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# Study of the Cosmetic Potential Uses of Plants from Mayotte as Skin Care Agents through the Screening of Their Biological Activities

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**Abstract:** (1) The island of Mayotte, located in the Indian Ocean, possesses remarkable biodiversity. In a previous ethnobotanical study, we already highlighted 69 different plant species used in traditional medicine. Among those, 21 were traditionally employed for skin care by the local populations. The present study aimed to better understand the traditional use of those plants by investigating their in vitro biological activities and, more specifically, their anti-lipoxygenase, antioxidant and anti-tyrosinase properties. (2) These three activities were, respectively, determined by lipoxygenase inhibition, DPPH-reducing potency, and anti-tyrosinase activity assays. (3) Results revealed high biological activities for several plant species, with some of them displaying one strong single activity, while others had at the same time anti-lipoxygenase, antioxidant and anti-tyrosinase effects. (4) Those in vitro biological activities agreed with the traditional use of those plants by the local population. It also highlights the high potential of those species from Mayotte in the development of new cosmetic ingredients for the treatment of many skin affections, such as eczema.

**Keywords:** Mayotte; cosmetics; skin care agents; traditional uses; anti-inflammatory; anti-tyrosinase; antioxidant



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# 1. Introduction

The island of Mayotte is part of the Comoros archipelago located in the Indian Ocean. One of the particularities of this island is its localization in a geographical area where the climate is appropriate for the development of exceptional biodiversity. Indeed, it has been reported that 25% of the earth's biodiversity is present in the Indian Ocean area [1]. It was around 1820 that the interest in tropical islands from a botanical point of view began to grow, as De Candolle [2] notably suggested that "… 'Islands' flora is worth studying due to the peculiar characteristics shown by their vegetation or to the fact that the study areas are clearly defined, thus the studies can be done thoroughly … ". Since then, this affirmation has been confirmed many times in numerous insular areas of the world. However, Mayotte was not one of them, as very little research has been done about this small island [2].

On the other hand, the history of the island of Mayotte and, notably, the successive settlement of people from African Bantu and Arab-Muslim origins [3] explains that the use of traditional medicine is still very common and deeply ingrained in the habits of the inhabitants [4–6]. Indeed, as these people have a strong bond to their cultural heritage, traditional cosmetic practices are still used nowadays. The conservation of this knowledge is based on an oral transmission that passes from one generation to another [7,8]. Mayotte has, therefore, much to offer when it comes to ethnobotanical studies [9].

During a previous ethnobotanical field study on the Island of Mayotte, we identified 69 plants used in traditional medicine for the treatment of many different conditions [10]. Among these plants, we noted a large number of uses related to skin care, whether to treat skin problems or for cosmetic purposes [10]. As the skin is the first protective layer of

the human body, it withstands all types of stress, either endogenous, such as the effect of aging, or exogenous, such as sun exposition. These stresses are responsible for many skin imperfections and afflictions [11]. It did not take long for humanity to understand that these deleterious effects could be thwarted using what nature has to offer. Therefore, plants have been used to heal but also for aesthetic uses through makeup and perfume for as long as humanity can remember. These practices are deeply linked to man's evolution [12,13].

In the present work, we focused more particularly on 21 plants traditionally used for skin care by the inhabitants of Mayotte based on our previous ethnobotanical study. Indeed, pharmaceutical industries are continuously searching for new bioactive molecules. The main purpose of the present study was, therefore, to investigate the potential of these plants for use as an ingredient in cosmetics through the study of three targeted biological activities: anti-lipoxygenase activity linked to anti-inflammatory properties, antioxidant activity, and anti-tyrosinase activity related to the treatment of pigmentation issues.

The inflammatory triggers can be divided into infections, tissue injuries, as well as tissue stress and malfunction. In the present work, we will focus on the second and third types of affection since the anti-inflammatory properties are meant to be paired with cosmetic practices. Among the different pathways linked to the appearance of inflammatory reactions, the research for lipoxygenase (LOX)-inhibitory compounds is crucial as LOX is responsible for the synthesis of leukotrienes, which are pro-inflammatory molecules [14]. Concerning antioxidant activity, we focused on the reaction involving DPPH (2,2-Diphenyl-1-picrylhydrazyl), which has previously been described as a good indicator of the antioxidative power [15]. This specific test assay indeed detects the scavenging potency of free radicals through the scavenging activity of the stable DPPH free radical. Finally, the tyrosinase inhibition capacity of plant extracts was used to evaluate their potential to treat skin pigmentation issues. Indeed, tyrosinase is the key enzyme in the biosynthesis of melanin that plays a major protective role against skin photocarcinogenesis, such as melanoma. Melanin is also responsible for phenomenon known as hyperpigmentation and hypopigmentation, often visible in the form of darker or lighter spots, zones or body parts [16]. The search for natural chemical agents capable of modulating pigmentation metabolism is, therefore, of great interest.

# 2. Materials and Methods

# 2.1. Reagents

Acetone ( $\geq$ 99%, technical), methanol ( $\geq$ 98.5%, technical), K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, pure ethanol, NaOH, and H<sub>3</sub>BO<sub>3</sub> were purchased from VWR chemicals (Leuven, Belgium). DPPH (2,2-Diphenyl-1-picrylhydrazyl), mushroom tyrosinase (EC 1.14.18.1), kojic acid, TROLOX (( $\pm$ )-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), linoleic acid, Tween 80, L-DOPA (3,4-dihydroxiphenilalanine) and *Glycine max* lipoxygenase (EC 1.13.11.12) were purchased from Sigma-Aldrich (Darmstadt, Germany).

#### 2.2. Plant Extracts Preparation

Based on the results of the previous ethnobotanical survey conducted by M. Saive in 2014 on the island of Mayotte [10], 21 different plant species were collected, and a specimen was stored for each sample at the CBNM herbarium in Mayotte [10]. All available organs at the time of the harvest were collected, leading to a total of 89 samples (see Table 1).

**Table 1.** Recapitulative table presenting the acetone extracts obtained from the different plant species and organs with the biological activities investigated.

Species	Plant Organs	Anti-Lipoxygenase Activity	In Vitro Tests DPPH-Reducing Potency	Anti-Tyrosinase Activity
Acalypha hispida	Dried flower	Х	Х	Х
,	Dried leaf	X	X	X
	Dried root	x	X	X
	Dried wood	x	X	X
	Fresh leaf	X	X	X
Acalypha wilkesiana	Dried leaf	Х	Х	х
	Dried root	x	X	X
	Dried wood	x	X	X
	Fresh leaf	X	X	X
Adansonia digitata	Dried seed	Х	Х	х
ū	Dried wood	X	X	X
Aloes mayottensis	Mucilage	Х	Х	x
	Dried leaf	x	X	X
	Fresh leaf	X	X	X
Cananga odorata	Dried flower	Х	Х	Х
	Dried leaf	X	Χ	X
	Dried root	x	X	X
	Dried wood	X	Χ	X
	Fresh flower	X	X	X
Erythroxylum corymbosum	Dried leaf	Х	Х	X
	Dried root	x	X	X
	Dried wood	x	X	X
	Fresh leaf	Х	Х	x
Erythroxylum lanceum	Dried leaf	X	X	x
	Dried root		X	
	Dried wood	X	X	X
	Fresh leaf	X	X	X
Kalanchoe pinnata	Dried leaf	X	X	x
	Dried root	x	X	X
	Dried stem	X		X
	Fresh leaf		X	X
Lantana camara	Dried fruit	X	X	X
	Dried leaf	x	X	
	Dried root		X	X
	Dried wood	x	X	X
	Fresh leaf	X	X	X
Lawsonia inermis	Dried leaf		Х	x
	Dried root	X	X	X
	Dried wood	X	X	X
	Fresh leaf	X	X	X
Leea guineensis	Dried fruit	Х	Х	x
	Dried leaf	X	X	X
	Dried root	X	X	X
	Dried wood	X	X	X
	Fresh leaf	X	X	X

Table 1. Cont.

Species	Plant Organs	Anti-Lipoxygenase Activity	In Vitro Tests DPPH-Reducing Potency	Anti-Tyrosinase Activity
Litchi chinensis	Dried leaf	Х	Х	х
	Dried root	x	X	X
	Dried wood	X	X	X
	Fresh leaf	X	X	x
Litsea glutinosa	Dried leaf	Х	Х	х
	Dried root	x	X	X
	Dried wood		x	
	Fresh leaf	X	X	
Myristica fragrance	Dried seed	Х	х	X
Pandanus mayottensis	Dried aerial root	Х	Х	Х
· ·	Dried fruit	x	x	X
	Dried leaf		x	X
	Dried wood	X	Χ	X
	Fresh fruit	X	Χ	х
	Fresh leaf	X	X	X
Paullinia pinnata	Dried aerial root	Х	Х	х
,	Dried leaf		X	X
	Dried liana	X	X	Х
	Dried root	X	X	X
	Fresh leaf	X	X	X
Persea americana	Dried leaf	X	Х	X
	Dried pit	X	X	X
	Dried root	X	X	X
	Dried wood	X	X	X
	Fresh leaf	X	X	X
	Fresh pit	X	X	x
Sesamum indicum	Dried seeds	X	X	X
Syzygium aromaticum	Dried leaf		X	X
= y = y & v = v = v = v = v = v = v = v = v = v	Dried root		X	X
	Dried wood		X	x
	Fresh leaf		X	x
Tamarindus indica	Dried leaf	X	X	X
111111111111111111111111111111111111111	Dried root	X	X	X
	Dried wood	x	X	x
	Fresh leaf	X	X	x
Zingiber zerumbet	Dried flower	X	X	X
	Dried leaf	X	X	X
	Dried rhizom		X	X
	Floral water	x	X	X
	Flower stem	X X	X	X
	Fresh flower	X X	X X	
	Fresh leaf	X X	X X	X
	Fresh rhizom			X
		X	X	X
	Stem	X	X	X

All samples were dried at 40  $\pm$  1 °C in a drying oven for 48 h. When the vegetal material allowed it, the samples were powdered using an analytical grinder IKA A11 (Staufen, Germany). The global size of the ligneous parts of the vegetal material was reduced using shears. Once the size allowed it, the samples were then ground using a laboratory hammermill mounted with a 6 mm mesh. The ground samples were kept vacuum-packed at  $-22\pm1$  °C until extracted. The extraction process was carried out using

a Soxhlet apparatus with acetone as a solvent. Acetone is indeed recognized as a solvent of choice for the extraction of bioactive compounds and, notably, antioxidants [17]. Two g of dried and ground samples were weighed and poured into the extraction chamber. Thirty mL of acetone was used [18], and the extraction lasted for 6 h. Once the extraction was completed, the crude extract was evaporated using a rotary evaporator (Heidolph, Laborota 4003, Schwabach, Germany). The dried crude extracts were kept at  $-22 \pm 1$  °C until used.

## 2.3. Lipoxygenase Inhibition Evaluation

The lipoxygenase inhibition capacity of plant extracts obtained by Soxhlet extraction with acetone was assessed by a spectrophotometric method described by Tanoh et al. (2019) with some minor modifications [19].

Lipoxygenase is sensitive to heat; therefore, all the following experimental steps were performed on an ice bath. A 0.1 mg/mL lipoxygenase solution ( $\geq \! 50,\!000$  U/mg) was prepared using distilled water. The 1 mM linoleic acid substrate solution was prepared as follows: 140 mg of linoleic acid, 18 mg of Tween 80, and 100  $\mu L$  of NaOH 5 mM were mixed and adjusted to 50 mL with distillate water. The enzyme solution, as well as the substrate, were divided into 1 mL aliquots and stored at  $-22 \pm 1$  °C until used, with only one thaw-out allowed.

As the expected product for the reaction was 13-HPOD, the optimal pH of the reaction matrix was obtained using a 0.1 M sodium borate buffer corrected to pH 9.5 using 5 M NaOH. To avoid false-positive results due to enzyme inactivation caused by acetone, pure ethyl alcohol was used for sample preparation. Five mL of pure ethanol was added to the dry extracts, which were then submitted to sonication and subsequently filtered using 0.45  $\mu m$  PTFE syringe filters (Whatman, Puradisc 13, Maidstone, UK). As the color of the crude extracts was an issue, all samples were diluted in the borate buffer from factor  $10^1$  to factor  $10^4$ . As oxygen is a co-substrate for the reaction, the borate solution was oxygenated for 30 min at room temperature prior to the reaction. The control was realized using 0.1 M borate buffer, leading to a non-inhibited reaction, which can be expressed as 0% of inhibition, and the blank was obtained by carrying out the experiment without the enzyme, which causes the absence of 13-HPOD synthesis and can be considered as 100% of inhibition.

The enzymatic reaction was performed by mixing 100  $\mu$ L of plant sample with 35  $\mu$ L of lipoxygenase and 800  $\mu$ L of the oxygenated buffer. For the control, 900  $\mu$ L of the oxygenated buffer was used. For the blank, 900  $\mu$ L of oxygenated buffer was used, but no enzyme was added prior to the incubation. The mixtures were left to incubate for 15 min at room temperature and then added into quartz absorption cells along with 35  $\mu$ L of the substrate solution. The synthesis of linoleic acid hydroperoxide was observed in real time as absorbance was measured every 20 s for 5 min at 234 nm. Each sample/control was done in triplicate. The relative activity was obtained by putting the slopes of the samples against the slopes of the control, according to Equation (1):

% inhibition activity = 
$$\frac{\text{Control Slope } - \text{Sample Slope}}{\text{Control Slope}} \times 100$$
 (1)

where the control slope is the slope of the control (0% inhibition) at the linear part of the reaction, and the sample slope is the slope of the samples at the linear part of the reaction, defined by an  $R^2$  higher than 0.9.

# 2.4. DPPH-Reducing Potency Evaluation

The evaluation of the DPPH-reducing potency of the plant samples was based on a protocol adapted from M. S. Blois (1958) [20]. A 2  $\times$  10<sup>-4</sup> M DPPH solution was prepared using technical-grade methanol. Once prepared, this solution was kept at 4  $\pm$  1 °C in the dark and was used within the following 7 days.

The positive control for this test was a  $2\times 10^{-3}$  M TROLOX solution prepared with methanol. The blank was likewise performed with methanol. For the sample analysis, 5 mL of methanol was added to the dry extracts, which were obtained by Soxhlet extraction with acetone. The crude extract was retrieved by sonication. The solution was then filtered using 0.45  $\mu$ m PTFE syringe filter (Whatman, Puradisc 13, Maidstone, UK) and submitted to successive dilutions (10 to 10,000 times) in methanol. The analysis went as follows. A 1:1 DPPH:sample/blank/TROLOX mix was performed and left to incubate at room temperature for 10 min. Then the absorbance of the reaction mix was observed using an Ultrospec 9000 UV/Vis spectrophotometer from Biochrom (Cambridge, UK) at 517 nm. Each sample was measured in triplicate. The samples' relative activity was established by comparing the final absorbance with the absorbance obtained with the blank and with the TROLOX, as seen in Equation (2):

% reduction activity = 
$$\left(1 - \frac{\text{Abs TROLOX} - \text{Corrected value}}{\text{Abs TROLOX} - \text{Abs BLANK}}\right) \times 100$$
 (2)

where Abs TROLOX refers to the absorption at 517 nm of the positive control (100% of activity), and Abs BLANK refers to the absorption at 517 nm of the negative control (0% of activity). The corrected value is obtained by Equation (3):

$$Corrected value = Final abs - Sample abs$$
 (3)

where the final abs is equal to the absorbance (517 nm) of the reaction mix after 10 min, and the sample abs is equal to the absorbance (517 nm) of the sample at the studied concentration.

# 2.5. Anti-Tyrosinase Activity Evaluation

To evaluate the anti-tyrosinase activity of the different plant extracts obtained by Soxhlet extraction with acetone, the protocol used was based on and adapted from Rangkadilok et al. (2007) [21].

When working with tyrosinase, all reagents and solutions were kept on an ice bath to avoid any loss of activity due to temperature-linked enzyme degradation. A 625 U/mL tyrosinase solution was obtained by adding 4 mL of  $K_3PO_4$  0.05 M pH 6.5 buffer directly into the commercial vial containing the enzyme (25.000 U) and by vortexing it for 10 s. One mL was retrieved and diluted in the same buffer to reach the required concentration. The total solution was divided into 1 mL aliquots stored at  $-22\pm1\,^{\circ}C$  until used. The aliquots were only thawed once. A 1 mM L-DOPA solution was prepared using distilled water. For this analysis, the inhibition control was a 1 mM kojic acid solution in distilled water, and the blank was pure  $K_3PO_4$  0.05 M pH 6.5 buffer. As for the lipoxygenase evaluation, pure ethyl alcohol was used for the sample preparation. Five mL of pure ethyl alcohol was added to the dry extracts and submitted to sonication. The solution was then filtered using a 0.45  $\mu m$  PTFE syringe filter (Whatman, Puradisc 13, Maidstone, UK) and submitted to successive dilutions (from 10 to 10,000 times) in  $K_3PO_4$  0.05 M pH 6.5 buffer. As oxygen is a co-substrate for the reaction, the buffer was oxygenated for 30 min at room temperature prior to the observation of the reaction.

The enzymatic reaction was performed by mixing 100  $\mu$ L of sample/blank/control with 50  $\mu$ L of tyrosinase and 250  $\mu$ L of the oxygenated buffer. The mixture was left to incubate for 15 min at room temperature and then added into a quartz absorption cell along with 400  $\mu$ L of the substrate solution. The synthesis of dopachrome was observed in real time, and absorbance was measured every 15 s for 5 min at 475 nm. Each sample/control/blank was carried out in triplicate. The relative activity was obtained according to Equation (4):

% inhibition activity = 
$$\frac{\text{Blank Slope} - \text{Sample Slope}}{\text{Blank Slope}} \times 100$$
 (4)

where the blank slope is the slope of the blank (0% inhibition) at the linear part of the reaction, and the sample slope is the slope of the samples at the linear part of the reaction, defined by an  $R^2$  higher than 0.9.

#### 2.6. Statistical Analysis

For enzymatic inhibition assays, the relative activity was determined using the absorbance slope at its maximum intensity. For the DPPH-reducing potency evaluation assay, the relative activity was based on the absorbance variation after a 30-min reaction period at room temperature. In that case, the relative activity was obtained when comparing the positive control (TROLOX) with the sample results. Each assay was carried out in triplicate. For each activity and dilution, all samples were compared two by two using Tukey's grouping test (Statistical software Minitab 19 Minitab, State College, PA, USA) with the following parameters: the null hypothesis was that all averages were equal, the significance limit was  $\alpha = 0.05$ , the confidence interval was bilateral, and the error rate for the comparison was 5. This allowed the identification of samples or groups of samples showing significantly higher relative activities in comparison to the sample batch.

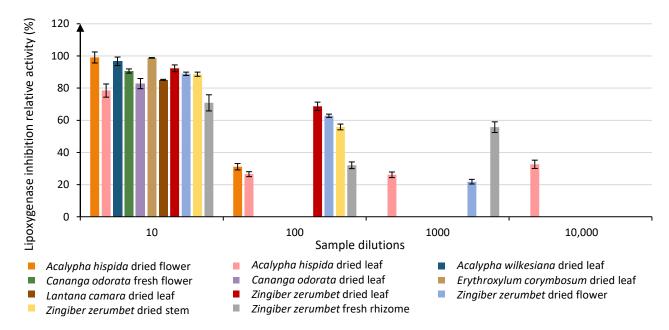
#### 3. Results and Discussion

# 3.1. Anti-Lipoxygenase Activity Evaluation

A total of 77 out of the 89 collected plant samples were tested for their ability to inhibit lipoxygenase activity, which therefore indicates their anti-inflammatory potential. The remaining samples were not included in this assay as they showed a strong absorbance at 234 nm. Serial dilutions of plant extracts were performed, allowing us to determine which species presents the highest relative activity and, therefore, which species contains the highest amount of active compounds. Moreover, species showing results above the limit of quantification (LOQ) up to the 10<sup>4</sup> dilutions are expected to contain several active compounds in their crude extracts or one compound with a high degree of efficiency. The LOQ was established using the relative standard deviation (% RSD) of control replicates immediately after the reagents were thawed. Those %RSD values were not higher than 10%, which is in concordance with standard procedures [22–24].

Out of the 77 samples, 17 absorbed too much at the first dilution but entered the evaluation process starting at the second dilution. Results showed that 27 plant extract samples were able to inhibit the lipoxygenase activity above the LOQ at a dilution of 10<sup>1</sup>. Out of those 27 + 17 samples, 9 were still above the LOQ at a dilution of  $10^2$ . Three of these were still above LOQ at a dilution of 10<sup>3</sup>, and only 1 sample remained above the LOQ at a dilution of 10<sup>4</sup> (Table 2). Results (Figure 1 and Table 2) highlighted that the 27 plant extracts investigated for their anti-lipoxygenase activity at a dilution of 10<sup>1</sup> can be classified into 17 different groups. Some of these extracts displayed strong anti-lipoxygenase activities up to 99.05%, and 2 species particularly stand out as being only classified in the group with the highest relative activity (group A): Acalypha hispida dried flower (99.05%  $\pm$  4.22) and Erythroxylum corymbosum dried leaf (98.83%  $\pm$  2.91). Results (Table 2) also showed that samples with the highest relative activities, which are classified in group A even if there is some crossing over up to group D, ranged from 90.64% to 99.05% of relative activity. These samples can be considered the most effective ones. For the plant extracts diluted 10<sup>2</sup> times, there is a drop-in activity implying a dose-dependent response as only 9 samples were above the LOQ. Out of these 9 samples, 5 groups were observed. The first group (group A) contained two samples with the following activities:  $68.73\% \pm 3.18$  (Zingiber zerumbet dried leaf) and 62.84% ± 1.26 (Zingiber zerumbet dried flower). Interestingly enough, samples from groups A and B only came from one plant species, Zingiber zerumbet, but from different organs of that plant. When looking at the plant extracts diluted 10<sup>3</sup> times, only 3 samples remained, which were classified into 2 groups, A and B. At this level of dilution, the only sample that was classified in group A was zingiber zerumbet fresh rhizome with  $55.73\% \pm 7.40$  of relative activity. Among the samples from this dilution, one that was not considered in the most effective category in the two first dilutions still showed activity

over the LOQ: *Acalypha hispida* dried leaf. Interestingly, only that extract remained above the LOQ with  $32.62\% \pm 2.58$  of relative activity at a dilution of  $10^4$ .



**Figure 1.** Evolution of the relative lipoxygenase inhibition activity of the most efficient plant extract samples through 4 different dilutions (10, 100, 1000 and 10,000). Each column represents the mean  $\pm$  SD (n = 3) per sample.

Through the different dilutions, a dose-response change can be observed for most plant extract samples (Figure 1) in their relative activities, except for *Acalypha hispida* dried leaves. This specific sample is indeed among the most active ones in the first dilution, though it then loses more than 50% of relative activity at the second dilution. It then remains around 30% of activity for the second, third and fourth dilutions as if the inhibition was caused by several compounds, some of these loosing activity as the dilution increases, while others are not impacted by the dilution. There is no simple explanation for these results, as the study of the enzyme inhibition potency of crude plant extracts containing many different molecules is not an easy process. Indeed, these molecules have many interaction options throughout the whole analysis process. They can react with one another but also with the substrate or with the enzyme. In the case of an enzyme interaction, several inhibition processes exist (competitive inhibition, noncompetitive inhibition, uncompetitive inhibition and mixed inhibition). Depending on the type of inhibition, the maximal speed of the reaction, the affinity between the enzyme and its substrate or both can be affected. As the inhibition evaluation was evaluated through kinetic observations, the inhibition phenomenon can also be affected by the incubation time. In addition, it can be due to other regulation modes and not be specifically linked to the enzyme inhibition process. All these phenomena render the study of inhibition arduous, and this is even more so when using an inhibition media as complex as a plant crude extract. This can lead to unexpected observations, such as the one observed for Acalypha hispida where the dilution does not seem to affect the results. Only an in-depth study of the different inhibition mechanisms occurring for this specific sample could help understand such a phenomenon. It is primordial to limit the uses of these results as a primary indication in a screening context.

**Table 2.** Lipoxygenase inhibition activity results for the plant extracts diluted 10, 100 and 1000 times (n = 3). Results were statistically analyzed using Tukey's grouping test (confidence level: 95%), and the observed group classifications (A, B, C . . . . . etc.) are shown in the present table.

Samples	Lipoxygenase Inhibition Activity (%)	Grouping Information Based on Tukey's Method
	Dilution 10	
Acalypha hispida dried flower	99.05	A
Erythroxylum corymbosum dried leaf	98.83	A
Acalypha wilkesiana dried leaf	96.70	A B
Zingiber zerumbet dried leaf	92.36	АВС
Cananga odorata fresh flower	90.64	A B C D
Zingiber zerumbet dried flower	88.92	B C D
Zingiber zerumbet stem	88.66	B C D E
Lantana camara dried leaf	85.19	C D E F
Cananga odorata dried leaf	82.82	D E F G
Leea guineensis dried root	79.94	E F G H
Acalypha hispida dried leaf	78.51	FGHI
Erythroxylum lanceum dried leaf	74.49	G H I
Litchi chinensis dried wood	72.50	НІ
Zingiber zerumbet fresh rhizome	70.85	ΙJ
Cananga odorata dried root	62.69	J K
Zingiber zerumbet fresh leaf	60.23	K L
Lawsonia inermis dried root	58.54	K L
Persea americana dried pit	51.57	L M
Zingiber zerumbet flower stem	45.04	M N
Litsea glutinosa dried root	42.55	N
Paullinia pinnata dried root	37.88	N O
Zingiber zerumbet fresh flower	33.70	O P
Acalypha hispida dried root	33.62	O P
Erythroxylum corymbosum fresh leaf	31.22	O P
Acalypha wilkesiana dried root	30.30	O P
Paullinia pinnata dried liana	27.87	P
Tamarindus indica dried root	9.21	
	Dilution 100	
Zingiber zerumbet dried leaf	68.73	A
Zingiber zerumbet dried flower	62.84	A
Zingiber zerumbet stem	55.90	В
Myristica fragrance dried seed	38.19	С
Zingiber zerumbet fresh rhizome	32.03	C D
Acalypha hispida dried flower	31.15	D
Acalypha hispida dried leaf	26.54	D
Litchi chinensis dried leaf	16.18	E
Persea americana dried pit	13.92	Е

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Samples	Lipoxygenase Inhibition Activity (%)	Group	ing Information Based on Tukey's Method
	Dilution 1000		
Zingiber zerumbet fresh rhizome	55.73	A	
Acalypha hispida dried leaf	26.13	В	
Zingiber zerumbet dried flower	21.81	В	

# 3.2. Antioxidant Activity (DPPH Test)

The antioxidant properties of the collected plant samples were evaluated through the DPPH test. The average values were compared two by two and grouped using Tukey's test. %RSD on replicates were not higher than 10%, which is in concordance with standard procedures [22–24].

For plant extracts diluted  $10^1$  times, 77 out of the 89 samples were above the LOQ. One could not be analyzed at this specific concentration as it impeded with the detector at the given wavelength (517 nm); the latter was re-integrated in the measurements for the following dilutions. These 77 samples were classified into 25 different groups (Table 3), and the group with the highest activity (Group A) showed activities ranging from 94.00% to 99.97%. Only one sample was only classified in that group: *Erythroxylum corymbosum* dried leaf (99.97%  $\pm$  0.06), as the other samples in group A presented some crossing over up to group L. At that dilution, a total of 26 samples were classified in Group A, which can be explained by the presence of compounds with known antioxidant properties, such as polyphenols, in many plants. The second and third dilutions will therefore allow a more precise classification of these samples.

Out of the initial samples and with the addition of the one removed from the plant extracts diluted 10 times, 56 plant extracts diluted  $10^2$  times remained above the LOQ. These samples were divided into 23 groups, and 19 samples were classified into group A, showing activities ranging from 92.58% to 98.76%. Only one sample was just in group A: *Acalypha hispida* fresh leaf (98.76%  $\pm$  1.08), as the others presented some crossing over up to group G (activities ranging from 86.63% up to 92.95%).

When looking at the plant extracts diluted  $10^3$  times, 21 samples remained above the LOQ and were classified into 9 groups. The group with the highest activities (Group A) contained 4 samples, of which 2 were only in group A: *Leea guineensis* dried leaf (45.91%  $\pm$  3.89) and *Litchi chinensis* dried leaf (43.10%  $\pm$  2.25). The other samples in group A presented some crossing over up to group B (activities ranging from 37.80% to 42.39%). A total of 5 samples were gathered among those high-relative-activity groups: *Lawsonia inermis* dried leaf, *Acalypha wilkesiana* fresh leaf, *Litchi chinensis* dried root, *Litchi chinensis* dried leaf, and *Leea guineensis* dried leaf (Figure 2).

Finally, no sample diluted  $10^4$  times showed results above the LOQ.

The present results, therefore, allowed the identification of plant species of interest for the isolation of antioxidant molecules. However, it should be noted that other tests could also be considered because of the complexity of oxidative stress and especially what might happen in the human organism.

**Table 3.** Antioxidant activity results (DPPH assay) for the plant extracts diluted 10, 100 and 1000 times (n = 3). Results were statistically analyzed using Tukey's grouping test (confidence level: 95%), and the observed group classifications (A, B, C . . . . . etc.) are shown in the present table.

Samples	Antioxidant Activity (%)	Grouping Information Based on Tukey's Method
		Dilution 10
Erythroxylum corymbosum dried leaf	99.97	A
Acalypha hispida fresh flower	99.96	A B
Erythroxylum lanceum dried root	99.94	A B
Lantana camara fresh leaf	99.91	A B
Erythroxylum corymbosum dried wood	99.89	A B
Erythroxylum corymbosum fresh leaf	99.68	A B C
Acalypha hispida fresh leaf	99.21	A B C D
Erythroxylum lanceum dried leaf	99.04	A B C D E
Acalypha wilkesiana dried root	97.83	A B C D E F
Erythroxylum lanceum dried wood	97.36	A B C D E F G
Myristica fragrans dried seed	97.02	A B C D E F G H
Acalypha wilkesiana fresh leaf	96.49	A B C D E F G H I
Cananga odorata dried root	96.35	A B C D E F G H I
Paullinia pinnata dried leaf	96.29	A B C D E F G H I
Cananga odorata dried flower	96.10	A B C D E F G H I J
Adansonia digitata dried wood	95.55	A B C D E F G H I J K
Leea guineensis dried root	94.86	A B C D E F G H I J K L
Syzygium aromaticum fresh leaf	94.86	A B C D E F G H I J K L
Leea guineensis dried fruit	94.51	A B C D E F G H I J K L
Persea americana dried root	94.50	A B C D E F G H I J K L
Paullinia pinnata dried liana	94.50	A B C D E F G H I J K L
Litchi chinensis dried root	94.28	A B C D E F G H I J K L
Acalypha hispida dried wood	94.28	A B C D E F G H I J K L
Leea guineensis fresh leaf	94.25	A B C D E F G H I J K L
Kalanchoe pinnata fresh leaf	94.21	A B C D E F G H I J K L
Litchi chinensis dried wood	94.00	A B C D E F G H I J K L
Persea americana dried pit	93.68	B C D E F G H I J K L
Paullinia pinnata dried aerial root	93.50	C D E F G H I J K L
Lawsonia inermis fresh leaf	93.33	D E F G H I J K L
Lawsonia inermis dried leaf	93.27	D E F G H I J K L
Pandanus mayottensis dried fruit	93.14	D E F G H I J K L
Syzygium aromaticum dried wood	93.05	D E F G H I J K L
Paullinia pinnata dried root	92.91	E F G H I J K L M
Litchi chinensis dried leaf	92.64	F G H I J K L M
Lantana camara dried leaf	92.40	F G H I J K L M
Leea guineensis dried leaf	92.31	F G H I J K L M

 Table 3. Cont.

Litchi chinensis fresh leaf   92.19	Samples	Antioxidant Activity (%)	Grouping Information Based on Tukey's Method
Parsen americana fresh pit	Litchi chinensis fresh leaf	92.19	F G H I J K L M
Paullinia pinnata fresh leaf	Leea guineensis dried wood	92.18	F G H I J K L M
Persea americana fresh leaf	Persea americana fresh pit	91.99	F G H I J K L M N
Litsea glutinosa dried bark   91.31   G H I J K L M N	Paullinia pinnata fresh leaf	91.90	F G H I J K L M N
Pandamus mayottensis fresh fruit 91.05 H I J K L M N  Acalypha vilkesiana dried leaf 90.98 H I J K L M N  Persea americana dried leaf 90.87 H I J K L M N  Cananga odorata dried leaf 90.87 H I J K L M N  Lausonia inermis dried wood 90.31 I J K L M N  Lausonia inermis dried wood 90.30 I J K L M N  Acalypha hispida dried flower 89.82 J K L M N  Acalypha hispida dried flower 89.82 J K L M N  Zingiber zerumbet fresh flower 89.78 K L M N  Zingiber zerumbet fresh leaf 86.66 M N O P  Tamarindus indica dried leaf 85.80 N O P Q  Kalanchoe pinnata dried leaf 82.38 O P Q R  Timarindus indica fresh leaf 80.39 P Q R  Litsea glutinosa dried leaf 80.13 Q R  Litsea glutinosa dried leaf 78.64 R  Pandamus mayottensis dried root 64.81 S T  Tamarindus indica dried leaf 59.18 S T  Tamarindus indica dried leaf 59.18 S T  Tamarindus indica dried leaf 50.25 U  Lingiber zerumbet dried leaf 47.57 U  Aloes Mayottensis dried leaf 31.24  V X  Zingiber zerumbet fresh leaf 31.24  V X  Zingiber zerumbet fresh leaf 31.24  V X  Zingiber zerumbet fresh leaf 21.87  W X  Zingiber zerumbet fresh leaf 21.87  W X  Zingiber zerumbet fresh leaf 21.87	Persea americana fresh leaf	91.87	F G H I J K L M N
Acalypha wilkesiana dried leaf   90.98	Litsea glutinosa dried bark	91.31	G H I J K L M N
Persea americana dried leaf         90.95         H I J K L M N           Cananga odorata dried leaf         90.87         H I J K L M N           Lawsonia inermis dried root         90.31         I J K L M N           Lawsonia inermis dried wood         90.30         I J K L M N           Acalypha hispida dried flower         89.82         J K L M N           Cananga odorata fresh flower         89.78         K L M N           Zingiber zerumbet fresh flower         89.61         K L M N           Persea americana dried wood         88.91         L M N O           Litsea glutinosa fresh leaf         86.66         M N O P           Tamarindus indica dried leaf         85.80         N O P Q           Kalanchoe pinnata dried leaf         80.39         P Q R           Acalypha hispida dried leaf         80.39         P Q R           Acalypha hispida dried leaf         80.13         Q R           Acalypha hispida dried leaf         80.13         Q R           Litsea glutinosa dried leaf         78.64         R           Pandanus mayottensis dried root         64.81         S           Litsea glutinosa dried dried dried wood         62.24         S           Litsea glutinosa dried dried wood         58.58         S T	Pandanus mayottensis fresh fruit	91.05	HI J K L M N
Cananga odorata dried leaf 90.87 H I J K L M N  Lawsonia inermis dried root 90.31 I J K L M N  Lawsonia inermis dried wood 90.30 I J K L M N  Acalypha hispida dried flower 89.82 J K L M N  Cananga odorata fresh flower 89.78 K L M N  Zingiber zerumbet fresh flower 89.61 K L M N  Litsea glutinosa fresh leaf 86.66 M N O P  Tamarindus indica dried leaf 85.80 N O P Q R  Tamarindus indica dried leaf 80.39 P Q R  Litsea glutinosa dried leaf 80.13 Q R  Litsea glutinosa dried leaf 80.13 Q R  Litsea glutinosa dried leaf 80.13 S C R  Litsea glutinosa dried leaf 80.13 S S C R  Tamarindus indica dried leaf 80.13 S S S S T  Tamarindus glutinosa dried leaf 80.13 S S S S T  Tamarindus glutinosa dried wood 62.24 S S S T  Tamarindus glutinosa dried wood 62.24 S S T  Tamarindus indica dried wood 62.24 S S T  Tamarindus indica dried wood 58.58 S T  Tamarindus indica dried wood 58.58 S T  Aloes mayottensis dried root 53.21 T U  Lantana camara dried fruit 51.92 U  Erythroxylum lanceum fresh leaf 50.25 U  Zingiber zerumbet dried flower 49.18 U  Pandanus mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh rhizome 26.45 V W  Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.87 W X	Acalypha wilkesiana dried leaf	90.98	HI J K L M N
Lawsonia inermis dried root 90.31 I J K L M N  Lawsonia inermis dried wood 90.30 I J K L M N  Acalypha hispida dried flower 89.82 J K L M N  Cananga odorata fresh flower 89.78 K L M N  Zingiber zerumbet fresh flower 89.61 K L M N  Persea americana dried wood 88.91 L M N O  Litsea glutinosa fresh leaf 86.66 MN N O P  Tamarindus indica dried leaf 85.80 N O P Q  Kalanchoe pinnata dried leaf 82.38 O P Q R  Tamarindus indica fresh leaf 80.39 P Q R  Acalypha hispida dried leaf 80.13 Q R  Litsea glutinosa dried leaf 8.644 R  Pandanus mayottensis dried root 64.81 S  Litsea glutinosa dried wood 62.24 S  Zingiber zerumbet dried leaf 59.18 S T  Tamarindus indica dried wood 58.58 S T  Aloes mayottensis dried root 53.21 T U  Erythroxylum lanceum fresh leaf 50.25 U U  Zingiber zerumbet dried leaf 47.57  Aloes Mayottensis dried leaf 47.57  Aloes Mayottensis dried leaf 47.57  Aloes Mayottensis dried leaf 47.57  Zingiber zerumbet fresh leaf 31.24  V W  Pandanus mayottensis fresh leaf 21.87  W X  Zingiber zerumbet fresh leaf 21.87  W X  Zingiber zerumbet fresh leaf 21.87	Persea americana dried leaf	90.95	HI J K L M N
Lawsonia inermis dried wood 90.30 I J K L M N  Acalypha hispida dried flower 89.82 J K L M N  Cananga odorata fresh flower 89.78 K L M N  Zingiber zerumbet fresh flower 89.61 K L M N  Persea americana dried wood 88.91 L M N O  Litsea glutinosa fresh leaf 86.66 MN O P  Tamarindus indica dried leaf 85.80 N O P Q R  Kalanchoe pinnata dried leaf 80.39 P Q R  Tamarindus indica fresh leaf 80.39 P Q R  Litsea glutinosa dried leaf 80.13 Q R  Litsea glutinosa dried leaf 78.64 R  Pandanus mayottensis dried root 64.81 S  Litsea glutinosa dried wood 62.24 S  Litsea glutinosa dried wood 62.24 S  Litsea glutinosa dried wood 58.58 S T  Alaes mayottensis dried root 53.21 T U  Lantana camara dried fruit 51.92 U  Erythroxylum lanceum fresh leaf 50.25 U  Zingiber zerumbet dried leaf 47.57 U  Aloes Mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.87 W X	Cananga odorata dried leaf	90.87	HI J K L M N
Acalypha hispida dried flower 89.82 J K L M N  Cananga odorata fresh flower 89.78 K L M N  Zingiber zerumbet fresh flower 89.61 K L M N  Persea americana dried wood 88.91 L M N O  Litsea glutinosa fresh leaf 86.66 M N O P  Tamarindus indica dried leaf 85.80 N O P Q  Kalanchoe pinnata dried leaf 80.39 P Q R  Tamarindus indica fresh leaf 80.39 P Q R  Acalypha hispida dried leaf 80.13 Q R  Litsea glutinosa dried leaf 78.64 R  Pandanus mayottensis dried root 64.81 S  Litsea glutinosa dried wood 62.24 S  Litsea glutinosa dried leaf 59.18  Tamarindus indica dried wood 58.58 S T  Tamarindus indica dried wood 58.58 S T  Aloes mayottensis dried root 53.21 T U  Lantana camara dried fruit 51.92 U  Erythroxylum lanceum fresh leaf 50.25 U  Zingiber zerumbet dried leaf 47.57 U  Aloes Mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.87 W X	Lawsonia inermis dried root	90.31	I J K L M N
Cananga odorata fresh flower  Zingiber zerumbet fresh flower  89.61  K L M N  Persea americana dried wood  88.91  L M N O  Litsea glutinosa fresh leaf  86.66  M N O P  Tamarindus indica dried leaf  82.38  O P Q R  Kalanchoe pinnata dried leaf  80.39  P Q R  Acalypha hispida dried leaf  80.39  P Q R  Litsea glutinosa dried leaf  78.64  R  Pandanus mayottensis dried root  61.31  Zingiber zerumbet dried leaf  59.18  Tamarindus indica dried wood  58.58  Aloes mayottensis dried root  53.21  Lantana camara dried fruit  51.92  Litsea flutinosa dried flower  49.18  V  Zingiber zerumbet dried leaf  31.24  V W  Pandanus mayottensis dried leaf  21.87  W X  Zingiber zerumbet fresh leaf  21.87  W X  Zingiber zerumbet fresh leaf  21.87  W X  Zingiber zerumbet fresh leaf  21.87	Lawsonia inermis dried wood	90.30	I J K L M N
Zingiber zerumbet fresh flower  Persea americana dried wood  Respectations fresh leaf  Robert Bescheler  Robert Beschele	Acalypha hispida dried flower	89.82	J K L M N
Persea americana dried wood 88.91 L M N O  Litsea glutinosa fresh leaf 86.66 M N O P  Tamarindus indica dried leaf 85.80 N O P Q  Kalanchoe pinnata dried leaf 82.38 O P Q R  Tamarindus indica fresh leaf 80.39 P Q R  Acalypha hispida dried leaf 80.13 Q R  Litsea glutinosa dried leaf 78.64 R  Pandanus mayottensis dried root 64.81 S  Litsea glutinosa dried wood 62.24 S  Litsea glutinosa dried wood 62.24 S  Litsea glutinosa dried wood 60.13 S  Zingiber zerumbet dried leaf 59.18 S T  Tamarindus indica dried wood 58.58 S T  Aloes mayottensis dried root 53.21 T U  Lantana camara dried fruit 51.92 U  Erythroxylum lanceum fresh leaf 50.25 U  Zingiber zerumbet dried flower 49.18 U  Pandanus mayottensis dried leaf 31.24  Zingiber zerumbet fresh leaf 31.24  Zingiber zerumbet fresh rhizome 26.45 V W  Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.67	Cananga odorata fresh flower	89.78	KLMN
Litsea glutinosa fresh leaf       86.66       M N O P         Tamarindus indica dried leaf       85.80       N O P Q         Kalanchoe pimnata dried leaf       82.38       O P Q R         Tamarindus indica fresh leaf       80.39       P Q R         Acalypha hispida dried leaf       80.13       Q R         Litsea glutinosa dried leaf       78.64       R         Pandanus mayottensis dried root       64.81       S         Litsea glutinosa dried wood       62.24       S         Litsea glutinosa dried root       60.13       S         Zingiber zerumbet dried leaf       59.18       S T         Tamarindus indica dried wood       58.58       S T         Aloes mayottensis dried root       53.21       T U         Lantana camara dried fruit       51.92       U         Erythroxylum lanceum fresh leaf       50.25       U         Zingiber zerumbet dried flower       49.18       U         Pandanus mayottensis dried leaf       31.24       V         Zingiber zerumbet fresh rhizome       26.45       V W         Pandanus mayottensis fresh leaf       21.87       W X         Zingiber zerumbet fresh leaf       21.67       W X	Zingiber zerumbet fresh flower	89.61	KLMN
Tamarindus indica dried leaf85.80N O P QKalanchoe pinnata dried leaf82.38O P Q RTamarindus indica fresh leaf80.39P Q RAcalypha hispida dried leaf80.13Q RLitsea glutinosa dried leaf78.64RPandanus mayottensis dried root64.81SLitsea glutinosa dried wood62.24SLitsea glutinosa dried wood60.13SZingiber zerumbet dried leaf59.18S TTamarindus indica dried wood58.58S TAloes mayottensis dried root53.21T ULantana camara dried fruit51.92UErythroxylum lanceum fresh leaf50.25UZingiber zerumbet dried flower49.18UPandanus mayottensis dried leaf47.57UAloes Mayottensis dried leaf31.24VZingiber zerumbet fresh rhizome26.45V WPandanus mayottensis fresh leaf21.87W XZingiber zerumbet fresh leaf21.87W X	Persea americana dried wood	88.91	LMNO
Kalanchoe pinnata dried leaf82.38O P Q RTamarindus indica fresh leaf80.39P Q RAcalypha hispida dried leaf80.13Q RLitsea glutinosa dried leaf78.64RPandanus mayottensis dried root64.81SLitsea glutinosa dried wood62.24SLitsea glutinosa dried dred froot60.13SZingiber zerumbet dried leaf59.18S TTamarindus indica dried wood58.58S TAloes mayottensis dried root53.21T ULantana camara dried fruit51.92UErythroxylum lanceum fresh leaf50.25UZingiber zerumbet dried flower49.18UPandanus mayottensis dried leaf47.57UAloes Mayottensis dried leaf31.24VZingiber zerumbet fresh rhizome26.45V WPandanus mayottensis fresh leaf21.87W XZingiber zerumbet fresh leaf21.87W XZingiber zerumbet fresh leaf21.67W X	Litsea glutinosa fresh leaf	86.66	M N O P
Tamarindus indica fresh leaf 80.39 P Q R  Acalypha hispida dried leaf 80.13 Q R  Litsea glutinosa dried leaf 78.64 R  Pandanus mayottensis dried root 64.81 S  Litsea glutinosa dried wood 62.24 S  Litsea glutinosa dried root 60.13 S  Zingiber zerumbet dried leaf 59.18 S T  Tamarindus indica dried wood 58.58 S T  Aloes mayottensis dried root 53.21 T U  Lantana camara dried fruit 51.92 U  Erythroxylum lanceum fresh leaf 50.25 U  Zingiber zerumbet dried leaf 47.57 U  Aloes Mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh rhizome 26.45 V W  Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.87 W X	Tamarindus indica dried leaf	85.80	N O P Q
Acalypha hispida dried leaf 80.13 Q R  Litsea glutinosa dried leaf 78.64 R  Pandanus mayottensis dried root 64.81 S  Litsea glutinosa dried wood 62.24 S  Litsea glutinosa dried root 60.13 S  Zingiber zerumbet dried leaf 59.18 S T  Tamarindus indica dried wood 58.58 S T  Aloes mayottensis dried root 53.21 T U  Lantana camara dried fruit 51.92 U  Erythroxylum lanceum fresh leaf 50.25 U  Zingiber zerumbet dried flower 49.18 U  Pandanus mayottensis dried leaf 47.57 U  Aloes Mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh rhizome 26.45 V W  Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.67	Kalanchoe pinnata dried leaf	82.38	O P Q R
Litsea glutinosa dried leaf78.64RPandanus mayottensis dried root64.81SLitsea glutinosa dried wood62.24SLitsea glutinosa dried root60.13SZingiber zerumbet dried leaf59.18S TTamarindus indica dried wood58.58S TAloes mayottensis dried root53.21T ULantana camara dried fruit51.92UErythroxylum lanceum fresh leaf50.25UZingiber zerumbet dried flower49.18UPandanus mayottensis dried leaf47.57UAloes Mayottensis dried leaf31.24VZingiber zerumbet fresh rhizome26.45V WPandanus mayottensis fresh leaf21.87W XZingiber zerumbet fresh leaf21.87W X	Tamarindus indica fresh leaf	80.39	P Q R
Pandanus mayottensis dried root         64.81         S           Litsea glutinosa dried wood         62.24         S           Litsea glutinosa dried root         60.13         S           Zingiber zerumbet dried leaf         59.18         S T           Tamarindus indica dried wood         58.58         S T           Aloes mayottensis dried root         53.21         T U           Lantana camara dried fruit         51.92         U           Erythroxylum lanceum fresh leaf         50.25         U           Zingiber zerumbet dried flower         49.18         U           Pandanus mayottensis dried leaf         47.57         U           Aloes Mayottensis dried leaf         31.24         V           Zingiber zerumbet fresh rhizome         26.45         V W           Pandanus mayottensis fresh leaf         21.87         W X           Zingiber zerumbet fresh leaf         21.67         W X	Acalypha hispida dried leaf	80.13	Q R
Litsea glutinosa dried wood         62.24         S           Litsea glutinosa dried root         60.13         S           Zingiber zerumbet dried leaf         59.18         S T           Tamarindus indica dried wood         58.58         S T           Aloes mayottensis dried root         53.21         T U           Lantana camara dried fruit         51.92         U           Erythroxylum lanceum fresh leaf         50.25         U           Zingiber zerumbet dried flower         49.18         U           Pandanus mayottensis dried leaf         47.57         U           Aloes Mayottensis dried leaf         31.24         V           Zingiber zerumbet fresh rhizome         26.45         V W           Pandanus mayottensis fresh leaf         21.87         W X           Zingiber zerumbet fresh leaf         21.67         W X	Litsea glutinosa dried leaf	78.64	R
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Zingiber zerumbet dried leaf59.18S TTamarindus indica dried wood58.58S TAloes mayottensis dried root53.21T ULantana camara dried fruit51.92UErythroxylum lanceum fresh leaf50.25UZingiber zerumbet dried flower49.18UPandanus mayottensis dried leaf47.57UAloes Mayottensis dried leaf31.24VZingiber zerumbet fresh rhizome26.45V WPandanus mayottensis fresh leaf21.87W XZingiber zerumbet fresh leaf21.67W X	Litsea glutinosa dried wood	62.24	S
Tamarindus indica dried wood58.58S TAloes mayottensis dried root53.21T ULantana camara dried fruit51.92UErythroxylum lanceum fresh leaf50.25UZingiber zerumbet dried flower49.18UPandanus mayottensis dried leaf47.57UAloes Mayottensis dried leaf31.24VZingiber zerumbet fresh rhizome26.45V WPandanus mayottensis fresh leaf21.87W XZingiber zerumbet fresh leaf21.67W X	Litsea glutinosa dried root	60.13	S
Aloes mayottensis dried root 53.21 T U  Lantana camara dried fruit 51.92 U  Erythroxylum lanceum fresh leaf 50.25 U  Zingiber zerumbet dried flower 49.18 U  Pandanus mayottensis dried leaf 47.57 U  Aloes Mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh rhizome 26.45 V W  Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.67 W X	Zingiber zerumbet dried leaf	59.18	S T
Lantana camara dried fruit51.92UErythroxylum lanceum fresh leaf50.25UZingiber zerumbet dried flower49.18UPandanus mayottensis dried leaf47.57UAloes Mayottensis dried leaf31.24VZingiber zerumbet fresh rhizome26.45VPandanus mayottensis fresh leaf21.87W XZingiber zerumbet fresh leaf21.67W X	Tamarindus indica dried wood	58.58	S T
Erythroxylum lanceum fresh leaf 50.25 U  Zingiber zerumbet dried flower 49.18 U  Pandanus mayottensis dried leaf 47.57 U  Aloes Mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh rhizome 26.45 V W  Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.67 W X	Aloes mayottensis dried root	53.21	T U
Zingiber zerumbet dried flower 49.18 U  Pandanus mayottensis dried leaf 47.57 U  Aloes Mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh rhizome 26.45 V W  Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.67 W X	Lantana camara dried fruit	51.92	U
Pandanus mayottensis dried leaf47.57UAloes Mayottensis dried leaf31.24VZingiber zerumbet fresh rhizome26.45V WPandanus mayottensis fresh leaf21.87W XZingiber zerumbet fresh leaf21.67W X	Erythroxylum lanceum fresh leaf	50.25	U
Aloes Mayottensis dried leaf 31.24 V  Zingiber zerumbet fresh rhizome 26.45 V W  Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.67 W X	Zingiber zerumbet dried flower	49.18	U
Zingiber zerumbet fresh rhizome26.45V WPandanus mayottensis fresh leaf21.87W XZingiber zerumbet fresh leaf21.67W X	Pandanus mayottensis dried leaf	47.57	U
Pandanus mayottensis fresh leaf 21.87 W X  Zingiber zerumbet fresh leaf 21.67 W X	Aloes Mayottensis dried leaf	31.24	V
Zingiber zerumbet fresh leaf 21.67 W X	Zingiber zerumbet fresh rhizome	26.45	V W
	Pandanus mayottensis fresh leaf	21.87	W X
	Zingiber zerumbet fresh leaf	21.67	W X
Kalanchoe pinnata dried root 18.23 X Y	Kalanchoe pinnata dried root	18.23	ХҮ
Cananga odorata dried wood 16.65 X Y	Cananga odorata dried wood	16.65	ХҮ
Lantana camara dried root 16.39 X Y	Lantana camara dried root	16.39	ХҮ
Acalypha wilkesiana dried wood 15.35 Y	Acalypha wilkesiana dried wood	15.35	Y

Table 3. Cont.

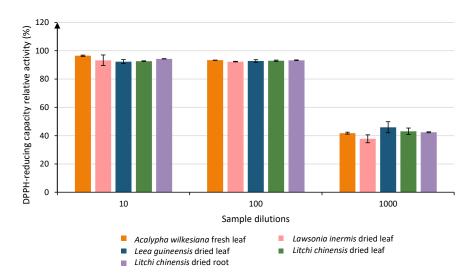
Samples	Antioxidant Activity (%)	Grouping Information Based on Tukey's Method	
Lantana camara dried wood	12.50		Υ
		Dilution 100	
Acalypha hispida fresh leaf	98.76	A	
Erythroxylum corymbosum fresh leaf	98.54	АВ	
Acalypha hispida dried flower	96.86	АВС	
Erythroxylum corymbosum dried leaf	95.77	A B C D	
Acalypha wilkesiana dried leaf	94.99	A B C D E	
Leea guineensis dried fruit	94.61	A B C D E F	
Leea guineensis dried root	94.55	A B C D E F	
Syzygium aromaticum fresh leaf	94.36	A B C D E F	
Leea guineensis fresh leaf	93.92	A B C D E F	
Lawsonia inermis fresh leaf	93.91	A B C D E F	
Acalypha wilkesiana dried root	93.71	A B C D E F	
Litchi chinensis dried wood	93.47	A B C D E F	
Persea americana dried root	93.34	A B C D E F	
Acalypha wilkesiana fresh leaf	93.30	A B C D E F	
Acalypha hispida fresh flower	93.27	A B C D E F	
Litchi chinensis dried root	93.26	A B C D E F	
Litchi chinensis dried leaf	92.95	A B C D E F G	
Leea guineensis dried leaf	92.76	A B C D E F G	
Persea americana dried leaf	92.58	A B C D E F G	
Persea americana fresh pit	92.32	BCDEFG	
Lawsonia inermis dried leaf	92.31	B C D E F G	
Syzygium aromaticum dried wood	91.79	CDEFG	
Lantana camara dried leaf	89.71	D E F G	
Paullinia pinnata dried root	89.39	D E F G	
Paullinia pinnata dried leaf	89.20	E F G	
Persea americana fresh leaf	89.12	E F G	
Acalypha hispida dried leaf	88.36	F G H	
Lawsonia inermis dried root	86.63	G H I	
Paullinia pinnata dried aerial root	82.12	н і	
Cananga odorata dried leaf	81.67	I	
Paullinia pinnata dried liana	73.33	J	
Persea americana dried pit	65.31	K	
Litchi chinensis fresh leaf	62.58	K	
Lantana camara fresh leaf	55.48	L	
Erythroxylum lanceum dried wood	52.26	L M	
Adansonia digitata dried wood	49.57	L M	
Tamarindus indica dried leaf	48.75	M	

Table 3. Cont.

Samples	Antioxidant Activity (%)	Grouping Informati	on Based on Tukey's Method
Kalanchoe pinnata fresh leaf	40.24		N
Erythroxylum corymbosum dried wood	38.45		N O
Erythroxylum lanceum dried root	37.71		N O P
Persea americana dried wood	36.16		N O P
Cananga odorata fresh flower	35.01		N O P
Myristica fragrans dried seed	33.76		O P
Paullinia pinnata fresh leaf	31.38		P Q
Leea guineensis dried wood	26.62		Q R
Pandanus mayottensis fresh fruit	23.15		R
Kalanchoe pinnata dried leaf	22.45		R
Pandanus mayottensis dried fruit	20.57		R S
Erythroxylum corymbosum dried root	20.55		R S T
Litsea glutinosa fresh leaf	14.33		S T U
Pandanus mayottensis dried root	13.67		T U
Aloes Mayottensis dried leaf	12.01		U V
Erythroxylum lanceum fresh leaf	9.69		U V
Pandanus mayottensis dried leaf	8.07		UVW
Zingiber zerumbet dried flower	6.81		V W
Cananga odorata dried wood	3.13		W
		Dilution 1000	
Leea guineensis dried leaf	45.91	A	
Litchi chinensis dried leaf	43.10	A	
Litchi chinensis dried root	42.39	A B	
Acalypha wilkesiana fresh leaf	41.78	A B	
Lawsonia inermis dried leaf	37.80	ВС	
Syzygium aromaticum fresh leaf	36.53	С	
Acalypha hispida dried flower	35.97	С	
Acalypha hispida fresh leaf	27.45	D	
Acalypha hispida fresh flower	26.83	D	
Leea guineensis fresh leaf	26.48	D E	
Lawsonia inermis fresh leaf	26.38	D E	
Leea guineensis dried fruit	26.07	D E	
Persea americana dried leaf	26.02	D E	
Erythroxylum corymbosum dried leaf	22.67	D E F	
Tamarindus indica dried leaf	21.83	E F	
Syzygium aromaticum dried wood	18.49	F G	
Acalypha wilkesiana dried leaf	17.86	F G	
	16.39	G	

<b>—</b>	1 1		•		
13	n	Δ	- 4	. Co	nt.

Samples	Antioxidant Activity (%)	Grouping Information Based on Tukey's Method
Persea americana dried pit	7.81	Н
Erythroxylum corymbosum dried wood	3.06	н і
Leea guineensis dried wood	2.65	I



**Figure 2.** Evolution of the relative DPPH-reducing capacity of the five most efficient samples at 3 different dilutions ( $10^1$ ,  $10^2$  and  $10^3$ ). Each column represents the mean  $\pm$  SD (n = 3) per sample.

### 3.3. Anti-Tyrosinase Activity

The anti-tyrosinase activity of the collected plant samples was evaluated through the observation of tyrosinase inhibition capacity. The activity averages of all samples were compared two by two and grouped using Tukey's test. The observed limit %RSD to establish the validity of the results was based in concordance with standard procedures, this being a %RSD of 10 [22–24]. Out of the 89 samples tested at four different dilutions (samples were respectively diluted  $10^1$ ,  $10^2$ ,  $10^3$  and  $10^4$  times), 6 samples could not be tested using the anti-tyrosinase protocol, as even when strongly diluted, they strongly absorbed at 475 nm, impeding an accurate reading.

For the 52 tested sample extracts diluted  $10^1$  times, Tukey's test highlighted 22 different groups above the LOQ (Table 4). Five samples were classified into the group with the best activity (Group A) and ranged from 89.74% to 98.57% of relative activity. Only one sample was only in this group: *Litchi chinensis* dried leaf (98.57%  $\pm$  7.09), as the others presented some crossing over up to group D (activities ranging from 80.07% to 89.82%). Together these groups counted for a total of 11 samples with interesting anti-tyrosinase potential.

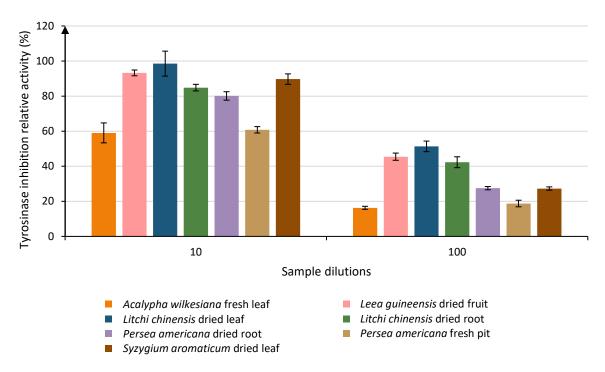
When the samples were diluted  $10^2$  times, 7 samples remained above the LOQ. These samples were divided into 4 groups, and only one sample was classified into the group with the best activity (Group A): *Litchi chinensis* dried leaf (51.36%  $\pm$  3.00). Group B contained *Leea guineensis* dried fruit (45.45%  $\pm$  2.08) and *Litchi chinensis* dried root (42.29%  $\pm$  3.11). Groups C and D had activities ranging from 16.26% to 27.49%. The two other dilutions did not allow for any significant observations. The evolution of the activity of the most active sample is shown in Figure 3.

**Table 4.** Anti-tyrosinase activity results for the plant extracts diluted 10 and 100 times (n = 3). Results were statistically analyzed using Tukey's grouping test (confidence level: 95%), and the observed group classifications (A, B, C . . . . . etc.) are shown in the present table.

Samples	Anti-Tyrosinase Activity (%)	Grouping Information Based on Tukey's Method
		Dilution 10
Litchi chinensis dried leaf	98.57	A
Leea guineensis dried fruit	93.28	A B
Leea guineensis fresh leaf	91.53	A B C
Leea guineensis dried root	89.82	A B C D
Syzygium aromaticum dried leaf	89.74	A B C D
Lawsonia inermis dried root	87.69	B C D
Syzygium aromaticum fresh leaf	86.22	B C D
Litchi chinensis dried root	84.83	B C D E
Persea americana dried leaf	82.05	C D E
Persea americana dried root	80.10	D E
Lawsonia inermis dried leaf	80.07	D E
Litchi chinensis dried wood	75.61	E F
Paullinia pinnata dried leaf	68.99	F G
Kalanchoe pinnata fresh leaf	66.18	F G H
Adansonia digitata dried wood	64.91	G H I
Paullinia pinnata dried root	63.91	G H I
Persea americana fresh leaf	62.84	G H I
Litchi chinensis fresh leaf	62.36	G H I
Persea americana fresh pit	60.80	G Н I J
Acalypha hispida fresh leaf	60.20	G Н I J
Lawsonia inermis fresh leaf	59.63	G Н I J
Acalypha wilkesiana fresh leaf	59.01	ні Ј
Paullinia pinnata dried aerial root	56.23	н і ј к
Lantana camara fresh leaf	54.99	I J K
Paullinia pinnata dried liana	51.78	J K L
Persea americana dried pit	51.64	J K L
Tamarindus indica fresh leaf	51.33	J K L
Erythroxylum lanceum dried wood	48.76	K L M
Persea americana dried wood	47.22	K L M N
Litsea glutinosa dried root	44.81	L M N
Pandanus mayottensis dried aerial root	44.07	L M N O
Erythroxylum corymbosum dried root	43.89	L M N O P

 Table 4. Cont.

Samples	Anti-Tyrosinase Activity (%)		Grouping	g Informatio	n Base	d on	Tu	key	's N	1eth	od				
Acalypha hispida dried flower	43.69				L	M	N	О	Р	Q					
Syzygium aromaticum dried wood	43.47				L	M	N	О	Р	Q					
Cananga odorata fresh flower	40.55					M	N	О	P	Q	R				
Acalypha hispida dried root	40.17					M	N	О	P	Q	R				
Aloes mayottensis mucilage	39.15					M	N	О	P	Q	R	S			
Zingiber zerumbet dried rhizome	38.77						N	О	Р	Q	R	S			
Paullinia pinnata fresh leaf	34.12							О	P	Q	R	S	T		
Tamarindus indica dried root	34.08								P	Q	R	S	T		
Cananga odorata dried root	33.85									Q	R	S	T		
Zingiber zerumbet fresh leaf	31.72										R	S	T	U	
Zingiber zerumbet flower stem	30.59										R	S	Т	U	
Zingiber zerumbet stem	29.61											S	T	U	
Erythroxylum corymbosum dried wood	29.40											S	Т	U	
Syzygium aromaticum dried root	28.46												Т	U	V
Cananga odorata dried wood	27.51												T	U	V
Zingiber zerumbet fresh rhizome	26.71												T	U	V
Zingiber zerumbet floral water	26.14												T	U	V
Zingiber zerumbet dried flower	24.76												T	U	V
Pandanus mayottensis fresh leaf	22.70													U	V
Aloes mayottensis dried leaf	18.85														V
		Dilı	ution 100												
Litchi chinensis dried leaf	51.36	A													
Leea guineensis dried fruit	45.45	В													
Litchi chinensis dried root	42.29	В													
Persea americana dried root	27.49	С													
Syzygium aromaticum dried leaf	27.28	С													
Persea americana fresh pit	18.69		D												
Acalypha wilkesiana fresh leaf	16.26		D												



**Figure 3.** Evolution of the relative tyrosinase inhibition activities of the most efficient samples through 2 different dilutions of 10 and 100 that were above the LOQ. Each column represents the mean  $\pm$  SD (n = 3) per sample.

## 3.4. Comparison of the Different Activities

In the present work, different organs of different plant species collected in Mayotte were tested for their in vitro anti-lipoxygenase, DPPH-reducing and anti-tyrosinase activities. While the results presented above showed the high potential of certain plant extracts for a single specific activity, the results (Table 5) also highlighted some plant samples displaying more than one interesting activity. Such observations may help in the selection of species for further works. Based on the limit of the analytical methods, the samples showing the best potency for a specific activity were compared with the two other activities.

Therefore, the tested plants can be classified in two categories: the ones that have one strong biological activity, such as *Lawsonia inermis* and *Zingiber zerumbet*, and the others that show multiple biological properties. In this second category, different plants can be highlighted, such as *Acalypha hispida* and *Litchi chinensis*. The plant species presented here are of high interest for the isolation of novel natural molecules from which originates the determined in vitro activities and that may be incorporated into cosmetic products. Interestingly, Saive et al. (2020) already identified a proanthocyanidin that is responsible for the anti-tyrosinase and antioxidant properties of *Litchi chinensis* root. In the same way, different compounds, notably belonging to the procyanidin and catechin families, were previously highlighted as interesting antioxidant molecules in *Persea americana* pit [25]. In addition, zerumbone, a sesquiterpenoid, has already been identified in the fresh rhizome of *Zingiber zerumbet* and is recognized for its anti-inflammatory properties [26]. By continuing in this perspective, new molecules could be identified and valorized for health purposes.

**Table 5.** Recapitulative table presenting a comparison of the extracts obtained from different plant species and organs for their investigated biological activities. The comparison is based on the plant organ extracts diluted 100 times for the 3 activities. + the plant organ extract has between 10% and 30% activity, ++ the plant organ extract has an activity superior to 30%, +++ the plant organ extract has an activity that is among the 3 best at the highest dilution, n/a means not applicable.

Sample	Status of the Plant Species in Mayotte	Anti-Lipoxygenase Activity	DPPH-Reducing Agent	Anti-Tyrosinase Activity n/a n/a		
Acalypha hispida dried flower Acalypha hispida dried leaf	Cultivated [27]	++ +++	++			
Acalypha wilkesiana dried leaf Acalypha wilkesiana fresh leaf	Cultivated [27]	n/a n/a	++	n/a +		
Cananga odorata dried leaf Cananga odorata fresh flower	Cultivated [10]	n/a n/a	++	n/a n/a		
Erythroxylum corymbosum dried leaf	Indigenous [28]	n/a	++	n/a		
Lantana camara dried leaf	Exotic [10]	n/a	++	n/a		
Lawsonia inermis dried leaf	Cultivated [10]	n/a	++	n/a		
Leea guineensis dried fruit Leea guineensis dried leaf	Indigenous [10]	n/a n/a	++ +++	+++ n/a		
Litchi chinensis dried leaf Litchi chinensis dried root	Cultivated [10]	+ n/a	+++	+++		
Persea americana dried root Persea americana fresh pit Persea americana dried pit	Cultivated [10]	n/a n/a +	++ ++ ++	+ + n/a		
Zingiber zerumbet dried flower		+++	+	n/a		
Zingiber zerumbet dried leaf	Exotic [10]	++	n/a	n/a		
Zingiber zerumbet dried stem Zingiber zerumbet fresh rhizome		++	n/a n/a	n/a n/a		

## 4. Conclusions

On the basis of previous ethnobotanical research in Mayotte, 21 different plants were selected for their potential as skin care agents. Different organs of these were collected and tested in vitro for their capacity to reduce the stable free radical molecule DPPH to inhibit the activity of tyrosinase and/or lipoxygenase in order to better understand why these plants are used in traditional medicine and to discover new biological sources of natural skin care agents.

When looking at the perspectives for future research, two interesting facts stood out. Firstly, 10 promising species were identified based on their in vitro biological activity for skin care. Among these species, some showed yet-unknown biological activities. These specific species could enter future research targeting activities such as topical anti-inflammatory activities. Such plants would be of interest in the development of treatments against dermatitis/eczema and could possibly help to replace the common use of corticosteroids. Secondly, some other species did not show an outstanding specific activity but an average potency for several activities, leading to more generalist applications such as anti-ageing and pigmentation issues or for a relieving balm. When looking at these two points of view, both are of interest: a strong, unique activity or a mild intensity but for multiple activities.

In conclusion, this study highlighted the interesting potential of many plants from Mayotte for their incorporation in cosmetic formulations. Among the 10 plant species of high interest highlighted in the present work, 6 are cultivated in Mayotte, 2 are indigenous

to this area, and 2 are exotic. Further studies are now needed, including in vivo tests, in order to confirm the activities shown here in vitro. Additionally, toxicological tests also should be conducted to ensure their innocuity. More studies must also be initiated to identify other molecules of interest. Moreover, if some of these plants begin to be used extensively for their properties, it would also be necessary to set up conservation measures in order to avoid their disappearance. In that case, the target collected organ of the plant will be very important as the collection of fruits, as an example, will not lead to species disappearance, while more attention should be paid when roots or leaves are collected.

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