American Journal of Applied Chemistry
2023; 11(6): 137-145
http://www.sciencepublishinggroup.com/j/ajac
doi: 10.11648/j.ajac.20231106.11
ISSN: 2330-8753 (Print); ISSN: 2330-8745 (Online)

Research/Technical Note

Antioxidant and Anti-Inflammatory Activities of the *Strychnos spinosa* Seeds' Citric Acid Esters Solution

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To cite this article:

Andry Tahina Rabeharitsara, Marie Florence Raharimalala, Diary Andriamanana Faharetana Tsiresy, Baholy Robijaona, Rakotomamonjy Pierre, Hanitriniaina Marie Ratsimba. Antioxidant and Anti-Inflammatory Activities of the *Strychnos spinosa* Seeds' Citric Acid Esters Solution. *American Journal of Applied Chemistry*. Vol. 11, No. 6, 2023, pp. 137-145. doi: 10.11648/j.ajac.20231106.11

Received: October 11, 2023; **Accepted**: October 28, 2023; **Published**: November 11, 2023

Abstract: The *Strychnos spinosa* seeds contains eriocitrin and neoericitrin which were potential antioxidant molecules. In addition, it contains ursolic acid, lupeol, teraxerol and β-sitosterol which were potential anti-inflammatory molecules. These molecules were extracted from the *Strychnos spinosa* seeds by esterification with citric acid moles as described in the bibliography in order to synthesize the citric acid ester of *Strychnos spinosa* seeds. Thus, in this manuscript, the potentiality of this ester solution to be an antioxidant and anti-inflammatory was tested using the spectrophotometry UV-visible method at the wavelength respectively λ=517[nm] and λ=550[nm] according to procedures described in this manuscript. For all tests, a principal-source solution was prepared using the citric acid ester of *Strychnos spinosa* seeds solution and appropriate solvents; thus from this solution was prepared the solutions at different concentrations to be tested. It was noticed that the concentration ratio between the active molecules and the molecules reacting with these active molecules during the antioxidant and anti-inflammatory tests influenced the quality of the tests. Thus, it was deduced that a low ratio allowed to a better quality results during the spectrophotometer UV-visible antioxidant and anti-inflammatory tests. In consequence, the quantities of the active molecules in the citric acid ester of *Strychnos spinosa* seeds necessary to the fifty percent diminution of the reacting or formed chemical molecules according to the tests respectively antioxidant tests or anti-inflammatory tests noted Ic-50 were deduced and allowed to evaluate and to compare their activities.

Keywords: *Strychnos spinosa's* Seeds, Flavonoids, Steroids, Antioxidant, Anti-Inflammatory, Ic-50

1. Introduction

The aim of this work is the logical continuation of the determination of active molecules in the *Strychnos spinosa* seeds. Indeed, it had been established that *Strychnos spinosa* seeds contain a non-negligible quantity of antioxidant molecules such as eriocitrin and neoeriocitrin and anti-inflammatory molecules such as eriocitrin, neoeriocitrin, ursolic acid, lupeol, teraxerol and β-sitosterol [1, 2]. Thus, tests of antioxidant and anti-inflammatory capacities of these molecules in citric acid esters solutions had been undertaken. The first part of this manuscript briefly describes the experimental conditions for extracting active molecules from *Strychnos spinosa* seeds by esterification reaction with citric acid molecules [1, 2]. The second part deals with the experimental conditions of anti-oxidant and anti-inflammatory tests, as well as the deduced results and their interpretations. First, for the anti-oxidant tests, the ratios

between the [dppH*]-[antioxidant tested molecules] concentrations were considered (4-2/3-3) in order to see their impact during the antioxidant tests. Then, the deductions from these results allowed to the choice of the ratios between the [anti-inflammatory tested molecules] / [nitroprusside] concentrations. Materials and chemicals used during experimentations were KERN precision scale, chronometer, glass spatula, iron spatula, magnetic stirrer, magnetic bar, flask-250ml, heating mantle-250ml, condenser, beaker-100ml, beaker-250ml, graduated burette, thick ash-free filter paper, glass funnel, pipette, spectrophotometer UV-visible, citric acid, distilled water, NaOH-0.05N, *Strychnos spinosa* seeds, helianthine, methanol, HBSS, dppH*-2,2 diphenyl-1-picrylhydrazyl, ascorbic acid, Greiss reagent, sodium nitroprusside, lamp incandescent 90W, dimethylsulfoxide.

2. Experimental Conditions for Extracting Active Molecules from *Strychnos spinosa* Seeds

The active molecules of *Strychnos spinosa* seeds had been extracted by esterification with citric acid molecules [3-5]. Indeed, on the one hand, the tri-acid functions of the citric acid molecule can enter into esterification with the alcohol functions of the active organic molecules of *Strychnos spinosa* and, on the other hand, the alcohol function function of the citric acid molecule can be esterified by the acid functions of the active molecules of the *Strychnos spinosa*; and in addition to these, the amine functions of the organic molecules of Strychnos spinosa can also react with the organic functions of the citric acid molecule [3-5]; in the end, a citric acid ester solution of *Strychnos spinosa* seeds is synthesized [1, 2] according to the experimental conditions presented in Table 1.

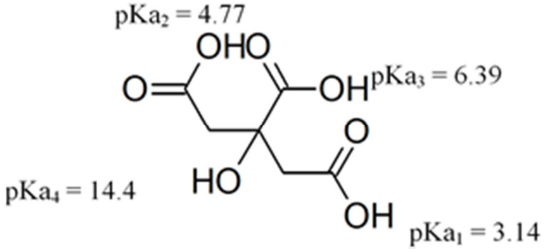

Figure 1. *Citric acid molecule with their organic functions.*

Table 1. *Experimental conditions of the Strychnos spinosa's citric acid esters synthesis.*

Citric acid weight [g]	13.1314
Citric acid quantitties [moles]	0.0683
Strychnos spinosa weight [g]	97.0007
Distillated water volume [ml]	150
Duration [mn]	165
Evaluated pH	1.97
Synthesized *Strychnos spinosa*'s citric acid esters Volume [ml]	995

The experimental conditions and esterification procedure were exactly the same as those described in the bibliography [1, 2], except that the reaction time had been extended in order to achieve full conversion of the citric acid molecules according to the conversion curve in the bibliography [1, 2]. In the end, a yellow citric acid ester solution of the active molecules of *Strychnos spinosa* seeds was synthesized. It should be remembered that the reflux assembly is used for this esterification. This assembly consists of a 250ml flask placed in a 250ml flask heater topped by a straight cooler [3-5]. Prior to esterification, the *Strychnos spinosa* seeds were ground and sieved through a fine-mesh kitchen sieve until a powder was obtained. After esterification, these powders disappeared and were replaced by fine soluble particles and more or less swollen seeds. Once synthesized, the citric acid ester solution from *Strychnos spinosa* was separated from the retained particles by filtration. The retained particles were then washed with ice-cold distilled water to remove any citric acid esters that may have been retained. In the end, the total volume of *Strychnos spinosa* citric acid ester solution collected was 995ml. This solution is very homogeneous, pale yellow in color with fine particles.

3. Experimental Conditions for the Antioxidant and Anti-Inflammatory Tests

3.1. Experimental Conditions and Results of the Antioxidant Tests

This antioxidant test permitted to evaluate the antioxidant capacity of one or many organic molecules isolated or in solution. This second case is studied in this manuscript where actives organic molecules were in solution as citric acid esters forms. This antioxidant test used the dppH* -2,2 diphenyl-1-picrylhydrazyl as reactant source of the radicals to be oxidized with the molecules to be tested. The evolution with time of the dppH* quantities in the solution to be tested corresponded to the leftovers of the dppH* which didn't react by hydrogen transfer with the active anti-oxidant molecules. The quantification of these dppH* was done by spectrophotometer-UV visible at the wavelength λ=517[nm] [6]. The solvent used should not absorb the dpph-H working-detection wavelength, and the use of volatile organic solvents should be avoided as far as possible, as their volatility could alter the measurement of concentrations. Thus, the organic solvent used during sample preparation is methanol. It should also be noted that all analyses require the preparation and testing of a sample solution called the "blank", which should contain all the reagents in the same proportions as the test samples, with the exception of the compound to be determined. The "blank" serves to eliminate the influence of solvent, impurities. In this dosing procedure, a low concentration of active-antioxidant molecules was expressly used which is of the order of 20.2882[µmol/l], compared with the concentration of dppH* which is of the order of 101.44[µmol/l]. These conditions were chosen with the only aim of ensuring that the oxidation reaction of dppH* was in

the majority, or at least roughly equal in quantity, to that of the active oxidizing molecules; in this case, for the last three samples (S25.5, S12.5, S8.5, S5 and S2.5), the concentration of which increases. Whereas for the first two samples (S110 and S75), the concentration is slightly higher for ascorbic acid oxidizing molecules (Table 2). Under these conditions, a gradual increase in active molecules should correspond to a gradual decrease in the majority of dppH* molecules to be oxidized. Each test lasts 30 minutes, during which the test samples are constantly shielded from light under opaque materials before being subjected to UV-visible spectrometry at wavelength λ=517[nm]. In this study, two antioxidant tests were carried out: the antioxidant test of a positive model-control molecule, ascorbic acid, and the citric acid ester solution of *Strychnos spinosa* to be tested. The following tables show the ascorbic acid concentrations and the concentrations of the anti-oxidant molecules eriocitrin-neoeriocitrin in the citric acid ester solution to be tested.

Table 2. *Experimental conditions for the antioxidant test of the positive control molecule ascorbic acid 3-3.*

Tested solution	S110	S75	S25,5	S12,5	S8,5	S5	S2.5
[ascorbic acid]$_{sample}$ [µmol/L]	110.146	75	25.5	12.5	8.5	5	2.5
sample volume [ml]	3	3	3	3	3	3	3
volume dpph* [ml]	3	3	3	3	3	3	3
volume total [ml]	6	6	6	6	6	6	6
[ascorbic acid]$_{sample}$ [µmol/L]	55.073	37.5	12.75	6.25	4.25	2.5	1.25
[ascorbic acid]/[dpph*]	1.086	0.739	0.251	0.123	0.084	0.049	0.025
[dpph*]/[ascorbic acid]	0.921	1.353	3.978	8.115	11.934	20.288	40.576

Table 3. *Experimental conditions for the antioxidant test of the positive control molecule ascorbic acid 4-2.*

Tested solution	S110	S75	S25.5	S12.5	S8.5	S5	S2.5
[ascorbic acid]$_{sample}$ [µmol/L]	110.146	75	25.5	12.5	8.5	5	2.5
sample volume [ml]	4	4	4	4	4	4	4
volume dpph* [ml]	2	2	2	2	2	2	2
volume total [ml]	6	6	6	6	6	6	6
[ascorbic acid]$_{sample}$ [µmol/L]	73.431	50	17	8.333	5.667	3.333	1.667
[ascorbic acid]/[dpph*]	2.172	1.479	0.503	0.246	0.168	0.099	0.049
[dpph*]/[ascorbic acid]	0.460	0.676	1.989	4.058	5.967	10.144	20.288

Table 4. *Experimental conditions for the antioxidant test of citric acid ester from Strychnos spinosa seeds containing the eriocitrine+neoeriocitrine 3-3.*

Tested solution	S110	S75	S25.5	S12.5	S8.5	S5	S2.5
[eriocitrin+néoeriocitrin]$_{sample}$ [µmol/L]	110.146	75	25.5	12.5	8.5	5	2.5
sample volume [ml]	3	3	3	3	3	3	3
volume dpph* [ml]	3	3	3	3	3	3	3
volume total [ml]	6	6	6	6	6	6	6
[eriocitrin+néoeriocitrin]$_{sample}$ [µmol/L]	55.073	37.5	12.75	6.25	4.25	2.5	1.25
[eriocitrin+neoeriocitrin]/[dpph°]	1.086	0.739	0.251	0.123	0.084	0.049	0.025
[dpph°]/[eriocitrin+neoeriocitrin]	0.921	1.353	3.978	8.115	11.934	20.288	40.576

Table 5. *Experimental conditions for the antioxidant test of citric acid ester from Strychnos spinosa seeds containing the antioxidant molecules eriocitrine+neoeriocitrine 4-2.*

Tested solution	S110	S75	S25.5	S12.5	S8.5	S5	S2.5
[eriocitrin+néoeriocitrin]$_{sample}$ [µmol/L]	110.146	75	25.5	12.5	8.5	5	2.5
sample volume [ml]	4	4	4	4	4	4	4
volume dpph* [ml]	2	2	2	2	2	2	2
volume total [ml]	6	6	6	6	6	6	6
[eriocitrin+néoeriocitrin]$_{sample}$ [µmol/L]	73.431	50	17	8.333	5.667	3.333	1.667
[eriocitrin+neoeriocitrin]/[dpph°]	2.172	1.479	0.503	0.246	0.168	0.099	0.049
[dpph°]/[eriocitrin+neoeriocitrin]	0.460	0.676	1.989	4.058	5.967	10.144	20.288

3.1.1. Preparation of the Ascorbic Acid Positive Model-Control Solution

A positive model-control solution is a solution of a molecule which anti-oxidant activity was described and cited by bibliographies like rosemary extracts, chlorogenic acid, ascorbic acid. In this study, an acid ascorbic solutions was used as positive model-control. Thus, chemicals used during these positive model-control analysis were methanol-90°, dppH solution 40[mg/l], different concentrations of the positive model-control solution named Sx[µmol/l], S110 – S75 – S25.5 – S12.5 – S8.5 – S5 et S2.5. The compositions of

these positive model-control is shown in the previous table 2 and table 3.

3.1.2. Preparation of the Antioxidant Test of Citric Acid's Ester of the Strychnos spinosa Seeds Solution

The aim is to prepare citric acid's esters solution to be tested which antioxidant molecules molar concentrations were the same as the positive model-control solution. Noticed that the concentrations of the antioxidant molecules in the *Strychnos spinosa* seeds such as eriocitrin and neoeriocitrin were shown in the bibliographies [1, 2]. Thus, the first step is to prepare the principal-source solution S110 then the preparation of the other solutions Sx [µmol/l] - S75 – S25.5 – S12.5 – S8.5– S5 et S2.5 could be deduced. The compositions of these citric acid's ester of the *Strychnos spinosa* seeds is shown in the previous table 4 and table 5.

3.1.3. Presentations and Discussions of the Antioxidants Tests Results

Table 6. *Experimental results for the antioxidant test of the ascorbic acid solution and the citric acid's ester from Strychnos spinosa seeds.*

	S110	**S75**	**S25.5**	**S12.5**	**S8.5**	**S5**	**S2.5**	**Test blank – dppH***
Tested solution ascorbic acid 3-3								
[dppH*] after 30mn [mg/ml]	0.0048	0.0026	0.0018	0.0795	0.0033	0.0176	0.0048	0.3778
Reacted dppH* Evolution [%]	98.7295	99.3118	99.5236	78.9571	99.1265	95.3415	98.7295	
Tested solution ascorbic acid 4-2								
[dppH*] after 30mn [mg/ml]	0.0034	0.0037	0,0046	0.0795	0.1201	0.1649*	0.2174*	0.4998
Reacted dppH* Evolution [%]	99.3200	99.2597	99.0796	84.0936	75.9704	67*	56.5*	
Tested solution citric acid's ester of the *Strychnos spinosa* seeds – anti-oxidants molecules eriocitrin-neoeriocitrin 3-3								
[dppH*] after 30mn [mg/ml]	0.1948	0.1717	0.0958	0.1656	0.1737	0.1591	0.1703	0.3778
Reacted dppH* Evolution [%]	54.9232	57.8878	54.0233	56.1673	74.6427	54.5527	48.4383	
Tested solution citric acid's ester of the *Strychnos spinosa* seeds – anti-oxidants molecules eriocitrin-neoeriocitrin 4-2								
[dppH*] after 30mn [mg/ml]	0.1922	0.1791	0.1674	0.164	0.1207	0.2271	0.2577	0.4998
Reacted dppH* Evolution [%]	75.8503	67.1869	66.5066	64.1657	61.5446	54.5527	48.4383	

The following figures showed the dppH* evolution of the tested solutions in comparison with the tested active molecules concentrations in the principal-source solution and in comparison with the tested active molecules in the spectrophotometer tested samples.

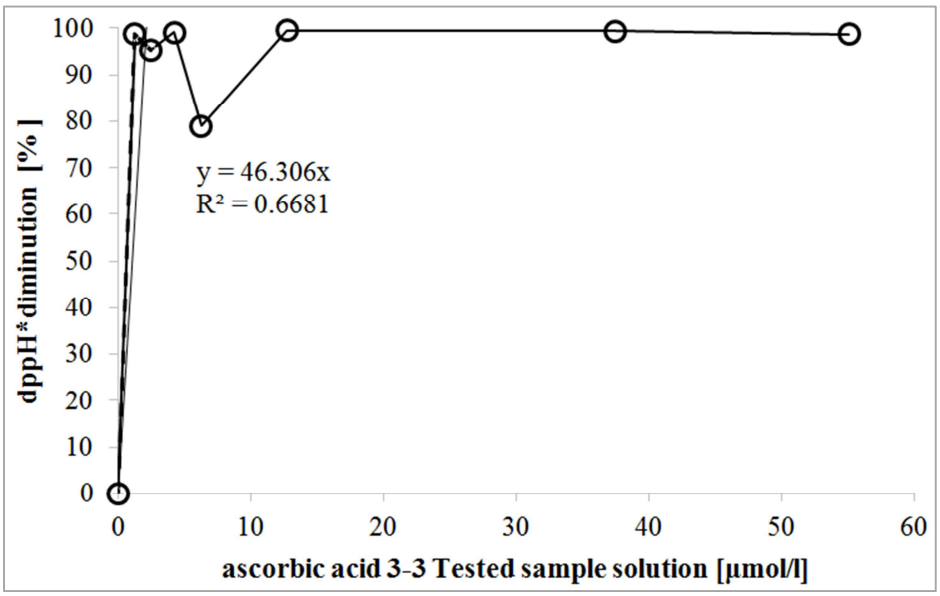

Figure 2. *Evolution of dppH* in comparison with ascorbic acid concentration in tested sample solution 3-3.*

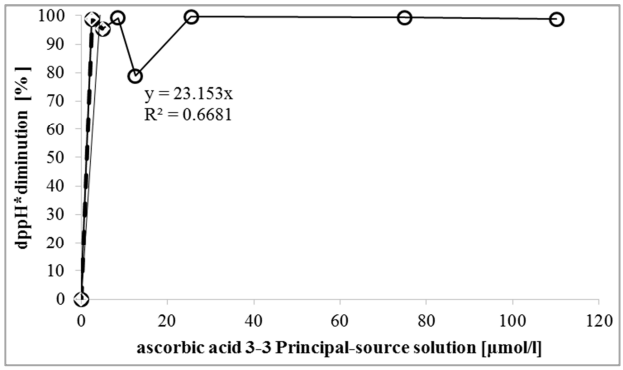

Figure 3. *Evolution of dppH* in comparison with ascorbic acid concentration in principal-source solution 3.*

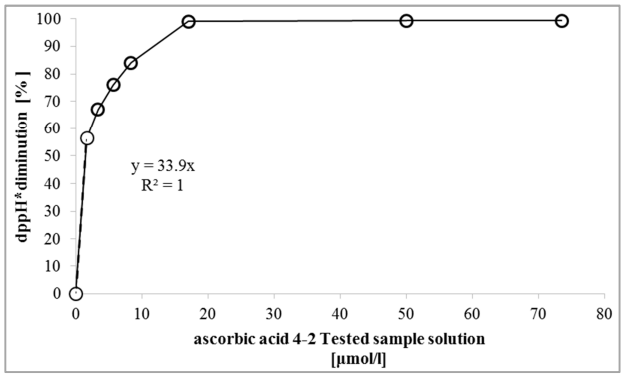

Figure 4. *Evolution of dppH* in comparison with ascorbic acid concentration in tested sample solution 4-2.*

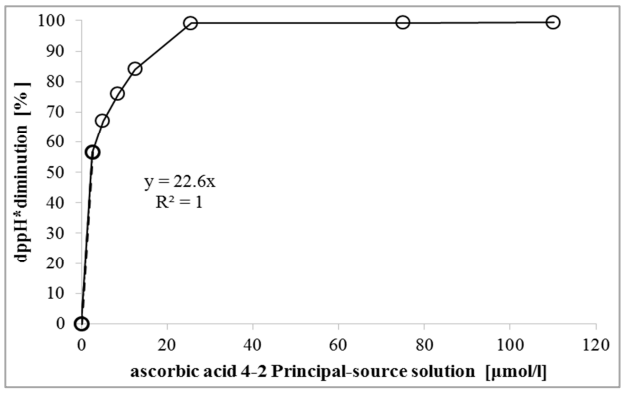

Figure 5. *Evolution of dppH* in comparison with ascorbic acid concentration in principal-source solution 4-2.*

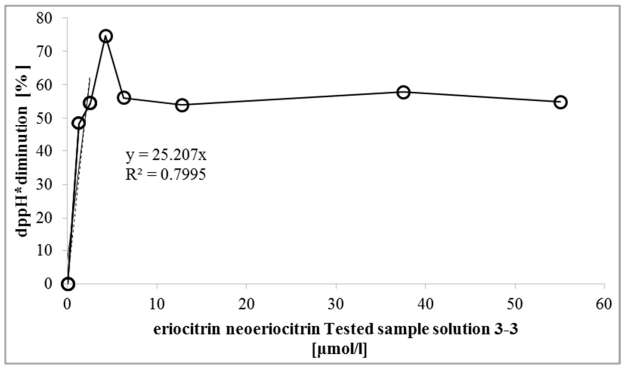

Figure 6. *Evolution of dppH* in comparison with eriocitrin neoeriocitrin concentration in tested sample solution 3-3.*

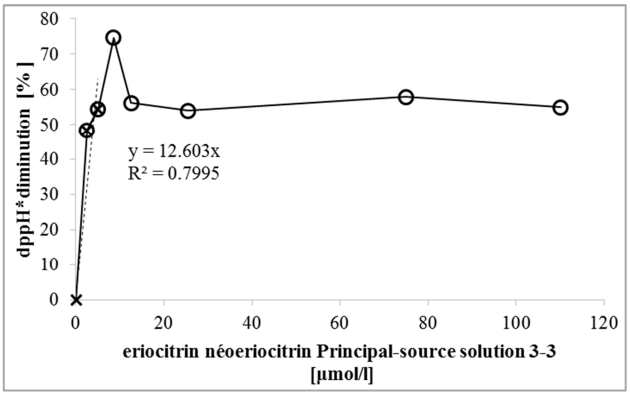

Figure 7. *Evolution of dppH* in comparison with eriocitrin neoeriocitrin concentration in principal-source solution 3-3.*

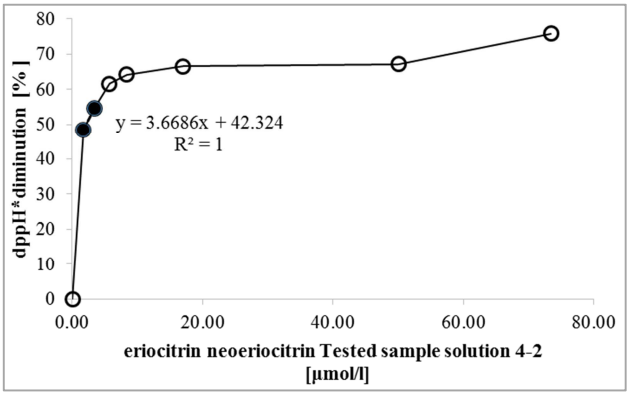

Figure 8. *Evolution of dppH* in comparison with eriocitrin neoeriocitrin concentration in tested sample solution 4-2.*

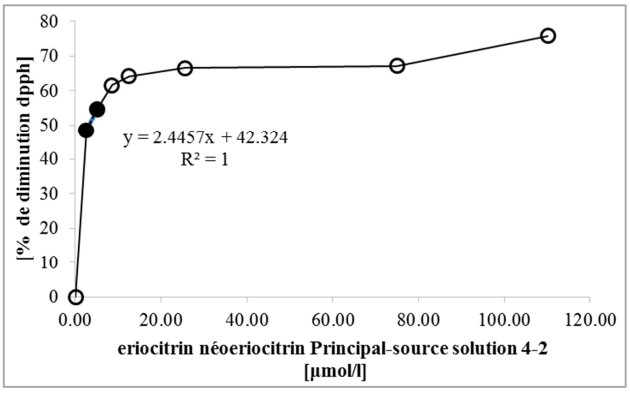

Figure 9. *Evolution of dppH* in comparison with eriocitrin neoeriocitrin concentration in principal-source solution 4-2.*

Firstly, when interpreting the effect of [dpph*]/[test solution] concentrations ratios [Table 2 - Table 5], it should be noted that when this ratio is low, initially in the case of 4-2 test samples compared with 3-3 test samples, the curves in general are clearer, especially at the initial points where test solutions concentrations are lowest. This explains the slight difference in IC-50 Index values for the same test sample with different [dpph*]/[test solution] ratios [Table 2 - Table 5]. The following Table 7 shows and summarizes the different Ic-50 values of the samples tested. Firstly, it should be noted that Ic-50 results relative to the concentration of active molecules in the principal-source solution are always

slightly higher than those relative to the concentration of active molecules in the test sample, given that the concentration of these active molecules is much greater in the test sample than in the principal-source solution. Consequently, Ic-50 results relative to the concentration of active molecules in the test sample are much more relevant than Ic-50 results relative to the concentration of active molecules in the principal-source solution even if their values are not so different. Next, the results of Ic-50s from the same sample, but with roughly equal ratios, are presented in Table 7. However, as already mentioned in the previous comments, a sharp increase in the initial dppH* content in relation to the active molecules leads to a slight disruption of the initial points, which is in favor of the 2-4 ratio.

Table 7. *Experimental IC-50 of the tested samples and their averages.*

Samples	Ratios	Ic-50 principal-source solution [µmol/l]	Ic-50 tested sample [µmol/l]	
Ascorbic acid	3-3	2.16	1.08	Average
	4-2	2.21	1.47	1.275
citric acid's ester of the *Strychnos spinosa* seeds – anti-oxidants molecules eriocitrin-neoeriocitrin	3-3	3.97	1.98	Average
	4-2	3.14	2.09	2.035

These results, deduced under the experimental conditions described above, clearly show that the presence of eriocitrin and neoeriocitrin in the citric acid ester's solution of *Strychnos spinosa* seeds [1, 2] induces highly significant antioxidant activities, with antioxidant activity in [µmol/l] still lower than that of ascorbic acid taken as a positive control. Indeed, the antioxidant activity of ascorbic acid is 1.59 times greater than that of the citric acid ester solution of *Strychnos spinosa* seed [1, 2]. These results also demonstrate the antioxidant activity of eriocitrin and neoeriocitrin molecules in the form of citric acid esters in the citric acid ester's solution of *Strychnos spinosa* seeds. Finally, these results confirm the antioxidant activity of eriocitrin and neoeriocitrin molecules described in the literature [7-26]. It should also be noted that eriocitrin has enormous potential as an anti-inflammatory agent. Anti-inflammatory tests were also carried out on the citric acid ester solution of *Strychnos spinosa* seeds [1, 2].

3.2. Experimental Conditions and Results of Anti-Inflammatory Tests

As a result of physical, chemical or biological aggression, such as injury, burns, microbial attack or cell malfunction, the body's anatomical and physiological barriers, i. e. the skin and mucous membranes, are crossed by harmful microbial foreign bodies. To deal with these, the body relies on immune cells capable of preventing their spread, or even eliminating them through a series of biochemical reactions. This is the body's first line of defense, known as the inflammation phenomenon or inflammatory reaction [27], of which there are two types: acute inflammation and chronic inflammation. These inflammatory processes involve a variety of molecules called inflammatory mediators, which are produced by immune system cells, mainly macrophages and mast cells, in response to stimuli and acting on specific targets, for example to inform the nervous system of the progress of inflammation. Inflammatory mediators include inflammatory cytokines such as TNF-α and anti-inflammatory cytokines, nitric oxide, lipid mediators and even oxygenated free radicals. Anti-inflammatories are substances that block the secretion or action of certain inflammatory mediators, and are used when the inflammatory reaction is prolonged abnormally (chronic inflammation), leading to tissue damage [28]. Anti-inflammatories can be classified into three groups: steroidal anti-inflammatories (cortisone and derivatives), non-steroidal anti-inflammatories (NSAIDs) and natural anti-inflammatories. Tests for anti-inflammatory activity are generally based on their effects on the above-mentioned inflammatory mediators; thus there is the anti-inflammatory test method measuring the effect of active extracts on NO production by inflammatory macrophages [27], the anti-inflammatory test method measuring the effect of active extracts on TNF-α production by inflammatory macrophages [27] and the anti-inflammatory test method measuring the NO scavenging activity of extracts [29]. The latter method is completely chemical and will be used in this study to measure the anti-inflammatory activity of citric acid ester extract from Strychnos spinosa seeds. The assay and titration of TNF-α and NO are carried out by the ELISA (Enzyme Linked ImmunoSorbent Assay) method [30-32] and the Griess method [33] respectively. The latter method, using Griess reagent as the reagent for NO detection on a UV-visible spectrophotometer at a wavelength of 550nm, is used in this study.

3.2.1. Experimental Conditions for Anti-Inflammatory Tests Measuring the No Scavenging Activity of Extracts

The first step was the preparation of the principal-source solution with 10.85[mg/ml] of anti-inflammatory molecules present in the prepared citric acid ester's solution of *Strychnos spinosa* seeds [1, 2] using dimethyl sulfoxide solution as solvent. From this stock solution, prepare the various solutions of different concentrations of citric acid ester molecules from *Strychnos spinosa* seeds to be tested, using HBSS of equal volume to the sodium nitroprusside solution prepared with HBSS 0.005N concentration. The sodium nitroprusside volumes used to prepare the four tests solutions are 20ml, 40ml, 80ml and 160ml and the principal-source solution used and introduced in each test solutions is 0.1ml. In addition to these tests solutions, the HBSS solution is used as blank during the spectrophotometer UV-visible test and the sodium nitroprusside 0.005N as reference. These test solutions are

placed in vials and then exposed to the air under a 90W incandescent lamp for two hours. Once the two hours have elapsed, switch off the lamp and add the Greiss reagent nitric oxide developer solution in volumes of 5ml, 10ml, 20ml and 40ml respectively to the 20ml, 40ml, 80ml and 160ml test solutions. Allow to incubate in the dark for 10 minutes, then measure the absorbance of the various test solutions under a 550nm UV-visible spectrophotometer to quantify the nitric oxide molecules. These experimental conditions are summarized in the following Table 8.

Table 8. *Experimental conditions of the citric acid ester's solution of Strychnos spinosa seeds scavenging test.*

Principal-source 10.85 [mg/l] solution volume [ml]	**0.1**	**0.1**	**0.1**	**0.1**
HBSS volume [ml]	160	80	40	20
nitroprusside solution volume [ml]	160	80	40	20
anti-inflammatoire molecules concentration [mol/l]	0.007860083	0.0157202	0.0314403	0.0628807
Ratio (anti-inflammatory/nitroprusside)	3.144033158	6.2880663	12.576133	25.152265
Nomenclature of solutions to be tested	4NFA	2NFA	1.1NFA	0.6NFA

3.2.2. Presentation and Discussions of Anti-Inflammatory Test Results

The results of the various analyses and anti-inflammatory tests on the citric acid ester solution of *Strychnos spinosa* seeds are shown in the following Table 9.

Table 9. *Anti-inflammatory tests results of the citric acid ester solution of Strychnos spinosa seeds.*

Nomenclature of tested solutions	**4NFA**	**2NFA**	**1.1NFA**	**0.6NFA**	**Nitroprusside**
anti-inflammatoire molecules concentration [mol/l]	0.007860083	0.0157202	0.0314403	0.0628807	
Ratio (anti-inflammatory/nitroprusside)	3.144033158	6.2880663	12.576133	25.152265	0.0095
Nitric oxides detected [mg/ml]	0.0167	0.1056	0.0947	0.0863	
percentage decrease in nitrite in comparison with the initial point recorded	-	1.0309278	11.246485	19.119025	

As the concentration of anti-inflammatory molecules in the test solutions increases, the concentration of nitric oxides decreases. This decrease is shown in the following Figure 10.

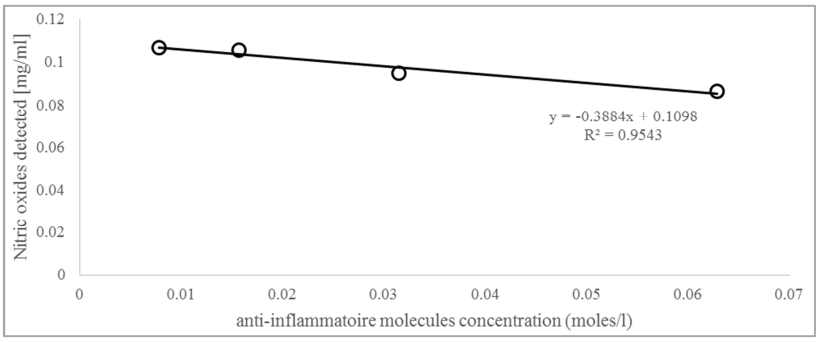

Figure 10. *Evolution of the nitric oxide detected in comparison with the anti-inflammatory molecules of the citric acid ester solution of Strychnos spinosa seeds concentration.*

The percentage reduction in nitric oxides compared with the initial experimental maximum nitric oxide quantity is shown in the following figure 11, from which it was deduced the anti-inflammatory concentration required to achieve a 50% reduction, which is rather equal to 0.1516 [mg/ml]. This value is the average of the values (Table 10) deduced by its two trend curves as shown in the following figure 11.

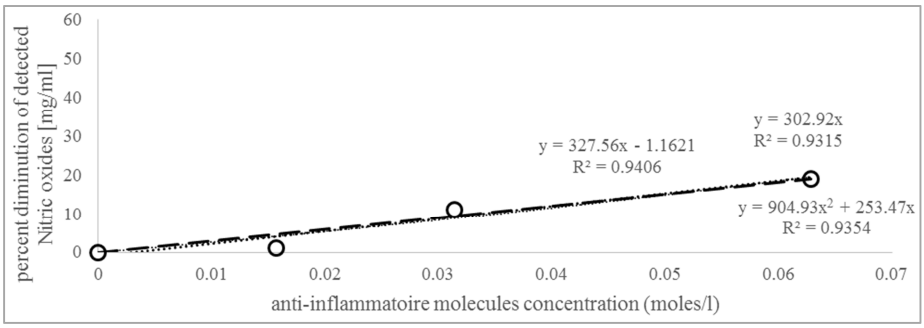

Figure 11. *Evolution of the percent diminution of the detected nitric oxides in comparison with the anti-inflammatory molecules of the citric acid ester solution of Strychnos spinosa seeds concentration.*

Table 10. *Evaluation of the Ic-50 average value.*

		Ic50 average	deviation
Ic50 straight line trend curve via origin	0.16506008		
Ic50 straight line trend curve	0.15619154	0.151612873	0.01622843
Ic50 polynomial trend curve via origin	0.133587		

4. Conclusion

The results of these studies indicated that the synthesized the citric acid ester of *Strychnos spinosa* seeds solution have potential antioxidant and anti-inflammatory properties. Whereas, it was noticed that the antioxidant activity of ascorbic acid is 1.59 times greater than that of the citric acid ester solution of *Strychnos spinosa* seed. But, all Ic-50 activities indicated that the citric acid ester of *Strychnos spinosa* seeds solution is very antioxidant its activities increased with its antioxidant molecules concentrations. Also, Ic-50 activities indicated that the citric acid ester of *Strychnos spinosa* seeds solution is an anti-inflammatory and its activities increased uniformly with its anti-inflammatory molecules concentrations. In general, the results showed clearly that the [dpph*]/[test solution] concentrations ratios have an impact on the curves of the antioxidant and anti-inflammatory tests, it was noted that when this ratio is low, initially in the case of 4-2 test samples compared with 3-3 test samples, the curves in general are clearer, especially at the initial points where test solutions concentrations are lowest.

Acknowledgments

Sincere thanks to the President of the Ecole Supérieure Polytechnique Antananarivo Polytechnics. And, sincere respect to the Chemical Process Engineering Chief Department (E. S. P. A) as well as the Chemical Engineering Laboratory staff and those who directly or indirectly contributed to the realization of this manuscript.

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