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# Determining of the Constituent Molecules in the *Strychnos spinosa* Pips by Extraction with Citric Acid Esterification Procedure

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**Abstract:** This publication treated the determination (qualitative) and the quantification (quantitative) of the steroidal and flavonoids constituent molecules in the *Strychnos spinosa* pips. These molecules were extracted by esterification between the *Strychnos spinosa* pips and citric acid molecules in a reflux assembly such as the evaluated total moles of the substance's organic functions, deduced by bibliographies values, and which could reacted with the citric acid molecules was in excess. Thus, the weight ratio between the *Strychnos spinosa* pips and citric acid was equal to 7.39. Seeing that the reflux assembly could be assimilated as a closed reactor composed with a beaker-250ml, the observed speed constant $k_{see}$ was equal to $4.3168\times10^{-2}$ [$L^2\times mol^{-2}\times mn^{-1}$]. Thereafter, the transesterification of the *Strychnos spinosa* citric acid esters solution by methanol was carried out with the volume ratio 2.82 and the extraction with dichloromethane followed by hplc-analysis permitted to determine the presence of virtuous steroidal and flavonoid molecules in the *Strychnos spinosa* pips respectively $4.25\times10^{-1}$ [$g_{steroïds}$ per $g_{Strychnos\ spinosa\ pips}$] and $3.89\times10^{-1}$ [$g_{flavonoids}$ per $g_{Strychnos\ spinosa\ pips}$]. The constituent molecules majority in the *Strychnos spinosa* pips were betulinic acid and eriocitrin which vertues as antioxidant, anti-inflammatory and anti-cancer were shown in bibliographies. Thus, it was important the perspective to valorize the *Strychnos spinosa* pips.

**Keywords:** *Strychnos spinosa* Pips, Betulinic Acid, Steroids, Eriocitrin, Neoeriocitrin, Flavonoids, Citric Acid, Esterification

## 1. Introduction

Seeing that no study was undertaken on the *Strychnos spinosa* pips, the aim of this manuscript was to show their constituent molecules. Thus, these molecules were extracted by esterification between the citric acid molecules and the grinded *Strychnos spinosa* pips followed by methanol transesterification and extraction with a polar solvent dichloromethane. These procedures were described on the literatures and the details were seen on the paragraphes §4 and §5. Once extracted, the dichloromethane-organic phase was analyzed through hplc according to two experimental conditions from the literatures which permitted to determine and to quantify first the steroidal molecules in the *Strychnos spinosa* pips and second their flavonoids molecules. The details of these experimental conditions and the results were shown in the paragraph §5.2.1 and §5.2.2. Then, the bibliographies studies shown that these constituent molecules in the *Strychnos spinosa* pips have many interesting activities, applications and vertues (§5.2.3). Some of them should be studied deeply not only to confirm their activities through the *Strychnos spinosa* pips but also in the same time, these studies permitted to valorize the *Strychnos* *spinosa* pips and their constituent molecules established and quantified in this publication.

## 2. The Citric Acid

### *2.1. Generalities and Applications of the Citric Acid*

Citric acid $C_6H_8O_7$ is a tricarboxylic acid α- hydrolyzed. It contains three acids with pKa such as pKa1 = 3.14, pKa2 = 4.77 and pKa3 = 6.39 and an α-alcohol function with pKa = 14.4 [1-3] (figure 1). By its reactivity, the citric acid was the object of several studies and was used in several fields such as the synthesis of citric acid polymers [4-6], the leaving creatures' molecules extraction by esterification with citric acid [7, 8], the trans-esterification between citric acid and oil to synthesize bio carburant [9, 10], the alimentary manufacturing [11-14] and the citric acid derivatives was used in water treatment [15, 16] and oil-hydrocarbon additives [17, 18].

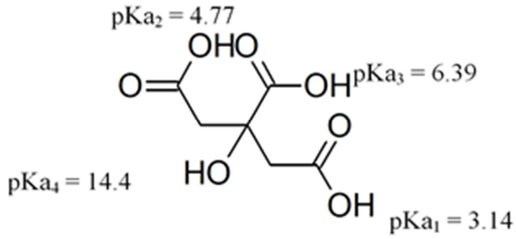


***Figure 1.*** *3-hydroxybutane-1, 2, 4-tricarboxylic acid (Citric Acid).*

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

| 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 |

### *2.2. Characteristics of the Citric Acid*

Citric acid is solid with monoclinic as crystal structure, white, odorless and excessively sour flavor [3]. Citric acid exists in hydrates forms, the monohydrate melts towards 343.15 °K and the anhydrous state melting point is 426.15°K. Citric acid is soluble in alcohol, ether, ethyl acetate and DMSO (Table 2) and insoluble in $C_6H_6$, $CHCl_3$, $CS_2$, and toluene. Its solubility in ethanol at 298.15°K is 62g/100g. Citric acid is very soluble in water and its solubility increases with the temperature as shown the following table (Table 3) [19].

***Table 2.*** *Citric acid physicochemical properties.*

| Physicochemical Properties | CITRIC ACID - $C_6H_8O_7$ |
|---|---|
| Appearance | Crystalline white solid |
| Crystal structure | Monoclinic |
| Molar mass | 192.12 [$g.mol^{-1}$] |
| Density | 1.665 [$g.cm^{-3}$] anhydrous<br>1.542 [$g.cm^{-3}$]monohydrate at 291.15°K |
| Melting point | 426.15°K anhydrous<br>343.15°K monohydrate |
| Boiling point | 448.15°K |
| Solubility in ethanol | 62g/100g |
| Solubility in water | 59.20% at 293.15°K (Table 3) |

***Table 3.*** *Evolution of the citric acid solubility in water (w/w) following to the temperature (°K).*

| T°K | 283.15 | 293.15 | 303.15 | 313.15 | 323.15 | 333.15 | 343.15 | 353.15 | 363.15 | 373.15 |
|---|---|---|---|---|---|---|---|---|---|---|
| Solubility (% g/100mg) | 54.0 | 59.2 | 64.3 | 68.6 | 70.9 | 73.5 | 76.2 | 78.8 | 81.4 | 84.0 |

## 3. The *Strychnos spinosa*

### *3.1. Generalities of the Strychnos spinosa*

The *Strychnos spinosa* is a specie of the genius Strychnos [table 4]. The *Strychnos spinosa* also named natal orange [20] is a tree to tropical and subtropical Africa and produced fruits only after good rains. The fruits are initially green and became yellow when they are ripe. Inside the fruits are tightly packed numerous hard white seeds which became hard brown sides when the fruit are ripe. This tree can be found growing singly in well-drained soils like along river banks. Its found and native for Ethiopia, Kenya, Madagascar, Mali, Mauritius, Seychelles, Sudan, Tanzania, Uganda and Zambia but exotic for South Africa and the United States of America. Seeds which contained alkaloïds and unripe fruit are toxic but the weet-sour fruit pulp is edible [21]. At Madagascar, there were fourteen (14) species of Strychnos such as five (5) species are endemic: S. bifurcata Leeuwenberg, S. diplotricha Leeuwenberg, S. mostueoides

Leeuwenberg, S. pentantha Leeuwenberg and S. trichoneura Leeuwenberg [22].

***Table 4.*** *Scentific classification of the Strychnos spinose.*

| Kingdom | Plantae |
|---|---|
| Clade | Tracheophytes |
| Clade | Angiosperms |
| Clade | Eudicots |
| Clade | Asterids |
| Order | Gentianales |
| Family | Loganiaceae |
| Genus | Strychnos |
| Species | S. spinosa |
| Binomial name | *Strychnos spinosa* Lamarck |

### *3.2. Researches and Studies About the Strychnos spinosa*

At Madagscar and around the world, many researches was done on many species of the genus Strychnos to study their applications and vertues [23]. The first research in the genus Strychnos was to determine the active molecules responsible for the tetanizing action of the Strychnos nux vomica L seeds, known in Asia since ancient times, and the paralyzing action of various Latin American Strychnos used by the Indians to make the poisons for their arrows. The results of these researches allowed to the discovery of a great wealth of active molecules in the leaves, seeds, stem barks and root barks of the genus Strychnos [23-25]. Among these molecules were alkaloids like strychnine [24, 25], Malagashanine, Malagashine and Strychnobrasiline [26, 27]. Traditionally, at Madagascar, the roots, stems and leaves of the *Strychnos spinosa* are used in decoction as antiblennorrhagic; its infused leaves was used in bath against skin diseases, especially scabies [23]. Nevertheless, no study had been undertaken on the seeds of the *Strychnos spinosa* to determine their active molecules and their vertues even if it was toxic [21]. That is why, the interest of this study consists in determining the active molecules of the *Strychnos spinosa* grains through an extraction procedure by esterification with citric acid molecules followed by transesterification-extraction and hplc analysis [13, 28].

## 4. Study of the Esterification Between the *Strychnos spinosa* Pips and the Citric Acid Organic Functions

### *4.1. Experimental Conditions*

As described previously (§2), the citric acid could react with other molecules of an organism mainly by esterification [7, 8] and results showed that their esterification together is an efficiency method for an organism's molecules extraction [13, 28, 29]. The aim was both citric acid molecules reacted with the organism's molecule, thus the total moles of the organism's organic functions should be in excess in comparison with the citric acid organic functions moles [13, 28, 29]. So, after bibliographies investigations it was elucidated that the seeds of the *Strychnos spinosa* used during these experimentations contained 1.79 [%] of fatty acids [30], and deduced by the literature the plant *Strychnos spinosa* contained approximately 60 [%] of terpénoïdes, 36 [%] of sterols and flavonoids, alkaloids, saponin and others [31].

Thus, the total fatty acid weight concentration in the pips of the *Strychnos spinosa* used during this experimentation and deduced after hexane extraction followed by chromatography analysis is 6.43E-5 $\frac{\text{[Total fatty acid moles]}}{[\text{g of } Strychnos\ spynosa]}$. As consequence of these different values and seeing that the used pips of the *Strychnos spinosa's* organic functions should be in excess in comparison with the citric acid organic functions moles, the moles of citric acid molecules used per gram of the *Strychnos spinosa* pips used during their esterification is 6.97E-4 $\frac{\text{[Citric acid moles]}}{[\text{g of } Strychnos\ spynosa]}$ equivalent to 7.4 grams of *Strychnos spinosa* pips per gram of citric acid. A reflux assembly was used to this esterification [7, 8] and the pH was carried out at 2.56; the following table 5 resume the experimental conditions and raw materials quantities used during the esterification between citric acid and the pips of the *Strychnos spinosa.*

***Table 5.*** *Experimental conditions during Citric acid esterification of the Strychnos spinosa pips.*

| Experimental conditions – Raw materials quantties | |
|---|---|
| *Strychnos spinosa* pips weight [g] | 97 |
| Water volume [ml] | 150 |
| Citric acid weight [g] | 13.13 |
| Temperature [°C] | 144 |
| pH | 2.55 |
| Citric acid concentration [mol/l] | 0.46 |
| Weight ratio [*Strychnos spinosa* pips/citric acid] | 7.39 |

### *4.2. Kinetics of the Esterification Between Strychnos spinosa Pips and Citric Acid Organic Functions*

During the esterification between the *Strychnos spinosa* pips and the citric acid organic functions, the protonic acid-$H^+$ play the role of catalyst [9-10, 29] and after recovering samples for few reaction times followed by their titration with NaOH-0.05N to quantify their citric acid rate [7, 8], the kinetic study of this reaction could be done. Especially, seeing that the *Strychnos spinosa* pips contained fatty acids, 5ml of hexane and 15ml of freezing distillated water was used during this titration to extract these citric acid fatty acids-esters and then recovered the aqueous phase for citric acid titration by NaOH-0.05N. Thus, the following table 6 contained the results of this kinetic study which confirmed that on this experimental condition where the used pips *Strychnos spinosa's* organic functions were in excess in comparison with the citric acid organic functions moles, these latest reactions increased sufficiently with reaction time.

***Table 6.*** *The citric acid quantities evolution with reaction time.*

| **Reaction time [mn]** | **0** | **1** | **5** | **15** | **30** | **60** |
|---|---|---|---|---|---|---|
| Citric acid quantities [moles] | 68.3413E-3 | 64.875E-3 | 46.125E-3 | 33.750E-3 | 15.750E-3 | 7.5E-3 |
| Citric acid conversion χ (%) | 0 | 5,47 | 32,51 | 50,62 | 76,95 | 89,03 |
| Citric acid concentration [mole/l] | 0,4556 | 0,4325 | 0,3075 | 0,2250 | 0,1050 | 0,0500 |

Noted that this esterification reaction between the pips' molecules of *Strychnos spinosa* in excess and citric acid molecules was done in a reflux assembly. So, the expression of the speed reaction is $v = k_{see} \times [citric\ acid]^{\propto}$. The resolution of this equation by the integral method [32] with considering the citric acid concentration evolution values in table 6, the kinetic constants $k_{see}$ and α could be deduced and calculated such as in this case, $\propto = 1$ and $k_{see} = 4.3168 \times 10^{-2}$ [$L^2 \times mol^{-2} \times mn^{-1}$] that is to say the reaction is first order in comparison with the citric acid reagent. Seeing that the reflux assembly could be assimilated as a closed reactor, the speed constant $k$ of this esterification reaction could be calculated by the expression from the integral method applied to the closed reactor such as the $t_{\frac{1}{2}} = 15\ [mn]$. In consequence, the value of $k$ is 667 [$L^2 \times mol^{-2} \times mn^{-1}$].

As said previously, seeing that not only the *Strychnos spinosa* pips contained fatty acids but also the weight concentration of fatty acid and the total moles of fatty acids molecules which reacted during the esterification were respectively 6.43E-5 $\frac{[\text{Total fatty acid moles}]}{[\text{g of } Strychnos\ spynosa]}$ (§ 4.1.) and 60.8413E-3 [moles], the estimated weight of *Strychnos spinosa* pips esterified during this esterification whith citric acid was

$$estimated\ weight\ of\ esterified\ Strychnos\ spinosa\ [g] = \frac{60.8413\text{E}-3\ [\text{moles}]}{6.43\text{E}-5\ [\frac{\text{moles}}{\text{g}}]} = 946.21\ [\text{g}] > 97\ [\text{g}]$$

That is to say, the totality 97 [g] of the S*trychons spinosa* reacted during this esterification-reaction with citric acid according the experimental conditions in the table 5. Indeed, it was noticed that at the end of the reaction, the *Strychnos spinosa* pips initially grinded became completely fine homogeneous powders.

# 5. Methanol Trans-Esterification of the *Strychnos spinosa* Pips' Citric Acid Esters Solution and Hplc-Analysis

According to the procedure in the bibliography [13, 28], after extraction of the molecules in the pips' *Strychnos spinosa* by reaction-esterification with citric acid molecules, the recovered solution must be trans-esterified with methanol using a reflux assembly followed by washing and extraction with cold-water and dichloromethane before the hplc-analysis [13, 28, 29].

## *5.1. Experimental Conditions of the Trans-Esterification with Methanol Followed by Extractions*

The following table 7 resume the experimental conditions of this trans-esterification followed by washing-extraction with cold water-hexane-dichloromethane according to the procedure described in bibliographies [13, 28, 29]. Then, the extracted dichloromethane-solution was analyzed by hplc to determine their sterol, terpenes and flavonoids rate such as their hplc-analysis experimental conditions according to the bibliographies [33, 34] were given in the next paragraph §5.2.

***Table 7.*** *Experimental conditions of the Trans-esterification with methano l of the Strychnos spinosa pips' citric acid ester solution.*

| | |
|---|---|
| Step1 – Trans-esterification with methanol using a reflux assembly | |
| *Strychnos spinosa* pips' citric acid ester solution [ml] | 141 |
| Methanol [ml] | 50 |
| Temperature [°C] | 144 |
| Balloon [ml] | 250 |
| Step2 – Washing extractions | |
| Freezing distillated Water [ml] | 38 |
| Hexane extraction [ml] | 171 |
| Dichloromethane extraction [ml] | 82 |

## *5.2. Sterol, Terpenes and Flavonoids Rate of the Strychnos spinosa Pips by Hplc-Analysis*

### *5.2.1. Hplc-Analysis of the Steroidal Molecules in the Strychnos spinosa Pips*

To determine and quantify the steroidal molecules in the pips of the *Strychnos spinosa*, a sample 80 [µl] of the extracted dichloromethane-solution was analyzed by hplc according to an experimental conditions deduced by the bibliography [33] such as the stationary phase is ODS, the mobile phase is isopropanol in acetonitrile 1:9, the column temperature is 20°C; the ultraviolet detector is 210 nm; the flow rate is 2 [ml/mn] and the mode is isocratic. Thus, the following chromatogram (figure 2) was given by the hplc and in comparison with the bibliography chromatograms [33] and after the inventories of the possible molecules products by the Trans-esterification with methanol of the *Strychnos spinosa* pips' citric acid ester solution according to the procedure described on bibliographies [13, 28, 29]; the following table 8 of the different pics and values which composed the chromatogram was deduced. In addition, seeing that the total dichloromethane extracted weight is 69.857 [g], the weight concentration of each molecules could be deduced and seen in table 8.

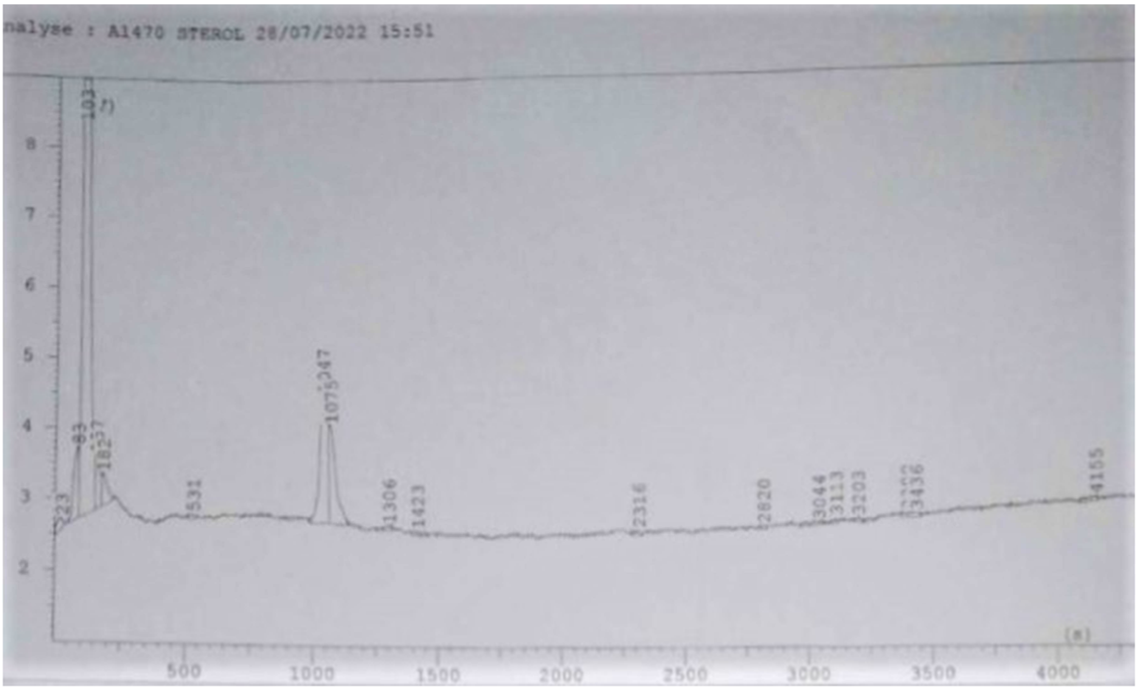


***Figure 2.*** *Chromatogram of the steroidal molecules in the Strychnos spinosa pips by hplc analysis.*

***Table 8.*** *Rate of the steroidal molecules in the Strychnos spinosa pips sample by hplc-analysis.*

| **molecules** | **surfaces** | **molar mass [g/mol]** | **% weight** | **moles/100g sample** | **molecules** | **moles/100g sample** | **moles/g of *Strychnos spinosa pips*** | **Weight g/g of *Strychnos spinosa pips*** |
|---|---|---|---|---|---|---|---|---|
| betulinic | 9 600 | 456.70 | 0.84 | 1.84E-03 | betulinic | 1.88E-01 | 1.36E-03 | 3.72E-01 |
| betulinic acid derivatives | 981 702 | 470.73 | 86.02 | 1.83E-01 | | | | |
| betulinic alcenes derivatives | 19 584 | 474.72 | 1.72 | 3.61E-03 | | | | |
| betulinic $-OCH_3$ | 593 | 470.73 | 0.05 | 1.10E-04 | | | | |
| ursolic | 742 | 456.70 | 0.07 | 1.42E-04 | ursolic | 3.64E-03 | 2.62E-05 | 1.17E-02 |
| ursolicic acid derivatives | 14 583 | 470.73 | 1.28 | 2.71E-03 | | | | |
| ursolic alcenes derivatives | 3 739 | 474.72 | 0.33 | 6.90E-04 | | | | |
| ursolic $-OCH_3$ | 520 | 470.73 | 0.05 | 9.68E-05 | | | | |
| stigmasterol | 541 | 412.69 | 0.05 | 1.15E-04 | stigmasterol | 1.15E-04 | 8.27E-07 | 2.69E-04 |
| lupeol | 62 266 | 442.72 | 5.46 | 1.23E-02 | lupeol | 1.26E-02 | 9.10E-05 | 2.42E-02 |
| lupeol $-OCH_3$ | 1 618 | 456.74 | 0.14 | 3.10E-04 | | | | |
| cycloartenol | 42 187 | 440.74 | 3.70 | 8.39E-03 | cycloartenol | 8.59E-03 | 6.18E-05 | 1.64E-02 |
| cycloartenol $-OCH_3$ | 1 032 | 454.77 | 0.09 | 1.99E-04 | | | | |
| teraxerol | 1 012 | 426.72 | 0.09 | 2.08E-04 | teraxerol | 2.08E-04 | 1.50E-06 | 3.83E-04 |
| β-sitosterol alkene derivatives | 964 | 432.72 | 0.08 | 1.95E-04 | β-sitosterol | 3.21E-04 | 2.31E-06 | 8.25E-04 |
| β-sitosterol | 595 | 414.71 | 0.05 | 1.26E-04 | | | | |
| Total | 1 141 278 | | 100 | 2.14E-01 | | 2.14E-01 | | 4.25E-01 |

### *5.2.2. Hplc-Analysis of the Flavonoid Molecules in the Strychnos spinosa Pips*

To determine and quantify the flavonoid molecules in the pips of the *Strychnos spinosa*, a sample 80 [µl] of the extracted dichloromethane-solution was analyzed by hplc according to an experimental conditions deduced by the bibliography [34] such as the stationary phase is ODS, the mobile phase is 0.01M phosphoric acid-methanol, the column temperature is 40°C; the ultraviolet detector is 285 nm; the flow rate is 0.6 [ml/mn] but the mode is isocratic. Thus, the following chromatogram (figure 3) was given by the hplc and in comparison with the bibliography chromatograms [34, 35] and after the inventories of the possible molecules products by the Trans-esterification with methanol of the *Strychnos spinosa* pips' citric acid ester solution according to the procedure described on bibliographies [13, 28, 29]; the following table 9 of the different pics and values which composed the chromatogram was deduced. In addition, seeing that the total dichloromethane extracted weight is 11.2967 [g], the weight concentration of each molecule could be deduced and seen in table 9. Noticed that: first, the neoeriocitrin and eriocitrin in common [derivatives-$OCH_3$] after trans-esterification on £(-O-)1 corresponded to the products of methanol trans-esterification after citric acid esterification of neoeriocitrin and eriocitrin on the sites in the blue circles (figure 4), second, the neoeriocitrin and eriocitrin in common [derivatives-$OCH_3$] after trans-esterification on £(-O-)2 corresponded to the products of methanol trans-esterification after citric acid esterification of neoeriocitrin and eriocitrin on the sites in the orange circles (figure 4) and third, the neoeriocitrin and eriocitrin in common [derivatives-$OCH_3$] after trans-esterification on £(-O-)1 corresponded to the products of methanol trans-esterification after citric acid esterification of neoeriocitrin and eriocitrin on the sites on red narrows (figure 4).

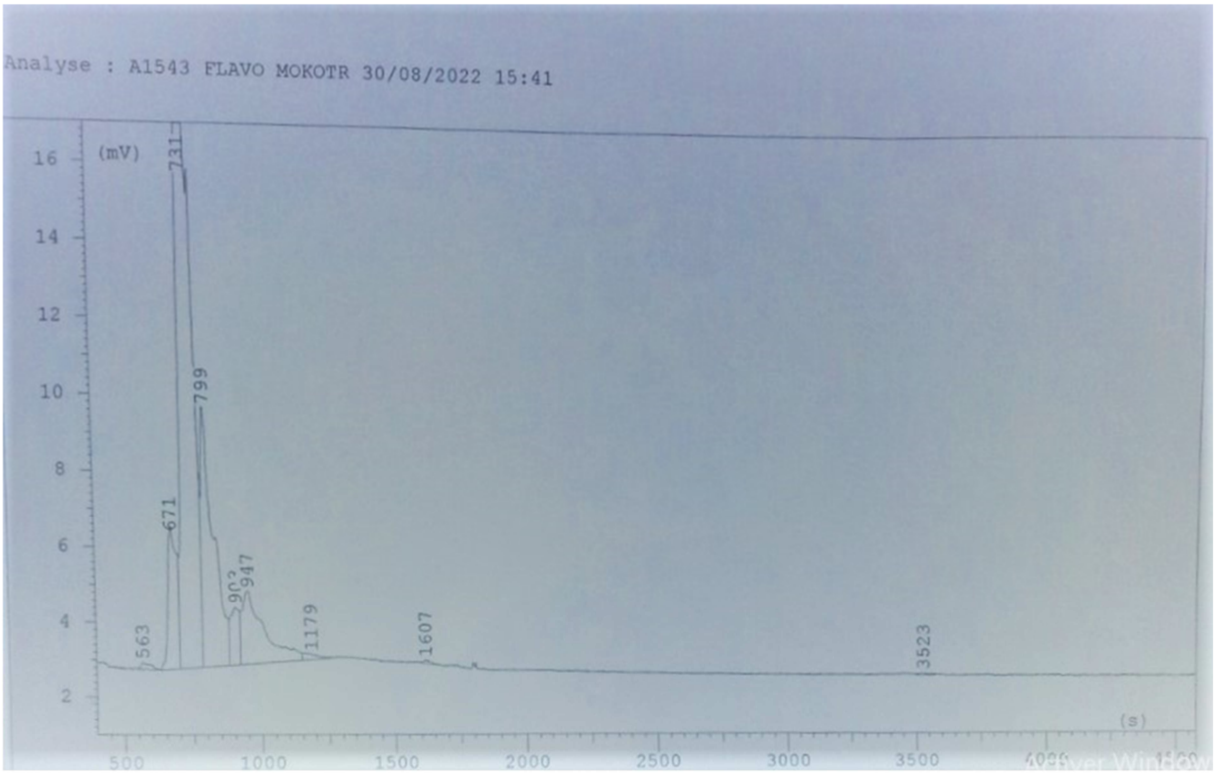


***Figure 3.*** *Chromatogram of the flavonoid molecules in the Strychnos spinosa pips by hplc-analysis.*

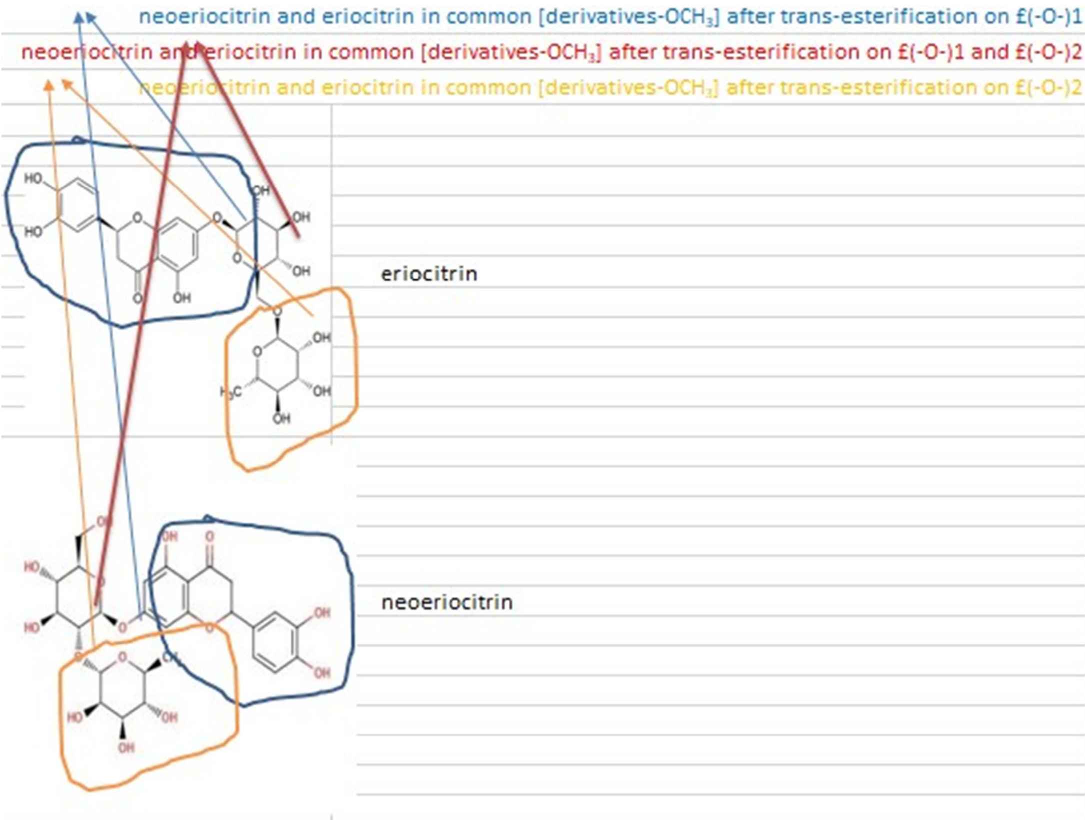


***Figure 4.*** *The neoericitrin and eriocitrin in common derivatives after trans-esterification with methanol [derivatives-$OCH_3$].*

***Table 9.*** *Rate of the flavonoid molecules in the Strychnos spinosa pips sample by hplc-analysis.*

| **molecules** | **surface** | **molar mass [g/mol]** | **% weight** | **moles/100g sample** | **molecules** | **moles/g *Strychnos spinosa* pips** | **weight g/g of *Strychnos spinosa pips*** |
|---|---|---|---|---|---|---|---|
| eriocitrin | 6 454 | 596.53 | 0.36 | 6.02E-04 | | | |
| eriocitrin-OCH3 (-C=O) | 136 186 | 611.57 | 7.58 | 1.24E-02 | eriocitrin | 4.23E-04 | 2.52E-01 |
| eriocitrin-OCH3 (OH) | 1 061 598 | 708.75 | 59.08 | 8.34E-02 | | | |
| neoeriocitrin | 339 878 | 596.53 | 18.92 | 3.17E-02 | | | |
| neoeriocitrin-OCH3 (-C=O) | 56 104 | 611.57 | 3.12 | 5.11E-03 | neoeriocitrin | 2.30E-04 | 1.37E-01 |
| neoeriocitrin-OCH3 (OH) | 183 827 | 660.70 | 10.23 | 1.55E-02 | | | |
| Neoeriocitrin and eriocitrin in common [derivatives-$OCH_3$] after trans-esterification on £(-O-)1 | 8 908 | 360.40 | 0.496 | 1.38E-03 | | | |
| Neoeriocitrin and eriocitrin in common [derivatives-$OCH_3$] after trans-esterification on £(-O-)1 and £(-O-)2 | 2 412 | 250.29 | 0.13 | 5.36E-04 | in common | 1.00E-05 | _ |
| Neoeriocitrin and eriocitrin in common [derivatives-$OCH_3$] after trans-esterification on £(-O-)2 | 1 493 | 206.24 | 0.08 | 4.03E-04 | | | |
| Total | 1 796 860 | - | 100 | 1.51E-01 | | 6.52E-04 | 3.89E-01 |

#### *5.2.3. The Active Molecules and Molecules Group of the Strychnos spinosa Pips*

In the following table 10 are established the steroidal and flavonoïds molecules in the *Strychnos spinosa* pips and their possible activities and benefits as seen in the bibliographies.

***Table 10.*** *The possible benefits and activities of the Strychnos spinosa pips according to their active molecules and molecules group.*

| **Steroidal molecules in the *Strychnos spinosa*** | |
|---|---|
| **Molecules** | **Activities and benefits** |
| Betulinic acid [36] | 1) Anti-cancer [37]<br>2) Antiviral [38]<br>3) Certain cases of leukemia [39]<br>4) Certain cases of brain cancer [40, 41] |
| Ursolic acid [42] | 1) Anti-cancer [43]<br>2) Lung, liver, kidney, brain protections [43]<br>3) Anabolic effects on the scrawny muscles [43]<br>4) Anti-inflammatory [43-45]<br>5) Anti-osteoporosis [46]<br>6) Anti-microbial [46]<br>7) Antiviral – Anti-bacterial VIH-HCV [47]<br>8) Inhibition of Escherichia coli biofilm formation [48]<br>9) Anti-viral for ADN-virus activities – Inhibitor for human elastase [49]<br>10)Inhibitor of acetylcholinesterase – potential effect on Alzeihmer disease [50]<br>11)Protect cardiovascular system [51]<br>12)Decrease abdominal weight and obesity in the mouse [51]<br>13)Increase glucose tolerance [52]<br>14)Inhibitior of atherosclerose induced by resistine, an adipokine linked with obesity [53-55] |
| Stigmasterol | Anti-cancer [56] |
| Lupeol [57] | 1) Anti-inflammatory [58, 59]<br>2) Anti-protozoal [60]<br>3) Anti-microbial [60]<br>4) Anti-tumor and chemopreventive [61]<br>5) Anti-cancer of skin and prostate [62-65]<br>6) Anti-giogenic and anti-cancer [66] |
| Teraxerol [67] | Anti-inflammatory [68] |
| B-sitosterol [69] | 1) Anti-hypercholesterolemia [70, 71]<br>2) Reduce and relieve the symptoms of prostate hypertrophy benign [72]<br>3) Contribute to improve the urinary flow and bladder emptying [72]<br>4) prevent cardiovascular disease [73-76]<br>5) Stimulate the production of lymphocyte and contribute to module the immune defense systems [77-82]<br>6) Anti-cancer [83, 84] |
| Eriocitrin [85] | 1) Antti-oxidant<br>2) Antitumor<br>3) Anti-allergic<br>4) Anti-diabetic<br>5) Anti-inflammatory |
| Neoeriocitrin | 1) Anti-oxidant [86, 87]<br>2) Anti-inflammatory [86] |

# 6. Conclusion

The results of this publication confirmed the efficiency of the esterification with citric acid method to extract and to quantify the constituent molecules of a substance [7, 28, 29] as it happens with the *Strychnos spinosa* pips. Thus, in this case, the *Strychnos spinosa* pips' molecules were esterified with citric acid molecules such as their evaluated constituent molecules were in excess with citric acid moles. It was noticed that the t1/2 (for 50% conversion of citric acid) was 15 [mn] considering the used reflux assembly as a closed reactor and the observed speed constant ksee was equal to $4.3168\times10^{-2}$ [$L^2\times mol^{-2}\times mn^{-1}$]. Thereafter, the citric acid esters solution was trans-esterified with methanol and the hplc-analysis of the extracted solution with dichloromethane showed not only many virtuous steroidal molecules such as betulinic acid, ursolic acid, stigmasterol, lupeol, cycloartenol, teraxerol and β-stigmasterol but also two virtuous flavonoïds such as eriocitrin and neoeriocitrin. The constituent molecules majority in the *Strychnos spinosa* pips were betulinic acid $3.72\times10^{-1}$ [g of betulinic per g of *Strychnos spinosa* pips] and eriocitrin $2.52\times10^{-1}$ [g of eriocitrin per g of *Strychnos spinosa* pips], and the bibliographies said their potentialities as anti-oxidant, anti-inflammatory and anti-cancer. Thus, some studies like anti-oxidant test, anti-inflammatory and anti-viral tests, anti-diabetic test, anti-allergic test and the capacity to limit and/or to stop the cancerous cells should be done on the *Strychnos spinosa* pips to confirm and/or to valorize their previous constituent molecules virtues and activities based on their rate in the *Strychnos spinosa* pips established and quantified in this publication.

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