

Composition and Anti-inflammatory Activity of Essential Oil from *Trichodesma afrecanum* (Boraginaceae) endemic in South-West of Algeria

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Abstract:

This present investigation was carried to study chemical composition and anti-inflammatory activities of essential oil of Algerian *Trichodesma africanum* (L) plants as a new natural source of essential oil. Plant material was collected from wild plant populations of *Trichodesma africanum* (L) (family Boraginaceae) growing on Gebel Taghit region in Bechar (Algeria). The essential oils obtained by water distillation from aerial parts fraish of *Trichodesma africanum* (L) yielded 1.6%. Thirty-five constituents representing 99.86% of the Algerian *Trichodesma africanum* (L) essential oil were identified. The major components of Algerian *Trichodesma africanum* (L) essential are (2E, 6E)-Farnesol (61.26%), Nonalactone< γ -> (20.28%). Minor component are Methyl farnesoate (0.01%), β -Costol (0.02%). The in vivo anti-inflammatory activity was evaluated using the model of formol induced pate edema.

Keywords: *Trichodesma afrecanum*; Boraginaceae; essential oil; anti-inflammatory activity.

1. Introduction

Trichodesma afrecanum (Linn.). is one of 2700 species in Boraginaceae family, is an erect, and annual herbaceous perennial herb, (Retief, 2002; Hegazy *et al.*, 2005; Benlakhdar *et al.*, 2021), about 1 m tall. It branches mainly from the base and has a fistular stem that is densely covered with stiff, thorny hairs about 2mm in length. The leaves of this plant are simple with entire margins, which are covered on both surfaces; the flower is small, pale and tied along the peduncle. The peduncle and calyx are dark pink to brown and covered with fine white hairs (Ahmed *et al.*, 2015, Jaradat *et al.*, 2016) it is known in the tropics and subtropics of Africa, Asia and Australia (Omar *et al.*, 1983; Yumkham *et al.*, 2019). *Trichodesma afrecanum* consisting of triterpenes, steroids, tannins, coumarins, flavonoids, phenolics, alkaloids (Alrawili *et al.*, 2020) glycosides and other constituents (Younis and Adam, 2011). *Trichodesma afrecanum* specie used in traditional medicine for wound healing, analgesic (Saive *et al.*, 2020) to treat snakebite poisoning, fever, skin diseases, dysentery, arthritis, diarrhea, diuretic (Ayodele *et al.*, 2015), anorexia, anti-cold and anti-microbial (Abdallah *et al.*, 2013; Górnas *et al.*, 2019). The purpose of this study was to investigation the chemical composition and anti-inflammatory activities of *Trichodesma africanum* volatile oils.

2. Materials and Methods

2.1. Plant material

Fresh aerial parts of *Trichodesma africanum* were collected from the region of Taghit 90 km of Bechar in South-West Algeria in April 2017 at the flowering stage (Figure 1). The botanical identification and the voucher specimen are conserved at Medicinal plant encyclopedia herbarium of the Bioactive Molecules and Chiral Separation Laboratory under accession number MPE15-4-E2.



Figure 1. Plant of specie *Trichodesma africanum* endemic in south-west of Algeria

2.2. Animals

Albinos Swiss mice weighing 23-36g allocated in different groups (n=6 per group) were used in the experiment of acute toxicity and evaluation of anti-inflammatory activity. Animals were obtained from Pasteur Institute Algiers. They were housed at 22 ± 2°C. The photoperiod is 12/24 hours.

2.3. Essential oil extraction

Essential oil was obtained by hydro-distillation (4h) from fresh aerial part (800g). The oil after preparation was submitted to GC/MS analysis.

2.4. GC-MS analysis

Gas chromatography-Mass spectrometry: The GC-MS analyses were carried out on a GC/MS BRUKER Chemical Analysis equipped with a DB-5 fused silica column (25 m×0.25 mm i.d.), Oven temperature was 40-240°C at a rate of 4°C min⁻¹, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm sec⁻¹, split ratio 1/60, flow rate 2 mL min⁻¹, Ionization energy 70 eV; scan time 1 sec, mass range 40-400 amu. The components of the oils were identified by comparison of their mass-spectra with those of a NIST Spectral Library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds. Kovat's indices were determined by co-injection of the sample with a solution containing homologous series n-alkanes (C9 -C40) and retention indices with (Adams, 1995).

2.5. Toxicity

The acute toxicity test in mice was performed. Male and female mice weighing 23-36 g were separated into test and control groups composing six

(n=6) animals in each group. The test was carried out using intra-planta (IP) doses of the essential oil of the *Trichodesma afrekanum* species: 25, 50, 150 and 200 mg / kg in body weight. The control group received only physiological saline (25 µl / kg). The experimental mice were allowed to eat; all were kept under regular observation for 48 h, for any mortality or behavioral change.

2.6. Study of anti-inflammatory activity

We checked the inhibitory action of the volatile oil on the edema caused by the injection of 1% of a solution of formalin in physiological saline (NaCl 0.9%) at the dose of 0.025ml/paw, according to the method of winter (Winter *et al.*, 1962). The measurements of the volumes of the left hind paw of each mouse were carried out before the induction of the edema and every 30, 60, 120 and 180 minutes after the injection of the formalin. 30 minutes before the injection of formalin, the different groups of mice received intra-peritoneal different treatments: The control group of 6 mice received physiological saline (0.9%). The two experimental groups of 6 mice each received the oil at the dose of 150 and 200mg / kg of body weight. A group of 6 mice received diclofenac intraperitoneally as a reference product at a dose of 25 mg/kg.

2.7. Statistical analysis

Data are presented as mean $\pm$ S.E.M values by Student's t test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Chemical analyses of essential oil

Thirty-five compounds were identified in the transparent volatile oil obtained of *Trichodesma afrekanum* grown wild in Algeria with a yield of 1.6% (w/w). As shown in (Table 1), the compounds presented about (99.86%) of total oil, of which (65.30%) are sesquiterpenes and for the monoterpenes are present at (6.91%), other constituents, are also identified during chemical analysis present with a rate of (27.65%). The majority compounds are (2E, 6E)-Farnesol (61.26%), Nonalactone< γ -> (20.28%). Minority compounds we distinguish some constituents which have a low quantity Menthene<1-p-> (0.02%), Isobaeckeol (0.06%), β -Costol (0.02%) and Methyl farnesoate (0.01%).

Table 1. Composition of the essential oil of *Trichodesma afrekanum*

N°	Compound	Rt	Area%	RI _{Exp}	RI _{Lit.}	References
01	α -phellandrene	3.95	2.45	1004	1004	(Babushok <i>et al.</i> , 2001)
02	Menthene<1-p->	5.65	0.02	1025	1026	(Adams , 2007)
03	Phenyl acetaldehyde	7.31	0.24	1046	1047	(Goodner , 2008)
04	2E-Octenal	8.21	0.55	1058	1058	(Üçüncü <i>et al.</i> , 2010)
05	Cineole 1,4-	12.24	0.38	1109	1110	(Davies ,1990)

06	Fenchol,endo-	12.70	0.24	1115	1115	(Babushok <i>et al.</i> , 2001)
07	Tans-Thujone	12.81	0.87	1117	1117	(Asta&Jurga, 2018)
08	Menthan-8-ol <i>p</i> -	16.27	0.64	1161	1162	(Davies ,1990)
09	Octyl acetate	19.99	1.10	1211	1211	(daSilva <i>et al.</i> , 1999)
10	Citronellol (β -)	20.27	0.43	1215	1215	(Davies ,1990)
11	Ethyl oct-(2E)-enoate	22.55	0.78	1249	1249	(Adams , 2007)
12	Linalyl acetate	23.25	2.31	1259	1259	Goodner , 2008)
13	Geranyl formate	26.20	0.27	1303	1303	(Babushok <i>et al.</i> , 2001)
14	Nonyl acetate	26.52	0.37	1309	1309	(Babushok <i>et al.</i> , 2001)
15	Nonalactone< γ ->	29.63	20.48	1361	1361	(Adams , 2007)
16	α -Methylbenzyl butyrate	29.87	0.48	1365	1365	(Adams , 2007)
17	Anastreptene	31.45	0.39	1391	1391	(Davies ,1990)
18	Copaene α -	31.85	0.21	1398	1398	(Davies ,1990)
19	Anthranilate<ethyl->	32.74	0.25	1415	1415	(Adams , 2007)
20	Terpinyl propionate	33.34	0.16	1426	1426	(Davies ,1990)
21	Thujopsene	33.57	0.66	1430	1430	(Davies ,1990)
22	Macrocarpene<ar->	38.65	0.36	1527	1526	(Adams , 2007)
23	Phenyl ethyl tiglate<2->	41.41	0.62	1584	1584	(Adams ,2003)
24	Gleenol	41.52	0.62	1586	1586	(Dioli <i>et al.</i> ,2011)
25	Fokienol	42.01	0.74	1596	1596	(Adams , 2007)
26	Hinesol	45.51	1.64	1638	1638	(Milton <i>et al.</i> ,1999)
27	Methyl epi-jasmonate«Z»->	48.98	0.74	1679	1679	(Adams , 2007)
28	(2E,6E)-Farnesol	55.11	61.26	1743	1743	(Babushok <i>et al.</i> , 2001)
29	Isobaeckeol	56.14	0.06	1754	1754	(Adams , 2007)
30	Santalol acetate «Z»- α ->	58.53	0.18	1778	1778	(Adams , 2007)
31	8- α -acetoxylemol	58.58	0.11	1779	1779	(Adams ,2003)
32	β -Costol	58.67	0.02	1780	1780	(Zito <i>et al.</i> , 2013)
33	Methyl farnesoate	59.08	0.01	1784	1784	(Adams , 2007)
34	Eudesmol acetate < β ->	59.84	0.05	1791	1792	(Adams , 2007)
	Isovalencenol<(E)->	59.96	0.17	1793	1793	(Adams , 2007)

Totale	99.86%
Sesquiterpènes	65.30%
Mono terpènes	6.91%
Autres	27.65%

3.3. Anti-inflammatory activity

The anti-inflammatory action was mediated in vivo by formalin-induced mouse paw edema. The results obtained were compared with those of diclofenac and the control. Edema is assessed by the size of the mice's paws throughout the experiment.

3.3.1. Formalin-induced edema

The injection of formalin into the left paw of the mouse, having received only physiological water, causes edema immediately after this injection. The increase in the volume of the mouse paw is 0.10 ± 0.03 , 0.16 ± 0.05 , 0.22 ± 0.05 and 0.25 ± 0.03 respectively at 30, 60, 120 and 180min (Table 1). In the presence of diclofenac and *Trichodesma afrecanum* oil, less significant increases in paw volume were observed (Table 2).

Table 2. Effect of the essential oil of the aerial part of *Trichodesma afrecanum* on formalin-induced mouse paw edema.

Group	Doses	30min	60min	120min	180min
Control	5ml/kg	0.10 ± 0.03	0.16 ± 0.05	0.22 ± 0.05	0.25 ± 0.03
EOTa	150mg/kg	0.09 ± 0.02	0.08 ± 0.02	0.05 ± 0.03	0.03 ± 0.02
EOTa	200mg/kg	0.07 ± 0.03	0.08 ± 0.02	0.05 ± 0.01	0.03 ± 0.01
Diclofenac	25mg/kg	0.07 ± 0.02	0.06 ± 0.02	0.03 ± 0.02	0.02 ± 0.02

EOTa= Essential Oil of *Trichodesma afrecanum*; data are expressed as mean $\pm$ standard error to mean. Significance levels in comparison to control values are * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
n = 6

The administration of the oil from the fresh aerial part of *Trichodesma afrecanum* at a dose of 150 mg / kg prevents very significantly ($p < 0.01$) and significantly ($p < 0.001$) the edema of the paw. Mice induced by formalin with 0.08 ± 0.02 , 0.05 ± 0.03 and 0.03 ± 0.02 ml at 60, 120 and 180min. The injection of diclofenac at a dose of 25 mg / kg by the i-p route significantly prevents the increase in the volume of the mouse paw. It is 0.06 ± 0.02 , 0.03 ± 0.02 and 0.02 ± 0.02 at 60, 120 and 180min after the formalin injection. Regarding the doses of *Trichodesma afrecanum* oil at 200mg / kg, the volume increases significantly different from those of physiological control. They are and 0.05 ± 0.01 , 0.03 ± 0.01 and 0.01 ± 0.01 ml at 60, 120 and 180min respectively.

3.3.2. Anti-inflammatory effect of the essential oil

The anti-inflammatory effects of the essential oil of *Trichodesma afrecanum* were evaluated initially in the paw edema model. The results in (Figure 2) indicate that the essential oil of *Trichodesma afrecanum* dose-dependently reduced the formol induced paw edema in mice when tested at the doses of 150 and 200 mg/kg b.wt. (30 min beforehand).

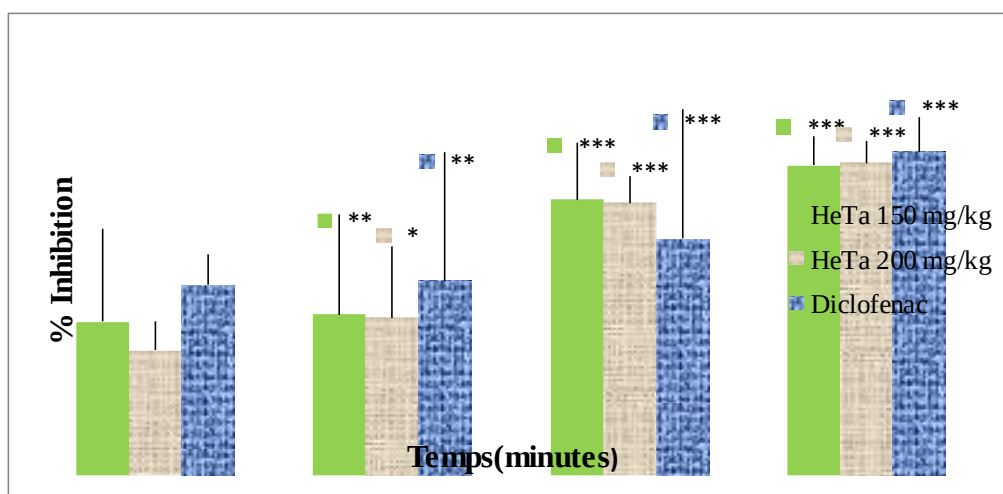


Figure 2. Percentages of inhibition of formalin-induced mouse paw edema of different doses of *Trichodesma afrekanum* essential oil.

The percentages of inhibition significant obtained for the group treated with the dose of 150 mg/kg were the following: 44.40 ± 27.87 ; 76.22 ± 15.54 and $85.86 \pm 7.85\%$ at 60, 120 and 180 min after formol injection, respectively. Also inhibited in a significant manner by treatment with the essential oil of, *Trichodesma afrekanum* 200 mg/kg, (30min beforehand, with inhibitions of 43.47 ± 19.66 ; 75.34 ± 7.30 and $86.34 \pm 6.35\%$ at 60, 120 and 180min after formol injection, respectively. Administration of diclofenac intraperitoneally at a dose of 25 mg / kg greatly reduced formalin edema ($p \leq 0.001$) after injection of the inflammatory agent. Maximum inhibition is obtained at 180 min, for which the percentage inhibition is 89.43 ± 9.50 .

4. Discussion

The essential oils obtained by hydrodisillation from the fresh aerial part of *Trichodesma africanum* (L) gave 1.6% w / w (fresh weight) and relatively higher than those obtained from the aerial part of *T. africanum* (Santos *et al.*, 2006; Diniz *et al.*, 2008), which were of the order of 0.3% by hydrodistillation, 1.2% by microwave, 0.43% by Ultrasonic-assisted and 0.2% respectively, We note that the best yield obtained by (Jaradat *et al.*, 2016) in their study on leaves of *T. afrekanum* (1.8%) by Microwave-ultrasonic. In the present study, we observed that sesquiterpene are the majority compounds compared to monoterpenes and non-terpene compounds, the same observation was reported in a study conducted by (Ahmed *et al.*, 2015) In the present study, the anti-inflammatory potential of oil from the fresh aerial part of *Trichodesma afrekanum* was evaluated in vivo on the paw of mice by creating edema by formalin. In control mice, sub plantar injection of formalin produced local edema, which gradually increased after injection of the phlogiston agent. The volume of the mouse's paw was measured at 30, 60, 120 and 180 minutes after injection of formalin. Diclofenac at a dose of 25 mg / kg reduced the volume of paw edema very and highly significantly by 0.06 ± 0.02 , 0.03 ± 0.02 and 0.02 ± 0.02 ml at 60, 120, and 120 minutes, respectively. At a dose of 150 mg / kg, *Trichodesma afrekanum* oil very and highly significantly inhibited the development of formalin-induced mouse paw edema from 60 minutes into the experiment, with a maximum reduction of $85.86 \pm 7.85\%$ at 180 minutes. On increasing the dose to 200 mg / kg, the oil showed anti-inflammatory activity these effects inhibited significantly and highly significantly.

5. Conclusion

The yield of essential oil of the fresh aerial parts of *Trichodesma afrecanum* was 1.61% (w/w). The essential oil analyzed by GC/MS was composed mainly of sesqui-terpenes; its main constituents being (2E, 6E)-Farnesol (61.26%), Nonalactone< γ -> (20.48). In the present work, the anti-inflammatory effect of *Trichodesmaafrecanum* essential oil was investigated. The results obtained show that the essential oil has anti-inflammatory effects. During the test of inhibition of the development of formalin-induced paw edema in mice, it can be concluded that the essential oil of the fresh air part has a significantly higher anti-inflammatory effect than that of the control. The evaluation of the anti-inflammatory effects of essential oil shows that this plant has a pharmacological power that supports its traditional use for the relief of various inflammatory diseases.

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