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Wound healing and analgesic effects of *Brocchia cinerea* essential oil in experimental animals

Zineb Lakache,[1] Hamza Aliboudhar,[2] Mohcene Sadallah,[3] Hinda Hacib,[1] Meryem Fekiri,[1] Amina Ihssane Zergat,[1] Hassina Tounsi,[1] Abdelkrim Kameli[1]

[1]Laboratory of Ethnobotany and Natural Substances, ENS-Kouba, Algiers; [2]USTHB, Laboratory of Functional Organic Analysis, Faculty of Chemistry, University of Sciences and Technology Houari Boumediene, El Bab-Ezzouar, Algiers; [3]Department of Biology, ENS-Kouba, Algiers, Algeria

Correspondence: Zineb Lakache, Laboratory of Ethnobotany and Natural Substances, ENS-Kouba, Algiers, Algeria.

E-mail: lakache.zineb@gmail.com

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Abstract

This study aimed to identify the main components of the essential oil extracted from *Brocchia cinerea* (Delile) Vis. via hydrodistillation and investigate its *in vivo* wound healing and analgesic properties. Thujone, santolina triene, camphor, and 1,8-cineole were among the compounds detected. Wounds were induced in mice and treated with essential oil, which resulted in accelerated wound healing and repair through topical application (88.1±1.1%). The study also evaluated the analgesic activity of the essential oil by administering intraperitoneal injections of acetic acid to mice. The results showed that *B. cinerea* essential oil at a dose of 400 mg/kg strongly inhibited pain, with a pain inhibition percentage of 95.5%. These findings indicate that the essential oil of *B. cinerea* has potential as a source of bioactive compounds that may have synergistic effects. Based on these results, the use of *B. cinerea* for therapeutic purposes in preventing pain and promoting wound healing is supported. These findings highlight the potential of *B. cinerea* in paving the way for future research aimed at the development of clinically valuable products.

Introduction

Arid regions pose a challenging and demanding environment for living organisms to survive due to their weak and unpredictable rainfall patterns, extreme temperature fluctuations, and persistent winds. Despite these adverse conditions, there are some areas in which spontaneous and diverse flora can thrive, benefiting from more favorable conditions.[1-6]

The arid regions' diverse plant life can provide valuable resources for scientific research in various fields such as medicine, pharmacology, and food. Such research can contribute to the economic growth of the local population. The Southeast region of Algeria is particularly rich in medicinal plants, including the spontaneous growth of a plant known as *Brocchia cinerea*.[4] This plant is well adapted to arid environments, and it is commonly found growing in sandy and desert areas.[7,8] *B. cinerea* is a compact, annual plant that typically emerges after the rainy season. It has a woolly appearance and reaches a height of 5 to 15 cm. The plant's stems can be upright or spreading, and the leaves are thick, whitish, and woolly with three to five obtuse teeth at the top. At the end of a short stem, small, yellow-gold pompom-like flowers are produced.[9]

B. cinerea belongs to the Asteraceae family, specifically from the Anthemideae tribe; previous chemical characterizations have revealed the presence of valuable bioactive compounds such as flavonoids, sesquiterpene lactones, and polyacetylenes.[10-13] *B. cinerea* contains a variety of secondary metabolites, making it a valuable source of natural compounds for traditional medicine. Studies have shown that this plant exhibits interesting biological activities, including anti-inflammatory, analgesic, disinfectant, antibacterial, antipyretic,[11,9] and larvicidal effects against *Anopheles labranchiae* larvae.[7] *B. cinerea* is highly valued in traditional medicine for its various medicinal properties, including treating digestive problems, rheumatism, urinary and pulmonary infections.[4,8,14] Additionally, it is commonly used to add flavor to traditional tea and preserve goat butter.[8]

Limited research has been conducted on the analgesic, antioxidant and wound-healing properties of alcoholic and aqueous extracts derived from *B. cinerea*. As far as we are aware, no study has been published evaluating the wound healing and analgesic activities *in vivo* of the essential oil from this species. This study aims to provide further insight into the pharmacological and medicinal properties of *B. cinerea* oil.

Materials and Methods

Plant material

The aerial parts (flowers, leaves and stems) of the *B. cinerea* plant were collected during the flowering season in February 2023 from the Ghardaïa region in Southeast Algeria. The harvesting site was situated at an altitude of 500 m within the semi-arid zone, with a latitude

of N 32°29′27 and longitude E 3°40′24. The identification of the plant was carried out by Professor Toumi from the Department of Biological and Environmental Sciences, ENS Kouba, Algeria. The plant specimens have been archived in the Herbarium of the Institute of Biology at the University of El Oued, Algeria, with the designated specimen number L.BIO39MN89. The collected plant material was air-dried naturally in a dark area at room temperature.

Hydrodistillation extraction

The essential oil of *B. cinerea* was obtained through hydrodistillation using the Clevenger method. Plant material was mixed with water in a flask and left for two hours. The resulting oil was then separated from the water through simple decantation, without the use of any organic solvents. The extracted oil was stored in a dark, cool place at 4°C in a tightly closed brown vial.

Gas Chromatography/Mass Spectrometry (GC/MS)

The chemical composition of the essential oil of *B. cinerea* was determined by Gas Chromatography-Mass Spectrometry (GC/MS) using a Gas Chromatography-Mass Spectrometry Flame Ionization Detector (GC-FID) system '7890A/5977B Mass Selective Detector (MSD) Agilent' and a fused-silica-capillary column with a non-polar stationary phase HP5MS (30 m × 0.25 mm × 0.25 µm film thickness). The GC analysis was carried out by injecting a volume of 0.2 µL into the splitless GC inlet, held at 250°C, and running a column temperature program of 60°C for 8 minutes, increasing at a rate of 4°C/minute to 250°C and held at 250°C for 25 minutes. The ionization mode used was an electronic impact at 70 eV. The identification of the chemical constituents was done by comparing the mass spectral fragmentation patterns with those stored in the database Adams 2017, NIST 2014 and Wiley, and by comparing the retention indices of the volatile extract constituents with those of the published index data.

Experimental animals

A total of 24 Swiss albino mice with a weight range of 20-25 g were obtained from the Pasteur Institute in Algiers and housed in standard cages with regulated environmental conditions at 25±1 °C and a 12-12 hours light-dark cycle. The mice were given a week to acclimate to the laboratory environment, during which they were provided with free access to water and a standard pellet diet. All experimental procedures were conducted in accordance with the guidelines established by the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research, as indicated by Agreement Number 45/DGLPAG/DVA.SDA.14.

Preparation of topical formulations

A mixture of 5 g of beeswax and 10 mL of soya oil was heated using the bain-marie to obtain a liquid mixture (cream). The essential oil was added to the cream (beeswax and soya oil) until it reached a concentration of 6% (v/w). The resulting topical formulations were packaged, labeled, and stored at 4°C until use. The selection of the 6% concentration in our study was based on previous *in vitro* attempts. Prior research and experimentation with different concentrations of the compound indicated that a 6% concentration exhibited the most promising results in terms of its effects on wound healing and analgesic properties. Therefore, considering the positive outcomes observed in previous *in vitro* attempts, we chose the 6% concentration as the optimal dosage for our study.

Wound healing evaluation

The mice used in this study were first anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally to create wounds. The dorsal region of each mouse was shaved and four wounds were created using a 5 mm punch, spaced approximately 1 cm apart. After surgery, the mice were housed individually to avoid any interference with the wound-healing process.[15] A total of 24 mice were randomly assigned into four groups with six to eight mice per group. Group I served as the control and received no treatment, while Group II received a commercial ointment of Fucidin cream. Group III received the 6% essential oil cream of *B. cinerea*, and Group IV received a cream containing beeswax and soya oil. All mice received

daily topical treatment with sterile swabs for 12 days, and wound healing was evaluated by measuring the wound closure during this period. Wound healing was measured using a ImageJ software, and the percentage of wound closure was calculated using the following formula:

$$\text{Percentage of wound closure} = \frac{(\text{Initial wound area} - \text{Current wound area})}{\text{Initial wound area}} \times 100$$

Acute toxicity

Different doses of the essential oil of *B. cinerea* were tested by diluting them in a physiological saline solution (0.9% NaCl).[16] The experiment involved five groups, each containing six mice and different doses were orally administered using a gastric tube. The first group served as the control and received only the physiological saline solution orally, while the other groups were administered varying doses of the essential oil (400, 600, and 1000 mg/kg). The mice were then observed for 72 hours to check for any signs of toxicity and mortality rate.

Analgesic activity (writhing test)

The analgesic activity of the essential oil of *B. cinerea* was evaluated using the method described by Collier *et al*.,[17] which involves assessing the ability of an analgesic substance to reduce the pain caused by an irritating substance that induces twisting movements in mice. For this study, five groups of six mice were formed, and different doses were orally administered using a gastric tube. The doses used were: a control group that received physiological water solution, a reference group that received aspirin solution at a concentration of 100 mg/kg, and two treated groups that received essential oil at doses of 200 mg/kg and 400 mg/kg, respectively. After one hour of oral administration, each mouse was intraperitoneally injected with 0.2 mL of a 3% acetic acid solution, and the number of writhings for each mouse was counted ten minutes after injection for a total of ten minutes.

The percentage reduction of pain was calculated using the following formula:

$$\% \text{ Reduction of pain} = \frac{(\Delta T - \Delta E)}{\Delta T} \times 100$$

Where, ΔT represents the mean number of writhings in the control group treated with physiological water 0.9% NaCl, while ΔE represents the mean number of writhings in the test group treated with the essential oil or positive control.

Statistical study

The mean values of the data were presented with the standard deviation (S.D). Statistical analysis was performed using a one-way analysis of variance (ANOVA) test, followed by the Tukey test to determine the significant differences between groups (*$p<0.05$, **$p<0.01$, and ***$p<0.001$).

Results

Hydrodistillation extraction

The essential oil obtained from *B. cinerea* aerial parts through hydrodistillation was a yellowish-brown color and yielded 0.42%.

Gas Chromatography/Mass Spectrometry (GC/MS)

The results showed the presence of 29 components, which made up 98.57% of the total oil. These components were identified and their retention indices and relative area percentages are presented in Table 1. The essential oil contained a high quantity of the major compound, thujone (34.02%), followed by santolina triene (16.25%), camphor (10.47%), 1,8 cineole (7.19%), cis-verbenyl acetate (4.29%), cis chrysanthenol (3.15%), α-terpineol (3.06%), α-pinene (2.82%), santolina alcohol (2.88%), camphene (2.25%), and α-thujene, borneol, terpinen-4-ol, and linalyl acetate, which were present in less than 1% of the essential oil. The identified components in the aerial parts consisted of oxygenated monoterpenes (62.72%), monoterpenes hydrocarbons (34.9%), and sesquiterpene hydrocarbons (0.95%).

Wound healing evaluation

The progress of wound healing can be measured by wound closure, which is the percentage reduction in the wound area over time. In this study, wound healing was compared among experimental groups over a 12-day treatment period, as shown in Figure 1. On day 6, Groups II (treated with Fucidin cream) and IV (treated with 6% *B. cinerea* cream) had significantly ($p<0.001$) higher percentages of wound closure than Group I (control) and Group III (beeswax and soya oil cream). Wounds treated with *B. cinerea* exhibited accelerated closure after 6 days of treatment (Figure 1 and Figure 2). By day 9, wounds treated with 6% *B. cinerea* cream had a greater percentage of wound closure (88.1±1.1%) compared to the Control group (64.2±2.1%) and beeswax and soya oil cream group (68.5±1.7%).

Acute toxicity

The findings indicated that the essential oil of *B. cinerea* did not cause any signs of toxicity or mortality in mice during the various trials, even when administered orally at a dose of 1000 mg/kg.

Analgesic activity (writhing test)

In this study, pain was induced in mice by administering intraperitoneal injections of acetic acid. Our findings revealed that the administration of *B. cinerea* essential oil at a dose of 400 mg/kg resulted in a higher writhing inhibition percentage of 95.5%, compared to the lower dose of 200 mg/kg, which exhibited a writhing inhibition percentage of 57.3%. (Table 2).

Discussion

Our findings revealed that the essential oil obtained from the aerial parts of *B. cinerea* yielded 0.42%. In contrast, other studies conducted on dried aerial parts of *B. cinerea* harvested from the Zagora and Smara regions in Southern Morocco reported extraction yields of 0.87% and 0.64%, respectively.[18,19] This disparity in extraction yields can be attributed to various factors,

including the degree of plant maturity, climate conditions, soil composition, harvesting time, extraction method, and the geographical position of the plant species.[20,21]

The essential oil from *B. cinerea* aerial parts was analyzed via GC-MS. Our findings revealed the presence of 29 components in the essential oil, which accounted for 98.57% of the total oil composition. The major compound identified in the essential oil was thujone, constituting 34.02% of the oil. Other significant compounds included santolina triene, camphor, and 1,8-cineole. These findings align with previous research conducted by Ghouti *et al*.[11] which also identified α-thujone as the dominant compound (32.35%) in *Cotula cinerea* Delile, a synonym of *B. cinerea*. However, the results are not consistent with those reported by Djellouli *et al*.[8] who studied the chemical composition of the essential oil of dry aerial parts of *B. cinerea* collected in the mountains and the desert of Lahmar city in Southwest Algeria, where they identified 33 components. The major compounds were (E)-citral (24.01%), cis-limonene epoxide (18.26%), thymol methyl ether (15.04%), carvacrol (15.03%) and transcarveol (13.79%). The majority of the identified compounds were oxygenated monoterpenes (95.40%), followed by monoterpene hydrocarbons (2.17%), oxygenated sesquiterpenes (0.68%), and hydrocarbon sesquiterpenes (0.41%). Larbi *et al*.[12] identified 40 components, which accounted for 88% of the total essential oil. The dominant components were trans-thujone (51.86%), santolina triene (10.6%), α-pinene (2.02%), sabinene (6.17%), 1,8-cineole (5.34%), δ-terpinene (1.57%), and camphor (2.63%).

Nonetheless, it is important to note that the chemical composition of the essential oil and its antioxidant activity can be influenced by various factors. One such factor is the extraction method, such as hydrodistillation, which may result in the degradation of bioactive compounds. During hydrodistillation, thermal degradation, hydrolysis, and solubilization of bioactive compounds in water can occur, leading to changes in their antioxidant capacity. Additionally, the presence of water in hydrodistillation can render several antioxidants unstable or subject them to enzymatic degradation within the moist plant material.[22]

Regarding the proposed mechanisms of action for wound healing of various monoterpenes, they include antimicrobial activity, which inhibits the RNA and protein biosynthesis of microorganisms, anti-inflammatory activity, which reduces the production of Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-α in mast cells, inhibits the release of leukotriene C4 (LTC4), and affects the release of thromboxane B2 (TxB2).[23] Additionally, they have antioxidant properties, which provide photoprotective effects and inhibit the production of

free radicals induced by UVB radiation. They are also characterized by their low toxicity and have effects on macrophage migration inhibitory factors and fibroblast growth.

α-Terpineol is a type of monoterpenoid alcohol that can be found in the essential oils of various species and is relatively non-toxic.[24,25] Its wound healing effect,[24] has been demonstrated, along with its anti-inflammatory activity through the inhibition of the cyclooxygenase (COX) enzyme and IL production.[26,27] α-Terpineol also acts as a nuclear factor κB (NF-κB) inhibitor and down-regulates the expression of IL-1β and IL-6,[28,29] while reducing TNF-α and NO production.[30] It has also been found to selectively inhibit ovine COX-2 activity,[27] inhibit neutrophil influx and exhibit strong antimicrobial and antifungal effects.[30-32]

Additionally, several major essential oils have been reported as antioxidant and anti-inflammatory agents, such as α-thujone, β-thujone, 1,8-cineole, camphor, and borneol. These compounds have been found to contribute significantly to the anti-inflammatory effect.[33]

In addition to its antioxidant properties, 1,8-cineole has been shown to have anti-inflammatory effects. It acts as an inhibitor for various inflammatory markers such as TNF-α, IL-6, IL-8, Leukotriene B(4), Prostaglandin E2 (PGE2), and IL-1β. Furthermore, it down-regulates the 5-lipoxygenase (LOX) and COX pathways, which are involved in inflammation.[34,35]

The various compounds present in the essential oil played a role in its ability to exhibit antioxidant and anti-inflammatory effects, as well as promote wound healing. The essential oil extracted from *B. cinerea*, containing santolina triene and limonene as its chemical constituents, has been reported to exhibit significant activities against human tumors, oxidant, and inflammatory processes.[36,37] Limonene itself possesses immune-stimulatory, analgesic, and anesthetic properties. Additionally, immune modulation and antiproliferative effects related to the anticancer activity of limonene have also been documented.[38] In addition, the presence of thujone and 2-bornanone has been noted, which are highly active against microorganisms.[39,40] The intriguing biological activities of this plant can be attributed to its chemical composition, characterized by a high content of oxygenated monoterpenoids, notably thujone and camphor. These compounds are renowned for their antiseptic properties and their effectiveness in treating respiratory ailments.[41]

B. cinerea is rich in phenolic compounds and possesses a notable anti-inflammatory capacity,[11,9] which is associated with its wound healing properties. The observed wound healing activity in this study can be attributed to the high percentages of key components, namely thujone, 1,8-cineole, and santolina triene. These components have been associated with wound-healing properties. Additionally, terpenes such as pinene, limonene, and sabinene, known for their antioxidant properties, may also contribute to the observed wound healing activity, despite their relatively low percentages in our essential oil.[42]

Our findings demonstrate that the essential oil of *B. cinerea* exhibited no signs of toxicity or mortality in mice across various dose levels. These results align with a study conducted by Chlif *et al*.[43] which showed that the administration of dry aerial parts of *B. cinerea* at dose levels of 200, 400, 600, and 800 mg/kg did not induce acute oral toxicity effects or mortality in Wistar rats. Additionally, Markouk *et al*.[7] reported that the oral administration of ether, ethyl acetate, and n-butanol extracts of *B. cinerea* at a dose up to 10 mL/kg in Wistar rats did not result in any acute toxicity.

The antinociceptive activity was assessed by inducing writhing in mice through the intraperitoneal administration of an acetic acid solution. Our findings demonstrated a strong inhibitory effect on pain upon administering *B. cinerea* essential oil at a dose of 400 mg/kg. In a previous investigation involving *B. cinerea* collected from Morocco,[43] the researchers demonstrated that the extract of dry aerial parts exhibited a higher percentage of writhing inhibition, with 43.15% and 50.71% observed at doses of 200 mg/kg and 400 mg/kg, respectively. On the other hand, the utilization of fresh aerial parts extract resulted in a writhing inhibition percentage of 32.14% and 45.51% when administered at doses of 200 mg/kg and 400 mg/kg, respectively.

The intraperitoneal injection of acetic acid leads to tissue damage, which triggers the release of various chemical mediators such as bradykinin, histamine, serotonin, acetylcholine, and prostaglandins. Prostaglandins, in particular, sensitize nociceptors to painful stimuli, resulting in later and widespread pain. This pain is demonstrated in mice through the stretching of the hind limbs and twisting of the dorsoabdominal musculature.[44] The essential oil of *B. cinerea* was found to inhibit abdominal contractions in a concentration-dependent manner, indicating its potential analgesic effect. This effect may be attributed to the inhibition of chemical mediator release.

Conclusions

The primary focus of this research was to investigate the correlation between the chemical makeup and effectiveness of the essential oil obtained from the aerial parts *B. cinerea* as well as its potential as a wound healing and analgesic agent. The outcomes of this study provide conclusive evidence that the essential oil from this plant can be a valuable natural alternative to contemporary medicine in pharmaceutical industries.

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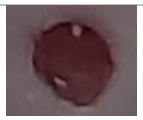
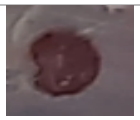
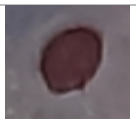
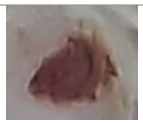
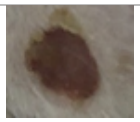
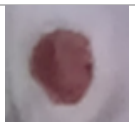
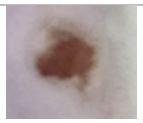
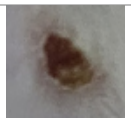
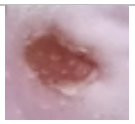
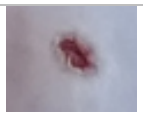
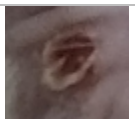
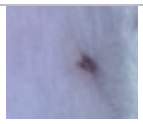
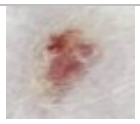
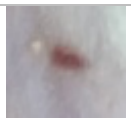
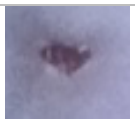
Days	6% *Brocchia cinerea*	Control	Fucidin cream	Beeswax and soya oil
0 Days				
3 Days				
6 Days				
9 Days				
12 Days				

Figure 1. Photograph of wound area after topical application of essential oil cream of *B. cinerea,* Fucidin cream and beeswax and soya oil cream in mice.

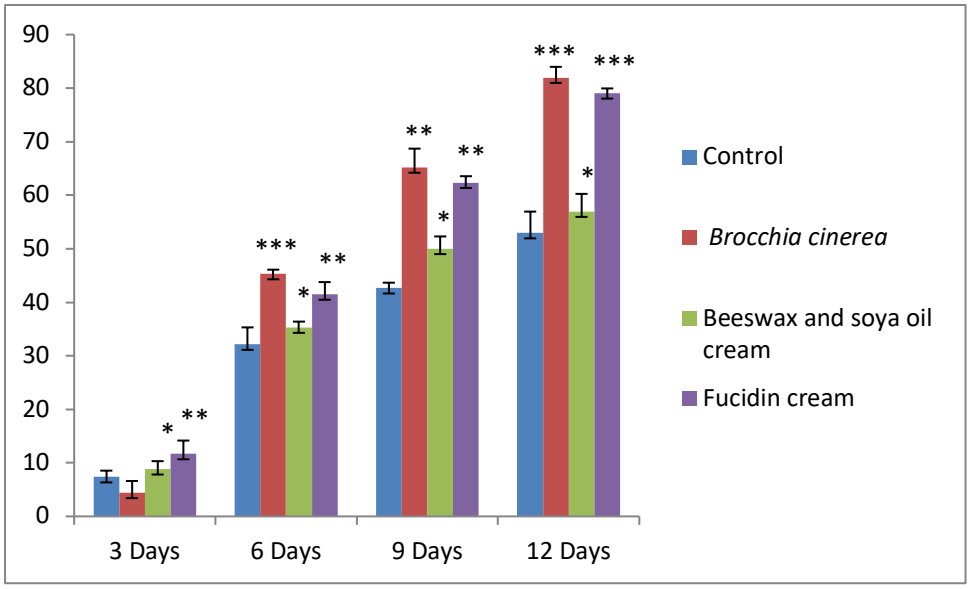


Figure 2. Percentage of wound closure in experimental groups during the treatment days of essential oil cream of *B. cinerea,* Fucidin cream, control and beeswax and soya oil cream in mice.* $p<0.05$, ** $p<0.01$, and *** $p<0.001$ are considered significant, compared to the control.

Table 1. Chemical composition of the *B. cinerea* essential oil.

No.	Compound[a]	RI[b]	RI[c]	Peak area (%)[d]
	Monoterpene hydrocarbons			**34.9**
1	Santolina triene	910	908	16.25
2	α-Thujene	931	929	1.73
3	α-Pinene	939	934	2.82
4	Camphene	953	944	2.25
5	β-Pinene	980	971	0.32
6	α- Terpinene	1018	1011	0.85
7	Limonene	1031	1025	0.14
8	1,8 Cineole	1033	1032	7.19
9	Santolina alcohol	1035	1034	2.88
10	y-Terpinene	1062	1051	0.47
	Oxygenated monoterpenes			**62.72**
11	Linalool	1098	1092	0.59
13	Thujone	1114	1119	34.02
14	Camphor	1143	1145	10.47
15	Cis Chrysanthenol	1163	1164	3.15
16	Borneol	1165	1168	1.34
17	Terpinen-4-ol	1177	1179	1.08
18	α-Terpineol	1189	1191	3.06
19	Carvacrol methyl ether	1245	1248	0.89
20	Linalyl acetate	1257	1263	1.21
21	Cis-Verbenyl acetate	1280	1285	4.29
22	Bornyl acetate	1287	1289	1.27
23	Carvacrol	1298	1304	0.11
24	Neryl acetate	1365	1374	0.85
25	Geranyl acetate	1383	1386	0.39
	Sesquiterpene hydrocarbons			**0.95**
26	β -Elemene	1389	1391	0.32
27	Caryophyllene	1418	1420	0.16
28	Germacrene D	1480	1486	0.35

29	Caryophyllene oxide	1583	1587	0.12
Total Identified (%)		**98.57**		

[a]Compounds identified according to their families on HP-5MS column; [b]Retention indices with respect to C5–C28 n-alkanes calculated on non-polar HP5-MS capillary column; [c]Retention indices given in the literature (NIST, Wiley or ADAMS on non-polar HP-MS or DB5-MS capillary column); [d]Percentage calculated from the peak areas of GC chromatogram on non-polar HP5-MS capillary column.

Table 2. Effect of *B. cinerea* on acetic acid-induced writhing in mice.

Treatment	**Dose (mg/kg)**	**Number of writhing**	**% Inhibition**
Control	-	89 ± 5.3	-
Aspirin	100	28 ± 2.6***	68.53
B. cinerea	200	38 ± 3.05***	57.3
B. cinerea	400	4 ± 2.7***	95.5

*** $p<0.001$ are considered significant, compared to control.

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