5 (continued)

Fig. 6 Evolution of weight (g) of rats treated with *C. cinerea* extract

effectiveness compared to controls whose diabetic rats not treated with this extract recorded a very high average glucose ( $2.92 \pm 0.23$  g/l) compared to treated individuals (D500 =  $1.04 \pm 0.13$  g/l) (Table 4). Idem, all the other biochemical parameters tested were very high in untreated diabetic individuals whose patients treated with the *C. cinerea* extract recorded values similar to or close to the control individuals (Table 4).

### Analysis of the correlation of doses and biochemical parameters

The analysis of the matrix revealed high correlation coefficients, 75.31% of which their variables were significantly correlated (Fig. 8). The eigenvalues representing the dosage of biochemical parameters of the groups studied on the axes are average, 86.7% for the first axis and 6.1% for axis 2,



**Fig. 7** Evolution of body weight (g) of treated and control rats during the experiment

**Table 4** Biochemical parameters of individuals

Lots	Glucose (g/l)	Lipids		TGO (UI/l)	TGP (UI/l)	Creatinine (mg/l)	Urea (g/l)
		Cholesterol (g/l)	TG (g/l)				
Control	0.79*** ±0.21	0.40 ±0.03	0.45 ±0.05	102.38 ±6.3	47.22 ±5.01	6.022 ±0.46	0.38 ±0.03
Diabetic	2.92 ±0.2	1.035 ±0.01	0.89 ±0.10	171 ±4.40	96.97 ±1.21	1.05 ±0.01	0.86 ±0.13
D <sub>500</sub>	1.04*** ±0.12	0.55 ±0.06	0.47 ±0.05	110.27 ±20.09	61.45 ±10.56	6.53 ±0.56	0.45 ±0.02
D <sub>250</sub>	2.38 ±0.25	0.84 ±0.01	0.515 ±0.01	137.5 ±0	74.35 ±2.5	7.91 ±0.34	0.78 ±0.11

TG triglycerides, GOT glutamic-oxaloacetic transaminase, GPT glutamic pyruvic transaminase, D<sub>500</sub> Dose 500 mg/ml, D<sub>250</sub> dose 250 mg/ml

\*\*\* Highly significant

thus contributing to the total variance. The total information explained by the first three axes from the PCA was 92.8%.

According to the statistical data illustrated in the figures above, the positive part of axis one was explained by triglyceride, GTP and GOT, while the total cholesterol was null. On the other hand, the negative part was presented by urea, glucose, and creatinine. The projection of the groups indicated the presence of a negative correlation between G1 and G3 with a disturbance of biochemical parameters. At the same time, G2 and G4 are positively correlated with these parameters (Fig. 8).

The analysis of ANOVA and PCA and the results obtained showed a significant relationship between groups 1 and 3 in stabilizing biochemical parameters. In contrast, the other groups (2 and 4) have an essential relationship in the disruption of these parameters (Fig. 9). The statistical data allowed us to ascertain the positive effect of the dose applied

by the extract of this plant in the third group, to decrease the vital values in the blood of the tested rats.

### Histological sections

A histological section of the heart made from untreated individuals (G1) showed gaps between fibers and enlarged blood vessels, unlike the treated individuals (G3, G4), Which Showed a decrease in vascular hypertrophy and partial restoration of fibers (Fig. 10).

Regarding the kidneys, the untreated batches showed some histopathological changes represented by an apparent dilation of Bowman's capsule, congestion of the blood vessels, congestion within the glomeruli and dilation of the tubules (Fig. 11).

At the level of the liver of G1, we noticed a deformation in the morphological appearance of the liver tissue in the

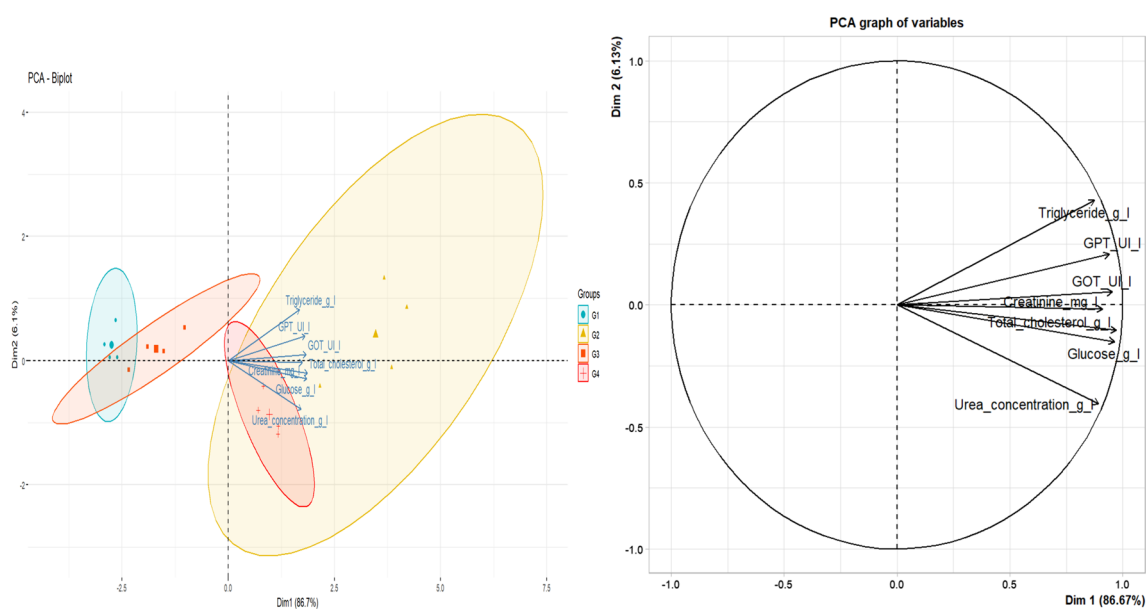


Fig. 8 Circle of correlations, projection of variables on the plane (1×2)

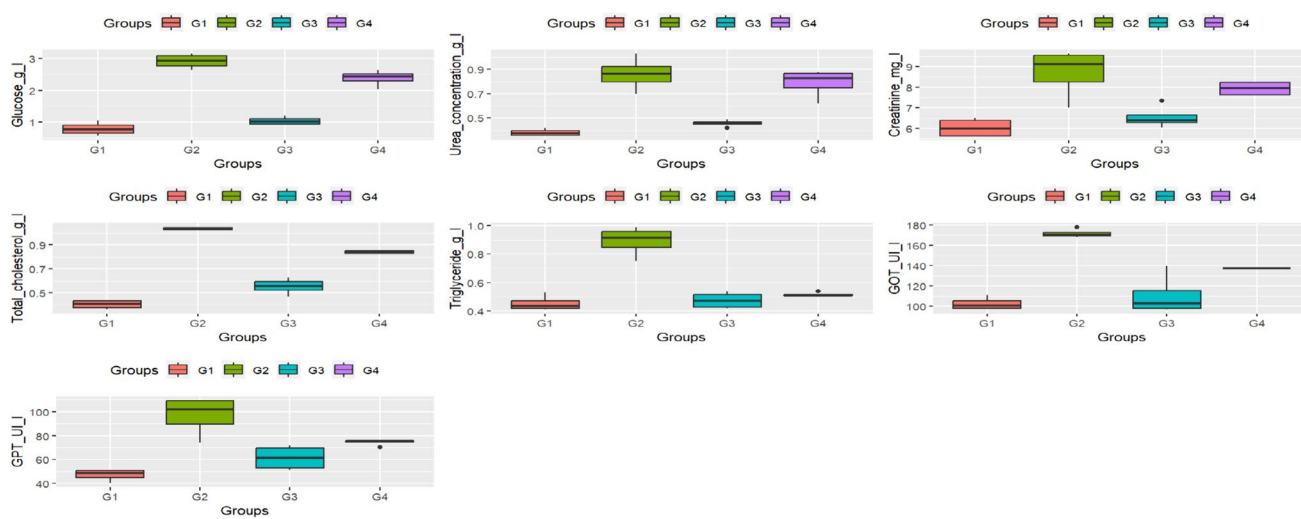


Fig. 9 Combination analysis between PCA and ANOVA of the biochemical parameters of the studied groups

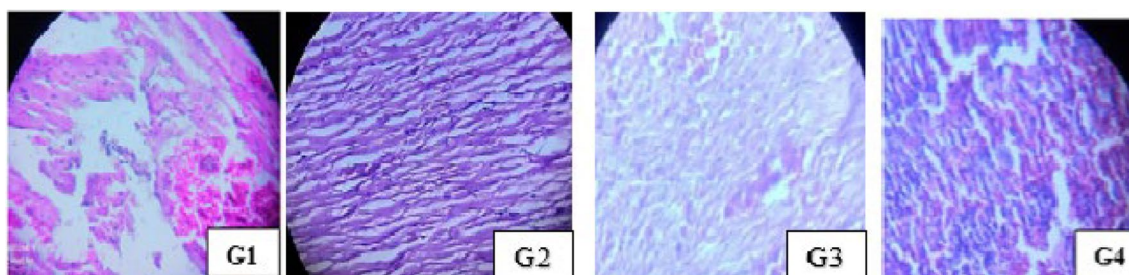


Fig. 10 Histological section of the heart of treated and untreated individuals

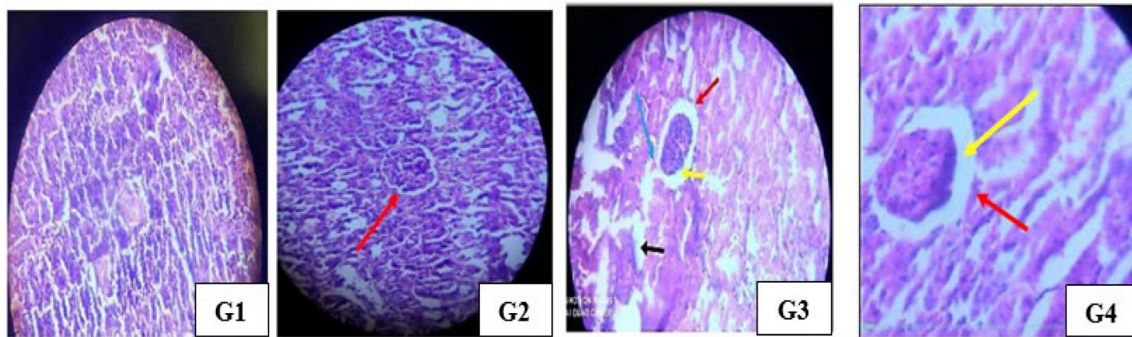


Fig. 11 Histological section of the kidney of treated and untreated individuals

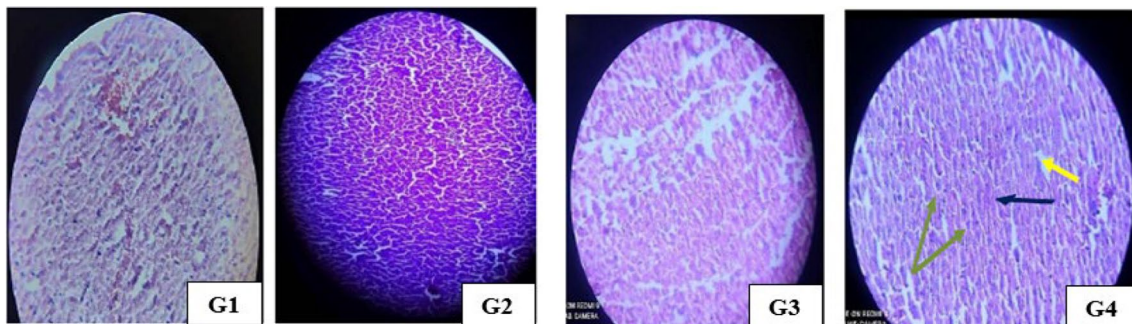


Fig. 12 Histological section of the liver of treated and untreated individuals

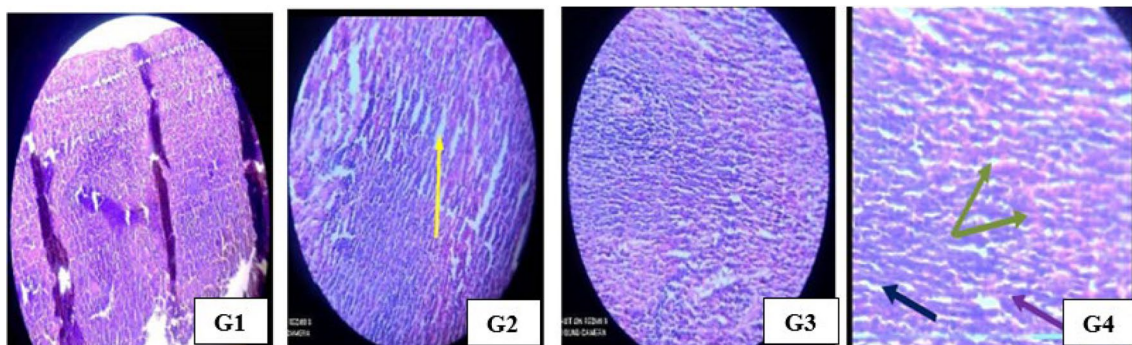


Fig. 13 Histological section of the spleen of treated and untreated individuals

form of blood congestion and fatty accumulation, in contrast to the treated G3 with mild blood congestion. As for the G4, there is no difference from the control (Fig. 12).

Concerning the spleen, we noted the presence of gaps in the cytoplasm of cells and between tissue cells and distortions in the shape of the nuclei, in contrast to the spleen of treated mice, which did not show any change in shape (Fig. 13).

### Evaluation of the antioxidant activity of *C. cinerea*

The present experiment, using the free radical trapping test (DPPH), allowed us to record the antioxidant potential of our extract by presenting an inhibitory concentration of  $0.54 \pm 0.023$  mg/ml. Ascorbic acid showed very powerful anti-radical activity with an IC<sub>50</sub> equal to  $0.06 \pm 0.005$  mg/ml. The lower the IC<sub>50</sub> value, the more powerful the extract is considered to be. On the other hand, the iron reduction test (FRAP) allowed us to record a lower reducing activity ( $0.51 \pm 0.012$  mg/ml) than that of standard ascorbic acid

( $0.11 \pm 0.014$  mg/ml). It was clear that our extract has considerable modest reductive power. The variation in the phenolic compounds present in our extract could explain this discrepancy.

### Anti-inflammatory activity in vitro and in vivo

Concerning the anti-inflammatory activity, in vitro, by the thermal denaturation method of egg white proteins, the results indicate a percentage of inhibition equal to 75.20% with concentrations of 1 mg/ml of the extract. In comparison, acetylsalicylic acid presented a rate of 89.64% at the same concentration. In addition, the aqueous extract's IC<sub>50</sub> value, obtained by linear regression, was 0.42 mg/ml, while the IC<sub>50</sub> of acetylsalicylic acid was 0.203 mg/ml.

The evaluation of the inhibition percentage of edema in rats showed that the aqueous extract of this plant has a significant anti-inflammatory activity (Fig. 14). In contrast, the recorded values were  $79.88 \pm 0.45\%$  and  $72.96 \pm 0.12\%$  for the two doses of the extract 500 mg/kg and 250 mg/kg, respectively, compared to the effect of two reference anti-inflammatories (diclofenac and aspirin) which, respectively, give inhibitions of  $89.07 \pm 0.18\%$  and  $92.03 \pm 0.73\%$ . The results showed that the extract (500 mg/kg) inhibits the edema caused in rats highly compared to the extract at a 250 mg/kg doses (Fig. 14).

## Discussion

Phytochemical analysis of the aqueous extract obtained from the aerial parts of *C. cinerea* revealed the presence of polyphenols and flavonoids, which constitute one of the most predominant secondary metabolites in many plants (Sato et al. 2011; Yao et al. 2019). The latter corresponds to that obtained by Belyagoubi-Benhammou et al. (2014). On the other hand, it is poor in alkaloids, terpenes and reducing sugars corresponding to that obtained by Amssayef et al. (2020) (Amssayef and Eddouks 2020) and Guaouguaou et al. (2023) (Guaouguaou and Es-Safi 2023). While the absence of tannins and saponins, despite their presence in each of Belyagoubi-Benhammou et al. (2014) and Amssayef et al. (2020) (Amssayef and Eddouks 2020) in significant quantities.

Regarding the quantification of polyphenols, our results were higher than those obtained by Agour et al. (2023) who found that the aqueous extract of *C. Cinerea* presented  $13.09 \pm 0.13$  mg EAG/g. While Belyagoubi-Benhammou et al (2014) found that the amount of polyphenols in this plant was  $22.22 \pm 0.41$  mg EAG/g.

This variation may be linked to the distribution of secondary metabolites, which change during plant development, environmental stress, and intense sunlight (America et al. 1984) Their behavior differs depending on their chemical structure and the environment in which they were found (acid–base) (Nunes et al. 2012; Suna et al. 2018).

The extract of *C. cinerea* showed superior antioxidant activity compared to the aqueous extract tested by Belyagoubi-Benhammou et al (2014) which found an inhibitory

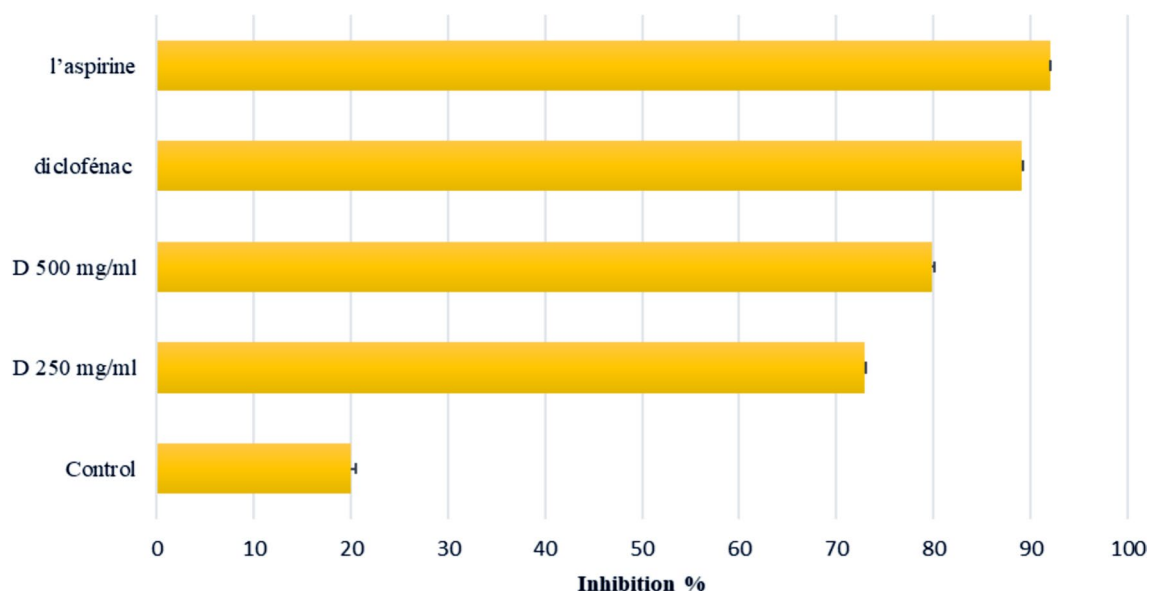


Fig. 14 Inhibition rate (%) of different standards in comparison with extract of *C. cinerea*

concentration equal to  $1.17 \pm 0.05$  mg/mL. In contrast, Agour et al. (2023) recorded lower antioxidant activity with  $IC_{50} = 0.049 \pm 0.217$  mg/ml. In another study, the ethanolic extract of this plant was 0.114 mg/ml. (Agour et al. 2023). Indeed, the methanolic extract of *C. cinerea* has anti-radical activity, revealing a mean  $IC_{50}$  of  $48.60 \pm 17.7$   $\mu$ g/mL (Moussa et al. 2023).

Regarding the effectiveness of iron reduction in our plant, it was lower than that obtained by Ben Moussa et al. (2023), who found a value of  $14.32 \pm 3.64$   $\mu$ g/mL (Moussa et al. 2023). While Agour et al. (2023) used an ethanolic extract for the same species, they found an  $IC_{50}$  of 0.248668 mg/ml. The mechanism of antioxidant action of polyphenols is based on their ability to transfer a hydrogen atom and donate electrons (Zheng and Wang 2001; Amaro-wicz et al. 2004). This reaction is related to the presence of hydroxyl groups (Dangles 2012). According to Khallouki et al (2015), the major phenolic compounds identified in the aqueous extract of *C. cinerea* by HPLC–ESI–MS were chlorogenic acid, cryptochlorogenic acid and luteolin-4'-O-glucoside. All compounds displayed powerful antioxidant capacities in the DPPH, FRAP. According to Amssayef et al. 2020 (Amssayef and Eddouks 2020), *C. cinerea* extract exhibits antioxidant activity in vitro.

The immune system interaction and antioxidant properties of flavonoid compounds are thought to contribute to their therapeutic potential (Hosseinzade et al. 2019). An expanding corpus of studies has examined the efficacy of different flavonoids in treating various types of epilepsy. Furthermore, the structure of flavonoids is essential and gives rise to their anti-inflammatory properties. Since the hydroxyl groups have a planar ring structure with unsaturation at C2–C3, their locations are crucial for imparting this characteristic. For flavonoids to continue having an anti-inflammatory action, the hydroxyl groups located at the B ring's 3' and 4' positions are essential (Al-Khayri et al. 2022). Therefore, it is conceivable to discuss flavonoids' anti-inflammatory effect on epilepsy treatment. A review published in 2023 by Shyam et al., have examined the anti-inflammatory activity of naringenin, kaempferol, quercetin, chrysin, myricetin, rutin luteonil and hesperitin by employing different methods (Rabidas et al. 2023). In addition, other publications have documented the antidiabetic activity of the flavonoids. It has been reported the antidiabetic effects of hesperidin and quercetin have been applied in various ways (Lu and Yip 2023). The study by Amssayef (2020) (Amssayef and Eddouks 2020) showed that the aqueous extract of *C. cinerea* has a significant hypoglycemic activity and reduced plasma triglyceride levels at a 20 mg/Kg dose.

Protein denaturation is among the causes of inflammation (Barros et al. 2008; Bagad et al. 2011). The production of autoantigens in inflammatory diseases may be due to protein

denaturation in vivo. The possible denaturation mechanism involves altering the electrostatic, hydrogen, hydrophobic and disulfide bonds, which maintain the three-dimensional structure of proteins (Barros et al. 2008). Leg edema induced by subplantar injection of carragenin is a classic model typically used to evaluate the acute anti-inflammatory effect in vivo of plant extracts (Chauhan et al. 2020). According to Bouyahya et al. (2022), *C. cinerea* extract possesses anti-inflammatory activity. The results of Refaey et al. (2022) show that *C. anthemoides* essential oils have anti-inflammatory properties thanks to their control over generating inflammatory mediators. The protective anti-inflammatory effect of the aqueous extract from *Inula Viscosa* from the family of Asteraceae at an oral dose of 200 mg/kg was also confirmed in vivo. They significantly reduced paw edema after 3 and 6 h of carrageenan stimulation, respectively (Lounis et al. 2018).

## Conclusion

The present study allowed us to determine, using both laboratory and live animal tests, the biological characteristics of *Cotula cinerea*, a medicinal plant indigenous to the Algerian Sahara. This plant is of significant therapeutic and commercial importance since it contains many beneficial chemicals, including many polyphenols. The results of our study reveal that *Cotula cinerea* possesses significant pharmacological capabilities, including anti-diabetic, antioxidant, and anti-inflammatory effects. These findings highlight the importance of this plant in traditional medicine. The aqueous extract of *Cotula cinerea* Del. includes bioactive compounds that can be used in pharmaceutical formulations. These compounds have essential preventative and therapeutic effects in regulating hypoglycemia in diabetes and function as potent anti-inflammatory agents. Subsequent investigations should prioritize isolating and characterizing the active constituents in *Cotula cinerea* by employing sophisticated methodologies such as mass spectrometry and NMR spectroscopy. Comprehensive investigations are necessary to comprehend the mechanisms of action, encompassing molecular pathways and biological targets. Clinical trials are essential for assessing the safety and effectiveness of *Cotula cinerea* extracts in humans. Furthermore, investigating the combined effects of this plant with other medical plants or medications could potentially improve its therapeutic efficacy. Conducting research on sustainable agricultural practices and creating different formulations to enhance usability and marketability is crucial. Finally, conducting an economic impact evaluation on the commercialization of products derived from *Cotula cinerea* can offer valuable insights into the potential financial benefits it might bring to both local and national

economies. Additional investigation in these domains is essential to completely exploit its therapeutic capacity and guarantee its secure and enduring utilization.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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