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Evaluation of the hypoglycemic and antimicrobial activities of the essential oil of *Myrtus nivellei* from Tamanrasset (southern Algeria)

Djamel BOUKHALFA * and Bachir NABTI

Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Algiers-1, Algeria.

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Abstract

The *Myrtus nivellei* Batt. & Trab. is a shrub native to the Mediterranean and endemic to the central Sahara in the Hoggar massif of southern Algeria, whose leaves are used by the Tuareg, in infusion mixed with tea, against diarrhea, fever, diabetes and for its anti-infective properties.

The hypoglycemic activity of the essential oil extracted from the leaves by hydrodistillation. has been demonstrated, with a significant decrease ($P<0.001$) in blood sugar levels and blood triglyceride levels in diabetic rats after the 2nd hour and up to the 3rd hour of administration (14% and 22%).

This drop in blood sugar was not observed in healthy rats treated with the essential oil.

The essential oil did not improve the weight of the rats, nor their cholesterolemia.

The antimicrobial activity of the essential oil has been studied for six microorganisms. *Bacillus subtilis* and *Candida albicans* are the most sensitive, they were inhibited from 2.5/1000 v/v. This oil is also active on *Staphylococcus aureus* at a concentration of 5/1000 v/v. This activity was weak against *Escherichia coli* and absent against *Pseudomonas aeruginosa* and no activity was detected against *Enterococcus faecium*.

It would be interesting to combine these evaluations with chromatographic and spectral studies to determine the compounds responsible for these activities.

Keywords: *Myrtus nivellei*; Essential oils; Toxicity; Hypoglycemic; Antimicrobial

1. Introduction

The Hoggar Myrtle, *Myrtus nivellei* Batt. and Trab., is an endemic shrub of the Hoggar Mountains in southern Algeria [1,2]. For centuries, the leaves and branches of the shrub have been used by the Tuaregs in their traditional medicine to prepare decoctions and infusions administered orally or applied topically to the skin to treat various ailments, such as gastrointestinal disorders, respiratory infections, menstrual pain, dermatological conditions, and eye disorders [3]. Numerous studies have been conducted on the pharmacological and phytochemical properties of this plant, which have confirmed the presence of active compounds in the Hoggar Myrtle, including flavonoids, phenolic acids, and terpenes, which could explain its therapeutic effects [4].

The objective of this work is to evaluate the hypoglycemic and antibacterial properties, as well as the acute toxicity, of the essential oil extracted from the leaves of the Hoggar Myrtle [5].

* Corresponding author: Djamel BOUKHALFA

2. Material and methods

2.1. Localisation, Period and Identification of the plant

The leaves of *Myrtus nivellei* were harvested in full bloom between the months of September and October on twigs at Oued Iguioui in the Tagmart region (45 km north of Tamanrasset : Latitude North : 23°13'20″ and Longitude East : 05°28' 36″) at 1600 meters altitude, the twigs also bore mature and immature flowers and fruits. The identification of the plant was carried out according to the new flora of Algeria and the southern desert regions (Quezel P. and Santa S. (1977), and the flora of the Sahara (Ozenda P., 1983), by Pr. Boukhalfa D. and Mr. Belghoul M. (biologist, ONPCA), it was confirmed by Mr. Abdelkrim H., professor of botany (National School of Agronomy of El Harrach) [4,6].

2.2. Extraction of essential oils

The vegetable matter, weighed beforehand (50gr), is placed in a glass column, which surmounts a balloon filled with water. The two-liter flask is filled to 2/3 with distilled water, a glass column surmounts it, and the whole is brought to 60 °C at atmospheric pressure. The steam flow is kept constant throughout the duration of the extraction. The mass of oil recovered is determined by weighing, the yield is expressed in relation to the mass of dry matter [4,7].

2.3. Assessment of the toxicity of the essential oil of *Myrtus nivellei*

The determination of toxicity is carried out by measuring the cumulative dose that causes 50% of deaths (LD_{50}) using the method of Miller and Tainter [8].

This study was conducted at the research and development center (CRD-SAIDAL) on 4 batches of 5 mice (weight = 20 ±2g) receiving increasing doses of the essential oil of *Myrtus nivellei*. These four groups of mice were monitored for 72 hours during which the number of deaths per group and their behavior were noted. The curve representing the evolution of mortality as a function of extract concentrations is established. The animals are fasted the day before the test, and water is not limited [9, 10].

The next day, increasing doses of the essential oil are administered to the animals (Table 1). The solution to be administered to the mice is prepared by diluting 2 ml of pure essential oil in 10 ml of Tween 80 (density of the essential oil = 0.9052).

The mice are fasted for 3 to 4 hours after administration and observed for 14 days to record the number of mice that died [9, 10].

Table 1 Doses of essential oil administered to the 4 groups of mice

N° of the Batch	**1**	**2**	**3**	**4**
Dose administered (gr/kg)	1.8	2.7	3.6	4.5
Volume of pure EO. administered (µl)	40	60	80	100
Volume of solution administered (ml)	0,2	0,3	0,4	0,5

2.4. Evaluation of hypoglycemic activity of essential oil

It is carried out by determining blood glucose levels in streptozotocin-induced diabetic Wistar rats. The study begins with an acute toxicity test [4,9]. Plasma measurements are performed according to technical data sheets on the SPINREACT automaton [11, 12].

- Diabetes induction : After an overnight fast (food deprivation for 16 hours but not water), diabetes is induced in rats by intraperitoneal injection of the STZ solution prepared at a dose of 55 mg/kg of body weight, which is a volume of 2 ml/kg equivalent to 0.49 ± 0.02 ml according to the weight of the rat [11, 12]. Non-diabetic groups of rats received the same volume of 0.1 M sodium citrate buffer pH 4.5 intraperitoneally [11, 12].
  After injection, water bottles were replaced with bottles containing a 5% glucose solution for 24 hours to overcome the hypoglycemia induced by STZ due to the destruction of pancreatic β-cells and the massive release of insulin [11, 12].
- Blood glucose measurement : 48 hours after STZ injection (time for diabetes development), diabetes was confirmed in STZ rats by measuring fasting blood glucose levels using a glucometer. Only rats with blood

glucose levels higher than 170 g/dl were considered diabetic and selected for this experiment [11, 12].

- Animal treatment : After induction of diabetes, all rats, diabetic and non-diabetic, are divided into four groups of six rats each and kept under the same conditions. 24 hours after diabetes confirmation, animal treatment begins:

  - Group I : Lot I: Diabetic + Essential oil of myrtle from the Sahara.
  - Group II: Diabetic control.
  - Group III: Healthy + Sahara myrtle essential oil.
  - Group IV: Healthy control or healthy control.

Table 2 Different steps of manipulation for the hypoglycemic test

Days	Manipulation
D1	Induction of diabetes by streptozotocin (STZ)
D3	Confirmation of diabetes by blood glucose measurement (>170 g/dl) Weighing of rats. Dosage of blood triglycerides
D4-j10	Treatment of animals: Group 1 and 3: the rats receive 1 drop (13 µl) of essential oil every day. Group 2 and 4: the rats receive 1 drop of distilled water daily. Animal weighing. Blood glucose test: Before administration (TO) After administration (T1, T2, T3, T4, T5).
D11	Dosage of blood triglycerides and cholesterol

Blood glucose levels are measured for all four groups before and 1 hour after each administration of essential oil.

The percentage reduction in blood glucose was calculated by the following relationship (Lamela et al., 1985) [13].

(%) reduction in blood glucose = (G0 – Gt) / Gt. 100 ; [G0 : blood glucose before essential oil administration ; Gt : blood glucose after essential oil administration] [11, 12].

Given the sample size, the results are interpreted based on the Student's t-test

2.5. Evaluation of antimicrobial activity

It is a qualitative in vitro evaluation of the action of essential oil of *Myrtus nivellei*; on strains selected according to their availability and their pathogenicity. These are young strains from the "American Type Culture Collection" (ATCC) [*Staphylococcus aureus (ATCC 6538); Pseudomonas aeruginosa (ATCC 9027); Escherichia coli (ATCC 9027); Bacillus subtilis* (ATCC 9372); *Candida albicans* (ATCC 24443); *Enterococcus feacium* (ATCC 6569)] [14, 15, 16].

- Re-isolation of the ATCC strains: by taking the most apparent colonies from the ATCC dishes using a Pasteur pipette previously sterilized by the blue flame of the Bunsen burner; then inoculation of the colonies taken from a nutrient agar, by deconcentration using the technique of the four cadrons. The boxes are placed in an oven at 37°C for 24 hours [14, 15, 16].
- Antimicrobial susceptibility testing: Starting from the re-isolated strains, use a sterile Pasteur pipette that has been sterilized by a blue flame from a Bunsen burner to pick the most individualized colonies and incorporate them into physiological saline solution. Adjust the turbidity ofthis suspension to 0.5 McFarland. Soak a sterile swab with the bacterial suspension, then squeeze it against the wall of the tube to eliminate excess suspension, and streak the Muller Hinton agar plates with tight strokes, rotating the plate and swab 60° three times, finishing by streaking the walls of the petri dish. Place the plates aside, close to the Bunsen burner, while preparing the antimicrobial discs (6 mm diameter cellulose discs cut from Whatman filter paper). Soak the

discs with Phlomis extract, then place them onto the culture medium agar. After a few seconds of contact, place the disc in the center of the petri dishes using sterilized forceps that have been sterilized by the blue flame from a Bunsen burner. Incubate the plates for 24 hours at a temperature of 37°C [14, 15, 16].

3. Results and discussion

3.1. Acute toxicity of *Myrtus nivellei* essential oil

After 14 days of observation, the results are presented in **Table 3**

Table 3 Mortality rates (%) by doses of E.O. administered to the 4 groups of mice

N° de lot	**1**	**2**	**3**	**4**
Dose administered (g /kg) (d=0.9052)	1.8	2.6	3.7	4.5
Number of dead mice	0	1	2	3
Mortality rate (%)	0	20	40	60

The behavior of the animals is monitored regularly for 48 hours after treatment. The number of deaths is recorded in each group after administration of the essential oil in order to determine the LD_{50} in each case using the Miller and Tainter log probability graph method (Figure 1). Therefore, the LD_{50} is equal to 4.1 g/kg.

Toxicity is manifested by a loss of balance, the mouse walks with difficulty, then loses consciousness until death. The *Myrtus* genus has not shown any toxicity to date, but it is advisable to be cautious in the use of essential oil [4,10], as it can cause headaches and lethargy [4].

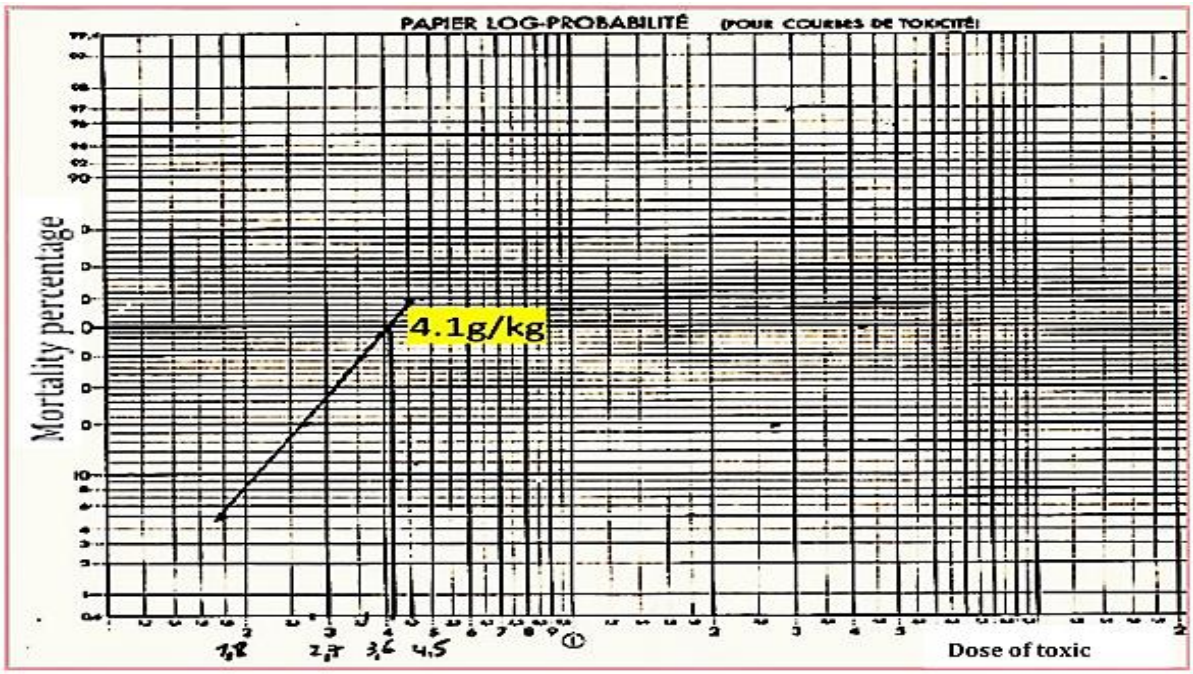

Figure 1 LD_{50} of the essential oil administered to mice (Miller and Tainter, 1944)

The essential oil of *Myrtus nivellei* leaves is non-toxic, with an LD_{50} of 4.1 g/kg; that is 0.44 ml for a 200 g mouse; therefore, 2.20 ml/kg. According to the Glossin Smith and Hodge scale [17], this E.O. could be moderately toxic: Probably lethal oral dose (human) Toxicity index or class. It is important to note that acute oral toxicity of the essential oil of *Myrtus communis* leaves gives an LD_{50} of 3.7 ml/kg in rats and 2.2 ml/kg in mice. However, even though it is non-toxic, it remains irritating to the skin [4, 10].

3.2. Evaluation of hypoglycemic activity

After (07) days of observation, the results are presented in **figure 2**:

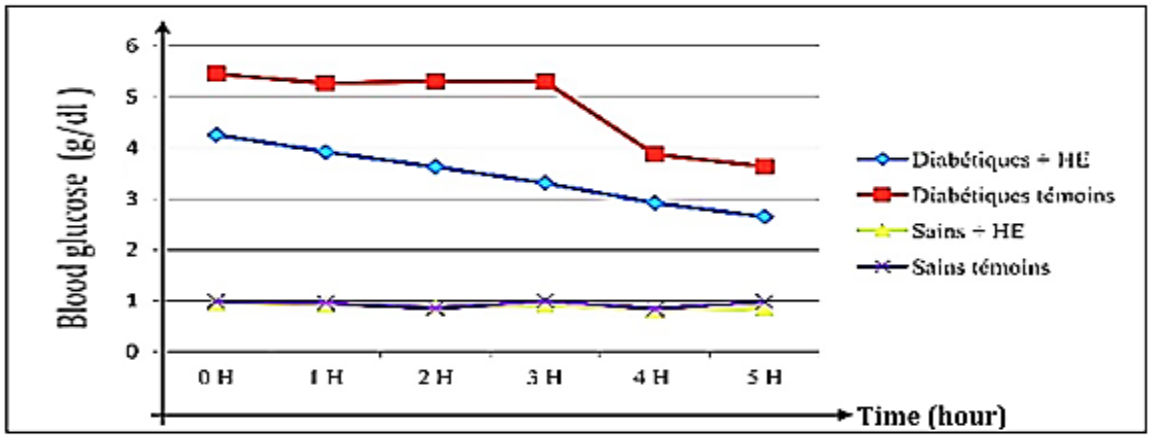

Figure 2 Variation in blood glucose levels over time in the 4 groups of rats

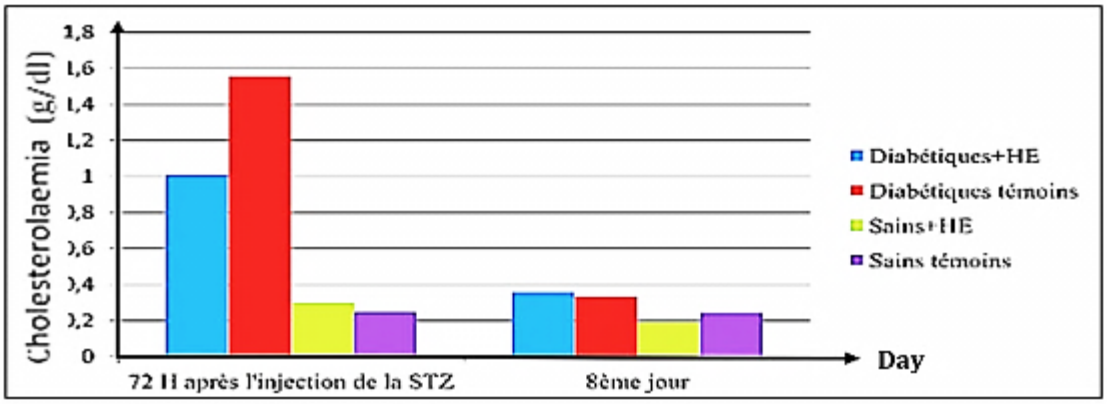

Figure 3 Variation in cholesterolaemia after 7 days of treatment with *Myrtus nivellei* EO

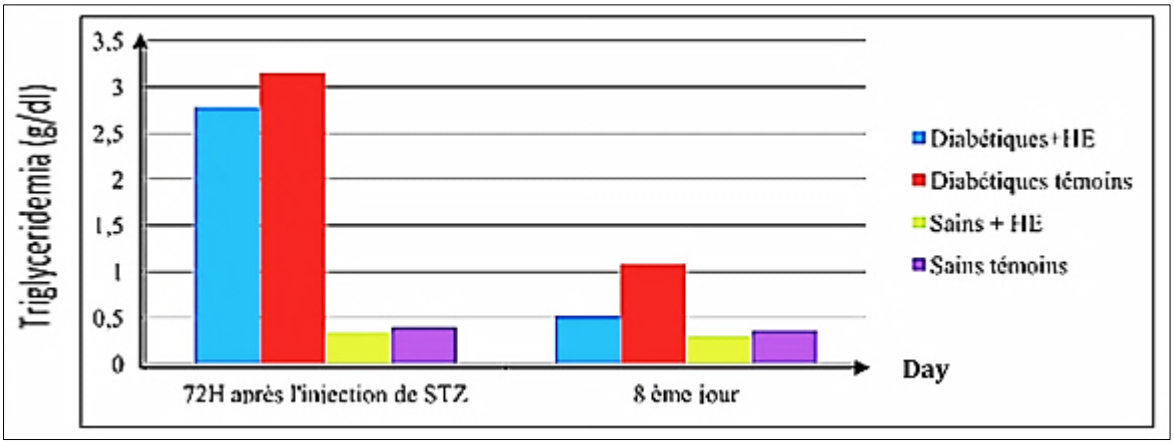

Figure 4 Variation in triglyceridemia after 7 days of treatment with E.O. from *Myrtus nivellei*

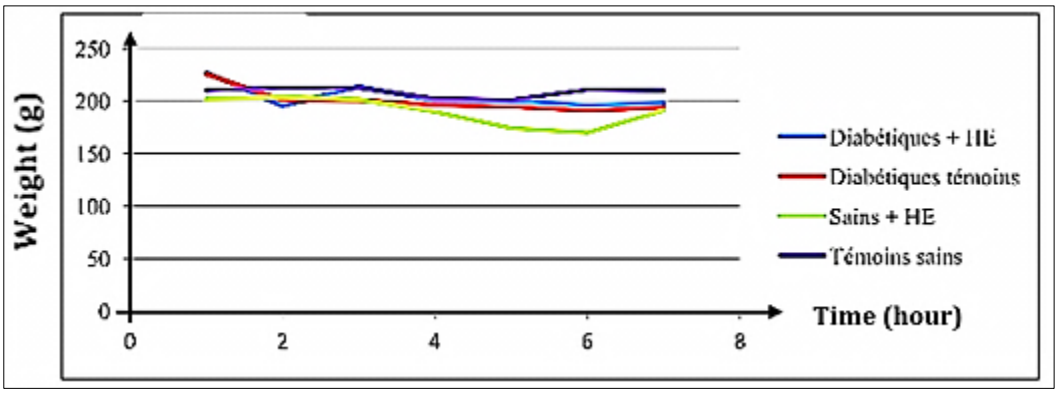

Figure 5 Evolution of weight in the four groups of rats

The values mentioned in face 1 are average values from three trials. On the second day after administration of the essential oil, and during the first trial, three out of six diabetic rats in the treated group were found dead. As the cause was not immediately clear, the decision was made to reduce the dose from 20µl to 13µl.

In the group of control diabetic rats (Group II), blood glucose levels remained constant until the third hour, where they significantly decreased (down to 3.87gr/dl) from the fourth hour. On the other hand, in the treated diabetic rats (Group I), a significant decrease in blood glucose levels was recorded during the second and third hour after the administration of the essential oil. It was 14.78% and 22.30%, respectively, compared to only 2.56% and 2.75% for the control diabetics.

In healthy rats treated with the essential oil (Group III), the most marked reduction in blood glucose levels compared to untreated healthy rats was recorded at the fifth hour (10.52%), but the Student's t-test did not show any significant difference. In vivo experimental studies have demonstrated the plant's ability to control the blood glucose levels of diabetics. Oral administration of a few microliters of *Myrtus nivellei* essential oil (13µl) to normal and diabetic rats was effective, as blood glucose levels decreased remarkably. Indeed, the essential oil of *Myrtus nivellei* used on streptozotocin-induced diabetic Wistar rats induced a significant decrease in blood glucose levels of 14.78% and 22.30% at the second and third hour after the administration of the essential oil. This anti-hyperglycemic effect has also been demonstrated by other authors, such as Sepici A [11] and Rebey, I. B [12].

The drop in blood sugar was accompanied by a strong decrease in blood triglyceridemia in diabetic rats after the 2nd hour until the 3rd hour of administration. This effect was not seen in healthy rats treated with the essential oil. The essential oil did not improve the weight of the rats, nor their cholesterolemia. Contrary to what was reported by Goetz 2012 [18], the administration of EO from the leaves of *Myrtus communis* for ten days is responsible for the relative increase in liver weight in rats from 10.8 and 28% at doses of 0.5, 1.0, 2.0 ml/kg.

Currently, it is not possible to identify the exact mechanism of the hypoglycemic effect; nevertheless, *Myrtus nivellei* has no effect on plasma insulin concentration. Therefore, the hypoglycemic activity of the plant could be extra-pancreatic [4,11,12].

3.3. Evaluation of the antibacterial activity

The essential oil of *Myrtus nivellei* has antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*. This activity is weak against *Escherichia coli*, while it is absent against *Pseudomonas aeruginosa*. For antifungal activity, only *Candida albicans* is sensitive to this oil, but there is no activity against *Enterococcus feacium* (Table 4)

This essence also has a strong inhibitory effect on *Candida albicans*. The MIC for *Staphylococcus aureus*, which is 0.5%, places this oil as bacteriostatic for this reference strain, and it is bactericidal for *Bacillus subtilis* (MIC = 0.25%); thus, confirming the results obtained in the qualitative study.

For species resistant to this oil, the MICs are quite high, they are 2%, 1%, 1% for *Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecium*, respectively, which is consistent with the results of the qualitative study. The MBC for *Escherichia coli* is greater than 2%, which proves the insensitivity of this strain to the essential oil of *Myrtus nivellei*. It is equal to 1% for *Pseudomonas aeruginosa and Enterococcus faecium* [16].

As for *Candida albicans*, it has a high sensitivity to this oil, with an MIC of 0.25% and an MFC of 1%, suggesting that the essential oil of *Myrtus* could possess a synergistic antifungal activity in combination with amphotericin against this fungus.

Table 4 Antimicrobial activity of *Myrtus nivellei* essential oil

Inhibition diameter Microorganisms	**Essential Oil (diameter in mm)**	**Control (-)**	**(Genta.)**	**CMI (%)**	**CMB (%)**	**CMF (%)**
P. aeruginosa ATCC 9027	<06	-	37	1	1	-
E. coli ATCC 4157	09	-	25	2	>2	-
B. subtilis ATCC 9372	12,5	-	34	0.25	0.5	-
S. aureus ATCC 6538	15	-	36	0.50	0.5	-
E. feacium ATCC 6569	<06	-	31	1	1	-
C. albicans ATCC 24433	17	-	25	0.25	1	1

The antimicrobial properties (antibacterial and antifungal) could be explained by the richness of the essential oil of *M. nivellei* in oxygenated (eucalyptol, α-terpineol…) and hydrogenated monoterpenes (dl-limonène) known for its effectiveness against microbial agents [11,15].

The antimicrobial activity of Moroccan Myrtle essential oil is mainly due to α-terpineol, myrtenol, and 1,8-cineole [19].

4. Conclusion

This study revealed that the essential oil of *Myrtus nivellei* is non-toxic, with an LD_{50} of 4.1 g/kg, which is equivalent to 0.44 ml for a 200 g mouse, or 2.20 ml/kg.

The hypoglycemic potential of *Myrtus nivellei* has been demonstrated in rats, confirming its use as an antidiabetic plant.

The study of antimicrobial properties showed that the essential oil of *Myrtus nivellei* has antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*, weak activity against *Escherichia coli*, and no activity against *Pseudomonas aeruginosa*.

For antifungal activity, only *Candida albicans* is sensitive to this oil, but there is no activity against *Enterococcus faecium*.

The inhibitory effect of this oil on bacterial and fungal development suggests prospects for application in the fields of the food, cosmetics and pharmaceutical industries.

It would be interesting to combine these evaluations with chromatographic and spectral studies to determine the compounds responsible for these activities.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors and all co-authors declare that they have no conflicts of interest in connection with this document, and the material described is not in the process of being published nor is it intended for publication elsewhere.

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