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Study of the Antioxidant Activity of Methanol Extract from *Ficus Sakalavarum* (Moraceae) in the Mice

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Abstract

Oxidative stress is one of the key factors in the onset of many chronic diseases such as diabetes, Alzheimer's disease, rheumatism, and cardiovascular diseases. Certainly, medicinal plants offer natural antioxidant molecules, but their effects and toxicity were less studied and unknown. Traditional Malagasy used the bark of *Ficus sakalavarum* to treat diabetes, however, there is a dearth of information on the antioxidant effect and the toxicity test of this plant species. The antioxidant activity of the methanol extract from the bark of *Ficus sakalavarum* was evaluated using the thediphenyl-picrylhydrazil (DPPH) free radical scavenging test. The toxicological evaluation of the extract was carried out by determining toxicity parameters and microscopic

observation of vital organs after administration of 4000 mg/kg of the methanol extract in mice. The methanol extract at 3.12 µg/ml and 25 µg/ml inhibited the DPPH free radicals between 8.37% and 43.93%, respectively. The median Inhibitory Concentration (IC₅₀) corresponded to 27.96 µg/ml, indicating strong antioxidant activity. No mortality was observed in the animal up to a dose of 4000 mg/kg. The signs of toxicity disappeared, and the animals' behavior returned to the normal after 24 hours of the treatment. This study justifies the antioxidant power of *Ficus sakalavarum* and represents a relevant approach for the development of a natural antioxidant phytomedicine.

Keywords: *Ficus Sakalavarum*, Antioxidant, Toxicity

1. Introduction

Oxidative stress is involved in a wide pathological spectrum and generate a significant impact on population health. Reactive oxygen species (ROS) can become toxic when the production was excessive and the quantities uncontrolled (Sanchez, 2017) [23]. This is a form of cellular aggression primarily caused by reactive oxygen species. Just like inflammation, oxidative stress is recognized as a key player in the onset and complications of many chronic pathologies, such as diabetes, Alzheimer's disease, rheumatism, and cardiovascular diseases (Bidié and *al.*, 2011) [9]. To protect themselves from the toxic effects of ROS, plant-derived molecules are able, to some extent, to limit the damage caused by free radicals by the antioxidant defense mechanisms (Hennebelle, 2006) [14].

Medicinal plants have always held an important place in the therapeutic arsenal, offering possibilities for effective and accessible treatments for populations. Moreover, the high cost of healthcare services and medications, as well as socio-economic factors, drive a large portion of the population to resort to medicinal plants for treatment (Agban and *al.*, 2013) [3]. However, the use of medicinal plants can be rejected simply out of prejudice under a scientific guise citing the lack of data or their toxicity (Fong and *al.*, 1977) [12]. Currently, the use of synthetic antioxidant molecules is being questioned due to potential toxicological risks (Tadhani and *al.*, 2007) [24]. However, interest in the search for natural plant-based antioxidants has significantly increased. Here we describe and evaluate the antioxidant activity and toxicity of the bark of *Ficus sakalavarum* (Moraceae) using the methanol extract, which is used in traditional Malagasy medicine to treat diabetes.

2. Materials and Methods

2.1 Plant Material

The bark of *Ficus sakalavarum* was harvested in the BOENY region, in northwestern Madagascar. The samples were dried in the shade and a well-ventilated area. Once dried, the bark was grounded. The obtained powders were stored in a dry place away from moisture and light until the usage.

2.2 Preparation of the Plant Extract

We conducted a series of extractions using solvents to increase polarity at the laboratory of the *National Center for the Application of Pharmaceutical Research (CNARP)*, Antananarivo.

300 g of the samples was macerated 3 times in 1300 ml of C₆H₁₂ during 3 days, filtered using filter paper and evaporated to dryness at 50°C using a rotary evaporator. Then, a second maceration was realized using the Marc underwent four times in 1200 ml of DCM during 4 days in order to solubilize moderately polar products. The macerates were filtered using filter paper, then evaporated to dryness at a temperature of 50 °C with a rotary evaporator. Finally, a third maceration was carried out 5 times in 1300 ml of MeOH, over a period of 5 days in order to solubilize the polar products as much as possible (Fig 1, P:4). After filtering the macerates, the process was undergoing as previously.

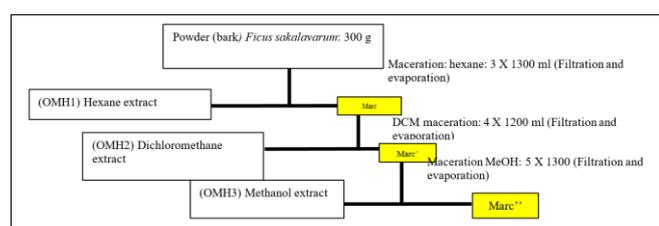


Fig 1: Extraction of *Ficus sakalavarum* bark powder with solvents of increasing polarity

2.3 Phytochemical Studies

The phytochemical screening was performed on the methanol extract of *Ficus sakalavarum* using Fong's method (Randimbivololona, 1996) [22]. It involves reacting the extract with specific reagents for each chemical family. The precipitation and the color change reveal the presence of the chemical families to be determined.

2.4 Experimental Animals

The biological study was conducted on Swiss breed mice, male and female, aged between 7 and 8 weeks, raised at the Laboratory of Biotechnology, Environment, and Health (LRBES) at the University of Mahajanga. The animals were fasted for 18 hours before the experiment. Then, the animals were divided into two groups of ten mice: the control mice were treated with distilled water, while the treated mice received the methanol extract of *Ficus sakalavarum*.

2.5 Evaluation of the Antiradical (Antioxidant) Effect

The antioxidant test was conducted according to the method of Awika and al (2003) [8] based on the ability of the methanol extract from the bark of *Ficus sakalavarum* to scavenge free radicals from diphenyl-picrylhydrazil

(DPPH). For this, a range of concentrations between 2 and 50 µg/ml of the methanol extract of *Ficus sakalavarum* was prepared in the methanol. A volume of 2.5 ml of this solution was mixed with 2.5 ml of a 4.5% DPPH solution, prepared in methanol. After homogenization, the mixture was incubated at room temperature, away from light. After 15 minutes of incubation, the absorbance of the solution was measured at $\lambda = 517$ nm against a "control" solution not containing the extract. The ability to trap free radicals was evaluated by determining the decrease of the percentage in discoloration (inhibition) of DPPH in the methanol solution using the formula:

$$\text{Inhibition rate} = \frac{(\text{Abs control} - \text{Abs test})}{\text{Abs control}} \times 100$$

With: Abs denotes the absorbance at the wavelength $\lambda = 517$ nm; Abs control: the absorbance of the control; and Abs test: average of the absorbance of each extract.

The IC₅₀ (concentration that inhibits 50% of DPPH free radicals) was subsequently determined using the linear regression curve equation representing the percentage of inhibition as a function of the concentration of *Ficus sakalavarum* bark extract. In this case, the extract was considered to have strong antioxidant activity if its IC₅₀ value was < 30 µg/ml. This activity was considered moderate if 30 µg/ml < IC₅₀ < 100 µg/ml (Ahmad and al., 2010) [4].

2.6 Evaluation of Acute Toxicity

The toxic effects of the methanol extract of *Ficus sakalavarum* bark were evaluated after oral administration of a high dose of 4000 mg/kg at a rate of 10 ml/kg to 30 males and females mice. The behavior and number of deaths of the animals were monitored during 7 days. During the first phase, the behavior of the animals was observed every hour until 6 AM, then the observation continued for 7 days, during which the mice had free access to food and water. During this period, signs of toxicity, notably changes in coat, motility, tremors, grooming, respiration, mobility, as well as death, were observed and noted (Etame and al., 2017) [10]. During the second phase, at the end of the seventh day of observation, the animals were sacrificed and a dissection was performed, isolating the vital organs (heart, stomach, pancreas, liver, kidney) to check for tissue damage. The observation was first done with the naked eye and then through microscopic analysis to provide details on the degree of tissue alteration. Finally, a comparative study on the histological and anatomical characteristics of vital organs was conducted between the groups of mice treated and not treated with the extract.

3. Results

3.1 Extraction Product

The extraction with solvents of increasing polarity allowed us to obtain three extracts with their respective yields (Fig 2, P:6), such as: the hexane extract, with a mass of 14.07 g, having a yield of R = 4.69%. The dichloromethane extract, with a mass of 3.927 g, having a yield R = 1.3%. Finally, the methanol extract, with a mass of 35.23 g, having a yield R = 11.74%.

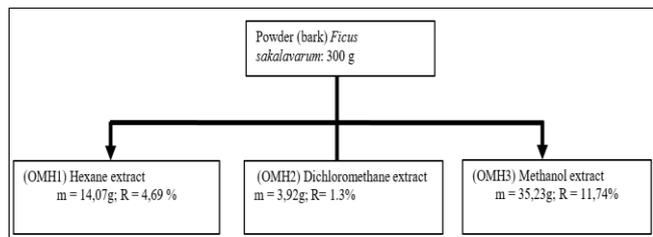


Fig 2: The hexane, DCM, and methanol extracts from the bark of *Ficus sakalavarum*

3.2 The Secondary Metabolites Present in the Methanol Extract of *Ficus Sakalavarum* Bark

The methanol extract of *Ficus sakalavarum* contains high levels (+++) of tannins, reducing sugars, steroids, and terpenoids. Furthermore, phenolic compounds, saponins, and coumarins are found in moderate concentrations (++) . It also contains flavonoids and alkaloids but in low concentrations (+) (Table 1).

Table 1: Secondary metabolites present in the methanol extract of *Ficus sakalavarum*

Phytochemical families	Tests	Results
Tannins	Gélatine + NaCl	+++
Reducing sugars	3 volumes of ethanol	+++
Steroids	Acetic anhydride + H2SO4	+++
Terpenoids	Acetic anhydride + H2SO4	+++
Anthocyanins	Concentrated HCl + water bath at 37° C	++
Saponins	Foam index	++
Phenolic compounds	Gelatin 1%	++
Coumarins	NH4OH at 10%	++
Flavonoids	Concentrated HCl	+
Alkaloids	DRAGENDORFF	+

3.3 Antiradical Activity of the Methanol Extract of *Ficus Sakalavarum* Bark

The results of the antiradical activity test of the methanol extract of *Ficus sakalavarum* bark are summarized in Table 2. The antiradical activity is concentration-dependent. The IC50 value obtained from the linear regression curve is 27.96 µg/ml (Fig 3).

Table 2: Effect of the methanol extract of *Ficus sakalavarum* bark on diphenyl-picrylhydrazil (DPPH) free radicals: Absorbance at 517 nm

Extract (µg/ml)	Absorbance (Mean ± e.s.m)	Inhibition of absorbance (%)
50	0.192 ±	82.873 ±
25	0.630 ±	43,930 ±
12.5	0.735 ±	34,521 ±
6.25	0.957 ±	14,752 ±
3.125	1.029 ±	8,370 ±

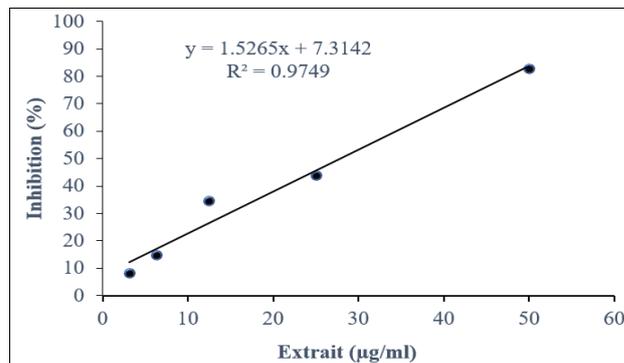


Fig 3: Anti-radical activity of the methanol extract of *Ficus sakalavarum* bark against the free radical of diphenyl-picrylhydrazil (Mean ± SEM, n = 3). y is the equation used for the determination of IC50 and R2, the correlation coefficient between the different tested concentrations and the observed antiradical activities

3.4 Toxicity of the Methanol Extract of *Ficus Sakalavarum* Bark

The oral administration of the methanol extract of *Ficus sakalavarum* bark at a dose of 4000 mg/kg in mice does not cause any mortality. Moreover, at the first hour of observation, piloerection, loss of appetite, as well as accelerated breathing persisted during four hours. After 5 hours, other signs of toxicity such as decreased motor activity, reduced reaction to noise, and diarrhea appear and persist until the 6th hour of observation. At the 24th hour of observation, these signs disappear and the animals gradually return to their normal behavior (Table 3). Additionally, microscopic observation (X 100) of histological sections of the heart, liver, and kidney of treated mice revealed no tissue lesions (Fig 4, P:9).

Table 3: Signs of toxicity observed during the toxicity evaluation of the methanol extract of *Ficus sakalavarum* bark

Signs of toxicity	Observation time (h/day)								72 hours to 6 days
	1	2	3	4	5	6	24	48	
Piloerection	+	+	+	+	-	-	-	-	-
Lack of Appetite	+	+	+	+	-	-	-	-	-
Motor Activity (dynamism)	N	N	N	N	N	N	N	N	N
Diarrhea	-	-	-	-	+	+	-	-	-
Intense breathing	+	+	+	+	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-
Number of deaths	0	0	0	0	0	0	0	0	0

-: without, + : with, N : normal

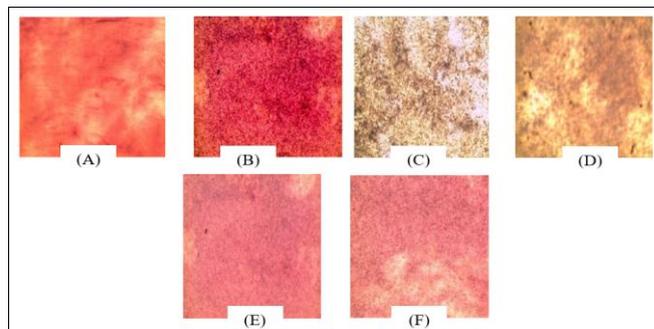


Fig 4: Microscopic observations of histological sections of the heart, kidney, and liver of mice treated (A, C, E) or not (controls: B, D, F) with 4000 mg/kg of the methanol extract of *Ficus sakalavarum* bark, respectively.

4. Discussion

Several factors can cause cellular oxidation, but the most cited is a high-fat diet. It seems that a high-fat diet promotes the excessive production of ROS (Reactive Oxygen Species) on one hand, and the weakening of antioxidant defense capacity on the other. Indeed, the alteration of the oxidant/antioxidant balance causes cellular damage, resulting, among other things, in an increase in lipid peroxidation, protein oxidation, and increased DNA damage. All these alterations lead to the induction of systemic oxidative stress (Marseglia and *al.*, 2015) [16]. Various scientific studies demonstrated through the use of DPPH that plants belonging to the genus of *Ficus* (Moraceae) present antioxidant properties, such as the phenolic compounds of *Ficus carica* (Aneta and *al.*, 2016) [6], the ethanolic extract of *Ficus elastica* leaves and its compounds (Ginting and *al.*, 2020) [13], the bark of *Ficus amplissima* (Rajan and *al.*, 2012) [21], the non-enzymatic and enzymatic antioxidants of *Ficus deltoidea* (Mansor and Mahmood, 2009) [15], *Ficus religiosa* (Arpana and *al.*, 2022) [16], etc. Moreover, according to Abas and *al.* (2006) [1], natural products with antioxidant activities with IC₅₀ < 30 µg/ml revealed a very strong antioxidant activity. In this study, the evaluation of the antioxidant activity of the methanol extract of *Ficus sakalavarum* revealed that this extract inhibited DPPH free radicals with an IC₅₀ = 27.96 µg/ml < 30 µg/ml, and presented a strong antioxidant activity. In general, the secondary metabolites, particularly phenolic compounds and flavonoids found in plants, are recognized as scavengers of free radicals such as hydroxyl radicals and superoxide anions. They also inhibit lipid peroxidation (Fatine and *al.*, 2022) [11]. Polysaccharides are also the source of antioxidant activities, regardless of their nature (Xu and Vanhoutte, 2012) [25]. Among the polysaccharides, sucrose is known for its antioxidant power. Their reducing groups are capable of neutralizing free radicals (Alaa and *al.*, 2023) [5]. Tannins also exhibit numerous biological activities, including antioxidant effects (Oroian and Escribe, 2015) [20]. As we have shown in our previous study, these secondary metabolites are also present in *Ficus sakalavarum* (Omary and *al.*, 2020) [18]. As a result, these secondary metabolites could be responsible for the antioxidant property of the methanol extract from the bark of *Ficus sakalavarum* highlighted in this study.

The results of the acute toxicity test also demonstrated that the methanol extract from the bark of *F. sakalavarum* does not alter the general behavior of mice up to a dose of 4000 mg/kg. These results showed that at this dose, the mice tolerated the plant extract and the LD₅₀ (lethal dose causing the death of 50% of the mice) of this plant could be significantly higher than 4000 mg/kg. Furthermore, it has been reported that a high LD₅₀ value would indicate a wide safety margin for the use of a product (Ondele and *al.*, 2015) [19]. Moreover, according to the Globally Harmonized System (GHS) for the classification of chemical products, such substances are classified in category 5 of compounds with relatively low acute toxicity (UNECE, 2021) [17]. Such a result was observed in the work of Adjoua and *al.* (2022) [2], on the toxicity study of *F. sycomorus* (Moraceae) In this study, the authors stated that the plant *Ficus sycomorus* with an LD₅₀ > 5000 mg/kg showed no toxic effects and was classified in category 5 of substances with relatively low acute toxicity. Given these results, the methanol extract of *Ficus sakalavarum* bark could also be classified in category

5 of substances with relatively low toxicity. This classification would also be further justified by the results of the microscopic observation of the tissues of the vital organs of the mice used in this study, which revealed no lesions or anatomical structural modifications.

5. Conclusion

Oxidative stress is recognized as the cause of the onset and complications of numerous chronic pathologies such as diabetes, Alzheimer's disease, rheumatism, and cardiovascular diseases. Plants, through their diverse content of secondary metabolites, can be used as antioxidant agents in the treatment of these pathologies. *Ficus sakalavarum* is one of these plants whose bark is traditionally used in the treatment of diabetes. The work carried out within the framework of this study demonstrated that the methanol extract of *Ficus sakalavarum* bark exhibited antiradical activity against DPPH free radicals. It inhibits these free radicals with an IC₅₀ value of less than 30 µg/ml, qualifying it as an extract with high antioxidant activity. It is not toxic up to a dose of 4000 mg/kg administered orally in mice, allowing it to be classified in category 5 of substances with low toxicity. The results of this study highlighted an understanding of the traditional use of *Ficus sakalavarum* in the treatment of pathologies related to oxidative stress. However, further studies are necessary to isolate and identify the active principle(s) involved in this observed antioxidant activity.

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