American Journal of Applied Chemistry
2024, Vol. 12, No. 6, pp. 112-134
https://doi.org/10.11648/j.ajac.20241206.11

Research Article

The *Crinum amabile's* Aerial Organs Fatty Acids, Steroids, Flavonoids, Study of Their Antioxidant and Anti-Inflammatory Activities

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Abstract

The *Crinum amabile* is a plant whose alkaloids medicinal virtues are well discussed in bibliographies. However, specific studigges on the molecules present in the aerial organs of *Crinum amabile*, as well as their virtues as antioxidant and anti-inflammatory are still few and far between. This led us to undertake studies to determine and quantify the fatty acid molecules, then the steroid molecules, then to determine the flavonoid molecules in the various organs systems of the *Crinum amabile* and also to determine their alkaloids quantities. The method used to extract the various molecules present in the different organs of the *Crinum amabile* is the esterification with citric acid molecules to get citric acid ester solutions of their bioactive molecules. The fatty acid molecules were extracted by soxhlet using hexane and identified by Phase Gas Chromatography; so, the fatty acid molecules present in the various organs of *Crinum amabile* are myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and arachidic acids whose antioxidant with anti-inflammatory chemical properties are very interesting. HPLC was used for steroids and flavonoids quantifications after transesterification and extraction of the active molecules in citric acid esters from the various aerial organs of the *Crinum amabile*. The analysis showed that the various aerial organs or organ systems of the *Crinum amabile* contained flavonoids and steroids that are respectively eriocitrin, hesperidin, neohesperidin, isorhoifolin, rhoifolin and betulinic acid, ursolic acid, betuline, lupeol whose antioxidant with anti-inflammatory chemical properties are also very interesting. These different bioactive molecules extracted into citric acid ester solutions of the various organs or organ systems of the *Crinum amabile* were subjected to dpph antioxidant test and NO scavenging anti-inflammatory test and the results were very promising, given that the organ system « anther-stamen fillet-pistil-perianth had the most effective IC50 for both antioxidant and anti-inflammatory activity, followed by organ system «stem-receptacle-leaf »and finally the organ «petal ». In addition, kinetic studies were carried out during the esterification reactions with citric acid of the various *Crinum amabile* organs, making it possible to deduce their kinetic constants and subsequently to show the effect of the rigidity of their structures and porosities on their antioxidant and anti-inflammatory activities at the level of their citric acid ester solutions. Determination of alkene content in the various *Crinum amabile* organ system stock solutions used in the anti-inflammatory tests confirmed and reported their effective role as carriers of reactive and bioactive molecules.

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Received: 29 October 2024; **Accepted:** 22 November 2024; **Published:** 12 December 2024

Keywords

Crinum amabile, Aerial Organs, Fatty Acids, Steroid, Flavonoid, Alkaloid, Antioxidant-DPPH Test, Anti-Inflammatory-NO Scavenging Test

1. Introduction

This publication begins with a detailed description of the various aerial organs of the *Crinum amabile* plant (see the paragraph §.2. of this manuscript), followed by the experimental conditions and the results of the evolution of their esterifications with citric acid molecules as a function of time (see the paragraph §.4. of this manuscript). Part of this publication describes the soxhlet extraction of fatty acids from the various organs of *Crinum amabile* and their identification by gas chromatography. Citric acid ester solutions from the various organs of *Crinum amabile* are then transesterified with methanol and extracted with dichloromethane to identify and quantify their steroid and flavonoid molecules by HPLC-high performance liquid chromatography (see the paragraph §.4. of this manuscript). Anti-inflammatory tests using the NO Scavenging method and antioxidant tests using the dpph method had been undertaken to ascertain the anti-inflammatory and antioxidant activities and potencies of citric acid ester solutions of the various aerial organ systems of *Crinum amabile*. Finally, a quantification of alkaloids in the various aerial organs of *Crinum amabile* was undertaken using the method developed in the manuscript by Bouzidi et al.; (see the paragraph §.5.of this manuscript).

The materials, chemicals and method used in carrying out the experiments in this manuscript are:

1) Materials: 250ml flask, 150ml soxhlet, 250ml beaker, measuring cylinders, burettes, magnetic stirrer, 250ml flask heater, KERN precision balance, magnetic rod, pipettes, decanter, 100W bulbs, hot plate, HPLC, CPG, UV-Visible spectrophotometer, aluminium foil, opaque bag.
2) Chemicals: Citric acid, NaOH, NH_4OH, H_2SO_4, HF, héxianthine, méthanol, éthanol, eau distillée, hexane, dichlorométhane, HBSS, nitroprusside, réactif de Greiss, dpph.
3) Plant: The species used is the *Crinum amabile* in kingdom of *Plantae Haeckel*, Clade *Angiosperms*, Clade *Monocots*, class *Equisetopsida*, order *Asparagales*, family *Amarylliaceae* Subfamily *Amaryllidoideae* Subtribe *Crininae* and genus *Crinum*.
4) Methods used: NO Scavenging Anti-inflammatory test – dpph antioxydant test – Esterification with citric acid to extract plant molecules.

2. The Various Aerial Organs of *Crinum Amabile* Studied in This Manuscript

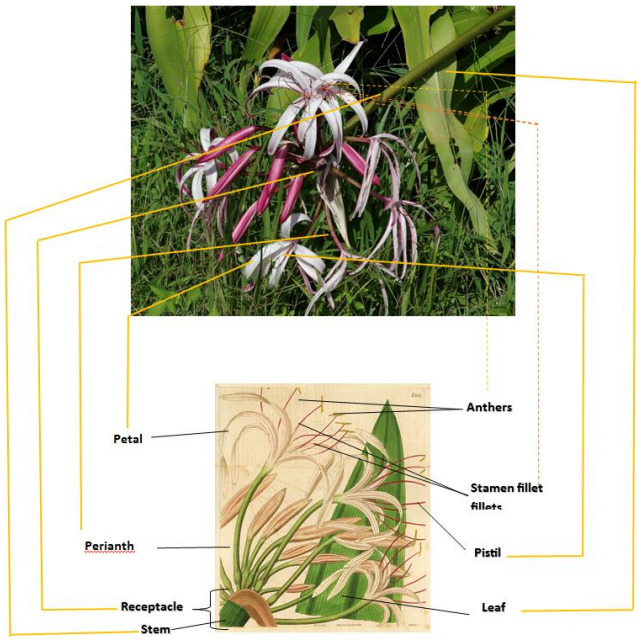

Figure 1. *The various aerial organs of the Crinum amabile.*

The *Crinum amabile* is a plant of the *Amarylliaceae* family, widely distributed in tropical and subtropical regions of the world [1]. It's a very pleasant ornamental plant, especially at the start of its flowering phase, with its particular mix of white and purplish-red colors, and its unique fragrance and scent. It is also characterized by the presence of a receptacle, a sort of intermediate site that links its strong stem, which can be up to 1.5 metres long and 5 to 10 cm wide, and the perianths anchored to it, a sort of secondary stem carrying the flowers of the *Crinum amabile*. The plant used in this study was grown, in the urban environment of the Antananarivo city - Madagascar, in Ivandry, whose geological coordinate is Ivandry Tananarive, -18.876680, 47.520937.

2.1. Extracting Active Molecules Procedure from the Aerial Organs of the *Crinum Amabile* for Use in Phytochemical Tests

Phytochemical tests on *Crinum amabile* concern the qualitative identification of chemical molecules and the main bioactive compounds present in the plant's various organs and responsible for its biological and medicinal properties. To do

this, the various molecules present in each different aerial organ of *Crinum amabile* were dissolved and extracted in 80 ° ethanol using the hydroalcoholic extraction method. First, these aerial organs were dried for a few days in the open air in an aerated chamber with dry air flow; then they were cut and/or ground into small pieces. Extraction is then carried out under reflux at 75 ℃, using a 250ml flask containing 51ml ethanol-80 ° for a maximum of 4.08g sample, for one hour. The recovered solution is filtered to retain the crushed pieces and recover only the hydroalcoholic filtrate-extract of the organs to be tested. This extract will then undergo the various phytochemical tests.

2.2. Phytochemical Test Procedures and Results on the Various Aerial Organs of the *Crinum Amabile*

The reagents used on each phytochemical tests and the results of these tests on the different aerial organs of the *Crinum amabile* were given in the following table 1.

Table 1. *Phytochermical reagents-procedures and results for the different aerial organs of the Crinum amabike.*

Experimental conditions and reagents used in phytochemical tests of *Crinum amabile* aerial organs	**Flavonoids**	**Protein-amino acid**	**Tannins**	**Saponin**	**Alcaloïds**	**Triterpene steroid**	**Coumarins**	**Anthocyanins**	**leuco anthocyanins**
Expected color	Red rose	Purple	Purple ring	Persistent moss	Precipitates Yellow to brown	Red ring	Yellow	Red rose	Purplish red
Extract volume [ml]	1	1	2	15	10	10	1	2	2
HCl concentrated	5 à10 drops	-	-	-	2 à3 drops	-	-	-	-
$MgCl_2$ – solution	A few drops	-	-	-	-	-	-	-	-
NaOH-5%	-	Mixed solutions	-	-	-	-	-	-	-
$CuSO_4$ – 1%	-		-	-	-	-		-	-
$FeCl_3$	-		A few drops	-	-	-	-	-	-
Distillated water				20 ml	-	-	-	-	-
Wagner reagent					5 drops	-	-	-	-
H_2SO_4 concentrated						1ml	0,1 à1ml	-	-
HCl – 25%								2	2
Petal	+	-	+	-	+	+	+	+	+
Stamen fillet	+	-	+	+	+	+	+	+	+
Pistil-Perianth	+	-	+	+	+	+	-	+	+
Stem	-	-	+	-	+	-	+	-	-
Anther	+	-	-	-	+	+	+	-	-
Receptacle	+	-	-	-	+	+	+	+	+
Leaf	+	-	+	+	+	+	+	+	+

3. Extraction, Identification and Quantification of Fatty Acids in the Aerial Organs of the *Crinum Amabile*

3.1. The Method of Fatty Acids Extractions from the Various Aerial Organs of the *Crinum Amabile*

First, each sample was air-dried for a few days in a dry airflow chamber; then they were cut and/or ground into small pieces, carefully weighed on the precision balance and placed in cotton fabric cartridges. Next, place the sample in the soxhlet siphon located below the soxhlet cooling-condenser system, which will be placed above the soxhlet flask containing the hexane extraction solvent, which volume respects the soxhlet capacity. The temperature of these soxhlet extractions of fatty acids from the various organs of *Crinum amabile* is 145 ℃, lasting from a minimum of 3H to a maximum of 5H, depending on the mass quantity of the samples and the nature of the organs to be extracted. At the end of extraction, the total extract recovered in the flask, after rinsing the various parts of the soxhlet with hexane, if necessary, is subjected to gentle evaporation in a vacuum evaporator at a moderate temperature of 45 ℃. The weight of fatty acid recovered is weighed on a precision balance, and the fatty acid content of each *Crinum amabile* organ is deduced from this method.

Table 2. *Fatty acid rate in various dry organs of the Crinum amabile.*

	Petal	Stamen fillet	Pistil-Perianth	Stem	Anther	Receptacle	Leaf
Hexane volume (ml)	100	145	125	145	75	113	125
Initial weight (g)	0.2908	0.8383	0.1815	0.9184	0.0414	0.3039	0.7934
Fatty acids weight (g)	0.0284	0.6894	0.0101	0.1094	0.0057	0.0328	0.2033
Yield (%)	9.7662	82.2379	5.5647	11.9120	13.7681	10.7930	25.6239
Ratio Yield/Initial weight [%. g^{-1}]	33.5838	98.1008	30.6597	12.9704	332.5632	35.5151	32.2963

Looking at the ratio of yield to initial organ mass, it's clear that the anther has the highest fatty acid yield value per gram of sample. (332.5632 – Table 2) followed by its supporting organ, the stamen fillets (98.1008 – Table 2). The leaf, petal, pistil-perianth and receptacle all have ratio values around 30.6 à 35.52 and whose receptacle holds the maximum value of 35.5151 (Table 2). Finally, the stem holds the minimum ratio value (12.9704 – Table 2). These results indicate and confirm that primary metabolites, including fatty acids, are synthesized in the leaves of the *Crinum amabile* via photosynthesis and glycolysis [2, 3]. Then, they are redistributed to its various organs via the phloem, which transports the raw sap rich in metabolites produced by the leaves to the different plant's consumer organs. While the xylem transports raw sap composed of water and mineral salts from the roots to the rest of the plant (asp, 2024), on these distribution pathways, the receptacle (35,5151 – Table 2) acts as an intermediate storage area between the stem (12.9704 – Table 2) and the upper organs stamen fillets, pistil, perianth, petal and anther - the metabolite's last delivery organ, which explains the very high maximum value of its yield/initial mass ratio, equal to 332.5632. (Table 2).

3.2. Identification and Quantification of Fatty Acids in Various Aerial Organs of the *Crinum Amabile*

Gas chromatographic analysis of the fatty acids in the various organs of *Crinum amabile* was carried out at the LCP pesticide control laboratory at Nanisana Madagascar. Analyzing the results of the various *Crinum amabile's* organ chromatograms led to the conclusion that the fatty acids present in different proportions were myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid. The weight concentrations of fatty acids in the various organs of the *Crinum amabile* are shown in the table 3 below:

Table 3. *Concentration massique en acide gras des différents organes aériens du Crinum amabile.*

Fatty acids	Anther [mg/g]	Stamen fillet [mg/g]	Pistil-Perianth [mg/g]	Petal [mg/g]	Receptacle [mg/g]	Stem [mg/g]	Leaf [mg/g]
myristic acid	8.02E+00	1.24E+00	6.34E-01	1.13E+00	0.00E+00	3.70E+00	0.00E+00
palmitic acid	4.43E+01	1.03E+02	2.32E+01	4.10E+01	2.10E+01	5.14E+01	1.09E-02
palmitoleic acid	9.71E-01	1.01E+00	2.49E-01	1.16E+00	8.20E-01	4.64E+00	4.45E-04
stearic acid	1.24E+01	9.22E+00	2.56E+00	5.12E+00	4.62E+00	8.11E+00	1.40E-03
oleic acid	3.63E+01	1.11E+02	2.28E+01	3.55E+01	2.57E+01	2.88E+01	9.48E-03
linoleic acid	3.22E+01	2.94E+01	5.85E+00	1.25E+01	2.21E+01	1.85E+01	4.67E-03
linolenic acid	1.56E+00	5.68E-01	1.47E-01	7.08E-01	9.00E-01	3.02E+00	2.58E-03
arachidic acid	1.86E+00	0.00E+00	2.21E-01	5.39E-01	5.17E-01	1.04E+00	2.61E-04

Based on Table 3 above, the following Table 4 shows the quantities of the different categories of fatty acids (SFA-saturated fatty acid; UFA-unsaturated fatty acid; MUFA-monounsaturated fatty acid; PUFA-polyunsaturated fatty acid) for the different organs of the *Crinum amabile*; a table that also allows to compare fatty acid categories. TFA is the total fatty acid.

Table 4. *Weight concentration of various fatty acid categories in aerial organs of the Crinum amabile.*

FATTY ACIDS CATEGORIES	Anther [mg/g]	Stamen fillet [mg/g]	Pistil-Perianth [mg/g]	Petal [mg/g]	Receptacle [mg/g]	Stem [mg/g]	Leaf [mg/g]	Average	Standard deviation	Average + Standard deviation
SFA - TOTAL	66.58	113.46	26.615	47.789	26.137	64.25	0.013	49.263	36.791	86.054
UFA - TOTAL	71.031	141.98	29.046	49.868	49.520	54.96	0.017	56.631	43.887	100.519
MUFA - TOTAL	37.271	112.01	23.049	36.66	26.520	33.44	0.010	38.423	34.876	73.299
PUFA - TOTAL	33.76	29.968	5.997	13.208	23	21.52	0.007	18.209	12.379	30.588
TFA-TOTAL	208.642	397.416	84.707	147.525	125.177	174.17	0.047			

Values in red are above the average, and values in green are above the sum of the standard deviation and the average.

The results in Table 4 show that for the *Crinum amabile*:

1) The stamen fillet contains the most total fatty acids - TFA (397.416 - Table 4), the most saturated fatty acid - SFA (113.46 - Table 4), the most unsaturated fatty acid - UFA (141.98 - Table 4) and the most monounsaturated fatty acid - MUFA (112.01 – Table 4) followed by anther and Stem.
2) The anther (33.76 - Table 4) contains the most polyunsaturated fatty acids - PUFA, followed by the stamen fillet (29.968 - Table 4), the receptacle (23 - Table 4) and the Stem (21.520 - Table 4)
3) The reproductive organs with the highest total fatty acid - TFA content are the stamen fillet (397.416-Table 4) followed by the anther (208.642 - Table 4)
4) The receptacle is an intermediate storage-passage site, especially for polyunsaturated fatty acids (23 - Table 4)

The stem is the transport organ for various fatty acids, with a significant content of saturated fatty acids - SFA (64,25 - Table 4) and polyunsaturated fatty acid - PUFA (21,52 – Table 4)

Furthermore, these weight concentration results for the various organs of *Crinum amabile* confirm the comments made in the paragraph §4.1 of this manuscript.

3.3. The Medicinal, Biological and Chemical Virtues and Properties of Fatty Acids in Aerial Organs of the *Crinum Amabile*

The virtues and biological activities of the fatty acids detected and quantified in the various organs of the *Crinum amabile* according to previous bibliographic research [4, 5] are presented in Table 5 below:

Table 5. The virtues, biological and chemical activities of fatty acids from the different aerial organs of the Crinum amabile.

FATTY ACIDS	VIRTUES, BIOLOGICAL AND CHEMICAL ACTIVITIES
myristic acid	Internal or external lubricant in plastics Anti-diabetic
palmitic acid	Surfactant, viscosifier, emollient, coemulsifier
palmitoleic acid	Anti-inflammatory
stearic acid	Protecting the cardiovascular system Improve insulin sensitivity Inhibit tumor and cancer cell proliferation
oleic acid	Anti-inflammatory - Anti-oxidant
linoleic acid	Anti-inflammatory - Anti-oxidant
linolenic acid	Anti-inflammatory - Anti-oxidant
arachidic acid	Additive in detergents, photographic materials and lubricants

In general, referring only to the results of the determination and quantification of its fatty acids (Table 3), The *Crinum amabile* is a plant whose various organs have antioxidant and anti-inflammatory properties. By summing all the fatty acids present in the various organs, the total quantity of each fatty acid in *Crinum amabile* can be determined (Table 6), this plant has anti-inflammatory and antioxidant properties, mainly through oleic acid (MUFA – C18 – 2.60E+2 mg/g – Table 6) and through the linolenic acid (PUFA – C18 – 1.21E+2 mg/g – Table 6). According to the Table 6, the quantity of palmitic acid (SFA – C16 – 2.84E+02mg/g) much higher than the average total fatty acid quantity, Thus, the fatty acid extract from *Crinum amabile* also has very important properties such as viscosifying agent, surfactant, emollient agent and co-emulsifying agent. These interpretations in no way detract from the virtues, biological and chemical activities of other fatty acids, especially those in exponent 1, such as myristic acid. (SFA – C14 – 1.47E+01mg/g – Table 6) and especially stearic acid (SFA – C18 – 4.20E+01mg/g – Table 6).

Table 6. Evaluation of the main virtues, biological and chemical activities and chemical activities of aerial Crinum amabile.

FATTY ACIDS	TOTAL QUANTITIES IN THE AERIAL *Crinum amabile* [mg/g]	VIRTUES, BIOLOGICAL AND CHEMICAL ACTIVITIES
Myristic acid SFA - C14	1.47E+01	Internal or external lubricant in plastics Anti-diabetic
Palmitic acid SFA - C16	2.84E+02	Surfactant, viscosifier, emollient, coemulsifier
Palmitoleic acid MUFA - C16	8.85E+00	Anti-inflammatory
Stearic acid SFA - C18	4.20E+01	Protecting the cardiovascular system Improve insulin sensitivity Inhibit tumor and cancer cell proliferation
Oleic acid MUFA - C18	2.60E+02	Anti-inflammatory - Anti-oxidant
Linoleic acid PUFA-2 - C18	1.21E+02	Anti-inflammatory - Anti-oxidant
Linolenic acid PUFA-3 - C18	6.91E+00	Anti-inflammatory - Anti-oxidant
Arachidic acid SFA - C20	4.18E+00	Additive in detergents, photographic materials and lubricants

4. Extraction, Identification, Quantification of Steroids, Flavonoids in the *Crinum Amabile's* Aerial Organs

4.1. Extraction Procedure of Steroids and Flavonoids in the Various Aerial Organs of the *Crinum Amabile*

Extraction by esterification with citric acid [6, 7] is a recent method used in our laboratory to extract the organic molecules present in the various organs of a plant or other living organism [4, 5, 8-11].

The experimental conditions for citric acid esterification of *Crinum amabile's* various organs studied are shown in Table 7 below. Kinetic studies were carried out to evaluate the evolution of citric acid conversion as a function of time, and 1ml samples were taken from the reaction medium at 1mn, 5mn, 15mn, 30mn, 60mn and eventually 90mn.

Table 7. *Experimental conditions for citric acid esterification of various organ molecules of the Crinum amabile.*

	Anther	Petal	Stem and receptacle	Stamen fillet pistil perianthe	Leaf
Sample weight (g)	0.2434	16.4352	1.0562	4.8627	0.0989
Citric acid weight (g)	0.3165	1.7438	1.3734	3.5771	0.1286
Distillated water (mL)	200	200	200	200	200

The evolution of conversions as a function of time for each organ of *Crinum amabile* is given in the following table 8:

Table 8. *Citric acid conversion as a function of time for the various organ systems involved of the Crinum amabile.*

	Reaction time (min)	Conversion (%)
Anther	1	9.38
	5	32.38
	15	55.15
	30	66.53
	60	91.12
Petal	2	15.34
	5	15.77
	15	41.33
	31	49.97
	60	58.52
	90	62.86
Stem and receptacle	1	47.79
	5	53.25
	15	58.65
	30	64.00
	60	69.30
Stamen fillet pistil and perianth	1	15.82
	5	20.23
	15	24.59
	30	28.92
	60	33.21
Leaf	1	55.40
	5	66.72
	15	77.92
	30	83.52
	60	89.07

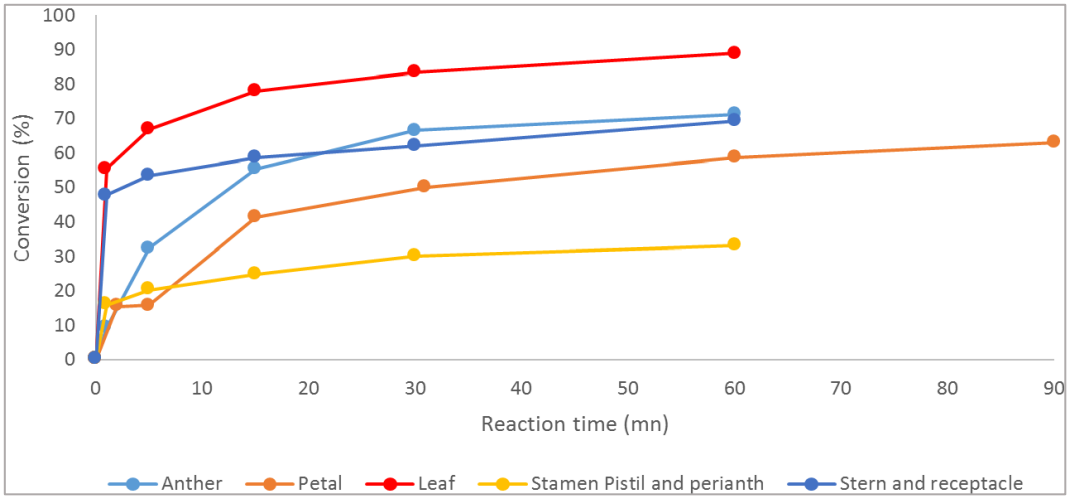

Figure 2. *Evolution of citric acid conversion as a function of time for various organ systems involved of the Crinum amabile.*

The curves in Figure 2 clearly show that:

1) Initial conversion and overall leaf conversion are very high compared to other organs. These results are undoubtedly due to the structure of the leaves, which feature micro- and even nano-porosities and channels through which photosynthesis takes place and, above all, through which the products of this photosynthesis are distributed to the other organs of the plant *Crinum amabile*. As a result, when these channels and porosities are reached, the contact surfaces and contact time between the citric acid molecules and the leaf molecules increase considerably, resulting in a very high initial and overall conversion rate in leaves compared to other organs. Previous studies had already shown the effect of these contact surfaces and sample size during the esterification of plants or vegetative substance with citric acid such as the *Garcinia dulcis*, the *Callistemon citrinus* and rice husks [12].
2) As in the case of leaves, initial conversion in stems and receptacles is very high (Figure 2), confirming the presence of micro- or even nano-porosities and channels in these organs, where contact surfaces and contact times between citric acid molecules and molecules in stems and receptacles will initially increase considerably. However, from the 5th minute onwards, global conversion and the slope of the curve are lower than in the leaves. For leaves, not only the channels and porosities are more rigid and functional in the long term, but for stems and receptacles they are less rigid and easily attacked by acidity. In fact, in the long term, the conversion of citric acid joins that of the anther (figure 2).
3) Initially, anther and petal conversions are on the same slope (figure 2); it's only from the 15th minute onwards that the slope is steeper on the anther than on the petal. These results mean that the organs had the same initial speed of citric acid esterification, but there was a noticeable difference in overall speed from the 15th minute onwards. As a result, it's possible that the anther, which is the tip of the stamen fillet extension, has porous structures and channels through which compounds and molecules from the stamen fillet flow, and vice versa.
4) From the 15th minute onwards, the speed of citric acid esterification at petal and leaf level is very similar, following the same slope. However, conversion in the petal is lower than in the leaf, confirming the assertion that there is also a quality difference between the structure of their canals and porosities. Leaves are much stronger and more resistant to acidity than petals.
5) The initial slope of citric acid conversion over time in pistils, perianths and stamen fillets lies between the slopes of the other organs (figure 2) and, above all, its global conversion from the 15th minute onwards is the lowest. These results suggest that in these organs there are pores and channels with a strong structure but whose size could be greater than those of the other organs, such as meso-pores or macropores, thus facilitating reactions between large molecules such as citric acid rather than esterification. In addition, it should be noted that the quantity of citric acid used during this esterification process is the largest at over 3.5g.

Using these kinetic data, the experimental conditions for the esterification reactions of *Crinum amabile*'s various organs and using the global kinetic equation for this citric acid esterification reaction, such as

$$v = k \times [Citric\ acid]^{\alpha} \times [Active\ molecules]^{\beta}$$

This enables to determine the partial order related to citric acid "α", the partial order related to the active molecules "β" and the speed constant "k" for the various organ systems involved of the *Crinum amabile*. The results and values of these different kinetic parameters are summarized in Table 9 below.

Table 9. *Determination of globall speed constants for esterification reactions with citric acid of molecules from various organ systems involved of the Crinum amabile.*

	Anther	Petal	Stem and receptacle	Stamen fillet pistil perianth	Leaf
K	+3.55	+0.66	+10.8	7.85E-4	8,89
A	+1.33	1.17	+1.87	+0.714	2.03
B	+0.25	+0.27	-0.131	-0.625	-0.478
Global order	+1.58	+1.44	+1.74	+0.09	1.55
Sample weight (g) (1)	0.2434	16.4352	1.0562	4.8627	0.0989
Citric acid weight (g) (2)	0.3165	1.7438	1.3734	3.5771	0.1286
Ratio (1)/(2)	0.7690	9.4249	0.7690	1.3594	0.7691

These values of the speed constants and partial orders confirm the interpretations and discussions on the evolution of conversion as a function of time.

These results of kinetic parameters confirm the results discussed at the beginning of this paragraph §4.1. of this manuscript and figure 2 such as:

1) Anthers, leaves and the organ system (stem, receptacle) of *Crinum amabile* have the highest global esterification speeds with citric acid (k values table 9).
2) Followed by petals with constant growth (figure 2)
3) And finally, the group of (stamen fillet, pistil, perianth) organs which in the long term will have a parallel growth but less important than that of the petal. (figure 2)
4) The overall decrease in speeds constants for the group of organs (stamen fillets, pistil, perianth) could be explained by the phenomenon described and explained above: "the structure of the stamen fillet, pistil and perianth are less rigid and easily attacked by acidity, leading to a long-term reduction in their overall speeds".

4.2. Steroids Identification and Quantification in Various Aerial Organ Systems Involved of the *Crinum Amabile*

After esterification with citric acid following the procedure described in paragraph §4.1 of this manuscript and the literatures referred to, the citric acid ester solutions from the various organs systems undergo transesterification with methanol, followed by a series of hexane extractions to remove fatty acids and their derivatives, and then dichloromethane extraction to recover and to quantify polar organic molecules and their derivatives, notably steroids and flavonoids. This procedure is widely presented in the following literature [8] (Rabeharitsara, Ravomialisoa, & Randriana, Synthesis of Capsicum chinense Citric Acid Esters-Its Methanol Trans-esterification Investigations with hplc Analysis and Its valorization as Gels-Crystals Ca-Salts, 2021) [13].

Determination of steroids and triterpenoid derivatives from the various organs systems involved of *Crinum amabile* was carried out by HPLC at the LCP pesticide control laboratory in Nanisana - Madagascar, using the experimental conditions for previous analyses described in the literature [4, 8, 11] which in turn referred to the experimental conditions and basic chromatograms given in the following literature [14].

Thus, the steroid molecules identified by High Performance Liquid Chromatography in the aerial organs of the *Crinum amabile* are:

1) Betulinic acid
2) Ursolic acid
3) Betulin
4) Lupeol

Their quantities in the various organs of Crinum amabile are given in table 10 below:

Table 10. *Steroid quantities in the various wet aerial organ systems involved of the Crinum amabile.*

Wet organs [mg/$g_{humides}$]	Anther	Petal	Stem Leaf Receptacle	Stamen fillet Perianth Pistil	Average	Standard deviation	Average + Standard deviation
Betulinic acid	1.86E+02	2.52E+01	3.94E+01	1.57E+01	6.66E+01	80.2100731	1.47E+02
Ursolic acid	1.33E+02	2.20E+01	4.54E+01	2.39E+01	5.61E+01	52.3696716	1.08E+02

Wet organs [mg/$g_{humides}$]	Anther	Petal	Stem Leaf Receptacle	Stamen fillet Perianth Pistil	Average	Standard deviation	Average + Standard deviation
Betulin	0	0	0	2.46E-01			
Lupeol	6.43E+01	0	0	0			
Total	4.11E+02	4.77E+01	8.53E+01	4.29E+01			

These results in table 10 show that:

1) The anther contains the highest total mass concentration of steroids (Table 10 – figure 6)
2) Lupeol (figure 5) is found only on the anther, while betulin is found only on the stamen fillets/perianths and/or pistil of the *Crinum amabile*

The steroids present on all *Crinum amabile* organs, but always in high mass concentrations on the anther, are betulinic acid and ursolic acid.

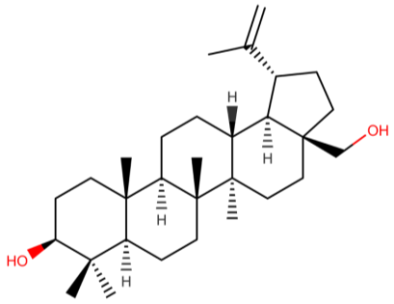

Figure 3. *The molecule of betulin.*

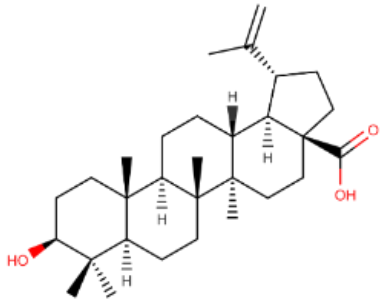

Figure 4. *The molecule of betulinic acid.*

Referring to the bibliography, which describes the oxidation reaction of betulin (figure 3) to betulinic acid (figure 4) [15] and the above results, betulin could be oxidized to betulinic acid in the stamen fillet and pistil and/or anthers; as the anther contains the most betulinic acid (1.86E+2 - Table 10), it is quite realistic that this oxidation reaction takes place mainly in the anthers, which have porous structures (see the paragraph §4.1. of this manuscript) and a condition of presentation in relation to atmospheric oxygen that is highly favorable to this oxidation reaction. Then, these betulinic acids, synthesized in high concentrations in the anthers, are redistributed throughout the plant's organs of the *Crinum amabile*.

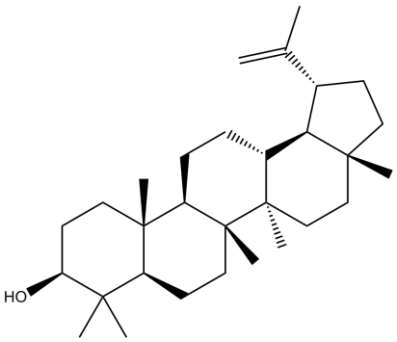

Figure 5. *The molecule of lupeol.*

Referring to the molecular structure of lupeol, and as it exists only on the anther, this molecule could also be chemically synthesized by dehydration followed by hydrogenation and/or enzymatic reduction of betulin at the anther site of the *Crinum amabile*.

The above results also demonstrate the manuscripts' assertion that the synthesis of different plant secondary metabolites can occur in various plant organs, depending on the type of metabolite and the function it performs and in response to specific needs for defense, attraction or interaction with the environment [2, 16].

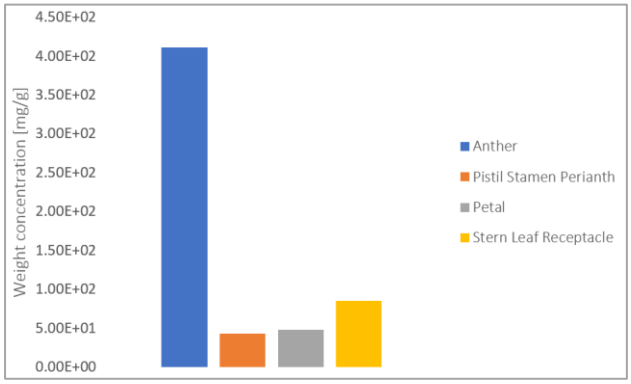

Figure 6. *The total quantities of the various steroids in the wet aerial organ systems involved of the Crinum amabile.*

The weight concentration of steroids is very high in the anthers and then on the stems, leaves and receptacle. This is normal, but somewhat exceptional for the anther of *Crinum amabile*, where the triterpenoids derivatives/steroid concentrations is very high, as the literature indicates that the synthesis of secondary metabolites can take place in different plant organs, depending on the type of metabolite and the function it fulfils. Here are a few examples:

1) Leaves: Terpenes and derivatives, which are often volatile molecules that can be odoriferous and play a role in defense against herbivores and pathogens, are often synthesized in leaves.
2) Root: Alkaloids, nitrogen compounds that can have toxic properties for herbivores by acting on their nervous systems, are often produced in roots.
3) Flower: Phenolic compounds including flavonoids, tannins and lignins, which attract pollinators with their colors and scents, are often synthesized in flowers
4) Bark: Certain secondary metabolites, such as tannins, are produced in the bark to protect the plant from infection and herbivores.

Each plant organ can thus contribute to the production of secondary metabolites in response to specific needs for defense, attraction or interaction with the environment. These compounds are essential for the survival and adaptation of plants in their natural environment, as a defense system against herbivores and pathogens, as an allelopathy system inhibiting the growth of competing plants, as an attraction system for pollinators and seed dispersers, and as a symbiosis system facilitating beneficial interactions with soil microbes.

4.3. Identification and Quantification of Flavonoids in the Various Aerial Organs System Involved of the *Crinum Amabile*

The dichloromethane extracts recovered during the procedure described at the beginning of paragraph §4.2. of this manuscript is still used to quantify flavonoids of the various aerial organs system involved of the *Crinum amabile*. [8, 13].

Determination of flavonoids in the various organs of Crinum amabile is carried out by HPLC at the LCP pesticide control laboratory at Nanisana Madagascar, using the same experimental conditions as for previous analyses described in the literature [8, 11, 13] which in turn referred to the experimental conditions and basic chromatograms given in the following literature [17].

Thus, the flavonoid molecules identified by High Performance Liquid Chromatography are:

1) Eriocitrin
2) Hesperidin
3) Neohesperidin
4) Isorhoifolin
5) Rhoifolin

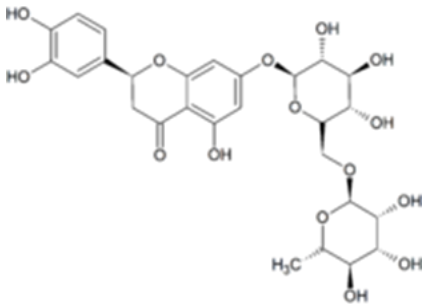

Figure 7. *The molecule of 'eriocitrin.*

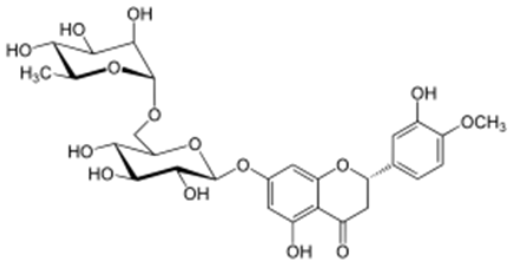

Figure 8. *The molecule of hesperidin.*

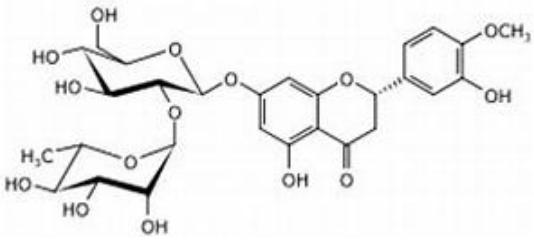

Figure 9. *The molecule of neohesperidin.*

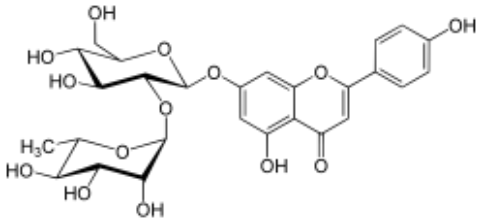

Figure 10. *The molecule of rhoifolin.*

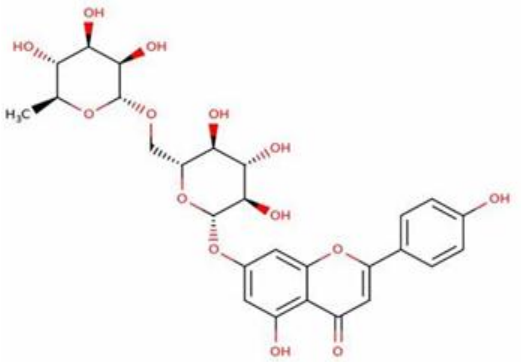

Figure 11. *The molecule of isorhoifolin.*

their quantities in the various aerial organs systems involved of *Crinum amabile* are given in the following table 11:

Table 11. *The quantities of flavonoids in the various aerial organs systems involved of the Crinum amabile.*

Wet organs systems [mg/g_{wet}]	Anther Stamen fillet Pistil Perianth	Petal	Stem Leaf and Receptacle	Average	Standard deviation	Average + Standard deviation
Eriocitrin	1.16E+02	4.51E+01	8.53E+01	8.21E+01	35.5559184	1.18E+02
Hesperidin	-	1.05E+00	-			
Neohesperidin	-	3.20E-01	-			
Ishorhoifolin	-	3.30E-01	-			
Rhoifolin	-	8.21E-01	-			
Total	1.16E+02	4.76E+01	8.53E+01	8.30E+01	34.248807	1.17E+02

These results in table 11 show that:

1) Eriocitrin is the most dominant flavonoid in the aerial organs of the *Crinum amabile* and is found in very high quantities in the anther-amine-pistil-perianth group and in the stem-leaf-receptacle group.
2) However, the petal contains the greatest variety of flavonoids, with rhoifolin being the dominant flavonoid in this organ.

These results confirm the assertions made in bibliographic reviews that it is the leaves, including the petals of the *Crinum amabile* not only attracts pollinators with its color and scent, but also synthesizes flavonoids. In fact, the petals of the *Crinum amabile* are marked by very pleasant colors and odors characteristic of the *Crinum amabile*.

Based on the structures of the different flavonoid molecules and the HPLC results, the different flavonoid molecules in the petal could well be synthesized by the transformation of eriocitrin either chemically, enzymatically or biochemically at the level of the petal.

4.4. The Virtues, Medicinal and Biochemical Properties of Steroids and Flavonoids in the Aerial Organs of the *Crinum Amabile*

The following Table 12 lists the various medicinal and biochemical properties of the various aerial organs of the *Crinum amabile*.

Table 12. *Virtues - Medicinal and biochemical properties of the molecules in the various aerial organs of the Crinum amabile.*

Aerian organs of the *Crinum amabile*	*Virtues - Medicinal and biochemical properties*
STEROL AND TRITERPENOÏDES DERIVATIVES	
Betulinic acid	Anti-inflammatory – Anti-VIH
Ursolic acid	Anticancer – Anti-inflammatory
Betulin	-
Lupeol	Anticancer – Anti-inflammatory
FLAVONOÏDES	
Eriocitrin	Anti-oxidant – Anti-inflammatory
Hesperidin	Anti-oxidant – Anti-inflammatory
Neohesperidin	-
Ishorhoifolin	Anti-oxidant
Rhoifolin	Anti-oxidant – Anti-inflammatory

It has been noted that almost all the molecules present in the various aerial organs of the *Crinum amabile* have antioxidant and anti-inflammatory properties. This led us to carry out anti-oxidant and anti-inflammatory tests on citric acid ester stock solutions from various aerial organs of the *Crinum amabile* on the paragraph §6 and the paragraph §7 of this manuscript.

5. Extraction and Quantification of Alkaloids in Various Aerial Organs of the *Crinum Amabile*

5.1. Description of Extraction and Quantification Method for Alkaloids in the Aerial Organs of the *Crinum Amabile*

Alkaloids were quantified in the various aerial organs of Crinum amabile using the procedure of Bouzidia et al. [18] which consists of four stages: delipidation, alkalinization, extraction with dichloromethane, purification and evaporation. The delipidation was carried out during the extraction, identification and quantification of fatty acids from the different organs of the plant *Crinum amabile*. Alkalinization consists of immersing delipidated samples in a 0.5N NH_4OH solution for 24 hours.

Dichloromethane extraction is performed on a Soxhlet. Extraction with dichloromethane takes a minimum of 4 hours and a maximum of 5 hours.

Purification is a step during which the preceding crude extract is successively and alternately purified and decanted with a sulfuric acid solution H_2SO_4 – 0.5N first, then with a solution of dichloromethane until exhaustion (i.e each purification-extraction operation is carried out three times in succession.) such that the ratio between dichloromethane and sulfuric acid in each purification is equal to

$$\text{Ratio} = \frac{V_{DCM}}{V_{H2SO4}} = 1.667$$

Note that:

After the first purification with sulfuric acid, the phase with the sulfuric acid at the top is recovered after settling for 30 minutes, then its pH is brought back to pH=9 with a 30% or 70% ammonia solution before undergoing purification with dichloromethane.

Once the second purification with dichloromethane has been carried out, the water molecules retained on the dichloromethane extract must first be removed by passing it through a before moving on to the final step of evaporating the total dichloromethane solution on a plate of sand moderately heated on a hot plate. Before evaporation, dry-weigh the evaporation beaker, then re-weigh after evaporation and deduce the mass of alkaloids extracted.

5.2. Quantification of Alkaloids in the Various Aerial Organs Systems Involved of the *Crinum Amabile*

The quantities of alkaloids in the various aerial organs systems involved of the *Crinum amabile* are given in table 13 below:

Table 13. *Experimental conditions and quantification of alkaloids from various aerial organs systems involved of the Crinum amabile.*

		Anther	Petal	Stamen fillet	Pistil Perianth	Leaf	Receptacle & Stem
Sample weight (g)		0.2908	0.1815	0.8383	0.0414	0.9184	1.2223
Alkalinization	NH_4OH – 0.5N(ml)	5	5	5	5	5	5
Extraction	Soxhlet capacity (ml)	150	150	150	150	150	150
	Dichloromethane volume (ml)	100	145	145	145	145	145
	Color of crude extract	Yellow	Red Brown	Brown orange	Brown orange	Dark green	Light brown
Purification	Sulfuric acid 0,5N volume (ml)	135	200	200	205	200	180
	Dichloromethane volume (ml)	675.135	1134.22	1134.22	1000.2	1134.22	900.18
Evaporation	Solution color on evaporation	White to red					
	Alkaloid crystal color	White to light brown					
	Alkaloid content (%)	55.56	5.02	1.54	19.12	1.82	0.32

The results show that alkaloid content is very high on the anther (55.56% - Table 13) and the pistil (19.12% - Table 13). In slight quantities on the petal (5.02% - Table 13) and in a very low content on the stamen fillet, leaf, receptacle and stem (≤1% - Table 13).

6. dpph - Antioxidant Test of the Various Aerial Organs Systems Involved of the *Crinum Amabile*

6.1. Procedure and General Principle of the dpph Antioxidant Test

The first step in the antioxidant test is to prepare a stock solution, of the various aerial organs systems involved of the of *Crinum amabile*, whose total molar concentration in anti-oxidant molecules is known from the bibliography and the previous work. This concentration of the stock solution was studied, prepared and set at 110.146 µmol/L [11, 19]. Three stock solutions were prepared: citric acid ester of the system "anther-pistil-perianth-stamen fillet", citric acid ester of the system "stem-receptacle" and citric acid ester of the petal, so three antioxidant tests were carried out. Then, from each stock solution of the various aerial organs systems involved of the *Crinum amabile*, Different concentrations of test solutions such as 75 µmol/l, 50 µmol/l, 12.5 µmol/l, 8.5 µmol/l, 5 µmol/l et 2.5 µmol/l are prepared with methanol as solvent. Since the dpph ° (2, 2'-diphenyl-1-picrylhydrazyl) test is based on the scavenging of the stable free radical dpph °by an anti-radical molecule, dpph ° accepts a hydrogen atom (H) from a scavenger molecule, such as the antioxidant molecules in the test solution, this results in a reduction of dpph ° to dppH2, accompanied by a change from violet to an increasingly light-yellow color - this is the decoloration of dpph °. The degree of color change is proportional to the concentration and potency of the antioxidant molecules contained, so this method is quick and easy to implement, and is carried out at room temperature, eliminating any risk of thermal degradation of the molecules tested [19, 20]. The dpph °solution used has a concentration of 40mg/L and is added to each test solution in the quantities given in the following tables 14-16 of the paragraph §6.2. of this manuscript giving the final test solutions in test tubes. The whole should be well covered and kept in the dark with aluminum foil and black-opaque sachet for 30mn before being analyzed with UV-visible spectrophotometer at 517 nm wavelength to quantify the remains of dpph ° in each test solution. A solution of dpph °was kept under the same experimental conditions as the solutions to be tested, thus serving as a blank such that:

$$dpph° \; Evolution \left(\% \; dpph°_{transformed-reduced}\right) = \frac{[dpph°]_{blank} - [dpph° \; remainder]_{after \; 30mn}}{[dpph°]_{blank}} \times 100$$

After plotting the evolution of the percentages of dpph ° transformed-reduced into dppH2 by antioxidant molecules in the test sample of the various aerial organs of the *Crinum amabile*, it is possible to deduce and to determine the IC50 of the test sample using the equation for the evolution of this curve in the vicinity of 50% dpph °Evolution.

6.2. Experimental Conditions and Antioxidant Test Results

6.2.1. Antioxidant Test of the *Crinum Amabile's* Organ System "Anther-Stamen Fillet-Pistil-Perianth"

Table 14. *Experimental conditions and results of the Crinum amabile's organ system "Anther-Stamen fillet-Pistil-Perianth" antioxidant test.*

Anther-Stamen fillet-Pistil-Perianth	75 µmol/l	50 µmol/l	12.5 µmol/l	8.5 µmol/l	5 µmol/l	2.5 µmol/l	Blank
Tested solutions volume [ml]	4	4	4	4	4	4	-
dpph °volume [ml]	2	2	2	2	2	2	4
Total tested sample solution [ml]	6	6	6	6	6	6	-
Tested sample anti-oxydant concentration [µmol/L]	50	17	8.33	5.67	3.33	1.67	-
[dpph °remainder] concentration after 30mn [mg/ml]	0.0226	0.0098	0.0341	0.0041	0.0132	0.0237	0.4998
dpph °Evolution (%) dpph °(transformed-reduced)	95.4782	98.0392	93.1773	99.1797	97.3589	95.2581	

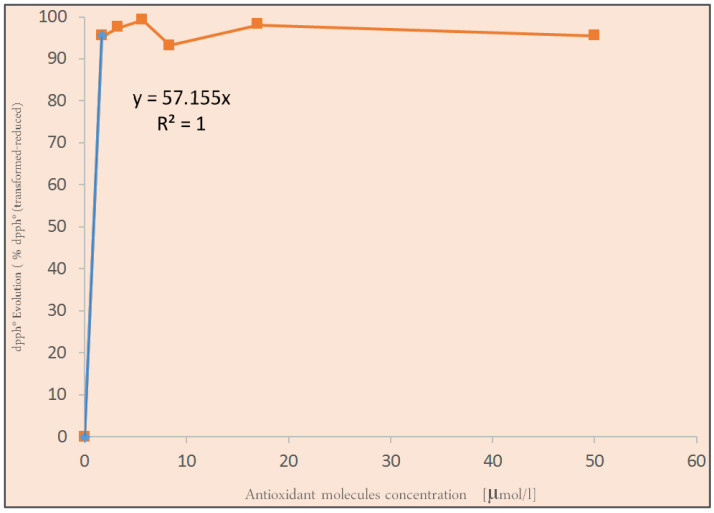

Figure 12. *dpph °Evolution according to the Antioxidant molecules concentration for the Crinum amabile's organ system "Anther-Stamen fillet-Pistil-Perianth"*

6.2.2. Antioxidant Test of the *Crinum Amabile's* Organ Petal

Table 15. *Experimental conditions and results of the Crinum amabile's organ petal.*

Petal	75 µmol/l	50 µmol/l	12.5 µmol/l	8.5 µmol/l	5 µmol/l	2.5 µmol/l	Blank
Tested solutions volume [ml]	4	4	4	4	4	4	-
dpph °volume [ml]	2	2	2	2	2	2	4
Total tested sample solution [ml]	6	6	6	6	6	6	-
Tested sample anti-oxydant concentration [µmol/L]	50	17	8.33	5.67	3.33	1.67	-
[dpph °remainder] concentration after 30mn [mg/ml]	0.039	0.042	0.0399	0.0475	0.0583	0.0589	0.4998
dpph °Evolution (%) dpph °(transformed-reduced)	92.1969	91.5966	92.0168	90.4962	88.3353	88.2153	

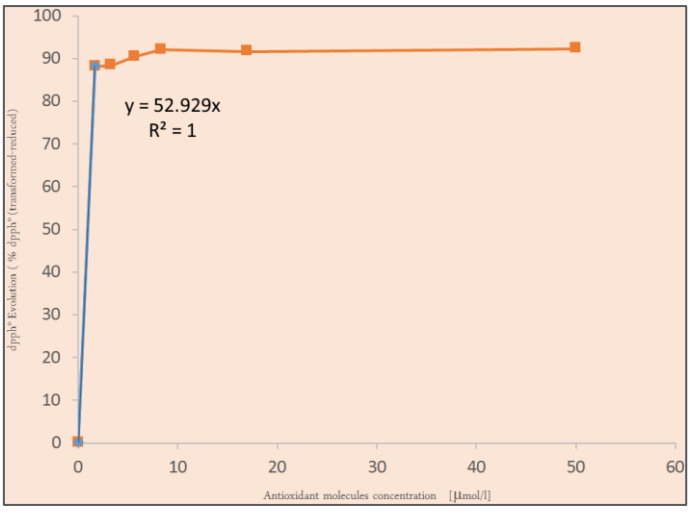

Figure 13. *dpph °Evolution according to the Antioxidant molecules concentration for the Crinum amabile's organ petal.*

6.2.3. Antioxidant Test of the *Crinum Amabile's* Organ System Stem-Receptacle-Leaf

Table 16. *Experimental conditions and results of the Crinum amabile's organ system "Stem-receptacle-Leaf".*

Stem-receptacle-Leaf	75 µmol/l	50 µmol/l	12.5 µmol/l	8.5 µmol/l	5 µmol/l	2.5 µmol/l	Blank
Tested solutions volume [ml]	4	4	4	4	4	4	-
dpph °volume [ml]	2	2	2	2	2	2	4
Total tested sample solution [ml]	6	6	6	6	6	6	-
Tested sample anti-oxydant concentration [µmol/L]	50	17	8.33	5.67	3.33	1.67	-
[dpph °remainder] concentration after 30mn [mg/ml]	0.0307	0.0385	0.023	0.0194	0.0599	0.0444	0.4998
dpph °Evolution (%) dpph ℃(transformed-reduced)	93.8575	92,2969	95,3982	96,1184	88,0152	91,1164	

Table 17. *Antioxidant activities IC50 of the various the Crinum amabile's organ system involved.*

Crinum amabile's organ system	"Anther-Stamen fillet-Pistil-Perianth" ASPP	petal	Stem-Receptacle-Leaf SRL
IC50 - [µmol/l]	0.8748	0.9447	0.9146

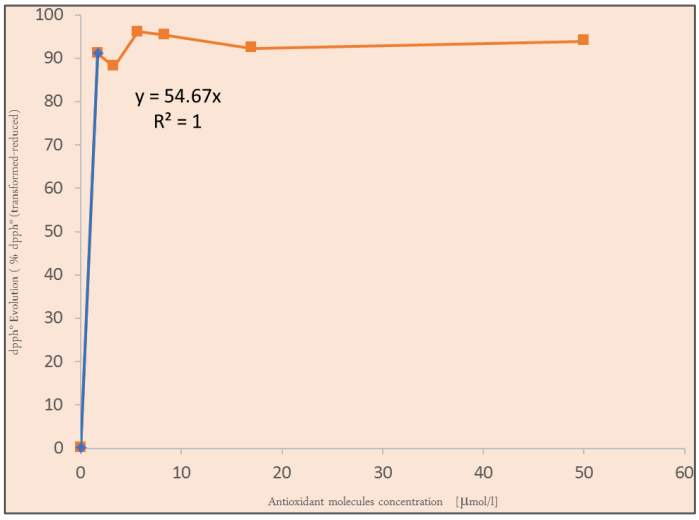

Figure 14. *dpph° Evolution according to the Antioxidant molecules concentration for the Crinum amabile's organ system "Stem-receptacle-Leaf".*

The antioxidant test results and IC50s in Table 17 show that it is the organ system of the *Crinum amabile* «Anther-Perianth-Stamen fillet-Pistil» is with the highest antioxidant activity (IC50-0.8748) followed by the organ system "Stem-Receptacle-Leaf" (IC50-0.9146) and finally the organ petal (IC50-0.9447). These results are entirely normal, given the different textures, porosities and, above all, antioxidant characteristics of the different organ groups and organs of the *Crinum amabile*. Indeed, it is the ASPP system of *Crinum amabile* that contains the highest quantity of antioxidant molecules, with increasingly crushed structures and high specific surface area in the citric acid ester solution, followed by the SRL system of *Crinum amabile*, which contains the second highest quantity of antioxidant molecules, even though its structure and porosity remain rigid in the ester solution. The petal's antioxidant activity is very close to that of the previous system, mainly due to its high specific surface area in the ester solution, since its structure and porosity are

not as resistant and attacked during esterification.

7. NO Scavenging Anti-Inflammatory Test of the Aerial Organs Systems Involved of the *Crinum Amabile*

7.1. Procedure and General Principle of the Anti-Inflammatory Test no Scavenging

Anti-inflammatories are substances that block the secretion or action of certain inflammatory mediators, and are used when the inflammatory reaction is abnormally prolonged (chronic inflammation) and leads to tissue damage. Inflammatory mediators include inflammatory cytokines such as TNF-α, nitric oxide, lipid mediators and even oxygenated free radicals. Anti-inflammatory activity tests are generally based on their effects on the inflammatory mediators mentioned above; for example, the anti-inflammatory test method measures the effect of active extracts on NO production by inflammatory macrophages [21], the anti-inflammatory test method measuring the effect of active extracts on TNF-α production by inflammatory macrophages [20] (Ibrahima, 2019) and the anti-inflammatory test method measuring the NO scavenging activity of extracts [22]. The steps of the anti-inflammatory test in this manuscript refer to previous works [11, 23]. The first step is to prepare a stock solution at 10.85 [mg/ml] concentration with citric acid esters of the various aerial organs systems involved of the *Crinum amabile* using distilled water solvent instead of the usual dimethylsulfoxide solvent. From this stock solution were prepared the different solutions of the various aerial organs systems involved of the *Crinum amabile* using HBSS at equal volume of the nutriprusside solution prepared with HBSS of concentration 0.005N; the volumes of nutriprusside to be used to prepare these test solutions are 20ml, 40ml, 80ml and 160ml, and the volume of stock solution to be used for each test solution is 0.025ml. In addition to these test solutions, add the 0.005N nutriprusside solution above as blank1-B1 and the HBSS solution as blank2-B2. These test solutions of the various aerial organs systems involved of the Crinum amabile, as well as the blanks, are placed in vials and then exposed to air under two 100W incandescent lamps for two hours. Once the two hours have elapsed, switch off the lamps and add the Greiss reagent nitric oxide revealer solution in volumes of 5ml, 10ml, 20ml and 40ml to the 20ml, 40ml, 80ml and 160ml test solutions respectively. Let them incubate in the dark for a maximum of 10 min, wrapped in aluminum foil while the spectrophotometer is being prepared, then measure the absorbances of the different solutions for the various aerial organs systems involved of the *Crinum amabile* under a 550nm UV-visible spectrophotometer to quantify the nitric oxide molecules transformed into nitrite/nitrate that are not blocked by the anti-inflammatory molecules in the test solutions. From the spectrometric measurements, the value of the percentage decrease in nitrite in relation to the initial nitrite-nitrate evaluated-E1 is deduced such as

$$D_1 = \frac{E1 - N_i - B1 - B2}{E1} \times 100$$

Knowing that E1 is evaluated from the trend curve of nitrite-nitrate evolution detected as a function of anti-inflammatory molecule concentrations [moles/l] plotted from the last three points and for a value of anti-inflammatory molecule concentration as close to zero as possible of the order of 1E-11 depending on the case. After plotting the evolution of the percentage decrease in nitrite-nitrate detected as a function of anti-inflammatory molecule concentrations [moles/l], it is possible to deduce and evaluate the IC50 of the sample tested, using the equation of the evolution of the curve in the vicinity of 50%.

7.2. Experimental Conditions and Anti-Inflammatory Test Results

7.2.1. Anti-Inflammatory Test of the *Crinum Amabile*'s Organ System "Anther-Stamen Fillet-Pistil-Perianth"

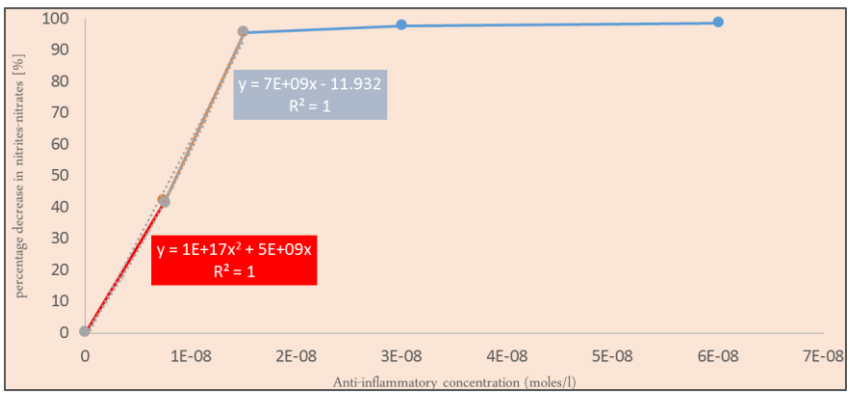

Figure 15. *Percentage diminution in nitrates-nitrites according to the concentration of the anti-inflammatory molecules of the Crinum ambile's organ system "Anther-Stamen fillet-Pistil-Perianth".*

Table 18. *Experimental conditions and results of the Crinum amabile's organ system "Anther-Stamen fillet-Pistil-Perianth" anti-inflammatory test.*

Stock solution 10.85[mg/l] volume [ml]	0.025	0.025	0.025	0.025	Evaluated Nitrates Detected For A A solution with None Concentration in Molecules Anti-Inflammatory (E1)	Blank-(B1) Nitroprusside	Blank-(B2) HBSS
HBSS volume [ml]	40	20	10	5		-	4
Nitroprusside solution volume [ml]	40	20	10	5		4	-
Nitroprusside quantities [moles]	0.0002	0.0001	0.00005	0.000025		-	-
Nitroprusside concentrations [moles/l]	0.0025	0.0025	0.0025	0.0025		-	-
Anti-inflammatoiry molecules concentrations [moles/l]	7.53E-09	1.51E-08	3.01E-08	6.01E-08		-	-
(Ni) - Nitrites-nitrates detected [mg/ml]	9.88E-02	0.0076	0.0037	0.0025	0.1697	0.0083	0.0001
(Di) - percentage decrease in nitrites-nitrates compared to initial Nitrites-nitrates evaluated-E1 [%]	4.18E+01	95.52	97.82	98.53	-	-	-

Table 19. *Determination of the Crinum amabike's organs system "Anther-Stamen fillet-Pistil-Perianth" IC50.*

		IC50 average Of the system "Anther-Stamen fillet-Pistil-Perianth"	**Standard-deviation**
IC50 straight line through origin	8.84743E-09		
IC50 straight line	8.84743E-09	8.75E-09	1.75185E-10
IC50 polynomial through origine	8.544E-09		

7.2.2. Anti-Inflammatory Test of the *Crinum amabile's* Organ Petal

Table 20. *Experimental conditions and results of the Crinum amabile's organ petal anti-inflammatory test.*

Stock solution 10.85 [mg/l] volume [ml]	0.025	0.025	0.025	0.025	Evaluated Nitrates Detected For A A solution with None Concentration in Molecules Anti-Inflammatory (E1)	Blank-(B1) Nitroprusside	Blank-(B2) HBSS
HBSS volume [ml]	40	20	10	5		-	4
Nitroprusside solution volume [ml]	40	20	10	5		4	-
Nitroprusside quantities [moles]	0.0002	0.0001	0.00005	0.000025		-	-
Nitroprusside concentrations [moles/l]	0.0025	0.0025	0.0025	0.0025		-	-
Anti-inflammatoiry molecules concentrations [moles/l]	7.53E-09	1.51E-08	3.01E-08	6.01E-08		-	-
(Ni) - Nitrites-nitrates detected [mg/ml]	2.05E-02	0.0076	0.0079	0.0001	0.04699	0.0083	0.0001
(Di) - percentage decrease in nitrites-nitrates compared to initial Nitrites-nitrates evaluated-E1 [%]	5.64E+01	83.83	83.19	99.79	-	-	-

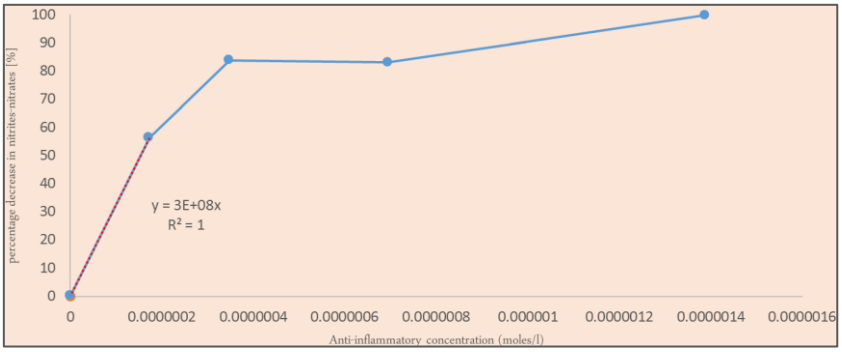

Figure 16. *Percentage diminution in nitrates-nitrites according to the concentration of the anti-inflammatory molecules of the Crinum amabile's organ petal.*

Table 21. *Determination of the Crinum amabike's organ petal.*

IC50 straight line through origin	1.667E-07

7.2.3. Anti-Inflammatory Test of the *Crinum Amabile'S* Organs System "Stem-Receptacle-Leaf"

Table 22. *Experimental conditions and results of the Crinum amabile's organ system "Stem-receptacle-Leaf" anti-inflammatory test.*

Stock solution 10.85[mg/l] volume [ml]	0.025	0.025	0.025	0.025		Blank-(B1) Nitroprusside	Blank-(B2) HBSS
HBSS volume [ml]	40	20	10	5	Evaluated Nitrates Detected For A A solution with None Concentration in Molecules Anti-Inflammatory (E1)	-	4
Nitroprusside solution volume [ml]	40	20	10	5		4	-
Nitroprusside quantities [moles]	0.0002	0.0001	0.00005	0.000025		-	-
Nitroprusside concentrations [moles/l]	0.0025	0.0025	0.0025	0.0025		-	-
Anti-inflammatoiry molecules concentrations [moles/l]	7.53E-09	1.51E-08	3.01E-08	6.01E-08		-	-
(Ni) - Nitrites-nitrates detected [mg/ml]	1.57E-02	0.0112	0.0033	0.0001	0.06603	0.0083	0.0001
(Di) - percentage decrease in nitrites-nitrates compared to initial Nitrites-nitrates evaluated-E1 [%]	7.63E+01	83.04	95.00	99.85	-	-	-

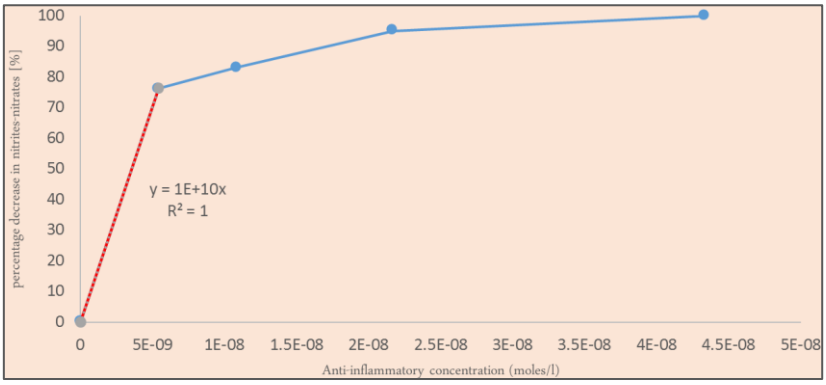

Figure 17. *Percentage diminution in nitrates-nitrites according to the concentration of the anti-inflammatory molecules of the Crinum amabile's organ system "Stem-receptacle-Leaf".*

Table 23. *Determination of the Crinum amabike's organs system "Stem-receptacle-Leaf" IC50.*

IC50 straight line through origin	5,00E-09

Anti-inflammatory test results show that it's always the *Crinum amabile*'s organ system «Anther-Perianth-Stamen fillet-Pistil» has the greatest anti-inflammatory activity (IC50-1.75185E-10) followed by the organ system "Stem-Receptacle-Leaf" (IC50-5,00E-09) and finally the organ petal (IC50-1,6667E-07). These results are entirely normal, given the differences in the nature of the structures and porosities, as well as the rigidities, of the different organ systems of the *Crinum amabile* discussed in the paragraph §4.1. of this manuscript. In fact, in the "Stem-Receptacle" system, in the "Stamen fillet-pistil" system and in the "Leaf" system, the initial conversions are all almost very high, which means that these organ systems of the *Crinum amabile* have rigid structures and porosities, and therefore surfaces favorable not only to esterification reactions with citric acid, (see in the paragraph §4.1. of this manuscript) [12] but also to anti-inflammatory and antioxidant activities. This is not the case with the petal, whose less rigid structures and porosities are easily attacked by the acidity of citric acid molecules.

To further investigate the effect of the nature of the structures and porosities of the various organ systems on their bioactive activities, the alkene concentrations in the stock solutions used in the anti-inflammatory tests on the various organ systems of the *Crinum amabile* were determined by hydrofluoric acid-HF with normality 0.0026N titration, an alkene assay procedure described in the bibliography [24]. Thus, 1ml of each anti-inflammatory test stock solution was taken and diluted in 30mL of distilled water in a 250ml beaker, then 4 to 5 drops of bromothymol blue color indicator were added, turning the solution color to be titrated to blue. Place the HF-0.0026N titrant solution in a burette and start dosing. When the solution turns green-yellow, the volume of HF-titrant corresponds to the alkenes on the accessible surfaces of the organ system structures and porosities of the titrated stock solution. Continuing the titration, when the solution turns completely to yellow, the difference between this final volume and the volume of the first turn to green-yellow corresponds to the alkenes in the rigid, difficult-to-access pore systems of the organ system of the titrated stock solution.

These alkenes play very important support-carriers roles [12, 25] for citric acid molecules and its derivatives with anti-inflammatory and antioxidant molecules. (Rabeharitsara, Ravomialisoa, & Randriana, Synthesis of Capsicum chinense Citric Acid Esters-Its Methanol Trans-esterification Investigations with hplc Analysis and Its valorization as Gels-Crystals Ca-Salts, 2021) [8, 13]. These alkene carriers enable better dispersion of the active anti-inflammatory and antioxidant molecules, thus enhancing their respective bioactive activities in the stock solution to be tested.

The results of the alkene concentration determinations for the various organ systems of the *Crinum amabile* are shown in the following table 24:

Table 24. *Alkene concentrations of different organ systems of the Crinum amabile.*

Alkene concentrations for different organ systems of the *Crinum amabile*	**Petal**	**Stem-Receptacle-Leaf**	**Anther-Pistil-Stamen fillet-Perianth**
Accessible surface alkenes [mol/L]	4,42E-03	5,46E-03	4,42E-03
alkenes in rigid structure & porosity mol/L]	2,34E-03	1,30E-03	4,68E-03

The results in Table 24 clearly show that the petal organ alone has a fairly large quantity of alkenes on the accessible surface compared with the other organs grouped into systems, confirming its less rigid structure and fairly open pore system compared with the other organs. On the other hand, for the other organ systems, alkenes in accessible surfaces are high for the stem-receptacle-leaf system, with a very low quantity of alkenes in rigid structure and porosity, certainly due to the rather large size of their pore system. The organ system of the *Crinum amabile* « Anther-Pistil-Stamen fillet-Perianthe » has the same quantity of alkenes on the accessible surface as the petal, with the highest quantity of alkenes in the rigid structure and porosities, thus demonstrating the nature of the porosities of the system's organs, not only large pores but also small pores, micropores and even nanopores, certainly in the anther, stamen fillet and pistil organs. Since the different quantities and qualities of alkenes in the various organ systems of *Crinum amabile* serve as carriers for the bioactive antioxidant and anti-inflammatory citric acid derivatives in their mother solutions, it's not surprising that the anti-inflammatory and even antioxidant activities of the organ system" Anther-Perianthe-Stamen fillet-Pistil" of the *Crinum amabile* (cf. Tables 17, 19, 21 and 23) are very strong, followed by

its “Stem-Receptacle-Leaf” and finally for its “petal”.

8. Conclusion

The *Crinum amabile* is a fairly representative and complete plant species for identifying the various aerial organs of plants. Gas chromatographic analysis of soxhlet extracts of *Crinum amabile's* various associated and non-associated organs using the solvent hexane showed that they contain not only Saturated Fatty Acids (SFA), but above all Unsaturated Fatty Acids (UFA) and Polyunsaturated Fatty Acids (PUFA) with antioxidant activities, anti-inflammatory activities, medicinal virtues and chemical properties, but in different concentrations, including myristic acid, palmitic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, linoleic acid and linolenic acid It has been observed that the highest concentration of total fatty acids is found in the Stamen fillet – 397.416 [mg/g] followed by the anther – 208.642 [mg/g] and the stem – 174.17 [mg/g] but the concentration of polyunsaturated fatty acids (PUFAs) is highest in the anther. The results confirm that these primary metabolites are synthesized in the leaves and possibly in the petals, whose total fatty acid concentrations are respectively 47.911 [mg/g] et 147.525 [mg/g]; and then distributed to the various aerial organs of *Crinum amabile*, with the receptacle serving as a passage organ. The results of the HPLC analyses confirmed the findings and comments made on the previous fatty acid results. The total concentration of flavonoids and steroids remains highest in the aerial organ system «anther-Stamen fillet-pistil-perianth »followed by the aerial organ system « Stem-Leaf-receptacle ». However, it is only in the petal that the flavonoids discovered are found in their entirety, which explains the color and pleasant fragrance characteristic of the *Crinum amabile* plant. Flavonoids and steroids discovered on the various aerial organs of the *Crinum amabile* are respectively eriocitrin, hesperidin, le neohesperidin, isorhoifolin and rhoifolin; then betulinic acid, ursolic acid, betulin and lupeol. Quantification of alkaloids in the various organs and organ systems of the *Crinum amabile* confirmed the alkaloid richness of this plant as stated in the various bibliographies. This level of alkaloids, which are frequently synthesized in roots, is found in very high proportions in anther - 55.56% and the lowest proportion is found at the level of the organ system «receptacle-stem »- 0.32%. These results further confirm the remarks made in the publication and litteratures concerning the distribution mechanism of metabolites in plants in general and especially yet in the *Crinum amabile*. In addition to all this, kinetic studies were carried out during the various esterifications with citric acid of different aerial organs, associated or not. The results of these kinetic studies were used to determine the kinetic constants of these different esterification reactions, and a relationship was found between these kinetic constants and the nature of the rigidity of the structures of the aerial organs of the esterified *Crinum amabile* and their porosities in relation to the acidity-acid attack of the citric acid molecules. The higher the speed constant and overall speed, the more rigid the structure and porosity with microporous and probably nanoporous of the organ-components, it’s the case for the organ system « stem-receptacle » and the organs leaf, anther. This is not the case for other organ systems, where the initial speed is high, but this speed rapidly decreases overall due to the fragility of their structures and porosities in relation to the acidity-acid attack of citric acid molecules during esterification reactions. It was also confirmed that alkenes act as support-carriers for bioactive molecules in the form of citric acid derivatives, enabling them to be dispersed effectively, thereby enhancing their anti-inflammatory and antioxidant activities. A positive correlation was found between the quantities of surface alkenes accessible, the alkenes in rigid structures and porosities, the nature and quality of the structures and porosities and their effects on the various reactions and bioactive activities of the reactive molecules, notably esterification reactions with citric acid, and the antioxidant and anti-inflammatory activities of the molecules in the citric acid ester solution.

Abbreviations

SPC	Scientific Process Control
SFA	Saturated Fatty Acid
UFA	Unsaturated Fatty Acid
HPLC	High Performance Liquid Chromatography
PUFA	Poly Unsaturated Fatty Acid
PNA	Poly Nuclear Aromatics
Dpph	2,2-diphenyl-1-picrylhydrazyl

Acknowledgments

Sincere thanks go to the Director of the Ecole Supérieure Polytechnique Antananarivo ESPA, and to the staff of the ESPA Chemical and Industrial Process Engineering laboratory of the Antananarivo University, Madagascar.

Author Contributions

Rabeharitsara Andry Tahina: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Rabezanahary Nilaina Finaritra Marie Angela: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft

Conflicts of Interest

The authors declare no conflicts of interest.

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