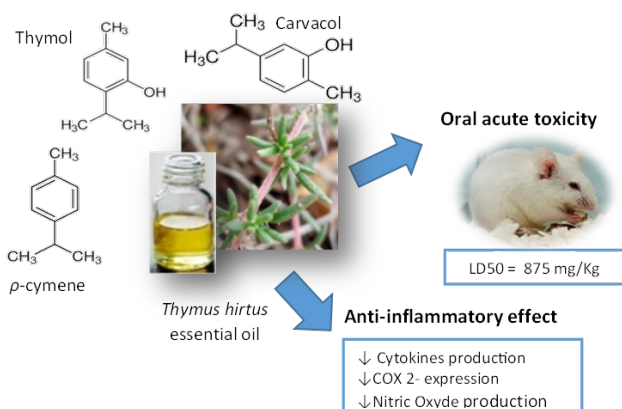


Chemical composition, oral acute toxicity and anti-inflammatory activity of *Thymus hirtus* Willd. essential oil.

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Abstract: This study aimed to determine the chemical composition of *Thymus hirtus* essential oil and to assess its acute toxicity and anti-inflammatory potential. This essential oil extracted by hydrodistillation from the leaves has a yield of 2.08 ± 0.5 %. The identification of its chemical composition was conducted using both GC-FID and GC-MS. Fifty-four constituents were detected, representing 98.53 % of the essential oil's composition. The major constituents were : thymol (48.5 %) and carvacrol (17.75 %). The oral acute toxicity of this essential oil was evaluated in mice by administering single doses ranging from 50 to 2000 mg/kg. The lethal dose (LD50) obtained was equal to 875 mg/kg. The anti-inflammatory activity was assessed using carrageenan-induced paw edema test in mice. A notable reduction of paw edema was obtained at dose of 50 mg/kg (50.9 %) and 100 mg/kg (65.6 %). The reported anti-inflammatory activity represents a significant initial outcome, underscoring the relevance of this work and paving the way for future investigations to validate and extend these findings.



Keywords: *Thymus hirtus*, essential oil composition, acute toxicity, anti-inflammatory activity

1 Introduction

The Lamiaceae family is widely used as source of spices and is a well-established source of functional ingredients¹⁾ within this family, the genus *Thymus* has attracted particular interest due to its bioactive properties related to food applications²⁾. *Thymus* is one of the most taxonomically complex genera, comprising 350 species and varieties of wild-growing evergreen perennials and subshrubs native to Southern Europe, North Africa, and Asia³⁾. Among the various biological properties reported for thyme, some are well-established, including antioxidant, insecticidal, antibacterial, antifungal, antiviral and anti-inflammatory activities^{4, 5)}. These activities are primarily attributed to its high content of polyphenols and other bioactive volatile compounds such as thymol, p-cymene, γ-terpinene, linalool, germacrene and borneol^{6, 7)}.

Thymus hirtus Willd. (*syn. Thymus wilddenowii* Boiss & Reut.), a Lamiaceae species bearing purplish-pink flowers, is an aromatic shrub of notable importance within North African *Thymus* taxa^{8, 9)}. Endemic to this region, it is traditionally incorporated into culinary preparations and employed in folk medicine for its antimicrobial, antispasmodic, and expectorant activities^{10, 11)}. Its essential oil displays a remarkably complex and variable composition, enriched in oxygenated monoterpenes and monoterpene hydrocarbons. Published analyses consistently report that compounds such as 1,8-cineole, camphor, α-pinene, terpinen-4-ol and linalool represent major constituents, with terpinen-4-ol and linalool reaching

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up to 33 % and 22 %, respectively^{12–15}). Regional studies, particularly in Tunisia and Algeria, reveal distinct chemotypes, some dominated by caryophyllene oxide, linalool and thymol, highlighting the strong influence of environmental factors such as geographic origin, climate and soil conditions on secondary metabolite profiles^{14,15}).

The anti-inflammatory activity exhibited by some *Thyme species* essential oils have been demonstrated by many previous studies^{16–18}). However, quantitative data regarding both the toxicity and anti-inflammatory properties of this endemic species remain lacking, highlighting a critical gap in the literature. Addressing this gap could provide valuable insights into its pharmacological potential and support the development of safe and effective natural therapeutics. Therefore, this study aims to characterize and evaluate the acute toxicity, as well as anti-inflammatory activities of *T. hirtus* essential oil.

2 Experimental procedures

2.1 Plant material and essential oil extraction

The leaves of *T. hirtus* were collected during the flowering stage in March 2025, from El Hamdania-Medea (Northeast of Algeria – latitude: 36.45,36',25"N; longitude: 2.73333 36° 27' 0" North, 2° 43' 60" East; altitude: 580 m). The identification was performed by *Dr Hammani* at *El-Hamma Botanical Garden* (Algiers). A voucher specimen has been preserved at the Herbarium of Biology Department at *Blida-1 University* (Voucher number: PAM-113). Dried leaves (100 g) were subjected to hydro-distillation for 3 hours using a *Clevenger-type* apparatus. The collected essential oil (EO) was dried over anhydrous sodium sulfate and stored in a dark glass vial at 4 °C.

2.2 Essential oil analysis

The chemical composition of the essential oil was analyzed using both gas chromatography with flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC-MS). GC-FID analysis was performed on a Hewlett-Packard HP 6890 system equipped with an HP-5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The injector and detector temperatures were set at 250 °C and 300 °C, respectively. A 1 µL aliquot of a 5 % v/v solution in n-hexane was injected in split mode (1:50). Helium was used as the carrier gas at 1.7 mL/min. The column temperature was programmed from 50 °C to 200 °C at 4 °C/min, then held at 200 °C for 5 min to ensure complete elution of late-eluting compounds.

GC-MS analysis was conducted on an Agilent HP 6890 GC coupled with an HP 5973 mass selective detector, controlled via HP Enhanced ChemStation software. The same HP-5MS column was used, with the injection port maintained at 250 °C in splitless mode. The oven temperature was programmed from 40 °C to 250 °C at 6 °C/min. Helium served as the carrier gas at 1 mL/min. Mass spectra were acquired in electron ionization mode (70 eV), scanning a mass range of 35–500 m/z, with source and quadrupole temperatures of 230 °C and 150 °C, respectively.

Identification of the essential oil constituents was achieved through comparison of retention indices with literature values and matching mass spectra against the Wiley 275.L library, with additional confirmation for sesquiterpene hydrocarbons using published spectra reported by Adams¹⁹).

2.3 Oral acute toxicity

Oral acute toxicity essay was conducted according to Tahraoui et al. protocol²⁰), with slight modifications. Healthy *Albino* mice of either sex, weighing between 20 and 25 g (*SAIDAL Antibiotical, Algeria*) were distributed into four groups of five mice each. The animals, housed in plastic cages, were kept at a temperature of 24 ± 0.3 °C for 12 hours. They had free access to tap water and regular rodent food, except for a brief fasting period before treatment. The EO was dissolved in a mixture of Tween 80 and physiological saline (v:v) and adjusted to a volume of 10 ml/kg per dose, and administered using a feeding probe at doses of 0, 50, 100, 500, 1000, and 2000 mg/kg body weight. Mice were monitored continuously for 1 hour after treatment for general behavioral changes, signs of toxicity and mortality, then intermittently for 4 hours, and subsequently over a 24 h period. Further observations continued for up to 14 days post-treatment, noting any behavioral changes, signs of toxicity, or death, in order to calculate the lethal dose 50 (LD50).

2.4 Anti-inflammatory activity

The anti-inflammatory activity of the EO was investigated on carrageenan-induced inflammatory paw edema described by Mansour et al²¹), with slight modifications. The EO was dissolved in a mixture of physiological saline solution (0.09 %) and Tween 80 (v:v), and administered using a feeding probe to a pretreated group of five mice at doses of 50 and 100 mg/kg. The negative control group received the same volume of the mixture solution (physiological saline (0.09 %) + Tween 80, v:v). One hour after administration, 0.1 ml of a carrageenan solution (0.5 %) was injected into the right paw

of each mouse in all groups. Before the carrageenan injection, the paw volume of each mouse was measured using a plethysmometer. The increase in paw volume due to carrageenan-induced inflammation was assessed at 1, 2 and 4 h post-injection. The anti-inflammatory activity of the EO was compared to that of 50 mg/kg Diclofenac sodium (*Voltum*[®], *Pharmalliance, Algeria*). The anti-inflammatory activity was quantified by calculating the paw edema rate and the inhibition rate of edema in the treated mice relative to the control mice, using the following formulas:

$$\text{Paw edema rate (\%)} = \frac{V_t - V_0}{V_0} \times 100$$

$$\text{Inhibition rate (\%)} = \frac{E_c - E_t}{E_c} \times 100$$

Where: V_t is the volume of the treated paw,
 V_0 is the volume of the untreated paw,
 E_c is the edema rate of the control group,
 E_t is the edema rate of the treated group.

2.5 Statistical analysis

For the anti-inflammatory activity, statistical comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey-post hoc test. The selection of these tests was based on the single-factor experimental design with independent and balanced replicates. All analyses were performed using GraphPad Prism version 9.5.1.

3 Results and discussion

3.1 Essential oil yield and chemical composition

The EO extracted by hydro-distillation from the leaves of *T. hirtus* has a yellowish color with a yield of 2.08 ± 0.5 % (w/w). A total of fifty-four compounds were identified, corresponding to 98.53 % of the total EO. The major components were : thymol (48.5 %), carvacrol (17.75 %), *p*-cymene (7.5 %), Bornyl acetate (4.12 %), limonene (3.18 %) and α -terpineol (2.15 %). This EO was particularly rich in oxygenated and hydrocarbon monoterpenes (**Table 1, Fig. 1**).

The extraction yield of *T. hirtus* EO in this study was lower than those reported by Guesmi et al.¹³⁾ for Tamerza (10.82 %) and Kairouan (5.75 %), but remained comparable to the yield observed in Guefsa (2.36 %) by the same authors.

Marked geographic and organ-specific variations have been documented in the chemical profile of *Thymus hirtus*. In Algerian populations, the essential oil is typically dominated by 1,8-cineole (34.6 %), followed by camphor (18.6 %) and α -pinene (9.5 %)¹²⁾. Studies conducted in Tunisia reveal distinct regional chemotypes: samples from Tamerza are enriched in terpinen-4-ol (33.3 %) and 1,8-cineole (14.1 %), whereas oils from Gafsa exhibit higher proportions of linalool (18.1 %) and camphor (13.0 %)¹³⁾. In Kairouan, 1,8-cineole (20.0 %) and camphor (19.2 %) constitute the major constituents¹³⁾. Beyond geographic influences, the chemical profile also varies across plant organs, with camphor prevailing in aerial parts and α -pinene being particularly abundant in the flowers¹⁵⁾.

The essential oil exhibited a high thymol content (48.5 %), a monoterpene with well-documented antimicrobial and anti-inflammatory activities^{22–24)}. Many authors reported that carvacrol and *p*-cymene are also known for their anti-inflammatory activity^{24–27)}. Moreover, thymol's activity may be further strengthened through synergistic interactions with compounds such as carvacrol and *p*-cymene^{28, 29)}. It is prudent to notify that chemical composition of EOs is subject to qualitative and quantitative variability, which can be influenced by the plant part and its phenological stage^{30,31)}. Such variability has also been linked to differences in biotopes and particularly environmental factors such as soil characteristics, solar exposure, and climatic conditions³¹⁾. In addition, several studies indicate that genetic traits and post-harvest parameters, including harvest timing as well as storage and extraction methods, further contribute to shaping essential oil composition³²⁾. Genetic traits and the plant's vegetative cycle further contribute to the observed chemical variability^{33,34)}. Considering these influences, robust analytical techniques such as GC-FID and GC-MS are essential for accurate profiling, quality control and comprehensive investigation of volatile compounds in essential oils^{35–38)}.

Table 1 GC-MS/FID analysis results of essential oil obtained from *T. hirtus* leaves

N°	Compound name	Retention indices (RI)		Peak area (%)	IM
		Lit*	Exp.		
1	Tricyclene	921	919	0.18	tr
2	α -thujene	924	921	1.35	tr, MS
3	α -pinene	932	943	1.10	tr, MS
4	camphene	946	955	1.05	tr, MS
5	Sabinene	969	972	0.33	tr
6	β -pinene	974	976	1.50	tr, MS
7	β -myrcene	988	1007	0.42	tr, MS
8	α -terpinene	1014	1018	0.40	tr, MS
9	p -cymene	1020	1019	7.50	tr, MS
10	Limonene	1024	1021	3.18	tr, MS
11	γ -terpinene	1054	1058	0.48	tr, MS
12	Cis-sabinene hydrate	1065	1062	0.11	tr, MS
13	Linalool	1095	1086	0.50	tr, MS
14	α -campholenal	1122	1120	0.35	tr
15	Trans-pinocarveol	1135	1138	0.14	tr
16	Trans-sabinene hydrate	1098	1097	0.12	tr
17	Camphor	1141	1143	1.05	tr
18	Camphene hydrate	1145	1146	0.25	tr
19	Borneol	1165	1163	0.50	tr, MS
20	Terpinen-4-ol	1174	1176	0.33	tr, MS
21	p -cymen-8-ol	1179	1182	0.10	tr
22	α -Terpineol	1186	1185	2.15	tr
23	Myrtenol	1194	1193	0.30	tr
24	verbenone	1204	1201	0.10	tr
25	Isobornyl formate	1235	1234	0.55	tr
26	Carvacrol-methyl ether	1241	1240	0.25	tr, MS
27	Piperitone	1249	1249	0.12	tr
28	Geranial	1264	1267	0.05	tr
29	Bornyl acetate	1284	1283	4.12	tr
30	Thymol	1289	1286	48.5	tr, MS
31	Carvacrol	1298	1293	17.75	tr, MS
32	α -copaene	1374	1376	0.52	tr
33	Geranyl acetate	1379	1382	0.24	tr
34	β -bourbonene	1387	1385	0.04	tr
35	β -elemene	1389	1393	0.12	tr
36	Z-Caryophyllene	1408	1401	0.30	tr
37	α -Gurjunene	1409	1414	0.35	tr, MS
38	E-Caryophyllene	1417	1422	0.3	tr, MS
39	Aromadendrene	1439	1440	0.18	tr, MS
40	α -humulene	1452	1449	0.05	tr
41	Allo-Aromadendrene	1458	1456	0.13	tr, MS
42	α -amorphene	1483	1481	0.08	tr
43	Valencene	1496	1494	0.03	tr
44	α -bulnesene	1509	1507	0.05	tr
45	γ -cadinene	1513	1511	0.04	tr

46	δ -cadinene	1522	1518	0.14	t _R
47	Palustrol	1567	1565	0.10	t _R
48	Caryophyllene-oxide	1582	1581	0.15	t _R , MS
49	α -epi-cadinol	1638	1636	0.18	t _R
50	α -cadinol	1652	1654	0.08	t _R
51	allohimachalol	1661	1658	0.04	t _R
52	Acorenone	1692	1693	0.2	t _R
53	Acorenone B	1697	1696	0.05	t _R
54	Isolangifolol	1728	1725	0.08	t _R
55	β -eudesmol acetate	1792	1791	0.25	t _R
Total identified				98.53 %	
Yield of essential oil				2.08 %	
Monoterpene hydrocarbons				17.49 %	
Oxygenated monoterpenes				77.58 %	
Sesquiterpenes hydrocarbones				2.58 %	
Oxygenated sesquiterpenes				0.88 %	

RI: Retention indices calculated against n-alkanes, IM: Identification method. t_R, identification based on the retention times

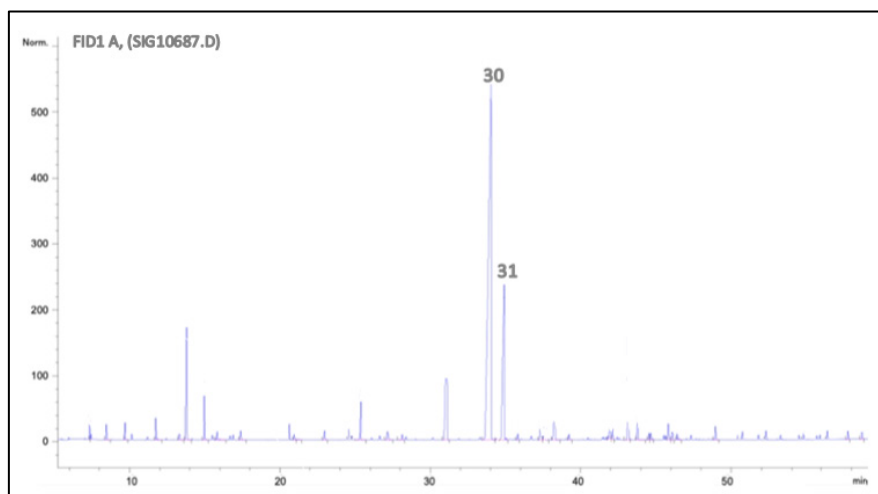


Fig. 1 Chromatographic profile of *T. hirtus* essential oil (peak numbers can be identified in **Table 1**).

The chemical profiles of essential oils provide key insights into their antimicrobial, antioxidant, and potential nutraceutical properties, as well as enabling the distinction of chemotypes within the *Thymus* genus^{36,–39}. GC-FID analysis of *T. hirtus* typically reveals a chemotype rich in thymol and carvacrol, while studies on related species, such as *Thymus vulgaris*, emphasize the genus's chemotypic diversity^{40,41}. When combined with GC-MS, these techniques allow precise structural identification of minor and side compounds.

A thymol–carvacrol chemotype of *Thymus hirtus* has not been reported previously. This study identifies it as a novel chemotype, providing a basis for its inclusion in future phytochemical classifications and for exploring its potential biological activities.

Table 2 Oral acute toxicity of *T. hirtus essential oil*

Doses (mg/kg)	Mortality percentage (%)	Signs of toxicity
0	-	-
50	-	-
100	-	-
500	20	Asthenia, anorexia, piloerection
1000	60	Asthenia, anorexia, piloerection, diarrhea, mild convulsion
2000	100	Asthenia, anorexia, piloerection diarrhea, severe convulsion

3.2 Oral acute toxicity

According to the acute oral toxicity results, there is a proportional relationship between the mortality rate and the acute toxicity of the administered EO compared to the control group (**Table 2**). Mice exhibited signs of piloerection, asthenia, significant anorexia, and death for some cases. At doses equal to 500 mg/kg, this pattern was similar to that observed in mice treated with higher doses (1000 and 2000 mg/kg), where additional symptoms such as diarrhea and convulsions were more severe. In contrary, treatment with doses of 50 and 100 mg/kg did not induce any clinical signs or mortality. The lethal dose (LD50) of this EO was equal to 875 mg/kg.

According to the Organization for Economic Cooperation and Development⁴²⁾, it can be deduced that this EO belongs to the class 4, corresponding to substances who has oral lethal dose (DL50) scales ranging between 300 and 2000 mg/kg, indicating that it is considered as toxic. This toxicity may be attributed to the high bioavailability of some toxic volatile compounds like : terpineol, gurjunene and limonene which are known for their significant toxicity⁴³⁾. Although, the doses of 50 and 100 mg/kg did not show any signs of toxicity. Nevertheless, long and medium-term toxicity of this EO should be necessary tested in vivo.

3.3 Anti-inflammatory activity

One hour after inducing inflammation with carrageenan, the negative control group continued to exhibit edema, while low inhibitory rates of EO at doses of 50 and 100 mg/kg during the first hour were about 23.9 % and 14.6 %, respectively, compared to 32.7 % of the positive control mg/kg) ($p < 0.0001$). After the fourth hour, these two groups treated with EO demonstrated a significant reduction in edema (**Table 3–5**), but still very lower than positive control paw edema's inhibition rate.

The carrageenan-induced paw edema test is commonly used to assess the anti-inflammatory activity of various bioactive compounds and to evaluate the effects of pro-inflammatory agents during the acute phase of inflammation⁸⁾.

Inhibitory edema rate is dose dependent (**Table 3–5**), this evidence allows us to suggest that the anti-inflammatory effects of EO are related to the inhibition of some intracellular signaling pathways involved in the effects of several inflammatory mediators. The present study demonstrated that the oral administration of this EO produces significant anti-inflammatory effects. Certain terpenes, particularly oxygenated monoterpenes, have been recognized for their role in managing inflammation⁴⁴⁾. Among them, thymol and carvacrol should be particularly mentioned. Numerous studies have highlighted the strong in vivo and in vitro anti-inflammatory synergetic effect of these two components as suppressors of pro-inflammatory activation in immune cells, such as macrophages, by inhibiting key signaling pathways (COX-2 expression, Nitric Oxide production), leading to reduced production of inflammatory cytokines^{45–50)}. Therefore, while thymol is a promising candidate for managing inflammatory disorders, more rigorous studies are needed to establish safe and effective dosing and to confirm its mechanisms in humans.

Thymus essential oils exert anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines (such as TNF- α , IL-6, IL-8, and IL-18) and reducing nitric oxide (NO) levels in immune cells. Key components like thymol and carvacrol are particularly effective, acting through pathways such as NF- κ B and 5-lipoxygenase inhibition and modulating microglial and macrophage responses^{51–54)}. The oils also enhance antioxidant enzyme activity, further supporting their anti-inflammatory action⁵³⁾.

Animal studies with *Thymus numidicus* EO, a close relative of *T. hirtus*, demonstrated significant reductions in inflammation and pain, with effects comparable to standard anti-inflammatory drugs at higher doses⁵⁵⁾. In vitro studies using *Thymus vulgaris* EOs showed potent inhibition of inflammatory mediators in human and murine immune cells^{53,54)}.

The anti-inflammatory potency of *Thymus* EOs depends on their chemical profile, which is influenced by plant species, growth stage, and extraction method. EOs rich in thymol and carvacrol generally show stronger effects. synergistic or antagonistic interactions among oil constituents can also impact efficacy^{51,52,55)}.

Table 3 Anti-inflammatory effect of *T. hirtus* essential oil

Time (h)	Negative control group*				Tested group**										Positive control group***			
					50 mg/ml				100 mg/ml				50 mg/ml					
	PER (%)				PER (%)				PER (%)				PER (%)					
	A1	A2	A3	Mean ±SD	A1	A2	A3	Mean ±SD	A1	A2	A3	Mean ±SD	A1	A2	A3	Mean ±SD	IR (%)	IR (%)
1h	20.17	20.5	20.83	20.5 ± 0.33	14.67	15.6	16.53	15.6 ± 0.93	17.38	17.5	17.62	17.5 ± 0.12	13.8	13.2	14.4	13.8 ± 0.6	14.62	32.67
2h	21.48	22.3	23.12	22.3 ± 0.67	17.52	17.9	18.28	17.9 ± 0.38	13.26	11.74	12.5	12.5 ± 0.76	6.8	7.42	6.18	6.8 ± 0.62	43.85	69.45
4h	15.35	15.7	16.05	15.7 ± 0.35	23.67	23.7	23.73	23.7 ± 0.03	5.4	6.42	4.38	5.4 ± 1.02	0.8	0.5	0.2	0.5 ± 0.5	65.55	96.77

PER: Paw Edema Rate, IR: Inhibition rate

* Negative control group receiving Physiological saline + Tween 80

**Tested group receiving the EO (50 mg/ml, 100 mg/ml)

***Positive control group receiving Diclofenac sodium (50 mg/ml)

Table 4 Percent Reduction of Edema in the analyzed groups

	Tested groups		Positive control group	
	50 mg/ml	100 mg/ml	Diclofenac 50 mg/ml	
	IR (%)	IR (%)	IR (%)	
1h	23.89 ^a	14.62 ^b	32.67 ^c	
2h	19.7 ^a	43.85 ^b	69.45 ^c	
4h	51 ^a	65.55 ^b	96.77 ^c	

Table 5 Summary of One-way ANOVA and Tukey's HSD post-hoc test results

IR after 1 hour

	Tested groups				Positif group	
	50mg/ml	Moyenne IR	1000mg/ml	Moyenne IR	50mg/ml	Moyenne IR
1h	27.27	23.89 ^a	13.83	14.62 ^b	31.58	32.67 ^c
	23.90		14.63		35.61	
	20.5		15.41		30.87	

ONE WAY ANOVA and Tukey HSD results after 1 hour

source	sum of squares SS	degrees of freedom v	mean square MS	F statistic	p-value	treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
treatment	489.5365	2	244.7682	39.4409	0.0004	A vs B	6.4429	0.0091625	** p<0.01
error	37.2357	6	6.2060			A vs C	6.1161	0.0116948	* p<0.05
total	526.7722	8				B vs C	12.5590	0.0010053	** p<0.01

IR after 1 hour

	Tested groups				Positif group	
	50mg/ml	Moyenne IR	1000mg/ml	Moyenne IR	50mg/ml	Moyenne IR
1h	27.27	23.89 ^a	13.83	14.62 ^b	31.58	32.67 ^c
	23.90		14.63		35.61	
	20.5		15.41		30.87	

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IR after 2 hours

	Tested groups				Positif group	
	50mg/ml	Moyenne IR	1000mg/ml	Moyenne IR	50mg/ml	Moyenne IR
1h	18.44	19.7 ^a	38.27	43.85 ^b	68.34	69.45 ^c
	19.73		47.35		66.73	
	20.93		45.93		73.27	

One way ANOVA and Tukey HSD results after 2 hours

source	sum of squares SS	degrees of freedom v	mean square MS	F statistic	p-value	treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
treatment	3,713.1427	2	1,856.5713	150.4574	7.4714e-06	A vs B	11.9077	0.0010053	** p<0.01
error	74.0371	6	12.3395			A vs C	24.5288	0.0010053	** p<0.01
total	3,787.1798	8				B vs C	12.6210	0.0010053	** p<0.01

IR after 4 hours

	Tested groups				Positif group	
	50mg/ml	Moyenne IR	1000mg/ml	Moyenne IR	50mg/ml	Moyenne IR
1h	54.2	51	64.82	65.55	94.79	96.77
	50.96		59.11		96.81	
	47.85		72.71		98.75	

One way ANOVA and Tukey HSD results after 4 hours

source	sum of squares SS	degrees of freedom v	mean square MS	F statistic	p-value	treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
treatment	3,283.0463	2	1,641.5231	81.2113	4.5212e-05	A vs B	5.6029	0.0173908	* p<0.05
error	121.2780	6	20.2130			A vs C	17.6369	0.0010053	** p<0.01
total	3,404.3243	8				B vs C	12.0340	0.0010053	** p<0.01

The findings obtained from the acute oral toxicity assessment and the in vivo anti-inflammatory assays remain preliminary, as they were based on basic experimental models that should be complemented by more comprehensive and in-depth investigations. Despite these limitations, the present results provide an original contribution, offering the first available insights into the safety profile and anti-inflammatory potential of this endemic species. Such initial evidence, although simple in design, introduces data that have not yet been explored in the scientific literature and lays the groundwork for future, more advanced evaluations.

4 Conclusion

The chemical composition, oral acute toxicity and anti-inflammatory effect of *T. hirtus* have been reported in this paper. Thymol and carvacrol were found to be the major components of this volatile fraction. The oral acute toxicity increased proportionally with increasing doses (LD₅₀ = 875 mg/kg). The interesting protector effect against inflammation was particularly registered for the dose of 100 mg/kg during the fourth hour. Future research should aim to deepen these findings by exploring the sub-acute and chronic toxicity of *T. hirtus* essential oil, as acute assessment alone cannot fully characterize its safety profile. Complementary pharmacological studies are also needed to clarify the molecular mechanisms underlying its anti-inflammatory effect and to determine whether synergistic interactions among its constituents, particularly thymol, contribute to the observed activity. Evaluating the oil in additional inflammation models and expanding the in vivo assays to include oxidative stress and immunomodulatory pathways would further strengthen its therapeutic potential. Moreover, standardized extraction procedures and chemotype comparisons across different geographical regions could help define the consistency and stability of its bioactive profile. Ultimately, these investigations would support the rational development of *T. hirtus* essential oil as a safe and effective natural alternative to conventional anti-inflammatory agents.

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Conflict of interest

The author declare no competing or conflict of interest in relation to the present research paper.

Contributions

MT :Writing and Editing-Original draft, Investigation, Methodology, Conceptualization, Data curation, Formal analys.

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