

Anticandidosic activity and acute toxicity of *Quercus suber* L. bark extracts

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Novelty statement

What New Information Does This Article Contribute?

- Cork oak bark extracts can be used to treat *Candida albicans* infections.
- LD50 was slightly toxic to mice according to Hodge and Sterner Toxicity Scale.

Abstract

The cork oak (*Quercus suber* L.), endemic essence of the Mediterranean Basin, is commonly used in traditional pharmacopoeia. The main objective of this work is to enhance the valorization of this plant species through the study of the anticandidosic activity of cork oak bark methanolic extracts in order to develop an efficient natural formulation for Candidiasis treatment. The anticandidosic activity of methanolic extracts of *Q. suber* bark stemming from decoction, maceration and Soxhlet methods of extraction in was tested on five different *Candida albicans* strains. Our results showed that all the tested extracts displayed an inhibitive activity, which varies according to the obtained extract and the tested strain. The best anticandidosic potential was observed with extracts obtained with Soxhlet method. The study of the acute toxicity showed that the lethal dose is 1150 mg/kg in mice, which remained moderately toxic according to Hodge and Sterner classification scale. Thus, this extract can be used in phytotherapy without danger in doses that are below 300 mg/kg of corporal weight. Based on these results, we can conclude that Cork oak bark extracts can be used to treat *Candida albicans* infections.

Keywords: Anticandidosic activity, *Candida albicans*, *Quercus suber* L., Methanolic extracts, Traditional pharmacopoeia, Acute Toxicity.

Introduction

Medicinal plants have always had an important place in the therapeutic arsenal of humanity. According to the World Health Organization (WHO), around 65-80% of the world population in developing countries, depend mainly on traditional herbal medicines for their primary health care, due to poverty and lack of access to modern medicine [1].

Thus, despite the progress of pharmacology and the remarkable advances in synthetic organic chemistry of the twentieth century, plants are widely used and even recommended by therapists and public health organizations such as the WHO [2].

More than 25% of drugs prescribed in industrialized countries such as France [4], Canada [5] and Brazil [6,7] come directly or indirectly from plants [3].

Morocco, by the richness and diversity of its flora, constitutes a true phylogenetic reservoir, with approximately 4500 species and subspecies of vascular plants, of which two thirds are endemic to Morocco and 8,69% and 14,28% of the total Moroccan flora are aromatic and medicinal plants [8].

This allows it to occupy a privileged place among the Mediterranean countries which have a long medical tradition and a traditional know-how based on medicinal plants [9]. Indeed, in Morocco, traditional medicine has always occupied an important place in the traditions of medication [10].

Cork oak (*Quercus suber* L.) is one of the plants most used as extracts with high curative power in traditional pharmacopeia. It constitutes an immense reservoir of biological activities, in particular its bark rich in polyphenolic substances.

It's an endemic species of the Mediterranean basin, but its range has been greatly reduced following strong climatic variations and especially human action [11].

Fungi are, alongside viruses, parasites and bacteria, the microorganisms most often implicated in infections and mycoses, and their treatment with chemical agents regularly leads to the selection of resistant fungal strains [12, 13].

Unfortunately, despite all the efforts made by medicine, we can see that infectious diseases have experienced a sharp increase in recent years. In terms of mycoses, candidiasis are frequent

opportunistic diseases in subjects living with HIV [14, 15, 16] and their overall incidence is 5,8% in seropositive subjects [17].

In addition, it is observed that the sensitivity to antifungal decreases in certain strains of *Candida* due to drug pressure, the phenomena of mutations and the strong progression of opportunistic infections [14].

Among all the yeasts isolated in the clinic, *Candida albicans* is the most frequently isolated yeast (66,5%) followed by *Candida tropicalis* (4,2%) [18]. Thus, candidiasis is currently classified among the serious infections that can cause a high rate of mortality and morbidity in immunocompromised and patients suffering from diabetes [19].

In pathology, *Candida albicans* is the cause in 70 to 80% of cases of human candidiasis. It is the agent of cutaneous, digestive, intestinal, genital, bronchopulmonary yeast infection [21].

This is in order to promote the Mâamora cork grove through the evaluation of the activity antifungal and the acute toxicity of methanolic extracts from the powder of the bark of the cork oak (*Quercus suber*) that the following axes have been addressed in this work :

- Determination of the anticandidosis activity of the bark of the cork oak obtained from the extraction by decoction, maceration and Soxhlet on the growth of five strains of *Candida albicans*.
- Comparison of the anticandidosis activity of the different extraction techniques and also the comparison of the degree of sensitivity of the different strains.
- Evaluation of the acute toxicity of the Soxhlet extract of the bark of the cork oak tree.

Material and methods

Plant material

The bark of the stem of *Quercus suber L.* was collected from the forest of Mâamora « canton A ». In the laboratory, we proceeded to a meticulous sorting in order to eliminate any foreign body. The material is then placed in an oven at 40°C for 48 hours for drying, then reduced to powder.

Fungal strains tested

The five strains of *Candida albicans* studied are affiliated with the Moroccan Coordinated Collections of Microorganisms (CCMM) of the Laboratory of Microbiology and Molecular Biology (LMBM) of the National Center for Scientific and Technical Research (CNRST). These strains come from various samples :

- L13 from oral swab
- L2 and L5 isolated from finger nail sampling
- L14 isolated from toe nail sample
- L12 isolated from the umbilical catheter.

Animal material

The acute toxicity study was carried out on white mice of the Balb/c type from the animal facility of the Animal Physiology Laboratory of the Faculty of Sciences of Rabat.

The mice are healthy, adult males, 3-4 months old, weighing 20-30g. Prior to testing, they were raised under ambient conditions of temperature, photoperiod and humidity. Access to food and water is unlimited.

Methods of obtaining extracts

Aqueous extracts

The aqueous extract of this essence was obtained by the decoction process : 5g of vegetable powder are brought to the boil in a flask for 20 min in 500 ml of distilled water and then filtered through Whatman paper.

Cold maceration

2g of powder were extracted with 500 ml of methanol and stirred for 24 hours. After filtration on Whatman paper, the marc is then transferred into 500 ml of the same solvent for 24 hours.

After four successive macerations for 4 days, the macerate obtained is filtered through Whatman paper, then the filtrate is evaporated under vacuum at 67,7°C using a Rotavapor.

The recovered extract is stored at 4°C.

Soxhlet extraction

The extraction is carried out in a Soxhlet. The principle is the same as for any extraction, but here the problem arises of the diffusion of the solvent into the solid phase, which can be slow. A very large number of successive extractions must be carried out to obtain satisfactory separation.

A quantity of 100g of powder of the bark of the cork oak *Quercus suber* is subjected to extraction with methanol in a Soxhlet for 32 hours, the time necessary for the total exhaustion of the plant material, and at the boiling point of the solvent used. The extract is then filtered, concentrated under reduced pressure using a rotary evaporator and stored at 4°C.

Calculating the yield of extracts

The collected extracts were weighed for performance and stored in a refrigerator at 4°C, in the dark.

The yield of the extracts is calculated by the following formula :

$$R = me / mi \times 100$$

R (%) : yield of extract

me : mass of the extract recovered (in mg)

mi : initial mass of dry plant matter (in mg)

Enumeration of *Candida albicans* colonies by the dilution method

The purpose of counting techniques (or enumeration) is to determine the concentration of yeasts contained in an initial preparation. They require one or more decimal dilutions (to tenths).

To do this, a range of successive dilutions, going 10^{-1} to 10^{-7} , was carried out. Each dilution requires a 9 ml tube of physiological water plus 1ml of suspension of the five strains to be treated.

Then, from each dilution, a volume of 1ml is taken and then added by the streaking technique to the surface of an agar medium (Sabouraud Dextrose Agar) at the rate of three repetitions for each dilution. The cultures are then incubated at 30°C, for 48 hours.

The counting is based on the concept of CFU (Unit Forming a Colony). Each cell unit will give rise to a colony. Thus, after incubation, the UFC of each strain is calculated by the following formula (23) :

$$N = \Sigma \text{colonies} / V_{\text{ml}} (1 + 0,1 n_2) \times d_1$$

N= Number of CFU per gram or per ml of initial product

Σ colonies = Sum of the colonies of the interpretable boxes

V= Volume of solution deposited (1ml)

n1 : Number of boxes considered at the first dilution selected

n2 : Number of boxes considered at the second dilution selected

d1 : Factor of the first dilution retained

Preparation of stock solution

A mass of 100 mg of extract was dissolved in 1 ml of sterile distilled water for the methalonic extracts.

Anticandidosis tests

To achieve the anticandidosis activity of the various extracts, we used the method of microdilution in liquid medium on sterile 96-well flat-bottomed plates (Bio-Rad) [24, 25].

The manipulation consists in depositing 100 µl of liquid Sabouraud medium in each well, then a series of successive dilutions at a rate of $\frac{1}{2}$ was established in order to obtain concentrations ranging from 50 mg/ml at 3,12 mg/ml. Five plates were used, each corresponding to a strain of yeast. Each well was then inoculated with 10 µl of yeast suspensions prepared beforehand.

The controls were placed in isolated wells by adding 10µl of the strains tested to 100 µl of the suitable medium without extract for the preparation of the negative controls and, by adding the culture medium alone, for the preparation of the positive controls.

We also tested a reference synthetic product : Fungizone (1µg/ml).

The microplates are sealed with parafilm under aseptic conditions and then incubated at 30°C, for a period of 48 hours.

Yeast growth was revealed by adding 10µl to each well of Tetrazolium (MTT: 3-(4,5-mg/mldimethylthiazol-2-yl)-2,5diphenyltetrazoliumbromide) previously prepared at 0,4 mg/ml. The microplate are reincubated at 30°C, for 24 hours. The absence of staining in the wells indicates an inhibitory effect on yeast growth.

The concentrations used correspond to the MIC (Minimum Inhibitory Concentration) obtained in studies on the antifungal activity of various synthetic products against the species *Candida albicans* (26, 27).

Fungicidal or fungistatic profile

The determination of the fungicidal or fungistatic profile requires seeding in 100µl streaks of the contents of the wells having a concentration greater than or equal to the MIC transferred to a Sabouraud culture medium. Incubation took place at 37°C for 24 hours. The fungistatic effect is a resumption of growth while the fungicidal effect indicates a complete lack of growth.

Soxhlet extract acute toxicity test

Toxicity tests therefore accompany biological activity test during the selection of new molecules. The toxicity can be assessed, among other things, by determining the LD50 (Lethal Dose 50).

The LD50 corresponds to the dose which induces the death of half of the animals tested in the same animal species, under determined conditions.

This determination is based on the evaluation of all-or-nothing responses : death or survival of the animals [28].

The choice of the initial dose was set on the basis of the recommendations of the OECD (Organization for Economic Co-operation and Development) guideline issued in 2001 which stipulates that in the absence of data on the toxicity of a substance test, the recommended

starting dose, for animals welfare reasons, is 300mg/kg. Our choice was therefore fixed on this dose as the starting concentration [29].

The mice are fasted on food for 3 to 4 hours and were divided into 4 groups with four male mice per group :

- Each mouse of batch 1 serving as control receives a volume of 20 ml/kg IP of physiological water NaCl 9% intraperitoneally.
- Each mouse in batch 2 receives a dose of 300 mg/kg of the methanoic extract of the bark of *Quercus suber* in a volume of 20 ml/kg IP.
- Each mouse in batch 3 receives a dose of 300 mg/kg of the extract of *Quercus suber* in a volume of 20 ml/kg IP (confirmation dose).
- Each mouse in batch 4 receives a dose of 2000 mg/kg of the extract of *Quercus suber* in a volume of 20 ml/kg IP (confirmation dose).

After administration of the extract, the treated animals were observed continuously every 30 minutes for the first 24 hours. Then, the animals are kept under observation for 15 days, during which the variation in body weight, signs of toxicity and the number of death are noted.

Calculation of the lethal dose LD50

The 50% lethal dose (LD50) is the dose of a substance capable of causing, by the route of administration chosen, the cumulative mortality of 50% of the animals in a population tested.

In the literature, several methods are used for its determination : methods of Dragstedt and Lang, Karber and Behrens, Miller and Tainter and Wilcoxon's methode.

To calculate the LD50, our choice fell on the method of Dragstedt and Lang [30].

In the vicinity of the LD50, on the curve representing the percentages of mortality as a function of the dose (figure 1), this point can be determined according to the following formula :

$$DL50 = \frac{50 (X_2 - X_1) + X_1 Y_2 - Y_1 X_2}{Y_2 - Y_1}$$

Y1 : Percentage of mortality corresponding to X1

Y2 : Percentage of mortality corresponding to X2

X1 : lower dose surrounding the LD

X2 : higher dose surrounding the LD

Results

Yield of extracts

The results of the calculation of the yield methanolic extracts according to the two extraction techniques developed are presented in Table 1 :

Table 1: Yields in % of the extract of the bark of *Quercus suber* according to the two extraction methods

	Aqueous extraction	Maceration	Soxhlet
Weight of plant matter (g)	5	200	100
Mass of pure extract (g)	0.96	32	17,5
Yield of extract (%)	19.2	16	17,5

For 200 g of plant material macerated in methanol, the bark of *Quercus suber* gave an extract yield of 16%.

For the 100g sample of powder of the bark of *Quercus suber* subjected to the extraction by methanol in Soxhlet, the result shows a yield of 17,5%, the extraction carried out by methanol in Soxhlet completely depletes the plant material in a few cycles with a saving of time.

Concerning the aqueous extraction obtained by decoction, which is the most widely used method in everyday life, the result shows a yield of 19.2%.

Determination of the number of colony-forming units of fungal strains

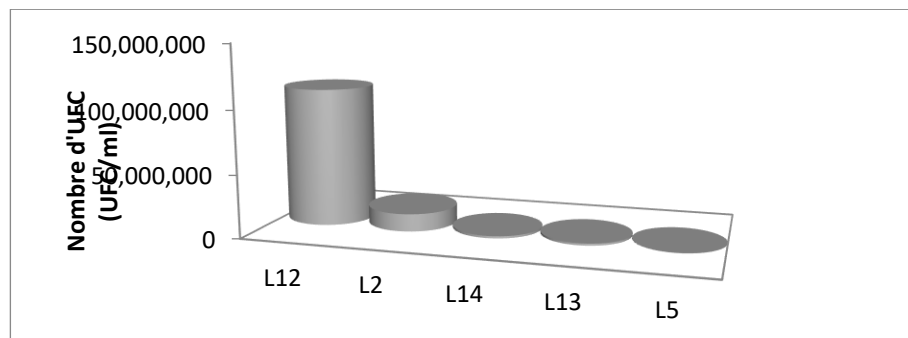


Figure 1: Number of colony forming units/ml of *C. albicans* strains. CFU : Colony forming units ; L2 : Strain taken from the fingernail ; L13 : Oral swab strain ; L5 : Strain take from the fingernail ; L12 : Sample strain from the umbilical catheter ; L14 : Toe nail sample strain.

The results of the counting of strains of *Candida albicans* are shown in figure 1. These reveal a large variation in the number of colony forming units (CFU) per milliliter of physiological water. The five strains studied being from the same species and placed under the same culture conditions, it can be said that the same number of CFU varies depending on the strains studied and therefore on their original substrate.

Indeed, we notice that the strains L2 and L12 reached the greatest number of CFU : 11.10^7 and 14.10^6 CFU/ml respectively, followed by the strains L14 and L13, 17.10^5 and 16.10^5 CFU/ml respectively. Then, the strain L5 which presented the lowest number of 8.10^4 CFU/ml.

Determination of the antifungal activity of *Quercus suber* extracts on the growth of *Candida albicans*

The antimycotic activity of the extracts of the powder of the bark of *Quercus suber* resulting from the different extraction techniques and the evaluation of the effectiveness of a synthetic chemical at the concentrations studied, were estimated by the absence or the presence of staining at the level of the wells.

On analysis of the results obtained, we note that all the strains studied are sensitive to the three extracts tested, but to varying degrees. Indeed, differences in effectiveness were noted depending on the dilutions of each extract and the method of extraction from which it is derived. However, the absence of staining at the first dilution for all the strains in all the tests carried out

reveal that no strain was able to develop at the highest concentrations (50 and 25 mg/ml) for the two extracts from the maceration and the Soxhlet.

And the analysis of the results of the MIC presented in figure 2, makes it possible to advance that the extract obtained by the decoction technique inhibits the growth of the L12 strain at the MIC 50 mg/ml, of L2, L5 and L13 strains at MIC 25 mg/ml and for L14 strain at MIC 12,5 mg/ml.

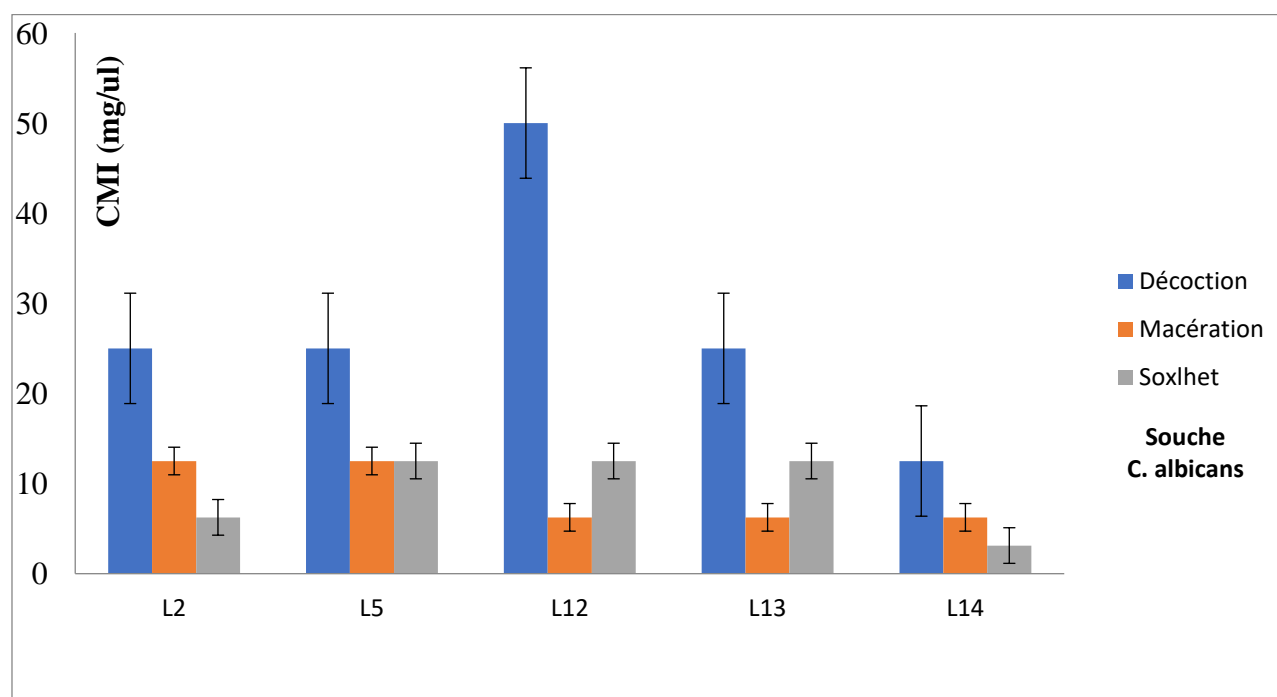


Figure 2: Minimum inhibitory concentrations 5MIC^o of the extracts of *Quercus suber* L. tested according to the five strains of *C. albicans*.

However, at the dilution 3,12 mg/ml, all the extracts tested were found to be inactive on all of the strains studied. And for the highest dilution 1,56 mg/ml, the extract obtained by the Soxhlet technique is inactive on the L14 strain.

Thus, the comparison of the effects of the three extracts tested shows that that extracted by the decoction is the least effective regarding all the strains.

Therefore, it can be said that all strains are sensitive to the treatment of the three extracts, but the L14 strain, isolated from a sample of toenail, was shown to be more sensitive than the others. The L2 and L5 strains from nail infections of the finger showed the same degree of sensitivity to the extract from decoction and maceration.

Also, strains L12, and L13 from neonatal infection and from oral sample, respectively, showed the potential for sensitivity to both extracts, from maceration and to Soxhlet.

Furthermore, the analysis of the effectiveness of the synthetic chemical Fungizone administered at doses indicates for the treatment of *Candida albicans*, showed ineffectiveness on the L13 strain. On the other hand, it was as effective on strains L2, L5, L12 and L14. By this fact, it can be said that the L13 strain is resistant to chemical treatment.

Fungicide/ Fungistatic tests

Table 2: Fungicidal (CMF) and fungistatic (CFS) effect of the tree extracts of *Quercus suber* on the five strains of *C. albicans*. CMF : Minimum fungicidal concentration ; CFS : Minimum fungistatic concentration ; L2 and L5: Strains taken from the finger-nail ; L12 : Sample strain taken from the umbilical catheter ; L13 : Oral swab strain ; L14 : Toe nail sample strain

	L2		L5		L12		L13		L14	
Extracts	CMF	CMS	CMF	CMS	CMF	CMS	CMF	CMS	CMF	CMS
Decoction	-	25	-	25	-	50	-	25	-	12.5
Maceration	-	12.5	-	12.5	50	6.25	-	6.25	-	3.12
Soxhlet	6.25	-	12.5	-	12.5	-	12.5	-	6.25	3.12

The **MIC** is the minimum inhibitory concentration. It's defined as the minimum concentration of the extract which inhibits fungal growth by more than 90%. It is determined by the technique of microdilution in liquid medium.

The **CMF** is the minimum concentration inducing a fungicidal effect, that is to say the concentration which completely inhibits the growth of the fungus by lysis of the fungal wall. The **CMS** is the minimum concentration inducing a fungistatic effect, i.e. inhibition of cell proliferation

Determination of the fungicidal/fungistatic profile for concentrations greater than or equal to the MIC (Table 2) revealed that Soxhlet extract has a fungicidal effect on all strains at varying concentrations, and a fungistatic effect on strain L14 at the dose 3,12 mg/ml.

For the decoction extract, all five strains presented MIC that coincide with the minimum fungistatic concentration.

Furthermore, the extract obtained from the maceration showed fungistatic activity on all strains and fungicidal activity on the L12 strain at a concentration of 50 mg/ml.

Acute toxicity test

Clinical signs

Upon administration of the dose of 300 mg/kg and 2000 mg/kg of Soxhlet extract of *Quercus suber* intraperitoneally (IP), the animals tested show in body appearance, physical activity and their usual behavior. Table 3 summarizes the disorders recorded during this experiment.

Table 3: Clinical signs observed after intraperitoneal administration of Soxhlet extract from *Quercus suber*

Dose	Observation
Control	Normal behavior
300 mg/kg	Short period of depression and sedation and a decrease in reflex. After about 5 to 10 minutes, the animals resumed their normal habits
2000 mg/kg	<p>Grouping of all the mice tested</p> <p>Decreased mobility</p> <p>Increased sleepiness</p> <p>Breathing difficulties</p> <p>Reduced sensitivity to pain and noise</p> <p>Loss of appetite leading to refusal to eat as well as paralysis of the hind limbs.</p>

Weight evolution

Taking into account the doses (Control – 300 mg/kg – 2000 mg/kg), the extract did not induce any significant change in mice at the dose of 300 mg/kg. However, at the dose of 2000 mg/kg, there is a significant decrease in weight and the death of all mice (figure 3).

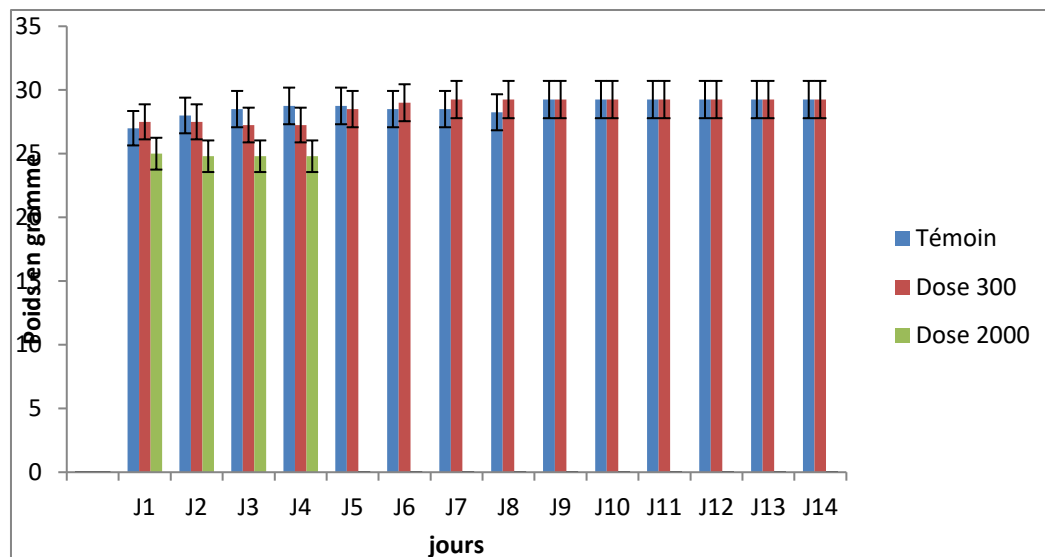


Figure 3: Evolution of the weight of the 4 mice after injection of the dose of 300 mg/kg and the dose of 2000 mg/kg IP of the extract of Soxhlet from *Quercus suber L.*

Calculation of the LD50

The curve representing the logarithm of the doses of the Soxhlet extract injected as a function of the percentage of mortality is a linear regression line.

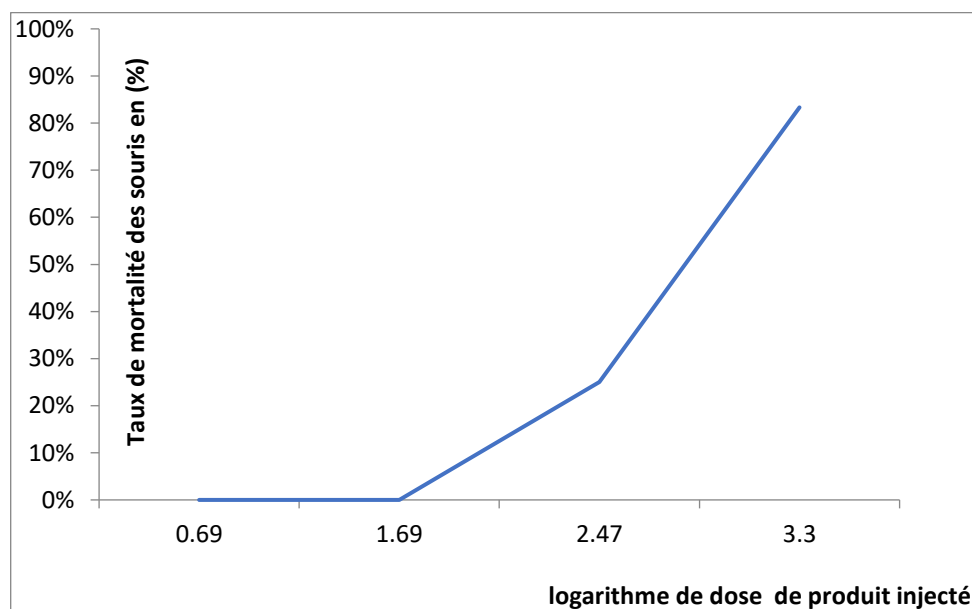


Figure 4: Acute toxicity curve of Soxhlet extract of quercus suber L. in mice (according to the method of Dragstedt and Lang)

The LD50 calculated by the method of Dragstedt and al. is around 1150 mg/kg (Figure 4).

According to Hodge and Sterner Toxicity Scale, this LD50 value (1150 mg/kg) indicates that the Soxhlet extracts of the bark of *Quercus suber*, administered intraperitoneally, is slightly toxic in mice.

Discussion

Two methanolic extracts were carried out in order to demonstrate the one which gave the best yield. Performance appraisal is very important ; it allows to appreciate the total extracts that can be obtained from each species and to determine the quantity of plant material must be removed, which would make the rational use, respectful of the environment and therefore sustainable of the species referred to Kpemissi [31].

In the literature, work on the bark powder of *Quercus suber* has only been done by the Soxhlet extraction technique. In our study, the yield obtained by this technique is of the order of 17,5% in comparison with 7% and 18,5% respectively advanced by [32, 33], during work carried out on the same plant species and the same extraction process.

Of the two techniques used, Soxhlet extraction gave the best yield (17,6%) for a period of 32 hours and with less consumption of plant material and solvent.

Extractions by maceration gave an almost similar yield, but it requires more time, more plant material and solvent.

Current research is focused on optimizing extraction techniques to obtain extracts of good quality and in short time. As a result, the Soxhlet extraction technique is more efficient compared to the maceration technique.

This work also highlighted the antifungal activity of the extracts obtained from the three extraction techniques on the five strains of *Candida albicans* studied. All the strains were found to be sensitive to the different treatments and the most sensitive in each case were not necessarily those with the lowest number of CFU. However, this sensitivity varies depending on the one hand on the extraction technique used and on the other hand on the concentration of the extract, depending on a dose-response relationship.

In general, extracts from the powder of the bark of *Quercus suber*, show very interesting activity against strains of *Candida albicans*. This inhibitory power varies depending on the extract obtained and the strain tested.

However, yhe maceration extract and the Soxhlet have shown an inhibitory effect on MIC which reach 6,25 and 3,12 mg/ml respectively.

Moreover, Akroum S., in 2016, suggested that the acetone extract of the powder of *Quercus suber* has an active effect in vitro and in vivo on *Candida albicans* [34].

Other species and parts of the plants of the genus *Quercus* have also been the subject of several studies on anticandidosis activity, particularly against *Candida albicans*. Thus, Didem and al [14] showed that the methanolic extracts of the leaves of *Q. suber*, *Q. cerris*, *Q. pubescens* and *Q. coccifera* have a high antifungal activity on the *Candida* species tested : *C. albicans*, *C. krusei* and *C. parapsilosis*.

The fungicidal/fungistatic profile also varies depending on how the extracts are extracted. The Soxhlet methanolic extract tested showed a fungicidal effect on all strains of *Candida albicans*. The fungistatic effect of the maceration and decoction extract observe dis temporary because it blocks the development of pathogens without eliminating them completely [37].

In this context, this work made it possible to determine the lethal dose 50% or LD50 of the Soxhlet extract of the powder of the bark of the cork oak (*Quercus suber* L.) in male mice and to highlight the existence of anticandidosis activity.

The study of the acute toxicity of the methanolic extract of the bark of *Quercus suber* showed that the statistical analysis of the parameter weight of the mice which received the dose of 300mg/kg of the methanolic extract of *Quercus suber* showed no significant difference. At the dose of 2000mg/kg, the weight was significantly reduced. So the extract did not induce any significant change in the mice at the dose of 300mg/kg, however at the dose of 2000mg/kg, there was a significant decrease and the death of all the batch of mice. The lethal dose is 1150mg/kg in male mice when administrated intraperitoneally.

By comparing this value with that of another study carried out in 1989 but by oral administration in mice and rats this substance can be considered as slightly dangerous [38].

In addition, at the end of this test for the evaluation of the doses of the methanolic extract of bark of *Quercus suber* ranging from 300 to 2000 mg/kg. This makes it possible to deduce a dose- response effect of this extract on the mice.

This result agree with those of Djyh Gb and al which showed that the intraperitoneal administration of the total aqueous extract of the bark of *Mansonia altissima* has a dose effect on mice and that the mortality rate increased by 30% for a dose ranging from 175 mg/kg to 200 mg/kg.

On the other hand, the study of the acute oral toxicity of the aqueous extract of the fruit powder of *Quercus suber* revealed an absence of mortality eveb for a high dose of 5000 mg/kg of body weight in all groups of rats after 14 days treatment [39].

The value of the LD50 in our work is lower than that advanced by Milrzaei and al, in 2013, which shows that the LD50 of the aqueous extract of the fruit powder of *Quercus suber* administrated intraperitoneally in rats is clearly greater than 5000 mg/kg. This difference can be explained by the part of the plant used, which suggests that the fruit of *Quercus suber* is less toxic than the rind [40].

Also, the LD50 value of *Quercus suber* in our experiment remains higher than those found in *Anogeissus leiocarpus* and *Mansonia altissima*, whose LD50s are respectively 290,81 and 186,5 mg/kg of body weight [41].

In addition, the various acute toxicity tests of several plants carried out by [41] have generally observed the same clinical signs of toxicity, namely a decrease in the motor activity of the animals demonstrated in our work.

This work has therefore contributed to the enhancement of the traditional uses exploited as a rapid and efficient approach to discover the medicinal properties of methanolic extracts from the powder of the bark of *Quercus suber* resulting from different extraction processes.

Each extract showed its anticandidosis power to varying degrees depending on the extraction technique used and the yeast strain studied. The Soxhlet extract proved to be the best, offering in addition to a strong anticandidosis activity even at high concentrations, a good yield in a short time and with a saving in plant matter and in energy. This process could therefore be extended on an industrial scale and be offered as an alternative solution to synthetic pharmaceutical products indicated today against fungal infections caused by *Candida albicans*. The isolation of the active ingredients of the extract would also be interesting in order to demonstrate the active molecule which is involved in the anticandidosis activity.

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