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Original article

Phytochemical Profile, Antibacterial Activity, and Antioxidant Potential of *Cotula cinerea*, a Libyan Medicinal Plant

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ABSTRACT

Libyan medicinal plants are an important source of therapeutic agents, and *Cotula cinerea*, a plant collected from the southwestern desert of Libya, is evaluated in this study for its phytochemical composition, antibacterial activity, and antioxidant potential for the first time. Soxhlet extraction using three solvents (n-hexane, chloroform, and methanol) was employed to compare the biological activities of the resulting extracts. The antibacterial activity was assessed using the disc diffusion method against *Staphylococcus aureus* and *Escherichia coli,* with ciprofloxacin as the positive control and DMSO as the negative control. Antioxidant activity was evaluated using the DPPH radical-scavenging assay, with ascorbic acid serving as the positive control. The analysis identified tannins, flavonoids, cardiac glycosides, terpenoids, and steroids, while testing negative for coumarins, anthraquinones, alkaloids, and saponins. None of the extracts exhibited antibacterial activity against *Escherichia coli.* However, the n-hexane extract showed the strongest activity against *Staphylococcus aureus* (23 mm) inhibition zone, followed by the chloroform extract (16 mm), while the methanolic extract demonstrated the lowest activity (11 mm). Only the chloroform and methanolic extracts demonstrated measurable antioxidant activity compared with the reference standard. This study provides the first phytochemical profiling and biological evaluation of *Cotula cinerea* growing in Libya. The study concludes that its antibacterial and antioxidant activities are solvent dependent.

Introduction

In recent years, there has been growing interest in medicines made from plants. This is mainly because many people consider medicinal plants to be safer. As a result, many studies and reviews have reported on the potential of medicinal plants and natural products, including their activity against bacterial infections, cancer, and many other diseases [1]. Herbal medicine is one of the most widely used forms of treatment, relied on by an estimated 3.5–4 billion people worldwide, especially in Africa, India, and China, according to the World Health Organization (WHO) [2]. The use of products made from medicinal plants has grown significantly over the past decade, leading to major progress in the field of phytomedicine [3]. Although about 35,000 plant species are used for medicinal purposes, only around 20% have undergone phytochemical analysis, and only about 10% have been tested for biological activity. This leaves a large number of medicinal plants still in need of scientific investigation [2]. Plants contain a wide variety of bioactive compounds, including alkaloids, flavonoids, lignins, tannins, terpenoids, polyphenols, vitamins, and other secondary metabolites. These active constituents make many plants strong candidates for antioxidant and antibacterial activity [4]. Phytocomponents, such as tannins, polyphenols, and flavonoids, are known to have antimicrobial effects, along with many other biological activities. They may also help lower the risk of cancer by slowing or preventing the development of cancer cells, partly through blocking or reducing the activity of enzymes such as COX-1, COX-2, and DNA topoisomerase I [5].

Cotula cinerea, belonging to the family Asteraceae, is a small annual plant that usually grows 5–15 cm tall, though it can sometimes reach 40 cm. It has a whitish-green stem covered with many fine hairs, giving it a woolly look. The leaves are also small, thick, and velvety, with three to seven lobes that look like slightly closed fingers. The plant produces small yellow flowers grouped in round, dome-shaped heads about 6–10 mm wide [6]. It is widely known in the Sahara region of North Africa by local names such as “Al-Gartoufa” and “Rabrouba.” The species is broadly distributed across arid and semi-arid regions of the Sahara, particularly in Morocco, Algeria, Egypt, and other parts of the Middle East and North Africa. It typically grows in sandy soils, including sand dunes and desert margins [7]. Historically, *C. cinerea* has been used in traditional Moroccan and Algerian medicine for its anti-inflammatory, analgesic, and antiseptic properties, as well as for the management of digestive disorders and to treat diabetes [7,8]. Furthermore, recent research has highlighted its promising therapeutic potential against colon cancer [9]. The *C. cinerea* plant has been documented in several locations within the Libyan Desert, including Al-Jufra [10], Al-Hamada Al- Hamra

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Region [11], and the Acacus Mountains, as noted in the current study. Despite the profound taxonomic diversity of medicinal plants in Libya, many species have yet to be comprehensively studied. To date, no documented investigations have characterized the phytochemical profile or biological activities of *C. cinerea* native to this region. Therefore, the present study aims to screen wild-growing *C. cinerea* collected from the Acacus mountains in the southern west of Libya and to compare its composition with published data from other parts of its natural distribution. In addition, this work evaluates the antioxidant and antibacterial properties of the plant extracts as a potential new source of biologically active natural compounds.

Materials and methods

Preparation of the plant

Collection and Identification

Cotula cinerea was collected from the Acacus mountains region in southwestern Libya on 26 January 2025. The plant was taxonomically identified and authenticated by Prof. Mohamed Abuhadra at the Herbarium Department, Faculty of Science, University of Tripoli, voucher number (6810746).

Drying and Processing

The collected plant material was shade-dried at room temperature. The dried sample was ground into a fine powder using an industrial mill. The resulting powder (58.3 g) was stored in airtight containers for further analysis.

Extraction procedure

A sequential Soxhlet extraction was performed on the powdered plant material (58.3 g) using 250 mL each of n-hexane, chloroform, and methanol (Analytical Grade). Each extraction stage lasted approximately 6-8 h until the siphoning solvent was colorless. The resulting extracts were filtered (Whatman No. 1) and concentrated to dryness using a rotary evaporator under reduced pressure at a temperature below 40 °C. The crude extracts were transferred to dried, pre-weighed glass vials and stored at 40 °C in airtight containers until further analysis.

Phytochemical screening

Preliminary phytochemical screening was performed to qualitatively characterize the secondary metabolites of *C. cinerea*. The presence of cardiac glycosides was determined via the Keller–Killiani test, while anthraquinone glycosides were evaluated using Borntrager's test. Tannins were identified through the addition of ferric chloride (5%), and saponins were detected based on the formation of persistent froth in the foam test. Furthermore, coumarins were identified via the alkaline reagent test, and flavonoids were screened using the sodium hydroxide test. Terpenoids and steroids were detected utilizing Salkowski's test and the acetic anhydride/sulfuric acid assay, respectively [12][5]. A positive reaction was recorded based on the appearance of specific color changes or persistent foaming as previously described in the literature.

Disc diffusion: an antibacterial method

Bacterial Strains and Culture Preparation

Staphylococcus aureus (Gram-positive) and *Escherichia coli (*Gram-negative) were used as test organisms. Each strain was streaked on nutrient agar and incubated at 37 °C for 24 h to obtain fresh colonies. Bacterial suspensions were prepared in sterile 0.85% saline and adjusted to the 0.5 McFarland turbidity standard to ensure a uniform inoculum.

Antibacterial Susceptibility Testing

The antibacterial activity of the plant extracts was evaluated using the disc diffusion assay on Mueller–Hinton agar. Each standardized bacterial suspension was evenly inoculated over the agar surface using a sterile swab. Sterile 6 mm filter-paper discs were impregnated with 20 µL of each extract, allowed to dry, and placed on the inoculated plates. Ciprofloxacin (5 µg) served as the positive control, and 10% DMSO as the negative [13].

Antioxidant Activity (DPPH Assay)

The antioxidant activity of the plant extracts was evaluated using the DPPH assay. The plant extracts and ascorbic acid (positive control) were applied onto a silica gel TLC plate and allowed to dry at room temperature. The plate was then sprayed with a 0.15% methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). A change in spot coloration from violet to yellow was considered indicative of free-radical scavenging activity [14].

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Results and Discussion

The *C. cinerea* plant, which was collected from the Acacus mountains region (Figure 1), has been subjected to an extraction procedure by using Soxhlet, and different residues were obtained based on the polarity of solvent which were used.

Figure 1. The Cotula cinerea specimens were collected from the Acacus region in Libya

The percentage of yield was calculated for each residue (Table 1). The highest extraction yield was achieved using methanol, whereas the hexane extract resulted in the lowest yield.

$$\text{Percentage Yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of Initial Plant Sample (g)}} \; X \; 100$$

Table 1. The yields of plant extractions

Weight (g)	**Hexane extract**	**Chloroform extract**	**Methanol extract**
	2.36	3.119	10.73
Yield (%)	4.05	5.35	18.4

The sequential extraction of 58.3 (g) plant sample weighted demonstrates a clear correlation between solvent polarity and extraction efficiency, with yields increasing significantly as polarity rises (Hexane < Chloroform < Methanol). The low yield from the non-polar hexane extract (4.05%) suggests a relatively small concentration of lipophilic compounds, such as waxes, fats, and fixed oils. In contrast, the chloroform yield (5.35%) indicates the presence of moderately polar compounds, including alkaloids and terpenoids. Ultimately, the substantial yield of the methanolic extract (18.40%) reveals that the plant is primarily composed of polar secondary metabolites, such as polyphenols, flavonoids, glycosides, and tannins. The preliminary phytochemical screening of the *C. cinerea* plant was carried out to investigate its phytochemical composition using several standard methods (Table 2). The Keller-Killiani test yielded a characteristic brown ring, indicating a positive result for cardiac glycosides, while the sodium hydroxide test produced a yellow coloration, confirming the presence of flavonoids. The ferric chloride test resulted in a dark green coloration, demonstrating a positive reaction for tannins. Furthermore, the presence of steroids was confirmed via the chloroform/sulfuric acid test, and terpenoids were identified using the Salkowski test. On the other hand, the extract tested negative for several other compounds. Borntrager's test for anthraquinone glycosides showed no color change, and the foam test produced unstable foam, suggesting the absence of saponins. Finally, the NaOH/KOH test failed to produce fluorescence under UV light, confirming a negative result for coumarins.

Table 2. The results of preliminary phytochemical screening of the C. cinerea plant

No	**Phytochemical Constituents**	**Tests**	**Result**
1	Cardiac Glycosides	Keller-killiani test	+
2	Anthraquinone glycosides	Borntragers test	-
3	Tannins	Ferric chloride test	+
4	Saponins	Foam test	-
5	Coumarins	UV test	-
6	Flavonoids	NaOH test	+
7	Steroids	Chloroform\Sulfuric acid	+
8	Alkaloids	Mayer's Reagent test	-
9	Terpenoids	Salkowski test	+

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The phytochemical profile observed in the current study of *Cotula cinerea* revealed a predominance of flavonoids, tannins, and terpenoids, while notably lacking alkaloids, saponins, and coumarins. These findings are largely consistent with previous literature, which characterizes this species by its rich concentration of phenolic acids such as luteolin-4´-O-glucoside, chlorogenic acid, and gallic acid, as well as sesquiterpene lactone [7]. Interestingly, the current study results support the geographic consistency of the species; similar metabolite profiles have been reported in samples collected across Algeria, Morocco, and Egypt. The absence of alkaloids in our findings is particularly significant, as it highlights the dominant coherence that *C. cinerea* typically lacks this class of compounds regardless of its regional origin [15]. The detection of tannins, terpenoids, and flavonoids was particularly noteworthy, as these compounds are well recognized for their biological activities, including antidiabetic, anti-inflammatory, antimicrobial, and antioxidant effects [7]. The extracts of *C. cinerea* showed no antibacterial activity against *Escherichia coli*, as all inhibition zones measured 6 mm, which was identical to the negative control. Meanwhile, significant inhibition was detected against *Staphylococcus aureus*, with inhibition zones ranging from 11 mm to 23 mm (Table 3). The n-hexane extract exhibited the highest activity (23 mm), approaching that of the positive control (24 mm). These results indicate that the *C. cinerea* extracts demonstrated antibacterial activity selectively against Gram-positive bacteria.

Table 3. The inhibition zones obtained for C.cinerea extracts against the tested bacterial strains

No.	Material	*E. coli*	*S.aureus*
1	*C.cinerea* methanol extract	6mm	11mm
2	*C.cinerea* chloroform extract	6mm	16mm
3	*C.cinerea* n-hexane extract	6mm	23mm
4	Ciprofloxacin -positive control	26mm	24mm
5	DMSO- negative control	6mm	6mm

In evaluating antibacterial activity, the extracts demonstrated selective effectiveness against Gram-positive bacteria. No activity was observed against *E. coli*, as all inhibition zones were equal to the negative control, indicating a lack of susceptibility. Conversely, clear zones of inhibition were recorded against *Staphylococcus aureus*, with the n-hexane extract exhibiting the strongest response. This pattern aligns with the typical resistance of Gram-negative bacteria, partly attributed to the outer membrane barrier and the comparatively higher susceptibility of Gram-positive species to plant-derived compounds. The substantial inhibition produced by the hexane extract suggests that some non-polar constituents of *C. cinerea* may play a significant role in its antibacterial potential [16]. The antioxidant activity of the *C. cinerea* extracts was evaluated using the DPPH assay. The hexane extract showed no antioxidant activity, as no color change was observed following application of the DPPH reagent. In contrast, both the chloroform and methanol extracts produced a distinct color change from violet to yellow, indicating notable free-radical scavenging activity. Their responses were visibly comparable to that of the ascorbic acid positive control, suggesting that these two extracts possess significant antioxidant potential.

Table 4. The following table summarizes the results of the antioxidant activity of C. cinerea

Extract	(DPPH color reaction)	Antioxidant Activity
n-Hexane extract	No color change	_
Chloroform extract	Violet → yellow	+++
Methanol extract	Violet → yellow	+++
Ascorbic acid (Positive control)	Violet → yellow	++++

The antioxidant assay using the DPPH method further supports the biological activity of the plant. The chloroform and methanol extracts produced a clear violet to yellow color change, indicative of free-radical scavenging activity, and their responses were comparable to the ascorbic acid positive control. In contrast, the hexane extract showed no antioxidant activity, consistent with the expectation that antioxidant compounds, often phenolic or flavonoid in nature, are more soluble in polar or moderately polar solvents. This observation corresponds well with the phytochemical findings, where flavonoids were detected and are likely contributing to the antioxidant performance of the polar extracts [8,17,18].

Conclusion

Overall, the combined findings indicate that *C. cinerea* contains bioactive constituents capable of exerting selective antibacterial activity against Gram-positive bacteria and notable antioxidant activity in its chloroform and methanol fractions. The absence of saponins, alkaloids, and anthraquinone glycosides suggests that other chemical classes, particularly terpenoids, essential oils, flavonoids, and possibly

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lipophilic compounds, may be primarily responsible for the observed biological activity. These results support the potential pharmacological value of *C. cinerea* and justify further fractionation and analysis to isolate the specific active compounds.

Conflict of interest. Nil

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