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Chemical Composition and Anti-inflammatory Activity of the Essential Oil of *Echium humile* (Boraginaceae) in vivo from South-West of Algeria

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Abstract

The objective of this work is to study and analyze the chemical composition and anti-inflammatory effect of the essential oil of *Echium humile*. The essential oil of the fresh aerial parts is obtained by hydrodistillation where 37 compounds are identified by GC-MS analysis. The major constituents are bicycloelemene (15.9%), pentacosane (8.4%), p-cymen-8-ol (5.8%), β-phellandrene(4.9%), trans-thujone (4.1%). The minor constituent is camphene hydrate (0.5%). The anti-inflammatory effect of the essential oil at the doses of 150 and 200 mg/kg compared with the control and the reference drug (Diclofenac) on local inflammation by formalin-induced mouse paw edema revealed considerable anti-inflammatory properties of this oil.

Keywords: Boraginaceae , *Echium humile* , essential oil, anti-inflammatory activity

1. Introduction

The Boraginaceae family incorporates more than 2700 species and 200 genus usually found in cosmopolitan, living spaces particularly in tropic (Ceramella *et al*., 2019 ; Ahmad *et al*., 2018 ; Tarimcilar *et al*., 2015). This family is medicinally used as antimicrobial, antitumor, anti-inflammatory (Dresler *et al*., 2017), antioxidant, immomodulatory, emolient, sedative and antianxiolytic (Zarghami *et al*., 2018) . *Echium humile* Desf. (syn, *Echium pycnanthum* ssp.) (Mahklouf *et al*., 2018) (common name : Hemimiche, Ouacham(Vipérine) (Slimani *et al*., 2018 ; Laallam *et al*., 2011), is a wild plant species of Boraginaceae family, commonly found in dry spaces and desert , well-known as a traditional remedy, usually used to treat liver disease , digestive ailments and hepatitis (Chaouche *et al*., 2012 ; Miara *et al*., 2018). The *Echium humil* with several flowering stems and dense transparent bristles. (Ozenda., 1977) , The bibliography search results in a lack of studies on the chemical composition and biological evaluation of essential oil of *E. humile*, so we decided to extract the volatile oil and analyzed these chemical constituents followed by a study of its anti-inflammatory activity. The anti-inflammatory activity of the essential oil of *E. humile* is like that of *cordia verbenceae* in general (Benzie and Strain, 1996).

2. Materials and Methods

2.1. Plant material

Fresh aerial parts of *Echium humile* were collected from the region of Bechar (Latitude : 31°58′20″ N ; Longitude : 2°16′20″ W ; 949 m) in South-West Algeria in April 2017 at the flowering stage. 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 MPE14-3-E1

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 (8h) from fresh aerial part (1200g). The oil after preparation was submitted to GC/MS analysis.

2.4. GC-MS analysis

GC/MS data of the *Echium humile* essential oil were carried out using a BRUKER Chemical Analysis, equipped with an DB-5 capillary column (25 m x 0.25 mm ; film thickness 0.25µm). The oven temperature was

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held at 60°C for 5 min and then 60-220°C at 4°C/min ; carrier gas helium with a flow rate of 2ml/min., injector temperatures were set at 240°C, split ratio 1/40. Ionization energy 70 eV ; masse range 40-400 amu. Compounds of the *Echium humile* essential oil were identified by comparison of their retention indices (RI) to n-alkanes (C9 -C40) and retention indices with NIST Spectral Library and literature.

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 sixe (n=6) animals in each group. The test was carried out using intra-planta (IP) doses of the essential oil of the *Echium humile* 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-peritoneally different treatments: The control group of 6 mice received physiological saline (0.9%) at the dose of 10µl/g. 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 10µl/mg.

2.7. Statistical analysis

Data are presented as mean ± S.E.M values and analyzed by ANOVA followed by Student's *t* test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Chemical analyses of essential oil

Thirty-seven compounds were identified in the light yellowish volatile oil obtained of *Echium humile* grown wild in Algeria with a yield of 1.2% (w/w). As shown in (table 1), the compounds presented about (83.5%) of total oil, where the major compounds were bicycloelemene (15.9%) and pentacosane (8.4%), p-cymen-8-ol (5.8%), β-phellanddrene (4.9%), trans-thujone (4.1%), whereas the minor component is camphene hydrate (0.5%). The oil comprises eleven monoterpenoids (22.2%), thirteen sesquiterpenoids (33.7%), three diterpenoids (6.0%), one sesterpenoids (8.4%) and nine non-terpenes (13.2%). The unidentified compounds represent (4.1%) of oil.

3.2. Toxicity

Intra-peritoneal administration (IP) of 25, 50, 150 and 200 mg / kg of essential oil to the different groups of mice did not show any particular clinical sign, and no death was observed (100% survival rate) during the 48 hours of observation. The absence of dead mice and undesirable effects during the 48 hours of observation indicates that the tested essential oil is not toxic by intra-peritoneal (IP) administration in a single dose.

3.3. Anti-inflammatory activity

The study was designed to assess the anti-inflammatory activity of the essential oil of the *Echium himule* plant. The anti-inflammatory action was carried out *in vivo* by edema formalin-induced mouse paw administration of oil at a dose of 150mg / kg significantly prevents the paw edema induced by formalin. The increase in percentage of inflammatory edema of the paw is 40±7%; 60±9%; 46.6±6.33% and 33.3±6.33% compared to the control group treated with physiological saline where the increase in edema is 60±5.17%; 80±8.67%; 86.6±8.33% and 93.3±3.83% after 30, 60, 120 and 180 minutes after injection of formalin respectively. At 200 mg / kg, the essential oil of *Echium humile* shows better prevention of paw edema formalin-induced versus dose 150 mg / kg. The increased percentages of inflammatory edema of the paw are less important. They are only 38.46±5%; 46.15±5.67%; 30.76±3.50% and 23.07±2.33% after 30, 60, 120 and 180 minutes respectively after injection of formalin (Table 2).

Assessment of percentage of inhibition shows that the essential oil of the *E. humile* has a greater anti-inflammatory activity in the fourth phase of this process. A better inhibition of inflammatory edema of the mouse paw was observed after 180mn (Figure 1) with a percentage of inhibition of 75.27±20.78%. After 120 minutes the essential oil shows an inhibition of 64.4±9.9% which is equal to the reference group treated with diclofenac. Hence the administration of the essential oil in the prevention of inflammatory edema with formalin by intra-planta administration has proved effective, in a dose-dependent manner (150 and 200 mg / kg). However this anti-inflammatory effects was weak in the initial period of edema but important later on (180mn).

Table1. Composition of the essential oil of *Echium humile*

N°	Compound	Rt	Area (%)	RI_{Expe}	RI_{Litu}	Reference
1	β-phellandrene	5.5	4.9	1041	1042	(Goodner, 2008)
2	β-Ocimene, (E)-	5.7	0.6	1047	1047	(Babushok *et al*., 2011)
3	Cis-sabinene	6.5	1.5	1067	1068	(Davies, 1990)
4	Terpinolene	7.0	0.6	1081	1081	(Davies, 1990)
5	Cis-thujone	8.0	0.8	1105	1106	(Tunalier *et al.* , 2002)
6	Menth-2-en-1-ol cis-p-	8.2	1.4	1111	1111	(Davies, 1990)
7	Trans-thujone	8.4	4.1	1116	1117	(Tunalier *et al*., 2002 ; Asta *et al.*, 2018)
8	Camphene hydrate	9.7	0.5	1148	1148	(Babushok *et al*., 2011)
9	Pinocarvone	10.2	0.8	1160	1160	(Babushok *et al*., 2011)
10	1-Nonanol	10.7	0.8	1173	1173	(Babushok *et al*., 2011)
11	Borneol	10.8	1.2	1177	1177	(Davies., 1990)
12	p-cymen-8-ol	11.1	5.8	1184	1183	(Babushok *et al*., 2011)
13	Isopulegyl acetate	13.9	1.1	1257	1258	(Davies, 1990)
14	p-Anisyl alchohol	14.9	2.8	1282	1282	(Babushok *et al*., 2011)
15	Dihydrocarvyl acétate	16.4	0.6	1319	1319	(Davies, 1990)
16	Bicycloelemene	16.8	15.9	1331	1333	(Babushok *et al*., 2011)
17	α-cubebene	17.5	1.1	1348	1348	(Goodner, 2008)
18	Longifolene	19.7	3.1	1405	1405	(Babushok *et al*., 2011 ; Santos *et al*., 2006)
19	γ-elemene	20.9	1.4	1444	1444	(Santos *et al*.,2006)
20	α-Patchoulene	21.4	0.9	1456	1457	(Babushok *et al*., 2011)
21	δ-cadinene	23.7	1.0	1526	1526	(Asta *et al*., 2018)
22	trans-Nerolidol	24.6	3.4	1552	1553	(Davies, 1990)
23	Germacrene B	24.7	1.4	1555	1554	(Deoliveira *et al*., 2007)
24	cis-Muurol-5-en-4-α-ol	24.9	2.2	1560	1560	(Khan *et al*., 2016)
25	Geranyl isovalerate	26.2	0.9	1599	1599	(Babushok *et al*., 2011)
26	Cadin-4-en-7-ol <cis->	26.5	1.3	1636	1636	(Adams, 2007)
27	Bisabolol<β->	26.7	0.9	1674	1675	(Adams, 2007)
28	Geranyl tiglate	26.9	2.5	1695	1695	(Adams, 2007)
29	β-Eudesmol, acetate	27.5	0.7	1792	1792	(Adams, 2007 ; Zito *et al*., 2013)
30	Hexadec-9-enoic acid,(Z)-	29.2	0.8	1950	1951	(Babushok *et al*., 2011)
31	α-Cadinol	33.7	0.7	2255	2255	(Tunalier *et al*., 2002)
32	Libocedrol	36.0	1.1	2345	2345	(Adams, 2007)
33	4-Epi-dehydroabietol	36.4	3.5	2357	2359	(Angelopouluo *et al*., 2001)
34	Ferruginol acetate< trans>	36.5	1.2	2362	2363	(Asuming *et al*., 2005)
35	Manoyl oxide	37.0	1.7	2379	2378	(Deoliveira *et al*., 2007)
36	Pentacosane	39.9	8.4	2498	2499	(Üçüncu *et al*., 2010)
37	5-ethylpentacosane	44.0	1.9	2669	2668	(Ramaroson *et al*., 1997)
38	Unknown	50.1	4.1	2951	-	-

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

Treatment groups (n =6)	Doses	30 min	60-min	120-min	180-min
Control (physiological saline)	5ml/kg	60±5.17%	80±8.67%	86.6±8.33%	93.3±3.83%
EO*Eh*	150mg/kg	40±7%	60±9%	46.6±6.33%	33.3±6.33%
EO*Eh*	200mg/kg	38.4±5%	46.1±5.67%	30.7±3.50%*	23.07±2.33%
Diclofenac	10µl/g	23.07±3.67%	35.4±6%	30.7±3.17%**	15.3±2%

EO*Eh*= essential oil of *Echium humile*; data are expressed as mean ± standard error to mean. Significance levels in comparison to control values are *p<0.01; **p= 0.001. n = 6

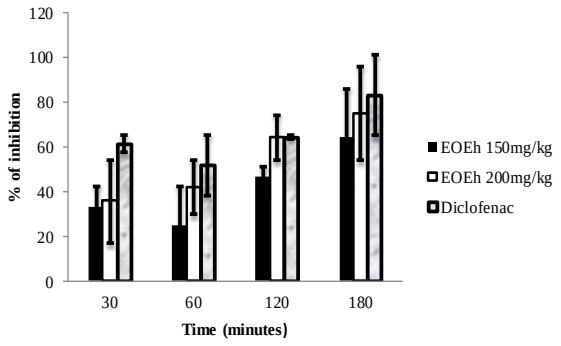

Figure 1. Percentage of inhibition of formalin-induced mouse paw edema of different doses of *Echium humile* essential oil.

Discussion

4.1. Volatile compounds composition

Essential oil of fresh flowering aerials parts of *Echium humile* has a light yellowish color and the yield was 1.2 % (w/w). This oil was averred to be rich in sesquiterpenoids and monoterpenoids. The identified sesquiterpene hydrocarbons were bicycloelemene (15.9%), longifolene (3.1%), germacrene B(1.4%) , γ-elemene (1.4%), α- cubebene (1.1%), δ-cadinene (1.0%), α-patchoulene (0.9%). The oxygenated sesquiterpens constituents in essential oil including geranyl tiglate (2.5%), cis-Muurol-5-en-4-α-ol (2.2%) , Cadin-4-en-7-ol

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<cis-> (1.3%) , Bisabolole<β>(0.9%), Geranyl isovalerate (0.9%), α-Cadinol (0.7%). On the other hand, the monoterpenoids with 22.2% , are mainly p-cymen-8-ol (5.8%), β-phellandrene (4.9%) and trans-thujone (4.1%). The other compounds are deterpenoids , sesterpenoids and non-terpenoids accounting for 6.0% , 8.4% and 13.2% respectively besides one unknown compound (4.1%).

4.2. Anti-inflammatory Activity

In our tests, the drug diclofenac is used as a reference substance. The anti-inflammatory potential of oil *was* evaluated in vivo on the paw of the mouse by creating an edema with formalin.

In the control animals, the sub-plantar injection of formalin produces local edema which gradually increases. The volume of the mouse paw was measured at 30, 60, 120 and 180 minutes after injection of formalin. Diclofenac, at 10 µl / kg, significantly reduces the volume of paw edema by 35.4±9.25%; 30.76±4.55% and 15.37±10.85% after 60, 120 and 180 minutes respectively.

At 150 mg / kg, the essential oil remarkably inhibited the development of formalin-induced edema of the paw from the first 60 minutes, with a maximum reduction of 64.30±21.93% after 180 minutes. However, the anti-inflammatory effect remains weaker than that of diclofenac (10 µl / kg ip) during the 180 minutes. By increasing the dose to 200 mg / kg, the essential oil showed strong anti-inflammatory activity. These effects are greater compared to those obtained with diclofenac (10 µl / kg ip).

The results obtained from anti-inflammatory tests show that the essential oil of *Echium humile* has an effect on inflammation and significantly reduces the formalin-induced paw edema, with a good dose – effect relationship, better inhibition is obtained at 200 mg / kg.

The effects of the essential oil of *Echium humile* manifested the same anti-inflammatory mechanism due to diclofenac, from the 120 minutes period. It is concluded that the essential oil of *Echium humile* contains compounds which has anti-inflammatory activity, such as diclofenac used as a reference substance in our tests. Also, the important effectiveness of this essential oil could be linked to the chemical profile, because it is rich in biologically active molecules such as sesquiterpenes, monoterpenes.

5. 5. Conclusion

The essential oil from the fresh aerial part of the *Echium humile* plant with a yield of 1.2% (w / w) and composed mainly of mono- and sesqui-terpenids. These majority constituents are bicycloelemene (15.9%); pentacosan (8.4%) p-cymen-8-ol (5.8%). The essential oil of *Echium humile* showed significant anti-inflammatory activity compared to the control and the animal reference group. It is the prime study determining the chemical composition and anti-inflammatory activity of the essential oil of the fresh aerial part of the species *Echium humile*.

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