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# Determination of the Fatty Acids in the *Epiphyllum oxypetalum's* Flower and Phylloclade Followed by the Study of Their Esterification with Citric Acid

**Andry Tahina Rabeharitsara*, Ny Idealy Elite Randriamanantena, Baholy Robijaona Rahelivololoniaina, Rakotomamonjy Pierre, Hanitriniaina Marie Ratsimba**

Chemical Process Engineering Department (E. S. P. A), Antananarivo University, Antananarivo, Madagascar

## Email address:

rabeharitsara_andrytahina@yahoo.fr (Andry Tahina Rabeharitsara), nyidealyrandria@gmail.com (Ny Idealy Elite Randriamalala), robijob111@gmail.com (Baholy Robijaona Rahelivololoniaina), kotomamonjypr@yahoo.fr (Rakotomamonjy Pierre), ratsimbamarie@yahoo.fr (Hanitriniaina Marie Ratsimba)

*Corresponding author

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**Abstract:** The fatty acids played important roles in the organism and process. Some saturated fatty acids composed and important for the functioning of many cell membranes. There was also saturated fatty acids which are antioxidant, antidiabetic, anti-carcinogen, anti-tumor and could protect the cardiovascular system by favoring the decrease of cholesterol rate. Some saturated fatty acids were used in process as emollient, flow agent, emulsifier, additives-surfactant, viscosity builder, co-emulsifier and internal/external lubricant in plastics. In addition, unsaturated fatty acids are in general antioxidant and anti-inflammatory. In consequence, the singularity of this manuscript aim is to identify and to quantify the fatty acids not only in the leaves-phylloclade of the *Epiphyllum oxypetalum* but also in its flower without pistil and etamines and in its pistil and etamines. Soxhlet assembly with hexane as solvent was used to extract the active fatty acids molecules in these different parts of the plant *Epiphyllum oxypetalum.* Chromatography phase gas analysis results gave the distribution of the fatty acids at their different parts-stems and affirmed the presence of lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids. It was noticed that the arachidic acid was seen only in the flower, stamens and pistils and the gadoleic acid is seen only in the stamens and pistils and allowed to consider their capacities to improve heart health, to reduce inflammation as anti-inflammatory, to improve cognitive function, to be anticarcinogenic and to be used as detergent additive. The exploitation of these chromatography phase gas analysis results permitted to give the weight concentration of the different fatty acids (mg/g) in relation to the grinded sample and in relation to the initial fresh sample for the *Epiphyllum oxypetalum*'s flower with stamens and pistils and for the *Epiphyllum oxypetalum*'s phylloclade-leaves. Then, the esterification of the *Epiphyllum oxypetalum*'s different parts with citric acid were carried out on a reflux assembly and followed with time reaction in order not only to extract their active molecules like flavonoids, terpénoïdes, alkaloids and fatty acids but also to determine each plant part's kinetics parameters seeing that their quantities were in excess in comparison with the citric acid quantities. The results showed that the initial kinetic constant k for the phylloclade and stamens/pistil respectively equals to 165,000 $[L^2 \times mol^{-2} \times mn^{-1}]$ and 173,844 $[L^2 \times mol^{-2} \times mn^{-1}]$ were very important than for the flower without stamens and pistil equals to 39,469 $[L^2 \times mol^{-2} \times mn^{-1}]$ which were certainly due to their porosities and micro-canals.

**Keywords:** *Epiphyllum oxypetalum*, Pistil, Stamen, Phylloclade, Fatty Acids, Esterification, Citric Acid, Kinetics Constants

## 1. Introduction

The singularity of this manuscript is to identify and to quantify the *Epiphyllum oxypetalum*'s fatty acids in the flower with pistils and stamens and to extract their active molecules by esterification with citric acid molecules. Thus, the first part of this manuscript treated the citric acid generalities and characteristics followed by the fatty acids and *Epiphyllum oxypetalum* generalities, roles and vertues. The fatty acids of the *Epiphyllum oxypetalum*'s different parts were extracted by soxhlet assembly using hexane as solvent. Then, they were identified and quantified by chromatography phase gas analysis in order to determine their weight concentrations in each *Epiphyllum oxypetalum*'s different parts. The second part is about the kinetic study of the *Epiphyllum oxypetalum*'s different parts esterification with citric acid molecules; these reactions were done in a reflux assembly composed with a balloon-250ml, a balloon heater and a condenser. In general, the materials and chemicals used during these different experimentations were KERN precision scale, chronometer, beaker-250ml, balloon 250ml, heating balloon mantle–250ml, condenser, magnetic stirrer, magnetic bar, soxhlet assembly, graduated burette, separating funnel, filter paper, glass funnel, pipette, mortar, citric acid, *Epiphyllum oxypetalum* phylloclade, *Epiphyllum oxypetalum* flower without pistil and etamine, *Epiphyllum oxypetalum* pistil and etamine, distillated water, hexane, NaOH-0.05N, NaOH-0.005N, helianthine.

## 2. Generalities of the Citric Acid and the Fatty Acids

### *2.1. The Citric Acid [1]*

Citric acid $C_6H_8O_7$ is a tricarboxylic acid α-hydrolyzed. It contains three acids with pKa such as $pKa_1 = 3.14$, $pKa_2 = 4.77$ and $pKa_3 = 6.39$ and a α-alcohol function with pKa = 14.4 [1-5]. By its reactivity, the citric acid was the object of several studies and was used in several fields like the cosmetics, the food one, the chemistry, the polymers and others [6-10], the leaving creatures' molecules extraction by esterification with citric acid. Noticed that the acid form is AH with pKa (AH). It was shown that if the pH ≤ [pKa (AH) – 2], the quantity of basic $A^-$ associated to the acid/base couple $AH/A^-$ is negligible in comparison with the AH quantity. And if the pH ≥ [pKa (AH) + 2], the quantity of acid AH associated to the acid/base couple $AH/A^-$ is negligible in comparison with the $A^-$ quantity [6]. For [pKa (AH) – 2] ≤ pH ≤ [pKa (AH) + 2], the basic $A^-$ and the acid AH forms coexist but if [pKa (AH) – 2] ≤ pH ≤ pKa (AH) the acid form AH dominates and if pKa (AH) ≤ pH ≤ [pKa (AH) + 2] the basic form $A^-$ dominates. Consequently, for the citric acid, the acids and basics forms according to the pKa and pH were showed in the following Table 1:

***Table 1.*** *Dominant Forms of "Citric Acid" According to the pH [1].*

| pH | Acid/base couple | pKa | Acid/Base reactions | Dominant forms | Dominant molecule/Ions |
|---|---|---|---|---|---|
| pH ≤ 3.14 | $AH_3/AH_2^-$ | 3.14 | $AH_3 \rightleftharpoons AH_2^- + H^+$ | $AH_3$ | Citric Acid |
| 3.14 ≤ pH ≤ 4.77 | $AH_2^-/AH^{2-}$ | 4.77 | $AH_2^- \rightleftharpoons AH^{2-} + H^+$ | $AH_2^-$ | Di-Hydrogenocitrate |
| 4.77 ≤ pH ≤ 6.39 | $AH^{2-}/A^{3-}$ | 6.39 | $AH^{2-} \rightleftharpoons A^{3-} + H^+$ | $AH^{2-}$ | Mono-Hydrogenocitrate |
| 6.39 ≤ pH | $AH^{2-}/A^{3-}$ | 6.39 | $AH^{2-} \rightleftharpoons A^{3-} + H^+$ | $A^{3-}$ | Citrate |

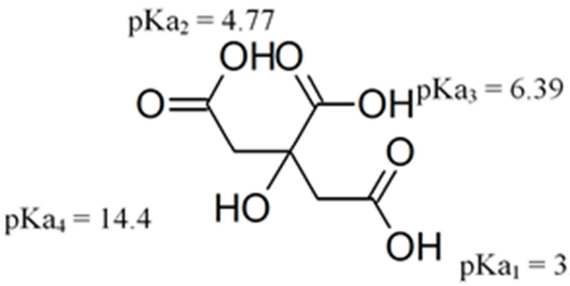


***Figure 1.*** *3-hydroxybutane-1, 2, 4-tricarboxylic acid (Citric Acid) [1].*

Fischer-Speier esterification also called carboxylic acids esterification is generally a liquid phase chemical reaction between an alcohol function and a carboxylic acid function which could be catalyzed by $H^+$ ions from acids functions on solution (Figure 2). This reaction is accompanied with water molecules formation according to the general reaction (Figure 3).

$$AH \rightleftharpoons A^- + H^+$$

***Figure 2.*** *$H^+$ ions catalysts from acids functions on solution.*

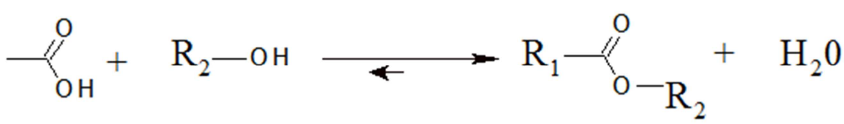


***Figure 3.*** *Esterification reaction general equation.*

Thus, tricarboxylic acids of citric acid could be esterified with three alcohol functions of species' organic molecules (Figure 4) and also species' organic molecules carboxylic acids could be esterified by the alcohol function of citric acid (Figure 5) if the solution pH is respected according to the general equations:

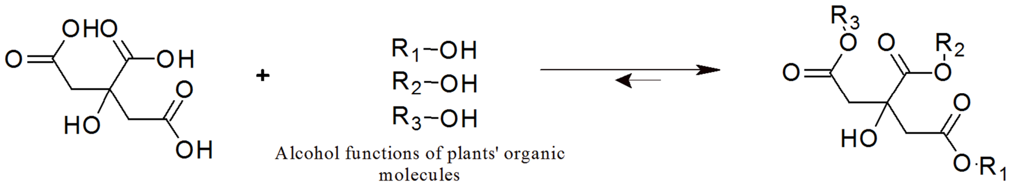


***Figure 4.*** *Tricarboxylic acids of citric acid esterification with three alcohol function of species' organic molecules.*

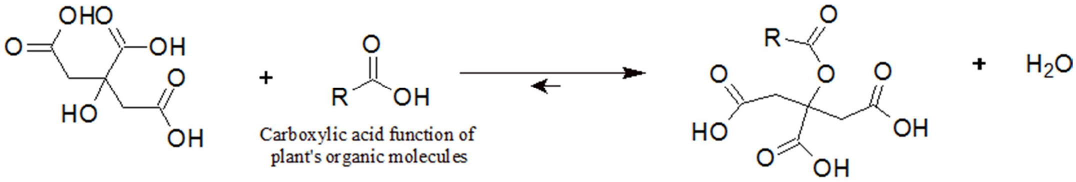


***Figure 5.*** *Acid of species' organic molecules esterification with the citric acid alcohol function.*

Also, tricarboxylic acids of citric acid could be in reaction with three amino-acids or amines functions of organic molecules if the solution pH is respected to obtain amide molecules according to the general equations (Figure 6 – Figure 7):

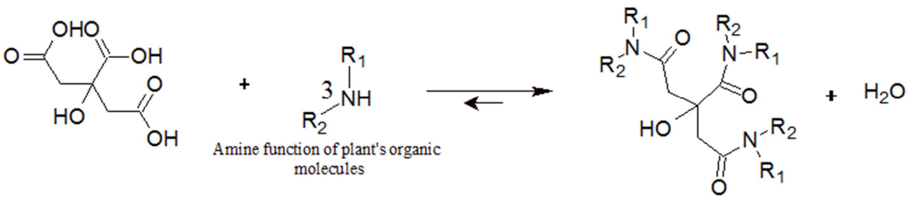


***Figure 6.*** *Amide formation by amine function and tricarboxylic acids of citric acid molecule reaction.*

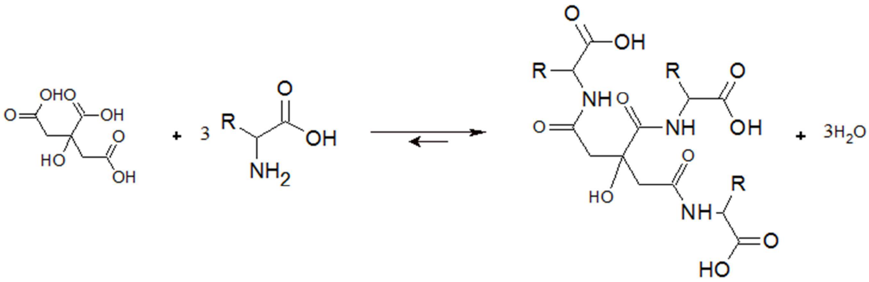

***Figure 7.*** *Amide formation by amino-acid and tricarboxylic acids of citric acid molecules.*

### *2.2. The Fatty Acids*

They are aliphatic carboxylic acids of general formula: $CH_3$-$(CH_2)_n$-COOH [10]. They are monoacids, linear, with an even number of carbon, either saturated or unsaturated. They differ from each other by the number of carbon atoms in the chain, generally between 4 to 30, by the number of unsaturations (double bonds) and their positions [12, 13].

#### *2.2.1. The Different Types of Fatty Acids*

***(i). Saturated Fatty Acids (SFA)***

The general formula of SFA is R-COOH such as R- is an alkyl composed with saturated aliphatic chain. They could be classified into four subclasses according to their chain length: short, medium, long and very long. There are several definitions in the literature for these subclasses of SFAs. The expert consultation recognizes that universal definitions are needed and recommends the following classifications [14]:

1. Short-chain fatty acids which aliphatic chains are composed with three (3) to seven (7) carbon atoms.
2. Medium-chain fatty acids which aliphatic chains are composed with eight (8) to thirteen (13) carbon atoms.
3. Long-chain fatty acids which aliphatic chains are composed with fourteen (14) to twenty (20) carbon atoms.
4. Very long chain fatty acids which aliphatic chains are composed with more than twenty (20) carbon atoms.

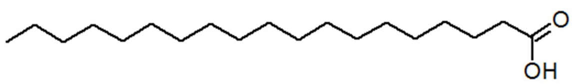

***Figure 8.*** *Saturated fatty acid $C_{19}H_{38}O_2$.*

***(ii). Unsaturated Fatty Acids (UFA)***

Many fatty acids contain one or more double bonds; they are said to be unsaturated. At the level of a double bond, two hydrogen atoms are missing on the same side of the molecule, the space thus freed creates a point of weakness in the chain which leads to an angulation [15].

We have two-2 types of unsaturated fatty acids:

Monounsaturated fatty acids which have a single double bond with the following general formula: $CH_3$-$(CH_2)_n$-CH=CH- $(CH_2)_{n'}$-COOH. They could be also noted like $C_N$:1(n-$X_\alpha$) or (ω-$X_\omega$) such as N is the number of carbon atom which composed the monounsaturated fatty acids; 1 indicated that it's monounsaturated and $X_\alpha$ indicated the position of the first carbon monounsaturated from the carboxylic acid (chemistry nomenclature) and $X_\omega$ indicated the position of the first carbon monounsaturated from the terminal $-CH_3$ ethyl group (biochemistry nomenclature). For example, the notation of the oleic acid which is a monounsaturated fatty acid is C18:1(n-9)/(ω-9).

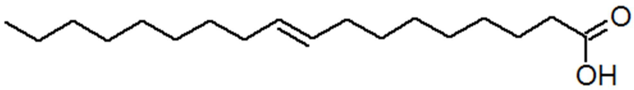


***Figure 9.*** *Oleic acid monounsaturated fatty acid* $C_{19}H_{34}O_2$ *- C18:1(n-9)/(ω-9).*

Polyunsaturated fatty acids which have more than one single bond. There are two families of essential polyunsaturated fatty acids; the first was named n-3 (or omega-3) which first unsaturation began on the third carbon atom from the terminal $-CH_3$ ethyl group and then every three carbon atom. For example, the eicosapentanoïc acid C20:5 (ω-3 omega-3) is a polyunsaturated fatty acid composed with 20 carbon atoms, 5 unsaturations every three carbon atoms.

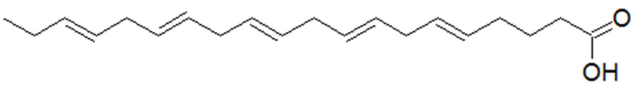


***Figure 10.*** *Omega-3 polyunsaturated fatty acid –* $C_{20}H_{30}O_2$*.*

The second families of the essential polyunsaturated fatty acids was n-6 (or omega-6) which first unsaturation began on the sixth carbon atom from the terminal $-CH_3$ ethyl group and then every three carbon atom. For example, the arachidonic acid.

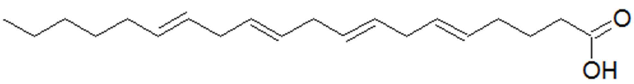


***Figure 11.*** *Omega-6 polyunsaturated fatty acid -* $C_{20}H_{32}O_2$*.*

Two fatty acids are at the origin of these families. These are α-linolenic acid, the precursor of omega-3, and linoleic acid which is the precursor of the omega-6 family.

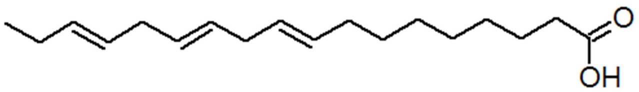


***Figure 12.*** *α-linolenic acid polyunsaturated fatty acid -* $C_{18}H_{30}O_2$*.*

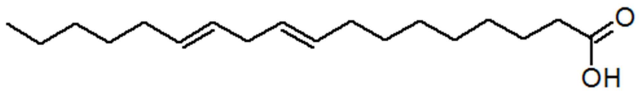


***Figure 13.*** *Linoleic acid polyunsaturated fatty acid -* $C_{18}H_{32}O_2$*.*

### *2.2.2. The Fatty Acids Properties*

#### *(i). Physical Characteristics of Fatty Acids*

The melting point of the fatty acids increases with its carbons number but it decreases when the unsaturation quantities increase. The fatty acids have a low density compared to water. Fatty acids are soluble in hexane and generally insoluble in water but the fatty acids with 2, 3, 4 and 6 carbons are slightly soluble in the water. The sodium and potassium salts of fatty acids are soap which were soluble in water [16]. Fatty acids absorb and attribute an electronic excitation or transitions with the ultraviolet (UV) like over organic compounds with high electronic density such as carbonyl groups, nitro groups, double and triple bounds, conjugated double bonds, …. The energy involved in electronic transitions correspond to the adsorption of photon in the visible (400nm – 750nm) and ultraviolet (200nm – 400nm) [17].

#### *(ii). Chemical Properties of Fatty Acids*

Fatty acids could react with sodium hydroxide or potash to give soap (figure 14).

$$R\text{-}COOH + NaOH \leftrightarrow R\text{-}COONa + H_2O$$

***Figure 14.*** *Reaction between fatty acids and sodiumhydroxide.*

Fatty acids' carboxylic acid could be esterified by an alcohol to give an ester and a water (figure 15).

$$R\text{-}COOH + R'\text{-}OH \leftrightarrow R\text{-}COO\text{-}R' + H_2O$$

***Figure 15.*** *Fatty acids esterification with an alcohol.*

Unsaturated fatty acids could be oxidized by a peracid, with great oxidizing power, even at low temperature to give an epoxide. At 50°C and under the mineral salt action, fatty acids give glycol. The air oxygen could also oxidize fatty acids' alkene into butyric acid, peroxides R-O-O-R and free radicals $R\text{-}O^{\cdot}$, $R\text{-}O\text{-}O^{\cdot}$ oxidizing agents responsible of the oil rancidity and highly toxic derivatives. Some procedures have been used to prevent and to eluate these radicals' formation [18-21] such as using the black citric acid polymers (PN) as molecular filter able to retain these previous free radicals and their derivatives in oils and decrease certainly their rancidity [22]. The cis-trans isomerization of natural unsaturated fatty acids is possible by chemical way. It is very slow at room temperature and faster when hot and eventually using a heterogeneous catalyst. It is one of the factors of rancidity of fats. In the same way, unsaturated fatty acids could be saturated (catalyst) by addition with dihydrogen using heterogeneous catalyst like platinum, palladium and nickel.

### *2.2.3. The Fatty Acids Roles in the Organism*

#### *(i). The Saturated Fatty Acids Effects on the Organism*

Saturated fatty acids composed many cell membranes. They are therefore important for the proper functioning of cells [23]. However, they tend to favour cholesterol deposits in the arteries and increase the risk of cardiovascular disease [24].

The myristic acid is a relatively rare fatty acid, it regulates the membrane proteins activities by acylation and it's also a hypercholesterolemia like the palmitic acid. The stearic acid has a nourish and protections properties for the skin because of its film gene effects, antiseptic and emollient. The stearic acid is particular than the over saturated fatty acids because it's also able to protect the cardiovascular system by favoring the decrease of the cholesterol rate. The stearic acid is also anti-carcinogen and anti-tumor. [25-28]. Lauric acid treated psoriasis which hydrate and repair skin with reducing redness and inflammation; then it is used in a lot of anti-aging skincare products. Lauric acid have also some anti-microbial properties, it calm xerosis cutis especially seen on the skin which appears to be abnormally dry and flaky [29]. Arachidic acid is a saturated fatty acid with a 20-carbon chain used for the production of detergents, photographic materials, lubricants and in the manufacture of pharmaceuticals.

***(ii). The Effects of Monounsaturated Fatty Acids on the Organism***

They are considered as protective elements against cardiovascular diseases. In fact, they are known to lower bad cholesterol (LDL cholesterol) and to increase good cholesterol (HDL cholesterol). they are also a source of energy [25]. Particularly, oleic acid have an antioxidant properties and it is the fattiest acid seen in the nature which help to prevent cancer and Alzheimer's disease and to lower cholesterol preventing atherosclerosis [30]. Among the omega-7 fatty acids, palmitoleic acid is the most interesting for our health such as it maintains skin and mucous membrane hydration so prevent dry skin, itching, wound healing, dry mouth, dry eyes, vaginal dryness. The palmitoleic acid contributes to healthy liver function by accelerating the satiety feeling and in term better weight control. It also helps to optimize blood cholesterol level by raising HDL and lowering LDL levels and finally it helps to reduce the risk of developing type-II diabetes [31]. Gadoleic acid have emollient properties, it doesn't block the skin's pore and readily absorbed by the skin.

***(iii). The Effects of Polyunsaturated Fatty Acids on the Organism***

In general, the polyunsaturated fatty acids (§2.2.1.(ii)) are involved in a large number of biological functions [25]:

1. Source of energy.
2. Fundamental constituents of phospholipids in cell membranes.
3. Precursors of molecules regulating cellular functions such as: prostaglandins and reproductive functions; thromboxanes and platelet functions.
4. Regulation of the expression of genes involved in their own transport and metabolism.

The linoleic acid is one of the three fundamental constituents of the phospholipid membrane. It modulates the enzyme, the transport, the receptor and the ionic channel activities in the inter/intra cellular signalization. The linolenic acid have an effect as an anti-inflammatory [32, 33].

#### *2.2.4. The Extraction and the Analysis of Fatty Acids*

At the laboratory scale, different methods are used to extract lipids from various matrices. These methods require the use of organic solvents such as hexane, chloroform, petroleum ether, etc.... Solid-liquid solvent extraction is the most commonly used method which consists of a weight transfer of the soluble active molecules to the used solvent. For the soxhlet method, the solvent is heated, the extraction is continuous and the solvent in contact with the sample is renewed-recycled [34]. It is also used to extract the fatty acids by esterification with citric acid followed by methanol transesterification for their analysis by gas chromatography, HPLC and/or GC-MS [35]. Indeed, the citric acid could react with other molecules of an organism mainly by esterification [10, 35, 1] and results showed that their esterification together is an efficiency method for an organism's molecules extraction [36-40].

## 3. The Epiphyllum Oxypetallum

### *3.1. Taxonomy [41]*

***Table 2.*** *Taxonomy of the Epiphyllum oxypetallum.*

| **Epiphyllum oxypetallum** | |
|---|---|
| **Kingdom** | **Plantae** |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Order | Caryophyllales |
| Family | Cactaceae |
| Sub Family | Cactodiae |
| Genus | Epiphyllum |
| Species | Oxypetallum |
| Binomial Name | Epiphyllum oxypetallum |

### *3.2. Phytochemistry and Activities of the Epiphyllum oxypetalum*

*Epiphyllum oxypetallum* contains phytochemicals such as saponins, steroids, glycosides, tannins, terpenoids and resins [42], phenolic compounds such as phenols, flavonoids, coumarins, quinones and xanthones [44, 45].

The leaves contain proteins, carbohydrates and lipids of which the fatty acids are oleic acid, nonadecanoic acid and hexadecanoic acid [46]. Terpenoids are other plant metabolites with antimicrobial, antifungal, antibacterial, and antiviral properties. The flavonoids act as an antibacterial agent. In addition to the flavonoids, tannins is one of the type of polyphenolic compounds that have antibacterial action. *Epiphyllum oxypetalum* has pharmacological activity as an anti-inflammatory [46, 47]. The preliminary phytochemical study of alcohol extract of leaves *Epiphyllum oxypetalum* plant has shown the presence of glycosides, saponins, steroids, phenols, proteins, resins, tannins and terpenoids which were responsible for its probable antioxidant and anticancer activity [45]. Secondary metabolite compounds such as phenolics, flavonoids, alkaloids, and steroids have antidiabetic activity. This plant is also reported to have a potential as a flavor, pesticide, hemolytic, and others [47].

# 4. Soxhlet Hexane Extractions of the *Epiphyllum oxypetalum*

Soxhlet hexane extractions were done over the leaves-phylloclades, over the flowers' stamen with pistil and over the outer reddish to amber tepals of the *Epiphyllum oxypetalum* was picked at Antananarivo on December 2022. These parts were dried separately before suffering the hexane soxhlet extractions in order to extract separately then to identify their fatty acids.

***Table 3.*** *Experimental conditions of the Epiphyllum oxypetallum's parts extraction.*

| Samples | Raw materials weight (g) | Water content [%] | Hexane volume (ml) | Extraction duration (min) |
|---|---|---|---|---|
| Flower without stamens and pistil | 1.2939 | 94,87 | 220 | 377 |
| Stamens and pistil | 0.3014 | | 243 | 385 |
| Leaves-phylloclades | 3.9192 | 80.01 | 230 | 373 |

## *4.1. Soxhlet Hexane Extraction of the Epiphyllum oxypetalum Procedure*

First, the raw material was weighed and wrapped in a Joseph paper and introduced into a cotton cloth which is introduced into the soxhlet. Second, the hexane is introduced into the flask and this assembly is putted into a heating mantle. Then, the heating mantle, the flask, the soxhlet and the right refrigerant is assembled and the extraction lasted 360 min at 70°C such as the hexane is incessantly evaporated and accumulated in the body of the soxhlet after condensation and transferred periodically into the flask through a siphon system. At the end of the extraction time, the flask with the hexane and the fatty acids are recovered, then the hexane is evaporated using a rotavapor to recover the fatty acids which were weighed in order to determine the extraction efficiency.

## *4.2. Experimental Results of the Epiphyllum oxypetalum Soxhlet Hexane Extraction*

The results on the table 4 showed that the fatty acid extraction efficiency was very important on the stamens and pistils which indicated the important quantities of fatty acids on this parts in comparison of the over parts. These results seem indicated that the fatty acids quantities decreased from the internal parts to the external parts of the *Epiphyllum oxypetalum* flowers. The following paragraphs showed the results of their fatty acids Chromatography Phase Gaz-analysis.

***Table 4.*** *Efficiencies of the Epiphyllum oxypetalum soxhlet hexane extraction.*

| Raw materials | Soxhlet siphon transfer numbers | Fatty acids weight (g) | Extraction efficiency (%) |
|---|---|---|---|
| Stamens and pistils | 5 | 0.0776 | 25.74 |
| Flower without stamens and pistil | 13 | 0.1827 | 14.12 |
| Leaves-phylloclades | 9 | 0.2183 | 5.57 |

## *4.3. Chromatography Phase Gas Analysis Results of the Epiphyllum oxypetalum Fatty Acids*

### *4.3.1. The Fatty Acid Contents of the Different Stems of the Epiphyllum oxypetalum*

The extracted fatty acids of the *Epiphyllum oxypetalum*'s different parts are suffered the chromatography phase gas to identify and to quantify their fatty acids which were presented in the following figure 16 to figure 18.

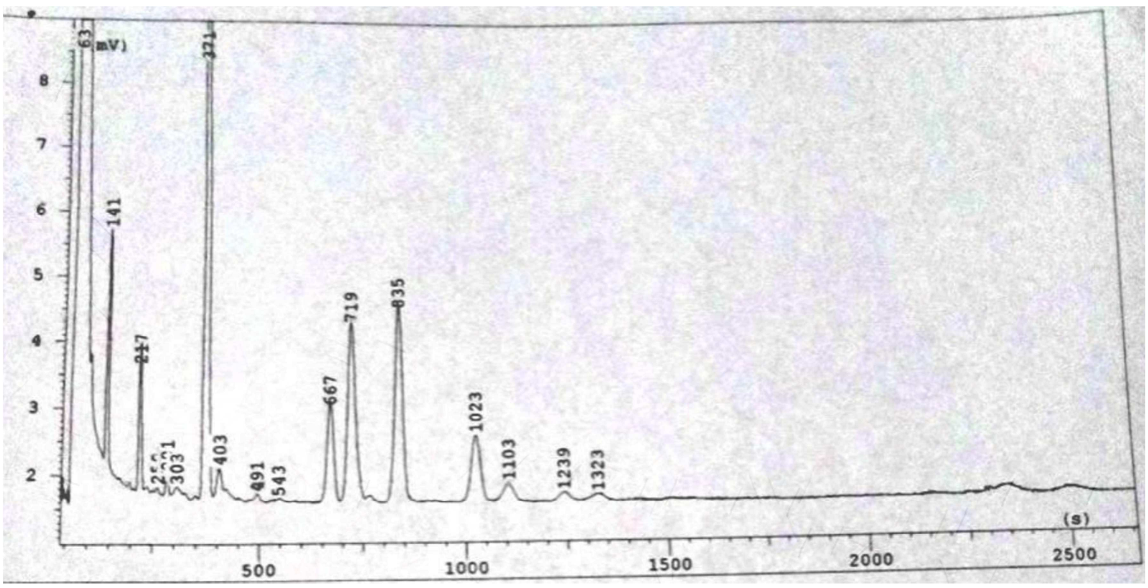


***Figure 16.*** *Chromatogram of the stamens and pistil's fatty acids.*

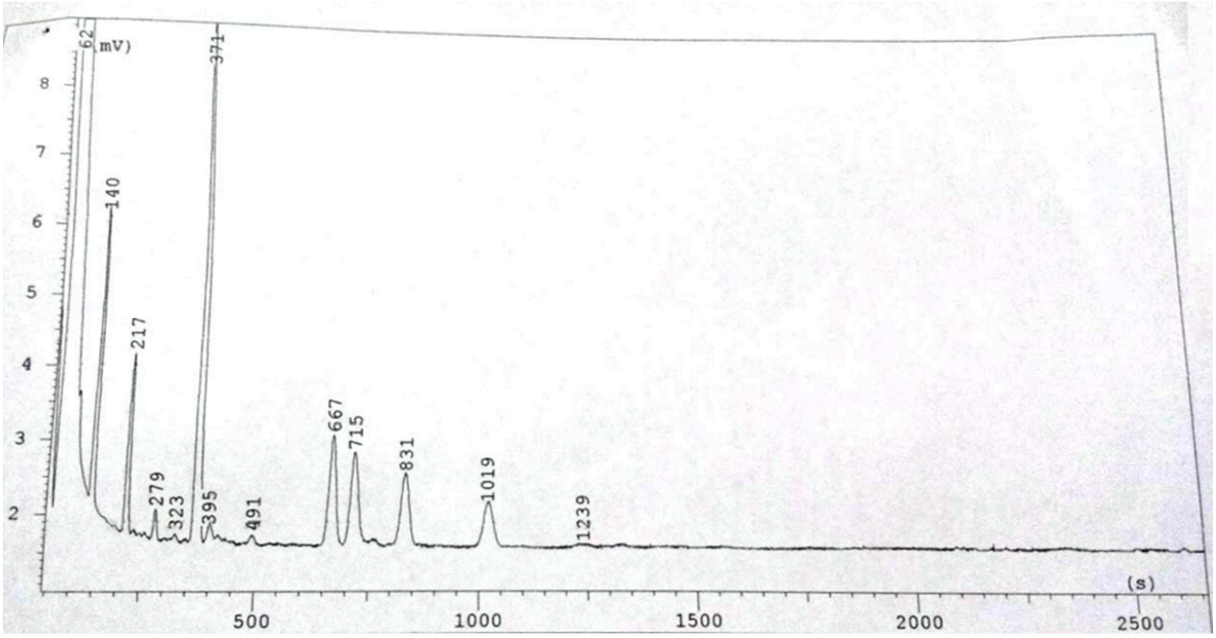


***Figure 17.*** *Chromatogram of the flower without stamens and pistil's fatty acids.*

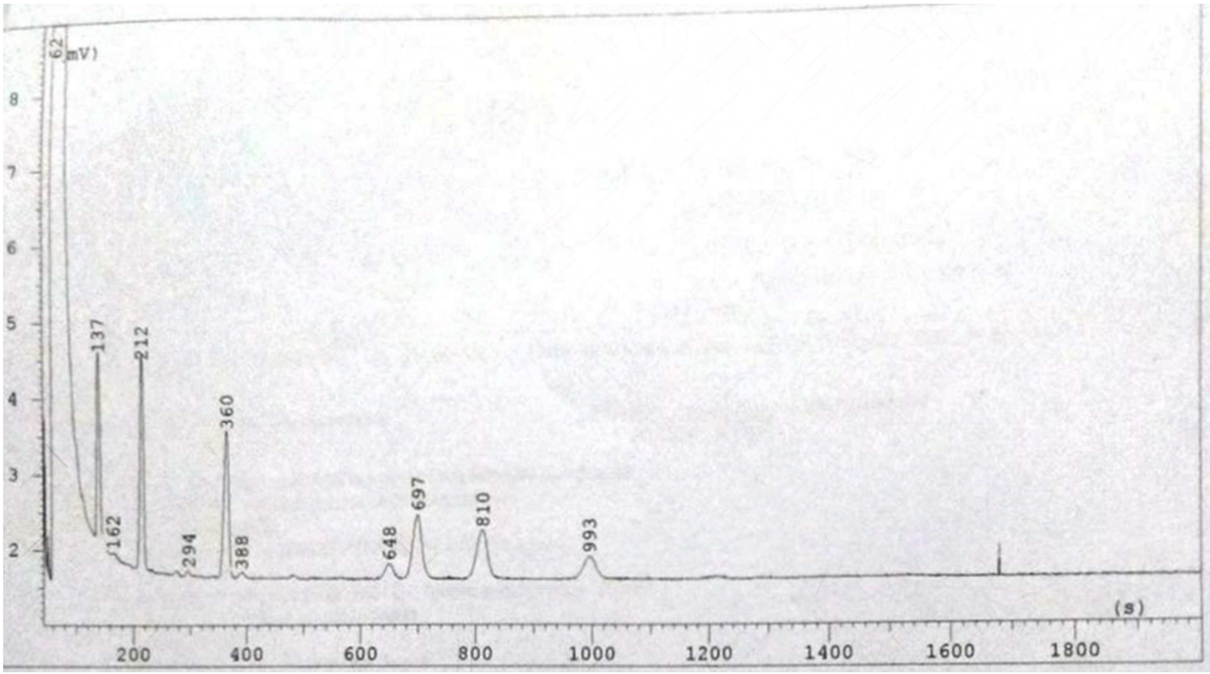


***Figure 18.*** *Chromatogram of the phylloclades' fatty acids.*

The following table 5 showed the fatty acids parts of the different stems which composed the *Epiphyllum oxypetalum* after chromatography phase gaz analysis. It could be deduced that the principal saturated fatty acids in the *Epiphyllum oxypetalum* were Lauric acid, Myristic acid, palmitic acid and stearic acid. But, it was noticed that the *Epiphyllum oxypetalum* contained non-negligible and valorous unsaturated fatty acids such as the oleic acid, the linoleic acid and the linolenic acid. It was noticed (Table 5) that the quantities of the principal saturated fatty acids were more important at the flowers without stamens and pistils (67,3438%) followed by the phylloclade-leaves (59.9413%) and the stamens and pistils (53,9274%). Then, the quantities of non-negligible and valorous unsaturated fatty acids were more important at the stamens and pistils (41.9086%) followed by the phylloclade-leaves (39.0574%) and the lowest at the flowers without stamens and pistils (29.9089%). Noticed in the same time that the arachidic acid was detected only on the flower without stamens and pistils and on the stamens and pistil. Whereas, the gadoleic acid is only on the stamens and pistils.

***Table 5.*** *Distribution of the fatty acids at the different stems.*

| R-Majority<br>B-Middle<br>S-Minority | Flower without stamens and pistils [%] | Stamens and pistils [%] | Phylloclades-leaves [%] |
|---|---|---|---|
| Lauric acid | 11.2274 | 5.2333 | 15.4227 |
| Myristic acid | 7.7420 | 4.0797 | 20.3580 |
| Palmitic Acid | 35.8207 | 37.2521 | 20.4490 |
| Palmitoleic acid | 2.0255 | 1.9926 | 1.0012 |
| Stearic Acid | 12.5537 | 7.3623 | 3.7116 |
| Oleic acid | 11.5564 | 16.8012 | 16.7880 |
| Linoleic acid | 10.7236 | 17.9653 | 14.6541 |
| Linolenic acid | 7.6289 | 7.1421 | 7.6153 |
| Arachidic acid | 0.7197 | 1.1117 | - |
| Gadoleic acid | - | 1.0593 | - |
| Colours quantities indices of major components | 2R-3B-2S | 3R-1B-2S | 2R-3B-2S |
| Colours quantities indices of *minor components* | 1R-1B | 2R-1B | 1S |

In the same time, it was detected the presence of minor components composed with interesting fatty acid molecules such as palmitoleic acid, gadoleic acid and arachidic acid sited in the majority at the stamens and pistils followed by the flower without stamens and pistil and finally very few at the phylloclades-leaves. Let's noticed also that the gadoleic acid was only detected at the stamens and pistils and the arachidic acid is detected only at the flower with stamens and pistils.

***Table 6.*** *Weight concentration of the Epiphyllum oxypetalum's flower with stamens and pistils.*

| Fatty acids | Weight concentration of the different fatty acids (mg/g) in relation to the grinded sample | Weight concentration of the different fatty acids (mg/g) in relation to the initial fresh sample |
|---|---|---|
| Lauric acid | 11.4547 | 0.5876 |
| Myristic acid | 8.4381 | 0.4328 |
| Palmitic Acid | 60.0868 | 3.0822 |
| Palmitoleic acid | 3.2673 | 0.1676 |
| Stearic Acid | 14.5381 | 0.7457 |
| Oleic acid | 24.8627 | 1.2754 |
| Linoleic acid | 25.7908 | 1.3223 |
| Linolenic acid | 11.8903 | 0.6099 |
| Arachidic acid | 1.6232 | 0.0833 |
| Gadoleic acid | 1.2131 | 0.0622 |

***Table 7.*** *Weight concentration of the Epiphyllum oxypetalum's phylloclades-leaves.*

| Fatty acids | Weight concentration of the different fatty acids (mg/g) in relation to the grinded leaf sample | Weight concentration of the different fatty acids (mg/g) in relation to the initial leaf sample |
|---|---|---|
| Lauric acid | 8.5905 | 1.7172 |
| Myristic acid | 11.3394 | 2.2668 |
| Palmitic Acid | 11.3901 | 2.2769 |
| Palmitoleic acid | 0.5577 | 0.1115 |
| Stearic Acid | 2.0673 | 0.4133 |
| Oleic acid | 9.3509 | 1.8693 |
| Linoleic acid | 8.1624 | 1.6317 |
| Linolenic acid | 4.2417 | 0.8480 |

### *4.3.2. The Weight Concentrations of the Epiphyllum oxypetalum's Fatty Acid*

From this table 5 and the experimental esterification condition was deduced the weight concentration of the different fatty acids in relation to the grinded samples; the weight concentration of the different fatty acids in relation to the initial fresh sample used during the esterification respectively for the *Epiphyllum oxypetalum*'s flower with stamens and pistils sample (Table 6) and the *Epiphyllum oxypetalum*'s phylloclades-leaves sample (Table 7).

These results on the table 6 and table 7 confirmed that were discussed on the pargraph 4.3.2 and showed that the weight concentrations of the palmitic acid and the stearic acid were more important on the *Epiphyllum oxypetalum*'s flower with stamens and pistils whereas the weight concentrations of the lauric acid and the myristic acid were more important on the *Epiphyllum oxypetalum*'s phylloclades-leaves. The weight concentrations of the unsaturated fatty acids were slightly superior on the *Epiphyllum oxypetalum*'s phylloclades-leaves but arachidic acid and gadoleic acid exist only on the *Epiphyllum oxypetalum*'s flower with stamens and pistils.

The majority of fatty acids have many virtues for the human body and they are also used as raw materials in many industrial production process. The following table 8 give an overview of the *Epiphyllum oxypetalum*'s fatty acids benefits and virtues.

***Table 8.*** *The benefits and virtues of the Epiphyllum oxypetalum's.*

| **Epiphyllum oxypetalum's fatty acids benefits** | |
|---|---|
| **Fatty acids** | **Benefits and virtues** |
| Lauric acid | Additive in Shampoo and soap process [35]<br>Antioxidant [48]<br>Antidiabetic [48]<br>Improve sperm quality [48] |
| Myristic acid | Flow agent and emulsifier [49]<br>Surfactant in soaps, detergents and textiles [49]<br>Internal or external lubricant in plastics [49]<br>Antioxidant capacity as myristic acid acylated derivative of phloridzin form [50] |
| Palmitic acid | Surfactant, viscosity builder, emollient, co-emilsifier [51] |
| Palmitoleic acid | Stronger anti-inflamatory [52] |
| Epiphyllum oxypetalum's fatty acids benefits | |
| Stearic acid | Protect cardiovascular system [53-54]<br>Improve insulin sensibility [53-55]<br>Inhibit the proliferation of tumor's and cancer's cells [53-56] |
| Oleic acid | Anti-inflammatory [57]<br>Antioxidant [58]<br>Hypocholesterolemia and anti-atherogenic [35] |
| Linoleic acid | Anti-inflammatory [59]<br>Antioxidant [59]<br>Anticarcinogenic [60] |
| Linolenic acid | Anti-inflammatory [61]<br>Antioxidant [62] |
| Arachidic acid | Additive in detergents, photographic materials and lubricants [63] |
| Gadoleic acid | Improved heart health [64]<br>Reduced inflammation – Anti-inflammatory [64]<br>Improved cognitive function [64]<br>Anticarcinogenic [65] |

# 5. The Different Parts of the *Epiphyllum Oxypetallum* Esterifications with Citric Acid

## *5.1. Experimental Conditions of the Epiphyllum oxypetallum Esterifications with Citric Acid*

In order to extract and then to identify and to quantify the different molecules which composed the different parts of the *Epiphyllum oxypetallum*, their esterification with citric acid, a method well described on the bibliographies, were carried out using a reflux assembly [36-40]. In brief, the operating procedure of the extraction using esterification with citric acid was first the parts of the plant and the citric acid are weighed.

Secondly, the citric acid is diluted in a balloon 250ml with a distillated water and thereafter introduce the parts of the plant to be esterified. Third, put the balloon into a balloon heater 250ml at 141°C and put a condenser on top of the balloon to complete the reflux assembly. Start the chronometer and samples were taken at the reaction times chose to determine the kinetics reactions. The following table 9 in the paragraph §.5.1. showed the different experimental conditions for each *Epiphyllum oxypetallum*'s parts esterification such as for all operating conditions the organic molecules in the different parts of the *Epiphyllum oxypetallum* were in excess in comparison with the citric acid molecules. These conditions permitted to evaluate the kinetic constants of the citric acid.

***Table 9.*** *Operating conditions for the esterification of Epiphyllum oxypetallum with citric acid.*

| | Weight of organic material (g) | Weight of the citric acid (g) | Water volume (ml) | Calculated pH |
|---|---|---|---|---|
| Flower without stamens&pistils | 26.2327 | 0.4029 | 200 | 2.79 |
| Stamens and pistils | 6.2541 | 0.0961 | 200 | 3.10 |
| Phylloclades | 5.2353 | 0.0773 | 200 | 3.15 |

## *5.2. Kinetics of the Different Parts of the Epiphyllum Oxypetallum Esterifications with Citric Acid*

Samples of about 1ml of the solutions in the balloon 250ml are taken after 1min, 5 min, 15 min, 30 min, 60 min and 110 mn. And each sample was titrated to determine the rest of citric acid in the solution for each reaction time [9, 10]. In brief, the samples are diluted in a beaker 250ml with 15 ml of distillated water. Put, the NaOH-0.05 titrant solution in the buret and add3 drops of helianthin color indicator in the beaker 250ml. Begin the titration and the equivalent point is reached when the solution in the beaker became orange-yellow. In this experiment, the flower and the stamens&pistils samples were titrated with NaOH-0.05N whereas the green stem-phylloclade samples were titrated with NaOH-0.005N. The following table 10 showed the results and their exploitations.

***Table 10.*** *Results of the different parts of the Epiphyllum oxypetallum esterifications with citric acid.*

*Phylloclades*

| **Reaction time [mn]** | **0** | **0.25*** | **0.5*** | **1** | **5** | **15** | **30** | **60** |
|---|---|---|---|---|---|---|---|---|
| Citric acid quantities [moles] | 4.02E-04 | 3.18E-04 | 2.33E-04 | 6.39E-05 | - | 4.88E-05 | 3.25E-05 | 4.02E-08 |
| Citric acid conversion χ(%) | 0 | 21.03 | 42.06 | 84.11 | - | 87.88 | 91.92 | 99.99 |
| Citric acid concentration [mole/l] | 2.01E-03 | 1.59E-03 | 1.17E-03 | 3.20E-04 | - | 2.44E-04 | 1.63E-04 | 2.01E-07 |

*Flower without stamens and pistil*

| **Reaction time [mn]** | **0** | **0.25** | **0.5** | **1** | **1.5** | **5** | **60** | **110** |
|---|---|---|---|---|---|---|---|---|
| Citric acid quantities [moles] | 2.10E-03 | - | - | 1.59E-03 | 1.38E-03 | 7.94E-04 | 2.74E-04 | 2.10E-05 |
| Citric acid conversion χ(%) | 0 | - | - | 24.28 | 34.20 | 62.14 | 86.94 | 99 |
| Citric acid concentration [mole/l] | 1.05E-02 | - | - | 7.94E-03 | 6.90E-03 | 3.97E-03 | 1.37E-03 | 1.05E-04 |

*Stamens and pistils*

| **Reaction time [mn]** | **0** | **0.25*** | **0.5*** | **1** | **15** | **30** | **60** | **110** |
|---|---|---|---|---|---|---|---|---|
| Citric acid quantities [moles] | 5.00E-04 | 3.91E-04 | 2.83E-04 | 6.53E-05 | 4.98E-05 | 3.32E-05 | 5.00E-08 | - |
| Citric acid conversion χ(%) | 0 | 21.73 | 43.47 | 86.94 | 90.04 | 93.36 | 99.99 | - |
| Citric acid concentration [mole/l] | 2.50E-03 | 1.96E-03 | 1.41E-03 | 3.26E-04 | 2.49E-04 | 1.66E-04 | 2.50E-07 | - |

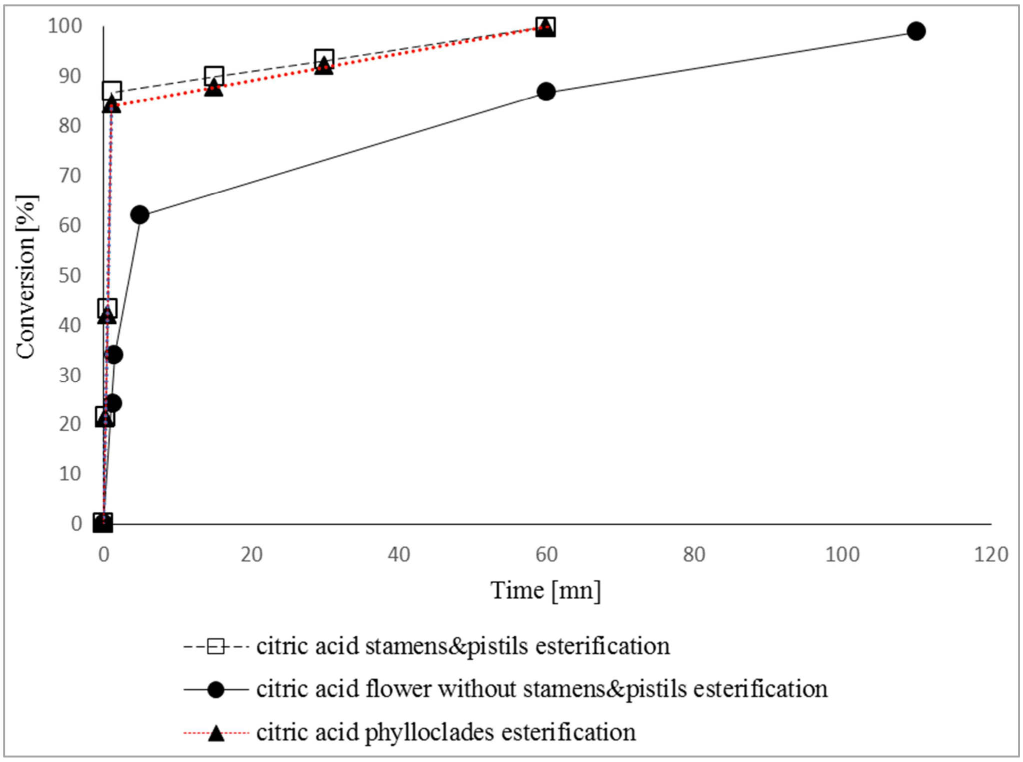


***Figure 19.*** *Comparison of the different parts of the Epiphyllum oxypetallum esterifications with citric acid.*

Noticed on the figure 16 that initially all the citric acid esterification was very important. But, for the flower without stamens & pistils the conversions from 1.5mn were lower than those for the stamens & pistils and phylloclades. It could be explained by the effect of the porosities and micro-canals generated by the micro-structure of the stamens & pistils and phylloclades-filament. Indeed, on the figure 19 it is seen that the conversion on the stamens & pistilts was slightly important than the conversion on the phylloclades; these results could indicate the best nature of the micro-canals on the *Epiphyllum oxypetallum*'s stamens & pistils.

As said previously, for all operating conditions the organic molecules in the different parts of the *Epiphyllum oxypetallum* were in excess in comparison with the citric acid molecules. Thus, the expression of the speed reaction is $v = k_{see} \times [citric\ acid]^{\propto}$. The resolution of this equation by the integral method [66] with considering the citric acid concentration evolution values in table 10, the kinetic constants $k_{see}$ and α could be deduced and calculated. The constant kinetic results for the different parts of the

*Epiphyllum oxipetalum* was presented in the following table 11. These results confirmed the best nature of the micro-canals on the *Epiphyllum oxypetallum*'s stamens & pistils.

***Table 11.*** *Kinetics constants for the esterification with acid citric of the Epiphyllum oxipetalum's different parts.*

| Parts of the Epiphyllum oxipetalum | $\propto value$ | $k_{see}$ [$L^2 \times mol^{-2} \times mn^{-1}$] | $k$ [$L^2 \times mol^{-2} \times mn^{-1}$] |
|---|---|---|---|
| Phylloclades | 1 | 0.5458 | 165,000 |
| Flower without stamens and pistil | 1 | 0.2781 | 39,469 |
| Stamens and pistils | 1 | 0.5729 | 173,844 |

# 6. Conclusion

The *Epiphyllum oxypetalum* contains lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. The arachidic acid is only detected on its flower without stamens and pistils and the gadoleic acid is only detected on its stamens and pistils. The important total weight concentration of unsaturated fatty acids oleic acid, linoleic acid and linolenic acid on the *Epiphyllum oxypetalum*'s flower with stamens and pistils in relation to the grinded sample and the initial fresh sample equal respectively to 62.54386954 (mg/g-grindesample) and 3.20823907 (mg/g-initial freshsample) indicated the anti-oxidant and anti-inflammatory potentialities of the *Epiphyllum oxypetalum*'s flower with stamens and pistils. Whereas, the high value of palmitic acid concentrations in the *Epiphyllum oxypetalum*'s flower with stamens and pistils equal to 60.08675636 (mg/g-grindesample) and 3.082199435 (mg/g-initial freshsample) warn that its consummation lead to hypercholesterolemia and cardiovascular disease. The active molecules on the *Epiphyllum oxypetalum*'s different parts citric acid ester solutions were determined and described explicitly in a dissertation and in general, the *Epiphyllum oxypetalum*'s flower with stamens and pistils could be valorized as anti-oxidant and anti-inflammatory cream.

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# Conflicts of Interest

The authors declare no conflicts of interest.

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