

## CHEMICAL COMPOSITION AND MOLLUSCICIDAL ACTIVITIES OF THE ESSENTIAL OILS FROM *Cotula cinerea* A SAHARAN MEDICINAL PLANT

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### ABSTRACT

Schistosomiasis is one of the 20 neglected tropical diseases (NTDs) recognized by the World Health Organization and remains a major public health problem in many developing countries. Natural molluscicides derived from medicinal and aromatic plants (MAPs) offer a promising and eco-friendly alternative. Sahara, with its vast botanical diversity and rich traditional knowledge, represents a valuable reservoir for the identification of new bioactive natural products for schistosomiasis control. The essential oil derived from the Saharan medicinal plant *Cotula cinerea* (Asteraceae), growing in Tindouf (Algeria), was analyzed for its chemical composition using gas chromatography/ mass spectrometry (GC/MS) and evaluated for its molluscicidal properties. Thirty-six compounds, representing 93.4% of the essential oil, revealed that the major constituents were trans-Thujone (34.8%), Camphor (11.9%), Santolinatriene (10.2%), 1,8-Cineol (7.4%), and Cis-Verbenyl acetate (6.4%). The essential oil is characterized by the presence of high content of oxygenated monoterpenes. The molluscicidal activity tested against adult *Lymnaea stagnalis* snails using WHO-recommended bioassay protocols showed that the essential oil of *C. cinerea* exhibited significant molluscicidal effects with the 24-h LC50 of 95.8 µg/mL and achieving 90% mortality at 114.2 µg/mL within 24 hours and 90% mortality at 87.3 µg/mL within 48 hours. The results support the potential use of essential oils from the Saharan medicinal plant as natural alternatives to synthetic molluscicides that cause serious environmental hazards.

**KEY WORDS:** Natural products; *Lymnaea stagnalis*; Oxygenated monoterpenes; Schistosomiasis; Algerian Sahara.

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## INTRODUCTION

The World Health Organization (WHO) acknowledges the significant role of traditional remedies and aims to support primary healthcare that includes medicinal and aromatic plants. Since ancient times, volatile essential oils and plant extracts have been used to preserve human health in most ancient civilizations (Cheriti, 2020). Thus, the importance of natural products to human health was emphasized by the award of the 2015 Nobel Prize in Physiology or Medicine to Satoshi Omura, William Campbell and Tu Youyou for their groundbreaking discovery of avermectin from a *Streptomyces* species and for its subsequent development as a treatment for river blindness and other parasitic diseases, that leading to the creation of the antimalarial drug artemisinin from the medicinal plant *Artemisia annua* (Wang et al., 2020).

Schistosomiasis is one of the 20 neglected tropical diseases (NTDs) recognized by the WHO and remains a major public health problem in many developing countries. Over 200 million people worldwide are affected, predominantly in endemic regions (78 countries) where exposure to contaminated freshwater is common. The disease is a water-borne parasitic disease caused by trematode worms of the genus *Schistosoma*, such as *S. haematobium*, *S. mansoni*, and *S. japonicum*, which involves two hosts: a freshwater snail as the intermediate host, where the parasite undergoes asexual reproduction, and humans as the definitive host, where sexual reproduction takes place (Siqueira et al., 2017; Pereira et al., 2020; Klohe et al., 2021). Clinically, there are two forms: i) Intestinal schistosomiasis typically causes abdominal pain, diarrhea, and blood in the stool, and may lead, in chronic stages, to hepatosplenomegaly, liver fibrosis, portal hypertension, and ascites, ii) Urogenital schistosomiasis is characterized by hematuria, dysuria, and in advanced cases, fibrosis of the bladder and ureters, kidney damage, and bladder carcinoma (Dahane et al., 2015a; Mendonça et al., 2025). Various studies indicate that, in men, genital involvement may result in testicular pain and hematospermia. In women, deposition of eggs in the cervix and lower genital tract causes intravaginal lesions, leading to genital itching, pain, bleeding, dyspareunia, infertility, and female genital schistosomiasis, which is recognized as a significant cofactor in HIV transmission in co-endemic regions (Mbabazi et al., 2011; Kokaliaris et al., 2022). Currently, praziquantel is the only widely used drug, despite its limited efficacy against juvenile parasites and the risk of resistance development. Thus, according to WHO recommendations, control strategies for schistosomiasis to interrupt transmission include improved sanitation, health education, and control of the intermediate snail hosts using molluscicidal agents (WHO, 2019). In this context, natural molluscicides derived from medicinal and aromatic plants (MAPs) offer a promising and eco-friendly alternative. Essential oils and plant extracts rich in bioactive compounds have demonstrated molluscicidal and antiparasitic properties in

various studies (Lahlou & Berrada, 2001; Lahlou, 2003; Matos Rocha et al., 2013; Machado et al., 2023).

Algeria, with its wide range of bioclimatic zones, from the Mediterranean coast to the arid expanses of the Sahara, boasts a rich and varied flora of about 4,450 taxa, of which 3,950 are indigenous with 6.5% endemic (Dobignard, 2013). Aromatic and medicinal plants hold a prominent place in Algerian traditions, reflecting a long-standing cultural heritage, playing key roles in folk medicine, culinary practices, and the livelihoods of rural communities. The Algerian Sahara region remains an important reservoir of diverse medicinal species with potential therapeutic benefits that have been largely unexplored. As highlighted by Ozenda (2004), the Asteraceae, Fabaceae, and Poaceae families dominate the Sahara flora. Specifically, the Asteraceae family accounts for 13.8%, 11.2%, and 7.9% of the total plant species in the Septentrional, Central, and Meridional Sahara, respectively.

Medicinal plants of Saharan ethnopharmacopoeia are invaluable resources for drug discovery due to their rich composition of bioactive compounds. Ethnopharmacological studies conducted since 1998 by the Phytochemistry and Organic Synthesis Laboratory (POSL) have provided valuable insights into the traditional medicinal practices in southwest Algeria (Boukhobza et al., 2019; Cheriti, 2019, 2000, 2020; Oughilas et al., 2019; Melkaoui et al., 2023; Benahmed & Cheriti, 2025). We are interested to deepen the investigation of the medicinal Saharan species, among these, *Cotula cinerea* from the Asteraceae family has gained prominence in the Algerian Sahara for its ethnomedical applications (Cheriti, 2000). This medicinal plant is traditionally prepared as an infusion or decoction. It is employed in the treatment of numerous ailments, including digestive disorders, colic, diarrhea, fever, inflammation, rheumatoid arthritis, headaches, migraines, cough, and respiratory infections (Leclerc, 1877; Ibn al-Baytar, 1992; Bellakhdar, 1997; Belboukhari et al., 2012). Additionally, biological studies have demonstrated the antibacterial, antiprotozoal, analgesic, and antipyretic properties of essential oils and extracts derived from *C. cinerea* (Fournier et al., 1989; Markouk et al., 1999a, 1999b, 1999c; El Bouzidi et al., 2011; Boukhobza et al., 2020).

However, as far as we know, nothing was found concerning the chemical composition and molluscicidal activity of *C. cinerea* essential oil growing wild in the Tindouf area (Extreme southwest of Algeria). Thus, as a part of our investigation into Algerian Sahara aromatic and medicinal plants, this is the first report on the chemical composition of the essential oils obtained from *C. cinerea*, and a search for eco-friendly and sustainable molluscicidal agents against freshwater snails, known vectors of schistosomiasis and other parasitic diseases, well known in arid zones, and which constitute a public health problem.

## MATERIAL AND METHODS

### *Plant material*

*Cotula cinera* (Delile), syn. *Brocchia cinera* (Delile), *Tanacetum cinereum* (Delile) DC., vernacular name Guertofa, Beida, Rabrouba, Chouhia, is a small annual spontaneous herb that features discoid capitula and hermaphrodite florets (Figure 1). This medicinal plant is widely found throughout the Sahara desert and belongs to the monotypic genera of Anthemideae (Sub-tribe: Cotulinae) that characterizes North African flora (Ozenda, 2004).

Aerial parts of *C. cinerea* were collected during the flowering period in February 2023 from Tindouf (Latitude: 27°40'00" N; Longitude: 8°09'00" W; Altitude: 433m), an area located in the extreme southwest of Algeria, which is characterized by a subtropical desert climate, with short, very hot, dry summers and cool, dry, clear winters. The identification of the plant was conducted by Pr. A. Marouf from the Department of Biology at University Center Naama, Algeria, and a voucher specimen is preserved at the Herbarium of the POSL Laboratory at Université Tahri Mohamed Bécharr (UTMB), Algeria, recorded under N° CA 07/10.

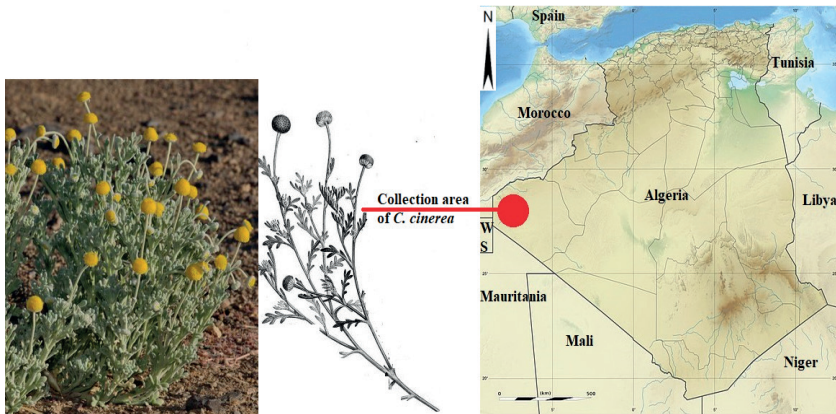


Figure 1. The medicinal plant *Cotula cinerea*, and the collection area in the Algerian Sahara.

## *Essential oil analysis*

We extracted the essential oil (EO) from 100 g of dried Saharan species *C. cinerea* through hydro distillation, using a Clevenger apparatus for 3 hours, in accordance with the 3<sup>rd</sup> Edition of the European Pharmacopoeia (Bruneton, 1999; Boukhobza et al., 2020). The obtained yellowish essential oil was dried using anhydrous sodium sulfate and stored in colored glass at 4 °C until analysis.

The analysis of the extracted essential oil from *C. cinerea* was performed by gas chromatography/ mass spectrometry (GC/MS) using a Shimadzu® GC-17A gas chromatograph equipped with a Shimadzu® QP5000 mass spectrometer, operating at an electron impact of 70 eV with an ion source temperature at 250 °C, scan a mass range of 40–400 m/z at a sampling rate of 0.5 scans/s. A 60 mm × 0.25 mm fused silica non-polar DB-5® capillary column with a 0.3 µm film thickness was used as described in our previous work (Boukhobza et al., 2020). The EO component identification was confirmed by comparison of mass spectral fragmentation patterns with the National Institute of Standards and Technology Mass Spectrometry Data Center (NIST MS) Library and comparing their retention indices (determined against the retention times of a homologous series of n-alkanes C-9 to C-24), with established literature data (Adams, 2007; Cheriti et al., 2007; Said et al., 2017; Boukhobza et al., 2020; Huda et al., 2024). The relative concentrations of individual components in the essential oil were calculated from peak areas without flame ionization detector (FID) response-factor corrections.

## *Molluscicidal activity*

We conducted our experiments using adult *Lymnaea stagnalis* snails measuring approximately  $2.50 \pm 0.50$  cm in length, sourced from various irrigation canals in the Boukais region (Southwest Algeria). These snails were identified and acclimatized for a 72-hour period under laboratory conditions as recommended by WHO and according to our previous work (Dahane et al., 2015b; WHO, 2019). Ten experimental animals were kept in Petri dishes containing 20 mL distilled water (pH =  $7 \pm 0.3$ ) at  $24 \pm 1$  °C. Dead animals were removed at each observation to avoid any contamination of the Petri dish water. 100 mg of EO was diluted in 1,000 mL of distilled water with 0.20 mL of the surfactant Tween 80. The solution was then dispersed by application of ultra-sound frequency, and various concentrations were prepared (5, 10, 20, 40, and 80 mg·L<sup>-1</sup>). Molluscicidal tests were performed in duplicate using ten snails for each test, which were exposed to different concentrations of essential oil, and mortality was observed for 24, 48, 72, and 96 h. Snail mortality was established by the contraction of the body within the shell, no movement, and discoloration of the body and shell. In the control test, the snails were kept

in an equal volume of distilled water under similar conditions but without EO. No mortality was recorded in the solvent control group throughout the experimental period, indicating that the solvent had no toxic effect on *L. stagnalis*, in accordance with our previous work (Dahane et al., 2015b). A stock solution of copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) ( $1 \text{ g.L}^{-1}$ ) was used for the preparation of the positive reference tests. The molluscicidal effects on *L. stagnalis* were expressed by LC50, LC90 (only after 96h). Mortality data were subjected to probit analysis to estimate LC50 and LC90 values with 95% confidence limits (CL) for each exposure period (24–96 h). The regression coefficient between exposure time and different concentrations and the values LC50 were determined as described in our previous work (Dahane et al., 2015b).

## RESULTS

The chemical composition of *C. cinerea* essential oils evaluated for their molluscicidal properties was analyzed to identify the bioactive constituents potentially responsible for the observed activity. We obtained a yield of 1.05% of yellowish oil with a pleasant odor characteristic of artemisia from *C. cinerea* collected in the Tindouf region (extreme southwest of Algeria).

The GC/MS analysis results are summarized in Table 1, which lists the components according to their elution order on the DB-5 capillary column.

*Table 1.* Chemical composition of essential oil from the Saharan plant *Cotula cinerea*.

N°	Components	Content (%)	RI*	RI**
1	Cis-Salvene	0.63	849	856
2	Santolinatriene	10.15	907	910
3	$\alpha$ -Thujene	0.65	929	931
4	$\alpha$ -Pinene	1.71	936	939
5	Camphene	1.96	949	953
6	$\beta$ -Pinene	1.68	975	980
7	a-Myrcene	0.28	987	990
8	a-Phellandrene	0.17	998	1002
9	$\alpha$ -Terpinene	0.43	1013	1018
10	Limonene	0.21	1028	1031
11	1,8 Cineol	7.38	1030	1033

12	Santolina alcohol	1.98	1033	1035
13	g-Terpinene	0.5	1057	1062
14	Camphenilone	0.11	1076	1082
15	Linalool	0.29	1094	1098
16	cis-Thujone	0.25	1105	1102
17	trans-Thujone	34.79	1117	1114
18	Camphor	11.86	1147	1146
19	Cis-Chrysanthenol	1.03	1160	1164
20	Borneol	1.51	1166	1165
21	Terpinen-4-ol	1.83	1180	1177
22	$\alpha$ -Terpineol	1.69	1192	1189
23	Carvacrolmethy ether	0.12	1247	1245
24	Linalyl acetate	0.46	1260	1257
25	Cis-Verbenyl acetate	6.42	1283	1280
26	Bornyl acetate	2.18	1289	1287
27	3-Thujanol acetate	0.15	1294	1295
28	Carvacrol	0.17	1301	1298
29	Nerylacetate	0.32	1368	1365
30	Geranyl acetate	0.27	1385	1383
31	$\beta$ -Elemene	0.11	1387	1389
32	trans-jasmone	0.17	1389	1391
33	$\beta$ -Caryophyllene	0.64	1421	1419
34	trans- $\beta$ -farnesene	0.12	1456	1454
35	GermacreneD	1.03	1484	1480
36	Caryophyllene oxide	0.14	1588	1583
Total Identified (%)		93.41		

RI\*: Retention indices measured (DB-5 column)

RI\*\*: Retention indices from the literature (Adams,2007)

The tested essential oils showed the most pronounced molluscicidal activity against the freshwater pond snail, *L. stagnalis*. Thus, the 24-h LC50 of the essential oil was 95.8  $\mu\text{g/mL}$  and achieving 90% mortality at 114.2  $\mu\text{g/mL}$  within 24 hours and 90% mortality at 87.3  $\mu\text{g/mL}$  within 48 hours (Table 2).

Table 2. Lethal Concentration ( $\mu\text{g} / \text{mL}$ ) of mortality accumulated by the essential oil of *Cotula cinerea* on *Lymnaea stagnalis* snail.

Time elapsed (h)	Lethal concentrations	
	LC50 ( $\mu\text{g}/\text{mL}$ ) (Lower-Upper limits) 95% CL*	LC90 ( $\mu\text{g}/\text{mL}$ ) (Lower-Upper limits) 95% CL*
24	95.81 (86.7 – 105.4)	114.21 (101.4 – 126.6)
48	72.45 (64.8 – 79.6)	87.26 (77.1 – 96.3)
72	39.20 (33.1 – 45.2)	58.12 (51.3 – 66.1)
96	27.97 (23.4 – 32.1)	38.24 (33.6 – 44.8)
Copper sulfate pentahydrate (Positive Control)	8.18 (6.3 – 10.1)	31.63 (27.5–36.8)

\*CL: Confidence Limits

## DISCUSSION

We identified in the GC/MS analyses of *Cotula cinerea* essential oils, thirty-six compounds, representing 93.4% of the essential oil (Table 1), revealed that the major constituents comprised predominantly by trans-Thujone (34.8%), Camphor (11.9%), Santolinatriene (10.2%), 1,8-Cineol (7.4%) and Cis-Verbenyl acetate (6.4 %), collectively accounting for 70.6% of the essential oil's composition, highlighting a significant presence of oxygenated monoterpenes. It is necessary to point out the presence of hydrocarbon monoterpenes and oxygenated monoterpenes with low percentages between 2.2 and 1.5 %, such as Bornyl acetate, Santolina alcohol, Camphene, Terpinen-4-ol,  $\alpha$ -Pinene,  $\alpha$ -Terpineol,  $\beta$ -Pinene and Borneol.

It was observed that all of the components reported in our study were found in *C. cinerea* from different regions of southern Algeria and Morocco, as indicated in Table 3. While some quantitative differences were observed, these discrepancies may be attributed to several factors, including the plant's growth stages, environmental conditions, and variations in geography and soil composition (Muller-Riebau et al., 1997).

*Table 3.* Major compounds detected in various *Cotula cinerea* essential oils from Algeria and Morocco.

Origin of specie		Major constituents (%)	References
Algeria	Tindouf (Extreme southwest)	trans-Thujone (34.79), Camphor (11.86), Santolinatriene (10.15), 1,8 Cineol (7.38) and Cis-Verbenyl acetate (6.42).	This work (2025)
	El-Oued (Southeast)	trans thujone (50.10), 1.8-cineole (8.74), sabinene (6.14), terpinen-4-ol (5.84), Camphor (4.90), Santolinatriene (4.00).	Mekhadmi et al. (2023)
	Brezina (North southwest)	trans-Thujone (36.11), Camphor (12.08), Santolinatriene (11.25), 1,8 Cineol (6.98).	Boukhobza et al. (2020)
	El Oued (Southeast)	trans-Thujone (51.86), Santalinatriene (10.6), Sabinene (6.17), Cineole (5.34).	Larbi et al. (2018)
	El Oued (Southeast)	3-Carene (30.99), Thujone (21.73), Santolinatriene (18.58), Camphor (6.21).	Chouikh et al. (2015)
	Ouargla (Southeast)	Thujone (47.72), Camphor (10.54), Santolinatriene (8.00), Eucalyptol (6.37).	Bouziane et al. (2013)
	Akka (Southeast)	Thujone (24.9), Lyratyl acetate (24.32), Camphor (13.55), 1,8-cineole (10.81).	Agour et al. (2022)
Morocco	Tata (Southeast)	Thujone (40.83), Camphor (16.58), Eucalyptol (10.99), Santolinatriene (9.93), 3-caren-4-ol acetoacetate (6.91).	Hamdouch et al. (2022)
	Al Nif (Southeast)	Thujone(26.05), cisChrysanthenyl formate (15.64), 2-bornanone (15.40), Santolinatriene (10.68), 1,8-Cineol (8.48).	Chlif et al. (2021)
	Zagora (Southeast)	trans-Thujone (41.4), cis Verbenyl acetate (24.7), 1,8-Cineole (8.2).	Kasrati et al. (2015)
	Zagora (Southeast)	trans-Thujone(41.4), cis-Verbenyl acetate (24.7), 1,8-cineole (8.2), Santolinatriene (7.2), Camphor (5.5).	El Bouzidi et al. (2011)

The results of this study (Table 2) highlight the potent molluscicidal activity of essential oils derived from Saharan medicinal plant *Cotula cinerea* against the snail *Lymnaea stagnalis*, an intermediate host of *Schistosoma haematobium*. We report that the evaluation criterion in the classification of molluscicidal plant species as active or inactive is that plant species that have a lethal concentration of 90% (LC90) below 400 ppm are considered bioactive. For essential oils to be effective as molluscicide candidates, they must cause 90-100% mortality of adult snails at concentrations of 100 µg/mL or lower, or achieving 50% mortality at concentrations below 40 µg/mL (Hostettmann et al., 1982; Marston & Hostettmann, 1985; Marston et al., 1993; WHO, 2019).

The pronounced molluscicidal activity observed in the tested essential oils of *C. cinerea* may be attributed to the synergistic effects of their major constituents, such as trans-Thujone, Camphor, Santolinatriene, 1,8-Cineol and Cis-Verbenyl acetate and Bornyl acetate. So, our results are consistent with prior reports highlighting the molluscicidal properties of plants rich in oxygenated monoterpenes and phenolic derivatives, which may penetrate the snail tegument, disrupt membrane integrity, and interfere with neuromuscular coordination. It is well known that these natural compounds induce oxidative stress, leading to enzyme inhibition and eventual mortality of snails (Marston & Hostettmann, 1985; Marston et al., 1993; Lahlou & Berrada, 2001; Pereira et al., 2020; Mendonça et al., 2025).

These results agree with previous investigations that have highlighted the molluscicidal properties of essential oils containing high levels of carvacrol, thymol, and 1,8-cineole, which are known for their neurotoxic and membrane-disrupting effects on freshwater snails by interfering with neurotransmission and cellular respiration (Pereira et al., 2020). Similarly, the abundance of oxygenated monoterpenes in the essential oil of *C. cinerea* may synergistically enhance toxicity through increased membrane permeability and oxidative stress. Due to their lipophilic nature, these compounds with hydrocarbon terpenes can easily penetrate the snail's tegument, leading to ionic imbalances, enzyme inhibition, and oxidative stress, which contribute to increased mortality. It is essential to consider the possible contribution of minor compounds or synergistic interactions between both major and minor constituents, given that the biological activity of essential oils often results from the combined effects of their various secondary metabolites (Bakkali et al., 2008; Pereira et al., 2020).

The findings of this study revealed for the first time that the essential oil extracted from the Saharan medicinal plant *C. cinerea*, collected in the Tindouf region (Extreme southwest of Algeria), is characterized by high content of oxygenated monoterpenes, and low LC50 and LC90 values, highlighting its potential as a promising natural candidate for the development of eco-friendly and sustainable molluscicidal agents. On the other hand, the abundance of local plants and their traditional use in Saharan communities may facilitate

their integration into community-based schistosomiasis control strategies. Nevertheless, further investigations are warranted to address aspects such as the relation between essential oil composition and molluscicidal activity, identification of bioactive compounds, standardization, and formulation stability.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

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