



Toxicological study of essential oils of three Moroccan aromatic plants: *Thymus vulgaris*, *Mentha spicata* and *Citrus limonum*

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Abstract

The aim of this work is to study the toxicological properties of three aromatic plants: *Thymus vulgaris*, *Mentha spicata* and *Citrus limonum*. This study was performed on albino mice by determining the median lethal dose orally. The mice were divided into six batches of ten each (5 males and 5 females) and received doses of plant species (added to an adjuvant) of 500 to 1000mg/kg. The results obtained show that the tested plant species have an oral toxicity.

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Introduction

Currently, the use of essential oils in several fields is expanding, mentioning cosmetics, perfumery and pharmaceutical industry. But the main obstacle to its development is the absence of legislation that organizes this area and, as a result, misuse of aromatic plants is found, posing threats to users' health.

Essential oils are presented, usually as "safe". But these natural substances are also powerful compounds (Degryse *et al.*, 2008). Because of their complex chemical composition, essential oils should be used with extreme caution because they can pose very serious dangers than of the independent random use (Benzeggouta, 2005).

The toxic effects of an essential oil vary considerably depending on its nature (Traoré, 2006). Generally, the search for the pharmacological activity of an essential oil starts with the evaluation of its acute toxicity in order to define the therapeutic limits of its use. For this, two types of studies are needed, namely, the efficacy study and the safety study in animals and humans (Buenz, 2006; Al Namer, 2014).

The aim of this work is to study the oral toxicity of essential oils extracted from moroccan aromatic plants: *Thymus vulgaris*, *Mentha spicata* and *Citrus limonum*.

Material and methods

Plant material

T. vulgaris samples were collected from Tafilet, *C. limonum* from the Agadir province and *M. spicata* from the Settat province.

The pickings were carried out during the month of April. The leaves of *T. vulgaris*, *M. spicata* and the fruits of *C. limonum* were cut by hand and placed in bags, transported immediately to the laboratory and stored at room temperature until use.

Biological model

We used 60 male and female albino mice (Swiss, strain), aged 2 months, weighing between 21 and 35 g

from the animal experimentation center of the Pasteur Institute of Casablanca.

These mice have free access to water and standard food and have been acclimatized to these conditions: a stable temperature of 25 °C and a humidity of 50%.

Preparation of essential oils

The oils were prepared by hydrodistillation using a Clevenger apparatus containing 2 liters of distilled water and surmounted by a column (60 cm in length and 2 cm in diameter) connected to a refrigerant. 500 g of the leaves of each plant was used to extract the essential oils of *T. vulgaris* and *M. spicata*. On the other hand, 700g of lemon bark were used for the preparation of its essence.

After removing traces of water with anhydrous sodium sulfate, the EO obtained were stored in opaque bottles and placed in a refrigerator at 4 °C until used.

Determination of median lethal dose (LD₅₀) in mice

After ten days of acclimation, six batches of ten mice each (females and males) were administered by oral route of oils tested with the following doses: 500, 750, 850, 900, 950 and 1000 mg/kg.

The control group received only physiological water. After administration of the EO to the mice, they were observed individually every hour for 14 days.

The behavior and clinical symptoms of the animals were noted throughout the experiment. The LD₅₀ were obtained from the TREVAN curves (Djyh *et al.*, 2010).

Results and discussion

Yields of hydrodistillation in essential oils

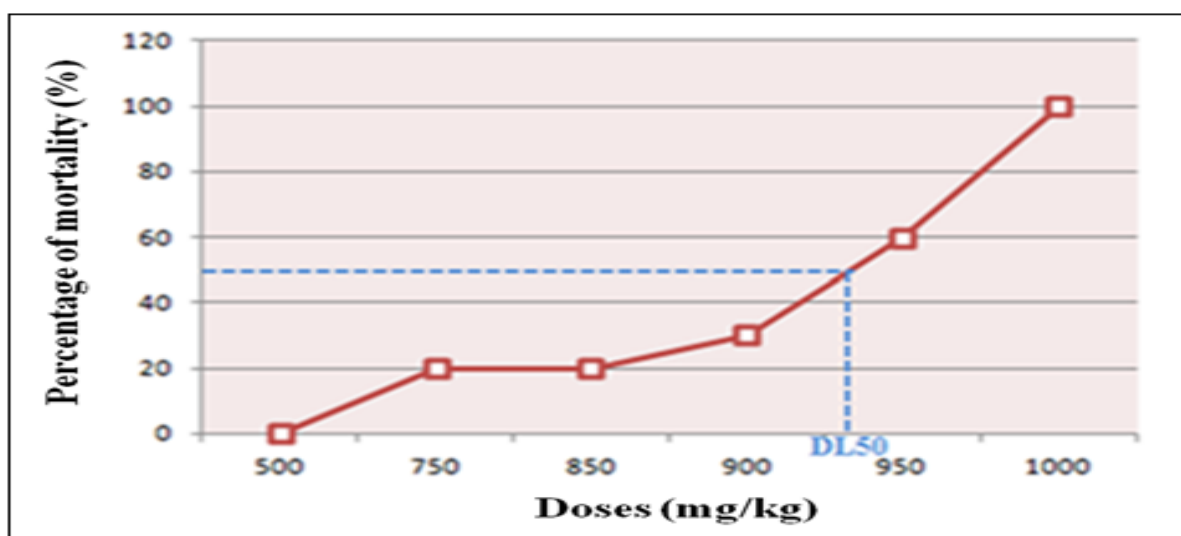
The yields of the hydrodistillation were estimated by the ratio of the masses of the EO and the dried vegetable matter. The yields obtained are shown in Table 1.

Table 1. Yields of hydrodistillation in essential oils of *T. vulgaris*, *M. spicata* and *C. limonum*.

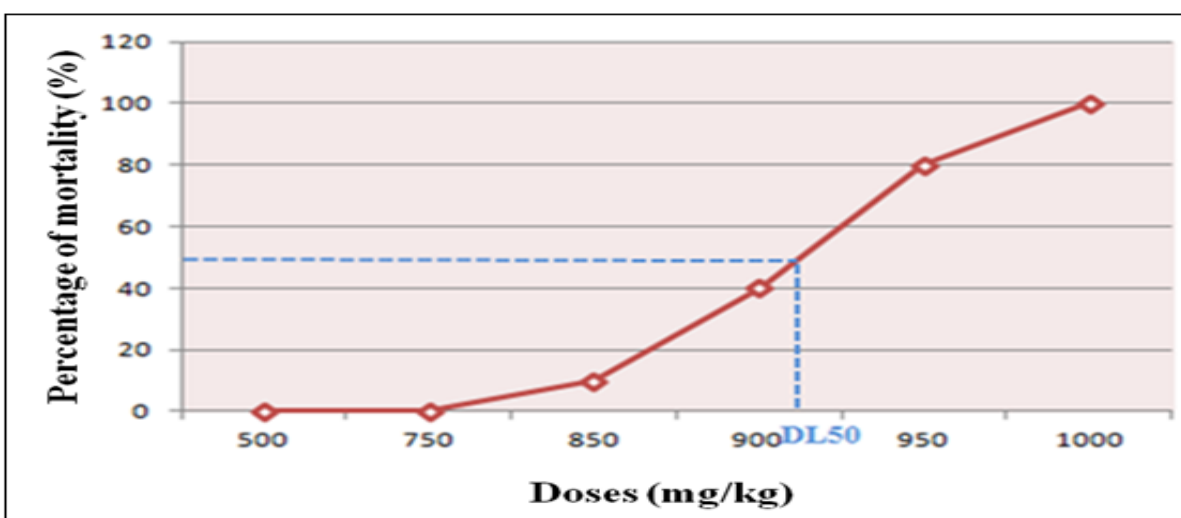
Plants studied	Yields of hydrodistillation (%)
<i>Citrus limonum</i>	0.75
<i>Mentha spicata</i>	0.72
<i>Thymus vulgaris</i>	0.65

The yields obtained are variable compared to those found by other authors. For *C. limonum*, the yield found was 0.75%, which is higher than that obtained by Makuba (2012), which is 0.5%. For *M. spicata* the yield obtained was 0.72%, while that Adjou and Soumanou (2013) found 0.96% and finally for

T. vulgaris the yield was 0.65%, which is lower than that found by Carolina *et al.* (2012), which is 1.76%. These variations may be due to abiotic factors, such as the specific climate of the source regions of the samples, geographical factors such as altitude, soil type and harvesting season.

**Fig. 1.** Percentage of mortality according to the gradual doses of essential oil of *T. vulgaris* administered orally.

The TREVAN curve of the effect of *M. spicata* EO on males and females mice (Fig. 2) showed that the median lethal dose was 911 mg / kg.

**Fig. 2.** Percentage of mortality according to the gradual doses of the essential oil of *Mentha spicata* administered orally.

Determination of median lethal dose (LD_{50}) in mice

The study of the acute toxicity of the EO studied showed no signs of toxicity during the first day, although weight loss and diarrhea in treated mice (males and females) were observed. This weight loss could be explained by a reduction in food

consumption (loss of appetite), which causes a decrease in the amount of food absorbed. For diarrhea, it can be explained by an irritating action that is due to oils. This irritation is due to the increase in cell permeability, resulting in considerable changes in the transport of water and electrolytes (Arseneault, 2000; Guergour *et al.*, 2012).

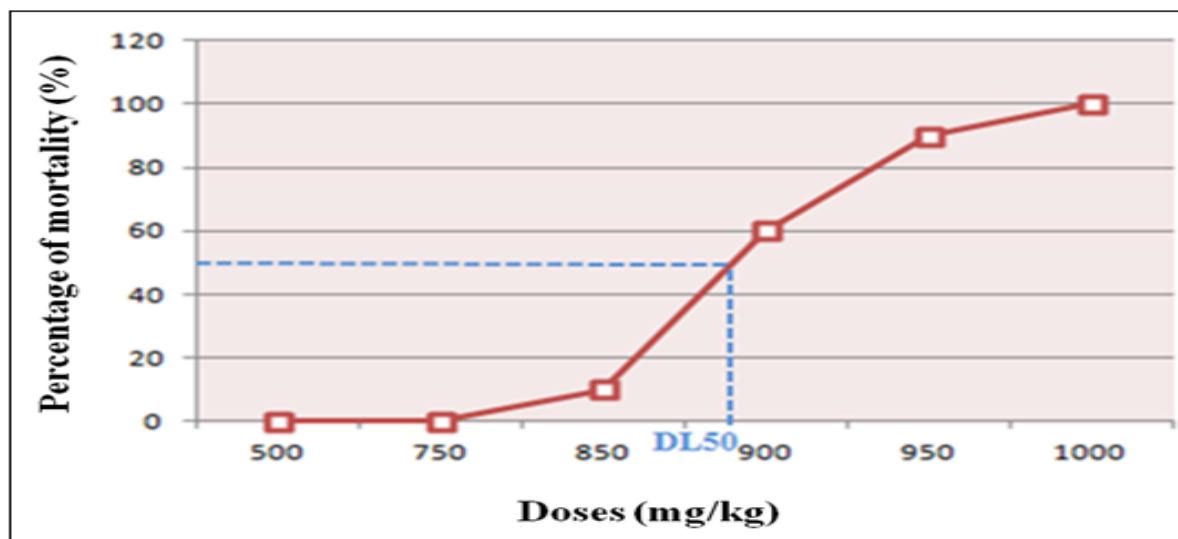


Fig. 3. Percentage of mortality according to the gradual doses of the essential oil of *Citrus limonum* administered orally.

After establishing the mortality rates for each dose of the EO tested, the median lethal doses were determined for each plant species.

From the TREVAN curve of the effect of *T. vulgaris* EO on male and female mice (Fig. 1) showed that the median lethal dose was 930 mg/kg.

From the TREVAN curve of the effect of *C. limonum* EO on male and female mice (Figure3), showed that the median lethal dose was 894 mg/kg.

The study of the acute toxicity of the three EO showed that median lethal doses were: 930 mg/kg for *T. vulgaris* EO, 911 mg/kg for *M. spicata* oil and 894 mg/kg for 1 of the *C. limonum*. According to Diezi (1989), substances with an LD_{50} between 50 and 500 mg/kg body weight are toxic and those with an LD_{50} greater than 5000 mg/kg are practically non-toxic. Referring to this classification, the oils studied are toxic by the oral route.

According to the literature, the toxicity of a substance varies according to the species (Morrisson *et al.*, 1968) and the route of administration. However, variations in LD_{50} as a function of the route of administration and of the species have already been reported by Lahlou *et al.* (2008) with the lyophilized aqueous extract of *Tanacetum vulgare*. Furthermore, Guergour *et al.* (2012) showed that the study of the acute toxicity of castor oil in female mice gave an LD_{50} = 5623.41 mg/kg allow classifying the extract of *Ricinus communis* L. in the category of products weakly toxic according to Frank's classification.

Conclusion

This work devoted to the study of the acute oral toxicity of essential oils extracted from Moroccan aromatic plants: *Thymus vulgaris*, *Mentha spicata* and *Citrus limonum*, showed that all three plant species are toxic orally. Further studies should be carried out to identify and elucidate the mechanisms of action of the different chemical constituents of the essential oils responsible for its toxic effects.

References

- Adjou ES, Soumanou MM.** 2013. Efficacité des extraits de plantes contre les moisissures toxigènes isolées de l'arachide. *Journal of Applied Biosciences*; 5555-5566.
- Al Namer RM.** 2014. Valorisation pharmacologique de *Rosmarinus officinalis* et de *Lavandula officinalis*: toxicité aiguë, potentiel psychotrope et antibactérien. Thèse de Doctorat. Université Mohammed V-Agdal, Rabat. N° 2707. 331 p.
- Arseneault Y.** 2000. Quelle est la place de l'huile de ricin dans le déclenchement de travail? Québec, pharmacie. 47: 746-748.
- Benzeggouta N.** 2005. Etude de l'activité antibactérienne des huiles infusées de quatre plantes médicinales connues comme aliments. Mémoire de Magister, Université de Constantine, Algérie, 110 p.
- Buenz EJ.** 2006. Developed countries should be the focus for effectively reducing chronic disease. *Journal Epidemiologie. Community Health*; **60(7)**, 562-3.
- Carolina F, Chini-Queiroz F, Kummer R, Fernanda Esteveao-Silva C, Dalva de Barros Carvalho M, Maria Cunha J, Grespan R, Aparecida C, Amado B, Kenji Nakamura R.** 2012. Effects of Thymol and Carvacrol, Constituents of *Thymus vulgaris* L. Essential Oil, on the Inflammatory Response. *Evidence-Based Complementary and Alternative Medicine*, 2.
- Degryse AC, Delpla I, Voinier MA.** 2008. Risques et bénéfices possibles des huiles essentielles. *Atelier santé environnement -IGS- EHESP*, 87 p.
- Diezi J.** 1989. Toxicology: Basic principles and chemical impact. In *Pharmacology: Fundamental Principles and Practice*, Slatkine M. (Ed). Academic Press: Genève; 33-44.
- Guergour H, Mahdeb D, Allouni N, Benouadah R, Bouzidi Z.** 2012. Toxicité aigue de l'huile de *Ricinus communis* L chez les souris blanches femelles *Mus musculus*. *Agriculture* N°3, 29-36.
- Morrisson JK, Quinton RM, Reinertth.** 1968. The Purpose and Value of LD₅₀ Determination. *Modern Trends in Toxicology*. Butterworths: London.
- Makuba Teddy S.** 2012. Contribution a la désinfection de l'eau par photosensibilisation avec des extraits de plantes. Thèse de Doctorat. Faculté des sciences de l'ULG. Université de Liège.
- Traoré MC.** 2006. Étude de la phytochimie et des activités biologiques de quelques plantes utilisées dans le traitement traditionnel de la dysménorrhée au Mali. Thèse de Doctorat. Université de Bamako, Mali, p. 175p.