Acute toxicity of essential oils of two Moroccan endemic species: *Thymus broussonetii* and *Thymus leptobotrys*

K. Elhabazi[1], R. Aboufatima[1], A. Bensalah[1], A. Collado[3], J. Sanz[3], A. Zyad[4], H. Ait Mouse[4], A. Benharref [2], A. Chait[1*]

[1]*Laboratoire de Pharmacologie, Neurobiologie et Comportement. Equipe de Pharmacologie et Comportement, Faculté des Sciences Semlalia. Université Cadi Ayyad, Marrakech Maroc.*
[2]*Laboratoire de Chimie des Substance Naturelles. Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech Maroc.*
3Instituto de Ciencias Agrarias-CCMA, CSIC, c/Serrano 115-dpdo., 28006, Madrid, España.
[4]*Laboratoire de Génie Biologique, Faculté des Sciences et Techniques; Université Moulay Sliman, Béni Mellal Maroc.*
[*]*Corresponding autor E-mail: chait@ucam.ac.ma*

Abstract

Thymus species essential oils are widely used in aromatherapy to treat several ailments. However, there is no report on their safety. In this study, we propose to investigate the acute toxicity of *T. leptobotrys* and *T. broussonetii* essential oils. These two species were selected on the basis of their frequency of medicinal use and commercial importance. Chemical analysis of these two species essential oil revealed that thymol, borneol, carvacrol and p-cymene were the main chemical constituents in *T. broussonetii*, whereas the essential oil of *T. leptobotrys* contains carvacrol (98 %) as the major component. In the acute toxicity assay, the animals showed no stereotypical symptoms associated with toxicity such as convulsion, ataxy, diarrhoea or increased diuresis. The calculated median lethal dose (LD_{50}) was estimated at 4.47 g/kg for *T. broussonetii* and 2.66 g/kg for *T. leptobotrys*.

Key words: *T. broussonetii, T. leptobotrys*, Essential oil, Chemical analysis, Acute toxicity, LD_{50}.

Introduction

Thymus species, the Mediterranean aromatic plants, are widely known and used in several countries as spice and food preservative as well as remedy for many health disorders. *T. broussonetii* and *T. leptobotrys* are two endemic species in Morocco. They are the most popular herbs used in folk medicine. Commonly, these plants were used as powder, decoction or infusion to treat digestive disorders, fever, cough, cold and dolorous processes (Bellakhdar, 1997).

Previous pharmacological studies have demonstrated analgesic (Elhabazi *et al.*, 2006a), anti-inflammatory (Ismaili *et al.*, 2002), anxiolytic and immunomodulators (Elhabazi *et al.*, 2006b) activities of the non volatiles extracts of *T. broussonetii*. While, it was shown that the essential oil of this species possesses antimicrobial activity due to the two phenolic compounds thymol and carvacrol (Lattaoui *et al.*, 1993). Whereas, there is no pharmacological and phytochemical investigations in the literature on *T. leptobotrys* extracts except our previous work demonstrating the analgesic effect of the non-volatiles extracts (unpublished data).

In this study, we have studied the acute toxicity and phytochemical profile of the essential oils extracted from two endemic Moroccan thyme species: *T. leptobotrys* and *T. broussonetii*. The essential oil of these two species are commercialised and used as medicine for several ailments and widely used in aromatherapy, but there is no investigation in the literature concerning their safety and acute toxicity. Such study is essential to prevent any overdose of this oil which may cause a serious health problem in humans.

Materials and methods

Animals

Swiss mice (25-35) g were used in these experiments. Animals were housed in standard cages with food and water *ad libitum* on 12-h light/12-h dark cycle. The air temperature was maintained at 22±2 ◦C.

Plant material and preparation of essential oil

The plants were collected in March 2003, in two regions in Morocco as followed: *T. broussonetii*: Essaouira, *T. leptobotrys*: Tiznite. Taxonomic identification of the plants was confirmed by Pr. Ouyahya Aicha from the Scientific Institute (Rabat). The plants specimens with their localities and other required fields records were recorded at the herbarium of the Scientific Institute, Mohamed V University, Rabat, Morocco. The essential oil was extracted by hydrodistillation using a Clevenger apparatus.

GC-MS analysis

The constituents of the essential oils were identified by gas chromatography/mass spectrometry (GC-MS) using an Agilent 6890 with 5973A detector. We used a methylpolisiloxane capillary column (25 m x 0.2 mm x 0.33 um), injecting 1 µl of a 1:100 oil solution in the split mode. The temperature program used was 70 to 250ºC, at 5º/ min.

Toxicity assay

Mice were randomly assigned to several groups with 10 animals in each group. The essential oil of each species was administered orally to the animals in increasing dosages 1, 2, 3, 4 and 5 g/kg body weight in a single dose. The control group was given an equal volume of water. The animals were observed for 7 days; the number of dead mice in each group was recorded daily to calculate the effective lethal dose (LD_{50}). The mice were also observed for other signs commonly associated with toxicity such as behavioural changes (excitation, twitches), motor coordination, piloerection, convulsion and diarrhoea. An evolution of body weight was also determined for groups receiving the dose 1 g/kg.

Data analysis

Data were analysed using SPSS program. The percentage of mortalities was converted to probits. The probit analysis was used to determine LD_{50} and LD_{90} (with their confidence limits) using the Leitchfield and Wilcoxon method (Leitchfield and Wilcoxon, 1949).

Results

Essential oils constituents

Table 1 presents the qualitative and quantitative results obtained from the GC-MS analysis of volatiles in *T. broussonetii* and *T. leptobotrys*. Relative concentrations were calculated directly from the total ion current trace,

and expressed as % of total volatile content. 13 compounds which represent a total of 88.4 % were identified in *T. broussonetii*, thymol (36.7 %) and borneol (21.9 %) being the two major constituents. Other important components are carvacrol (9.0 %) and p-cymene (7.56 %). In *T. leptobotrys* essential oil, carvacrol is the most abundant constituent and represents a 96.8 %. This oil also contains p-cymene (2.6 %) and α pinene (0.6 %). Other components listed in Table 1 such as thymol and borneol are present in low concentrations.

Table 1. Constituents of *T. broussonetii* and *T. leptobotrys* essential oil with their relative proportion in the pure oil

Constituents	% of total volatile content	
	T. broussonetii	*T. leptobotrys*
Aromadendrene	1.2	-
Borneol	21.9	traces
Camphene	2.8	-
Carvacrol	9.0	96.8
Caryophylene	-	traces
p-Cymene	7.6	2.6
Spathulenol	1.9	-
Thymol methylether	-	traces
Limonene	0.7	-
Myrcene	1.2	-
α-Pinene	-	0.6
ß-Pinene	0.7	-
Thymol	36.7	traces
α-Terpinene	0.7	-
δ-Terpinene	2.8	traces
α-Terpineol	1.2	traces

Acute toxicity of *T. broussonetii*

At 1 g/kg, the treated mice were immobilised approximately for 1 hour. After that, they find their normal activity. For the dose 2 mg/kg, there are cases of death whose the number depends on the time. Mostly, the death occurs at 2 h after treatment. Whereas, there is also cases of death occurring at 2 days after treatment. For the lethal doses, the animals remained immobilised until death. Nevertheless, the animals didn't show any additional clinical symptoms of toxicity such as convulsion, diarrhoea or motor incoordination. On the other hand, the survivors did not feed and therefore lost body weight (Table 2). After three or four days, the survivors recuperated the movement and recovered their normal state. The LD_{50} and LD_{90} were estimated at 4.47 (3.6-6.72) g/kg and 7.31 (5.64-13.54) g/kg.

Acute toxicity of *T. leptobotrys*

At 800 mg/kg, death cases were recorded. Just after treatment, the mice were immobilised and some signs of toxicity occurred such as acceleration of respiratory changes and piloirection. For all cases, the mice were immobilised until death. Additionally, the survivors also lost body weight (Table 2). The normal activity of the survivors was recuperated after four days. The LD_{50} and LD_{90} calculated were estimated at 2.66 (1.98-3.77) g/kg and 5.32 (4.09-8.66) g/kg.

Discussion

Examination of the two essential oils analysis make possible to highlight wide significant differences in the chemical composition between the two species. Carvacrol varies from 96.76 % in *T. leptobotrys* to 8.96 % in *T. broussonetii.* The two major components presents in *T. broussonetii* (thymol: 36.72 % and borneol: 21.9 %) are at very low concentration in *T. leptobotrys*. These results are in accordance with previous work which has found an extraordinary diversity in the composition of essential oil of Moroccan thyme species (Richard *et al.,* 1985). This variability could be explained by the fact that essential oil composition depends not only on the genetic factors but also on the biotopes

Table 2. Body weight evolution.

	Body weight (g)						
	j0	j1	j2	j3	j4	j5	j6
Lot 1 (control)	26±0.6	26.07±0.6	25.8±0.3	26.9±0.1	27.12±0.5	28.2±0.4	28.8±0.2
Lot 2 (*T. broussonetii*)	24.8±0.4	24.92±0.7	23.61±0.5*	24.38±0.5	24.89±0.6	25.15±0.3	25.82±0.7
Lot 3 (*T. leptobotrys*)	26.02±0.3	26.85±0.5	24.64±0.1*	25.16±0.4	26.86±0.9	27.08±0.9	27.8±1.4

(climatic conditions and soil composition). Besides, *Thymus sp.* are known to present chemical varieties (chemotypes) which differ markedly in their chemical composition (Thompson *et al.*, 1998; Ložienė *et al.*, 2002). On the other hand, our results showed that *T. leptobotrys* is more toxic than *T. broussonetii*. Knowing that *T. leptobotrys* essential oil contains predominantly carvacrol (96.8 %), we can suppose that this component is mainly responsible for this toxic effect. Whereas, *T. broussonetii* essential oil contains predominantly thymol (36.7 %) and borneol (21.9 %). It's known that carvacrol is more toxic which explains the high toxicity of *T. leptobotrys* essential oil. Additionally, our results showed that after treatment with the essential oil of both species, the mice remain immobilised during some time. This finding indicates that at high doses, the essential oil of the two thyme species exerted in animals a sedative effect. In a similar study, Ismaili *et al.*, (2002) have already showed that the administration of *T. broussonetii* total extract exerted a sedative action. Furthermore, it was shown that thymol modulates in a positive way the gabaergic receptors (Garcia *et al.,* 2006; Priestly *et al*., 2003). So, we can suppose that thymol could be responsible for the sedative effect observed after administration of thyme extracts at high doses.

In conclusion, this study showed that *T. broussonetii* essential oil contains 22 compounds. Monoterpenes are the most common. The main constituents are thymol, borneol, carvacrol and p-cymene. In *T. leptobotrys* essential oil, carvacrol is the most abundant compound. The acute toxicity assay revealed that *T. leptobotrys* is the most toxic possessing the low median lethal dose. Additionally, our finding showed that high doses of the two species essential oil produced a sedative effect and a loss in average body weight.

Acknowledgements

We gratefully acknowledge Pr. Ouyahya Aicha for the botanical identification of the two thyme species.

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