

## Original Research Article

# Analgesic and anti-inflammatory properties of aqueous extract of *Deverra scoparia* Coss and Dur obtained from Tamanrasset, Algeria

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### Abstract

**Purpose:** To determine the toxicity, anti-inflammatory and analgesic properties of *Deverra scoparia* Coss. & Dur. and the phenolic compounds involved

**Methods:** The aqueous extract of *Deverra scoparia* Coss. & Dur. was intra-gastrically administered at increasing doses (2, 4, 8, 12 and 16 g/kg) to mice of both sexes in order to study its acute toxicity. Analgesic activity was evaluated in mice and rats using acetic acid-induced writhing and formalin tests at doses of 250, 500 and 1000 mg/kg. Anti-inflammatory activity was assessed by carrageenan-induced paw oedema method, while the bioactive compounds in the extract were identified by high performance liquid chromatography (HPLC) analysis.

**Results:** The extract (1000 mg/kg) produced significant inhibition in acetic acid-induced writhing (71.18 %) and formalin (83.58 %) tests; and 73.52 % reduction of carrageenan-induced rat paw oedema test ( $p < 0.001$ ). HPLC analysis revealed the presence of two phenolic acids, viz, salicylic acid (21.81 mg/g dry weight) and rosmarinic acid (28.44 mg/g dry weight); and apigenin, a flavonoid aglycone (5.70 mg/g dry weight).

**Conclusion:** These results indicate that the extract of *Deverra scoparia* Coss. & Dur. is non-toxic even at a dose of 16g/kg. The extract possesses significant analgesic and anti-inflammatory potential which may be due to the presence of phenolic compounds (apigenin, salicylic acid and rosmarinic acid).

**Keywords:** *Deverra scoparia* Coss. & Dur., Acute toxicity, Anti-inflammatory, Analgesic, HPLC, Phenolic compounds

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## INTRODUCTION

For centuries, medicinal plants have been widely used for the treatment of various ailments [1]. Indeed, about 80 % of the people from developing countries depend on traditional remedies for their primary health care [2]. The

use of alternative medicines derived from natural sources has also spread to the industrialized nations. Indeed, a variety of herbal plants has been extensively used for the extraction of pure compounds used in the development of new drugs [3]. Pain is a disabling discomfort associated with many medical conditions. One of

the most important therapeutic priorities in handling pain involves pain control [4]. Synthetic drugs like non-steroidal anti-inflammatory drugs (NSAIDs), opioids and corticosteroids are the major clinically-used drugs for treating and reducing pain. However, their long term usage may induce toxic effects such as ulcers of gastrointestinal, nausea, hemorrhage, and kidney disorders [5]. In this regard, there is a need to search for medicinal plant-derived bioactive compounds with little or no side effects, for use as alternative analgesic and anti-inflammatory agents [6].

*Deverra scoparia* Coss. & Dur., a medicinal plant belonging to the *Apiaceae* family, is an endemic species of North African origin. Based on ethnobotanical evidence reported in the literature [7-9], the aerial parts of *Deverra scoparia* Coss. & Dur. have very important pharmacological properties [7,8]. This plant is used by the Algerian local population (Tergui) to treat several infectious diseases, digestive disorders and to calm abdominal pain [7]. However, extensive review of extant literature has revealed that no pharmacological studies have been so far been conducted on the acute toxicity, anti-inflammatory activity and the analgesic potential of *Deverra scoparia* Coss. & Dur. The present study was carried out to determine the anti-inflammatory and analgesic properties of *Deverra scoparia* Coss. & Dur. and the identity of the active compounds responsible for these activities.

## EXPERIMENTAL

### Plant material

*Deverra scoparia* Coss. & Dur. was collected in March, 2016 from Hoggar area located in Tamanrasset region (South of Algeria), and was identified by a taxonomist, Pr. Saida Ouafi, of Department of Biological Sciences of University of Sciences and Technology Houari Boumediene. A voucher specimen (number 38) was deposited at the herbarium of the National Institute of Agronomy, Algeria (INA). The aerial parts of the plant were shade-dried at room temperature ( $24 \pm 2$  °C) and preserved in paper bags, in order to avoid fungal contamination. The plant material (1Kg) was chopped into small pieces with a knife and then ground to a fine powder in an electric blender.

### Preparation of aqueous extract

An aqueous extract of *Deverra scoparia* Coss. & Dur. was prepared using infusion method. The powdered plant material was macerated, with

continuous stirring in boiling distilled water for a few minutes, and the resultant infusion was filtered through Whatman no. 1 filter paper.

### Experimental animals

Healthy male and female Wistar albino rats (mean weight =  $200 \pm 20$  g) and healthy male and female albino mice, *Mus musculus* (mean weight =  $20 \pm 5$  g) were obtained from Animal Laboratory of Biotic Unit (Saidal, Algeria). The animals were fed pellet diet from the National Office of Animal Feed (Bejaia-Algeria). The pellet feed and drinking water were provided *ad libitum*. The animals were also kept and maintained under standard laboratory conditions at a temperature of  $24 \pm 2$  °C, 12h light/dark cycle, and relative humidity in the range of 50 to 55 %. All experimental procedures were approved by the University Animal Experimentation Ethics Committee (approval ref no. 162/2011/8), and followed the recommendation of the care and use guide for laboratory animals [10].

### Acute oral toxicity test

The acute oral toxicity test of the aqueous infusion extract of *Deverra scoparia* Coss. & Dur. was determined as per OECD-423 guidelines, but with slight modifications [11]. Sixty albino mice were divided into six equal groups (n=10; 5 males and 5 females), one of which was used as a control. The mice were fasted for 12 h prior to the experiment but were allowed free access to drinking water. Different doses of the aqueous extract (2, 4, 8, 12 and 16 g/kg) were administered, each in a volume of 0.5 mL, while mice in the control group received 0.5 mL of sterile saline water (0.9 % NaCl). Close observations of behavioral changes in the mice was conducted up to 4 h following extract administration, and once every day until the fourteenth day. The behavioral changes of interest were salivation, diarrhea, tremors, ataxia, hyperactivity, lethargy, convulsions, sleep and coma. At the end of the observation period, the animals were anaesthetized, sacrificed and autopsied. The heart, lung, kidney and liver samples were excised and examined for any lesions in the treated groups, relative to the control group.

### Determination of analgesic activity

#### Acetic acid-induced writhing test

This study was performed using the abdominal writhing method described by Koster *et al* [12] and modified by Vogel [13]. Thirty albino mice of both sexes were randomly divided into five

groups each containing six mice. The aqueous infusion extract of *Deverra scoparia* Coss. & Dur. was administered separately by gastric gavage to three of the mice groups at doses of 250, 500 and 1000 mg/kg per body weight. The negative and positive control groups received sterile saline water (0.9 % NaCl) and Aspirin (500 mg/kg), respectively.

Thirty minutes after oral administration of the aqueous infusion extract and the drug, 0.6 % acetic acid (1 mL/kg) was given intraperitoneally to induce pain which was manifested in abdominal constrictions (writhes). After 5 min, each group of mice was monitored for the number of writhes within 15 min. The percentage of inhibition by plant extract and aspirin against acetic acid-induced abdominal writhing was used to evaluate the degree of analgesia (A) and was calculated for each group using Eq 1.

$$A(\%) = [(1 - W_e/W_t)] 100 \dots\dots\dots(1)$$

where  $W_e$  represents the mean value of writhes in treated groups and  $W_t$  represents the mean value of writhes in control groups.

#### Formalin test

Formalin test was carried out using the methods of Hunskaar *et al* [14] and Miranda *et al* [15]. Rats of both sexes were divided into five groups (six rats per group); three groups were orally treated (separately) with the aqueous infusion extract at doses of 250, 500 and 1000 mg/kg per body weight, Aspirin (500 mg/kg) and sterile saline (0.9% NaCl) were administered orally to the other two groups which served as positive and negative controls, respectively. Thirty minutes after treatment, 50  $\mu$ L of 2.5% formalin was injected subcutaneously into the dorsal surface of the right hind paw. The time spent in licking the injected paw was recorded by visual observation in the early phase (0 - 5 min) and late phase (15 - 30 min). The inhibition of licking (L) for both phases was calculated as in Eq 2.

$$L(\%) = [(1 - D_t/D_o)] 100 \dots\dots\dots(2)$$

where,  $D_t$  is the mean value of licking time in the treated groups, and  $D_o$  is the mean value of licking time in the control groups.

#### Determination of anti-inflammatory activity

The anti-inflammatory activity of the aqueous infusion extracted of the aerial parts of *Deverra scoparia* Coss. & Dur. was determined using the procedure previously described by Levy [16]. Albino mice were divided into five groups (six

animals per group). Group I, (negative control) mice were treated with 0.9% sterile saline, whereas mice in group II (positive control) were treated with diclofenac sodium (500 mg/kg). The remaining groups (III, IV and V) received the aqueous infusion extract at three different concentrations (250, 500 and 1000 mg/kg per body weight, respectively). One hour after the oral administration of all treatments, oedema was induced by injecting carrageenan suspension (0.05 mL, 1 % w/v) into the sub-plantar tissues of the right hind paw of all mice. After 3 h the animals were anesthetized with diethyl ether and sacrificed. The two posterior legs were excised at the tarsal joint and then weighed in an analytical balance.

For each mouse, the percentage oedema was calculated after recording the increase in the weight of the right posterior leg which received the carrageenan suspension, and the weight of the healthy left posterior leg. The percentage of oedema (O %) was calculated by determining the mean weight of both legs for each group, according to Eq 3.

$$O(\%) = \{[M(RLW) - M(LLW)]/M(LLW)\} 100 \dots\dots(3)$$

where, O (%) is the oedema percentage, M(RLW) is the mean weight of the right leg per group, and M(LLW) is the mean weight of the left leg per group.

The anti-inflammatory activity was calculated as the percentage inhibition of oedema between the test and control groups following the Eq 4

$$I\% = [(\%co - \%to)/\%co] 100 \dots\dots\dots(4)$$

where, I (%) is the inhibition percentage, %c o is the % of the control oedema and % to is the % of the test oedema.

#### HPLC analysis

HPLC analysis was carried out for the identification and quantification of phenolic compounds in the extract using Agilent series 1100 chromatography system coupled with UV Detector and bars of Diodes Surveyor, equipped with quaternary rapid separation pump. Separation was performed on Hypersil BDS-C18 column (250  $\times$  4.6 mm, 5 $\mu$ m). Analysis was carried out injecting 20  $\mu$ L, while the mobile phase consisted of two solvents: acetic acid (0.2% of acetic acid in water) and acetonitrile in a linear gradient for 30 min at a flow rate of 1.5 mL/min. The mobile phase started with 95 % of acetic acid, and ended with 100 % of acetonitrile.

## Statistical analysis

Data are expressed as mean  $\pm$  standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's test. Values of  $p < 0.001$  and  $p < 0.05$  were considered statistically significant.

## RESULTS

### Acute toxicity

The acute toxicity test was carried out in order to determine the toxicity of the aerial parts of *Deverra scoparia* Coss. & Dur. during the 14 days observation period, no behavioural changes, such as convulsion, hyperactivity, irritability diarrhea or mortalities were observed. The weights of the mice were not affected at any of the administered doses and all the vital organs (kidney, liver, lung and heart) were healthy and normal following necropsy. There were also no signs of cysts, swelling or hemorrhage.

### Analgesic activity

#### Acetic acid-induced writhing

**Table 1:** Effects of aqueous infusion extract of *Deverra scoparia* Coss. & Dur. and aspirin on acetic acid-induced writhing test in mice

Treatment/dose (mg/kg)	Number of writhings responses	Inhibition (%)
Control (normal saline)	59 $\pm$ 4.00	-
Aspirin (500)	31 $\pm$ 1.89*	47.45
Aqueous extract (250)	29 $\pm$ 1.41*#	50.84
Aqueous extract (500)	23 $\pm$ 1.09*#	61.01
Aqueous extract (1000)	17 $\pm$ 1.61*#	71.18

Data represent mean  $\pm$  SEM ( $n = 6$ );  $p < 0.001$  compared with control group; \* $p < 0.05$  compared with aspirin (One-way ANOVA followed by Tukey's multiple comparison test)

**Table 2:** Analgesic effect of aqueous infusion extract of *Deverra scoparia* Coss. & Dur. and aspirin on paw licking time in early and late phases of formalin test in rats

Treatment/dose (mg/kg)	Early phase		Late phase	
	Licking time (s)	Inhibition (%)	Licking time (s)	Inhibition (%)
Control (Normal saline)	119 $\pm$ 7.21	-	195 $\pm$ 7.74	-
Aspirin (500)	49 $\pm$ 4.69*	58.88	60 $\pm$ 1.88*	69.23
Aqueous extract (250)	42 $\pm$ 1.78*	64.70	50 $\pm$ 1.78*	74.35
Aqueous extract (500)	40 $\pm$ 1.41*	66.38	41 $\pm$ 1.09*	78.97
Aqueous extract (1000)	34 $\pm$ 1.67*	71.42	32 $\pm$ 1.41*	83.58

Data represent mean  $\pm$  SEM ( $n = 6$ );  $p < 0.001$  compared with control group; \* $p < 0.05$  compared with aspirin (One-way ANOVA followed by Tukey's multiple comparison test)

The results showed that administration of the different doses of the aqueous infusion extract of *Deverra scoparia* Coss. & Dur. significantly ( $p < 0.001$ ) reduced abdominal writhing in mice, when compared with the negative control group. All doses of the aqueous infusion extract showed strong significant analgesic action in the tested animals, by inhibiting the acetic acid-induced writhing dose-dependently, causing 50.84, 61.01 and 71.18 % of inhibitions at 250, 500 and 1000 mg/kg, respectively. In contrast, aspirin (500 mg/kg) produced only 47.45 % writhing inhibition effect (Table 1).

### Formalin test results

The evaluation of the analgesic activity was conducted using the formalin test. The results on Table 2 show that the extract produced significant and dose-dependent percentage inhibition of the number of writhes at the early and late phases, when compared with the control group ( $p < 0.001$ ). Maximum percentage inhibition was obtained at a dose of 1000mg/Kg (71.42 % in early phase), and (83.58 % in the late phase).

### Anti-inflammatory activity

Oral administration of the aqueous infusion extract of aerial parts of *Deverra scoparia* Coss. & Dur. to mice with carrageenan-induced hind paw oedema produced significant ( $p < 0.001$ ) anti-inflammatory activity at all doses tested (**Error! Reference source not found.**). There was a gradual decrease in oedema paw volume from 15.66 % at 250 mg/kg down to 8.68% at a dose of 1000 mg/kg, indicating that the effect of the extract was dose-dependent. Similarly, a significant increase ( $p < 0.001$ ) in percentage inhibition of carrageenan-associated oedema was produced at 250 (52.22 %), 500 (66.13 %) and 1000 (73.52 %) mg/kg while the reference drug diclofenac sodium exhibited 47.95 % inhibition.

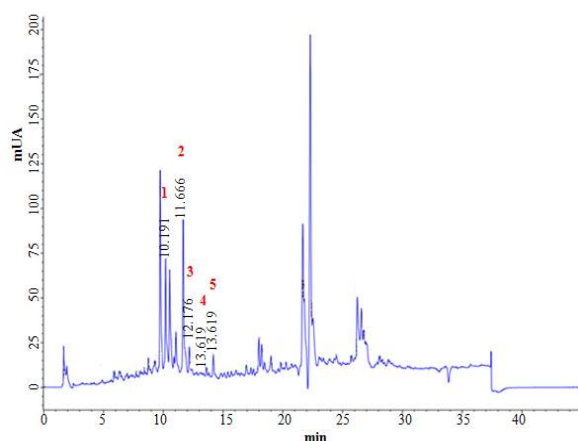
**Table 3:** Anti-inflammatory effect of the aerial parts of *Deverra scoparia* Coss. & Dur. and diclofenac sodium on carrageenan-induced hind paw oedema in mice (RLW right leg weight; LLW left leg weight)

Treatment/dose (mg/kg)	Mean weight (M)		Oedema (%)	Inhibition (%)
	RLW(g)	LLW(g)		
Control (normal saline)	0.1134 ± 0.009	0.0854 ± 0.004	32.78	-
Diclofenac sodium (500)	0.1113 ± 0.004 <sup>*</sup>	0.0955 ± 0.003 <sup>*</sup>	17.06	47.95
Aqueous extract (250)	0.1159 ± 0.003 <sup>*#</sup>	0.1002 ± 0.005 <sup>*#</sup>	15.66	52.22
Aqueous extract (500)	0.1141 ± 0.005 <sup>*#</sup>	0.1027 ± 0.004 <sup>*#</sup>	11.10	66.13
Aqueous extract (1000)	0.1151 ± 0.006 <sup>*#</sup>	0.1059 ± 0.004 <sup>*#</sup>	8.68	73.52

Data represent mean ± SEM (n = 6); <sup>\*</sup>p < 0.001 compared with control group; <sup>#</sup>p < 0.05 compared with aspirin (One-way ANOVA followed by Tukey's multiple comparison test)

### HPLC chromatograms

Phenolic compounds in the aqueous infusion extract of aerial parts of *Deverra scoparia* Coss. & Dur. were identified and quantified by HPLC. The profile (Figure 1) was detected at 365 nm, the wavelength used for the detection of several flavonoid compounds.

**Figure 1:** HPLC profile of phenolic compounds from the aqueous infusion extract of the aerial parts of *Deverra scoparia* Coss. & Dur. measured at 365 nm**Table 4:** Retention times and relative contents of phenolic compounds present in the aqueous infusion extract of *Deverra scoparia* Coss. & Dur.

Peak	Retention time (min)	Phenolic compound	Relative content (mg/g dry weight)
1	10.191	Salicylic acid	21.81
2	11.666	Rosmarinic acid	28.44
3	12.176	Luteolin	6.96
4	13.619	Kaempferol	3.63
5	14.186	Apigenin	5.70

The HPLC chromatogram revealed the presence of several compounds, five were identified by comparison with their retention times and UV absorbance of purified external standards. Three flavonoids (luteolin, apigenin and kaempferol),

and two phenolic acids (salicylic acid and rosmarinic acid) were identified. Table 4 shows the retention times and relative content of the bioactive phenolic compounds present in the aqueous infusion extract of the aerial parts of *Deverra scoparia* Coss. & Dur.

### DISCUSSION

Many people world-wide have depended, and still depend on the natural healing capacity of medicinal plants for their health care needs. Since some plants might have potential toxic effects, toxicity assays are an important aspect of the research on the medicinal plants [17]. In this study, the acute toxicity of the aerial parts of *Deverra scoparia* Coss. & Dur. was investigated. There were no toxicities or mortalities at doses ranging from 2000 mg/kg up to 16000 mg/kg. Thus, based on the toxicity scale of Hodge and Sterner, the aerial parts of *Deverra scoparia* Coss. & Dur. can be considered as non-toxic [18].

In addition, the present work was conducted to assess the analgesic and anti-inflammatory activities of aerial parts of *Deverra scoparia* Coss. & Dur. Acetic acid-induced writhing test is conducted in order to study any drug's peripheral analgesic effects. The analgesic effect of the aqueous infusion extract following intraperitoneal injection of acetic acid is due to the release of several chemical mediators such as histamines and prostaglandins, particularly prostaglandins E2 [19]. Prostaglandins activate the peripheral chemo-sensitive nociceptive receptors, leading to the induction of abdominal constrictions which are accompanied by extension of the forelimbs and elongation of the body [20]. The aqueous infusion extract of *Deverra scoparia* Coss. & Dur. at dose of 250 mg/kg, 500 mg/kg and 1000 mg/kg significantly reduced the number of writhes, surpassing the effect of the reference drug aspirin which was used at a dose of 500 mg/kg.

In addition, the aqueous infusion extract at different doses (250, 500 and 1000 mg/kg) showed significant reduction in the time spent in licking and biting (in both phases), when compared with the reference drug. These results are manifestations of the inhibitory effects of the plant extract on inflammation induced by injection of formalin into the intraplantar region of animal's hind paw. Two phases are involved in the model of nociception, with the neurogenic phase's nociceptive activity is mediated by the transmission of nociceptive impulses, while the anti-inflammatory phase occurs through the activation of central neurons at the spinal cord level [21] involving inflammatory mediators like bradykinin, histamine, serotonin and prostaglandin [1].

The present study shows that the aqueous infusion extract of *Deverra scoparia* Coss. & Dur. significantly reduced carrageenan-induced paw oedema, when compared to the reference drug, diclofenac sodium (500 mg/Kg). This inhibition of inflammation may be due to the blockage of the synthesis of some mediators such as histamines and prostaglandins by the plant extract [21]. In order to reveal the nature of the phytochemical compounds that could be responsible for these biological activities, HPLC analysis was performed. The results showed the presence of three potential bioactive molecules, two of which are phenolic acids, salicylic acid (21.81 mg/g dry weight) and rosmarinic acid (28.44 mg/g dry weight), and one flavonoid aglycone, (apigenin 5.70 mg/g dry weight). These compounds may account for the observed anti-inflammatory and analgesic activities of the extract. Many studies have demonstrated that salicylic acid exerts potential analgesic and anti-inflammatory effect by blocking the enzymes responsible for the production of prostaglandins and thromboxanes [22, 23]. Rosmarinic acid reduces inflammation by inhibiting prostaglandins biosynthesis [24], while apigenin inhibits inflammation by suppressing the expression of cyclooxygenase-2 (COX-2), a pro-inflammation enzyme [25].

## CONCLUSION

The aqueous extract of *Deverra scoparia* Coss. & Dur. possesses analgesic and anti-inflammatory properties. These pharmacological properties could be due to the presence of bioactive phenolic compounds such as salicylic and rosmarinic acids as well as apigenin.

## DECLARATIONS

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### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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