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Comparative Study of Phenolic Compounds Leaflets and Ribs of *Elaeis guineensis* Jacq (Arecaceae) Extracts and Evaluation of their Antioxidant Activity

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Abstract The present study aims was to appreciate: the phytochemical composed, and compared the phenolic compound contents in aqueous and ethyl acetate extracts of leaflets and rib of *Elaeis guineensis* and their antiradical activity. Qualitative phytochemical tests were used to detect the presence of bioactive molecules. The total Polyphenolic compounds content in each of aqueous and ethyl acetate extracts of leaflets and rib of *Elaeis guineensis* was made by using colorimetric Folin Ciocalteur method; Antiradical capacity was made by using the DPPH method. Results from phytochemical screening indicated the presence of flavonoids, tannins (cathechic and gallic), anthocyanin, leuco- anthocyans, in *Elaeis guineensis* leaves. The ethyl acetate extracts of leaflets and rib showed the high potential antioxidant capacity, polyphenol content, and flavonoid content. The high tannin content is showed in the ethyl acetate and aqueous extracts of leaflets but the high anthocyanin content is showed in the aqueous extracts of leaflets and rib. The important results obtained from this study can justify the use of this plant in traditional medicine for treatment of many diseases: arterial hypertension, malaria.

Keywords *Elaeis guineensis*, leaflets ,rib, ethyl acetate, aqueous, qualitative phytochemical, toxicity, antiradical activity

1. Introduction

Elaeis guineensis of the Arecaceae family is widely used in pharmacopeia and West African traditional medicine for treating various ailments: jaundice, internal bleeding and abdominal pain, fresh wounds [1], wound healing [2], malaria [3]. It is indicated as hypotensive aqueous decoction [4] and diuretic and saluretic propriety [5], cancer, headaches, skin infections, aphrodisiac… [6], Most of these diseases especially those called chronic (cancer, diabetes, cardiovascular disease ...) are linked to inflammation [7], particularly blood vessels which is now considered a major risk factor for heart disease such as high blood pressure and is an important source of oxygen radicals produced by the activation of phagocytic cells [8] that can be prevented by the antioxidants in several classes of secondary metabolites, the most studied currently are phenolic compounds known as good antioxidants by excellence [9]. Plant-based polyphenolic compounds have been reported to possess cardiovascular health benefits by intervening in the prevention and treatment of these diseases, [10]. The Improved knowledge of *E. guineensis*, especially on bioactive components of the leaf tissue will allow a correlation of its benefits and also would promote

the efficient use of this agricultural by product. In this vision we intend to evaluate, in the present study, the phytochemical composed, the bioactive molecules contents, the antiradical activity of aqueous and ethyl acetate extracts of leaves and rib of *Elaeis guineensis*.

2. Experimental Section

2.1. Material

Elaeis guineensis leaves were collected in Porto-Novo (kandévié) in July-August 2018. It was identified and authenticated. A voucher specimen (Ifangni: Adjakidje 4190) was deposited in the National Herbarium of Abomey Calavi University. The leaflets are speared to the rib with scissors and then dried for several days in the laboratory sheltered from the sun and then ground. After drying, the leaflets and rib are powdered. The powders obtained were then stored in glass jars in order to avoid any external contamination**.** They were used to obtain the aqueous and ethyl acetate extract.

2.2. Methods

2.2.1. Obtaining aqueous extracts

Elaeis guineensis leaves aqueous extract was prepared by decocting 50 g of leaflets or rib powder in 500 mL boiling distilled water for 30 min. The resulting mixture is then filtered on Watman paper (Ø = 185 mm). The filtrate was subjected to evaporation in a rotary evaporator (Büchi R 400 brand) at 40° C. Thereafter, the extract thus obtained was lyophilized by first freezing at -70° C in a deep freezer for 12 h and then dried in freeze-dryer.

Obtaining ethyl acetate extracts

50 g of leaflets or rib powder are mixed in 500 mL of ethyl acetate. The mixture is left stirring for 24 h and then filtered. The filtrate was evapored to a rotary evaporator (Büchi R 400 brand) at 40° C.

2.2.2. Preliminary phytochemical Analysis

Phytochemical screening which is a qualitative chemical analysis based on color reactions and precipitation of the major groups of chemical compounds in plants [11] was carried out to find out the phytoconstituents present in the *Elaeis guineensis* leaflets and rib aqueous extract and in their powder.

2.2.3. Quantification of the bioactive molecules

Polyphenolic content

Total polyphenol content (soluble and bound) was determined by the method described by [12]. Known volume of extract was taken and made up to 250 µL with distilled water and the sample was mixed with 625 µl of folin ciocalteau reagent and 500 µl of 20 % Na_2CO_3. The final reaction mixture volume was adjusted to 5 ml using distilled water. The reaction mixture was incubated in darkness for 30 minutes and samples were then centrifuged at 2000 rpm for 5 minutes and then the supernatant absorbance was measured at 760 nm. A calibration curve was constructed with different concentrations of gallic acid as standard. The results were expressed as mg of gallic acid equivalent/g of sample.

Flavonoids content

This was assayed following the method described by authors [13; 14] with slight modifications. 400 µL of extract was mixed with 500µL of distilled water. Then, 120µl of 5% of sodium nitrite was added to the mixture and allowed to stand for 5 minutes. 120 µL of 10% of $AlCl_3$ was added and whole solution mixed using a vortex. After 6 minutes, 800 µL of NaOH 1M were added and the mixture was incubated in darkness for 15 minutes. Solution of rutin was used as reference. The concentration values are directly read from the calibration curve established using the reference solution. The flavonoid content was expressed as mg of Rutin equivalent/1mg of sample.

Condensed tannins content

Condensed tannins were estimated using the method of author [15] modified by author [16]. A volume of 500µL of extract was added to 1.5 mL of vanillin solution initially dissolved in methanol for a final concentration at 4%; 1.5 mL of concentrated hydrochloric acid and 2 mL of methanol. The mixture was then incubated for 15 minutes and the absorbance taken at a wavelength of 500 nm. Condensed tannins were expressed as mg of tannic acid equivalent/mg of extract.

Anthocyans content

0.5 g of plant material are introduced into Erlenmeyer flasks, in the presence of 40 mL of HCl (2N) at room temperature. After a few minutes of contact, the flasks are placed for 40 minutes in a boiling water bath. After cooling, anthocyanins are extracted according to the method of Lebreton et al. [17].

For anthocyanins, the aqueous acidic phase is subjected 3 times with 6.5 mL of n-BuOH that extracts anthocyanidols whose color is red. Dosing is by scanning the spectrum from 480 to 600 nm and the maximum absorbance is noted. The content is calculated according to the formula proposed by Lebreton et al. [17].

$$T\ anthocyan = \alpha \,.\, Aext.\, \mathrm{M}\,.\mathrm{V}.\mathrm{D}$$

$$\varepsilon.\ \mathrm{m}$$

α: Correction factor (equal to 6) for the performance processing proanthocyans (17%)

Aext: Absorbance at the maximum absorption wavelength;

ε: Molar absorption coefficient of cyanidol (34700) ;

M: molar mass of leucocyanidol (= 306);

V: Volume of the butanol solution;

D: Dilution factor;

m: Mass of solids hydrolyzed plant material.

2.2.4. Total antioxidant capacity assay

Antioxidant activity was related to the capacity of plant extract to trap the free radical molecules. The technique applied for determination used the DPPH (2,2–diphenyl-1-picrylhydrazyl) method described by author [18]. 1.5 mL of DPPH solution (4mg of DPPH dissolved in 10 mL of methanol) was added to 0.75 mL of leaves extract at different concentration (from 0.5 mg/mL to 3.5 mg/mL). The mixture was then incubated in a dry bath room at room temperature for 15 minutes. The absorbance was measured at the wavelength of 517 nm using ascorbic acid as blank and standard. The total antioxidant capacity was expressed as Equivalent of Ascorbic acid per gram of aqueous extract (molEqAA/mg).

2.2.5. Statistical Analysis

The results are expressed as mean ± standard error of mean (SEM). Statistical processing was done on samples in independent series using ANOVA with software STATISTICA version 5.5. The results are considered statistically at probability level of $P < 0.05$.

3. Results and Discussion

3.1. Preliminary phytochemical Analysis

Results from phytochemical analysis of leaves extract of *E. guineensis* were presented in table 1 below.

Table 1: Phytochemical composition of *Elaeis guineensis*

Chemical compounds	Powdery of leaflets	Aqueous extract of leaflets	powdery of rib	Aqueous extract rib
Alkaloids	+	-	+	-
Cathechic tannins	+	+	+	+
Gallic tannins	+	+	+	+
Flavonoids	+ (flavon)	+ (flavon)	+ (flavon)	+ (flavon)

Anthocyanins	++	+	+	+
Leucoanthocyans	+	+	+	+
Quinone derivatives	–	-	-	-
Triterpenoids	+	+	+	+
Steroids	+	+	+	+
Cardiac glycosides	-	-	_	_
Saponins	+ (111,1)	+ (166,7)	+ (125)	+ (142,9)
Cyanogenic derivatives	–	-	-	-
Reducing compound	+	+	+	+
Mucilage	+	+	+	+
Coumarins	+	+	+	+
Free anthracene derivatives	+	+	-	-
C-heterosides	+	+	-	-
O-heterosides	+	+	-	-
Essentiel oil	+	-	-	-

(+) : indicates the presence and ; (−) indicates the absence of compound in plants

The phytochemical screening (table 1) of *Elaeis guineensis* (leaflets and rib) revealed the presence of flavonoids, tannins (cathetchic and gallic), saponins, anthocyanin, leuco- anthocyans, triterpenoids, steroids, mucilage, reducing compounds and coumarins, that are known to exhibit medicinal properties [21].

The phytochemical analysis of herbal drugs (leaflets and rib) and aqueous extracts (leaflets and rib) has similar results. We note a difference in some compounds such as alkaloids that are only present in the powders (leaflets and rib), the glycosides present in the powders of leaflets, and the volatile oil only present in the leaflets. This difference is due to their weak presence in the plant material or the method used to identify them. Gallic and catechic tannins constitute the majority of the leaflets followed anthocyanins and leuco-anthocyanidins. The reducing compounds, saponins, C-glycosides and free anthracene are few in majority in powder or aqueous extracts of the leaflets and ribs.

These results are similar to those obtained on methanol extract from the leaves of *E. guineensis* by Sreenivasan *et al*, [22]. It also similar to those obtained by N'Diaye *et al*., [23] unlike alkaloids, on the aqueous extract of the leaves of *E. guineensis*. On the other hand, Anna *et al* [24] reported the presence only of tannins and alkaloids in aqueous-alcoholic extract from the leaves of *Elaeis guineensis* used on antiplasmodial activity. Several factors could explain the differences observed in the major chemical groups of *E. guineensis*. According to Sofowora [25], these differences could be related to the age of plant, time of harvest, climate, type of soil culture and the method of extraction which brings up the concept of chemotype known plants. These differences can also be explained by the increased sensitivity of most methods of phytochemical screening, using the TLC characterization associated with tube reactions compared to ours. The presence of these secondary metabolites in the leaflets and rib of *Elaeis guineensis* has justified the claim by traditional medicine for the use of this plant in the treatment various diseases.

3.2. Polyphenols, flavonoids, condensed tannins and anthocyanins contents

By using molecule's absorbances, some important bioactive families were quantified. Indeed, total phenolic compound, flavonoid content and condensed tannins content were determined and gallic acid, rutin, catechin were used respectively as standard for each of family. Figure 1 presented the respective contents of total phenolic, flavonoids, condensed tannins.

The contents were expressed in mg equivalent of each standard per mg of plant extract. The respective contents of total phenolic, flavonoids, condensed tannins for leaflets ethyl acetate extract were 881.490 ± 1.459; 404.05 ± 5.689; 59.891 ± 1.351; for rib ethyl acetate extract were 294.597 ± 0.004; 215.956 ± 1.901; 21.227 ± 0.317; for leaflets aqueous extract were 176.085 ± 2.236; 124.815 ± 1.419; 49.83 ± 1.348 and for rib aqueous extract were 36.166 ±

0.661; 2.566 ± 0.001; 31.409 ± 1.043. The results showed that leaflets ethyl acetate extract have a high level of polyphenols and flavonoids but leaflets aqueous extract have a high level of condensed tannins.

The contents of anthocyanins show that leaflets and rib aqueous extracts have respectively the higher content of anthocyanins 1.417 ± 0.03 mg quercetol/g, 0.417 ± 0.006 mg quercetol/g but leaflets and rib ethyl acetate extracts have respectively the lower contents (0.215 ± 0.03 mg quercetol/g) (0.165 ± 0.08 mg quercetol/g).

Results showed significant differences ($p < 0.05$) in total phenolics content among the four samples. Our findings are in agreement with the result of investigator [26] who reported that the methanol extract of *Elaeis guineensis* has a content of an order of 56.7 mg EqCAT/g of extract. The content of polyphenols, flavonoids, condensed tannins are higher in leaflets than at the ribs. The unequal distribution of polyphenols in different organs of a plant has been reported by several authors [27; 28]. This unequal distribution of flavonoids might be explained by the fact that photosynthesis takes place in the leaves of the plant. Indeed, flavonoids provide protection for plant tissues against the harmful effects of solar radiation. This study may provide a justification for his diuretic and natridiuretic proprieties of the leaves aqueous extract [5] and the various uses that are made of plants in traditional medicine particular in the treatment of hypertension, inflammation and diabetic.

Polyphenols are secondary metabolites of plants and are very important because of their antioxidant activity by chelating metal ions having a redox activity, inactivating the free radicals of the lipid chains and preventing the conversion of hydroperoxide in oxygen reactive radicals.

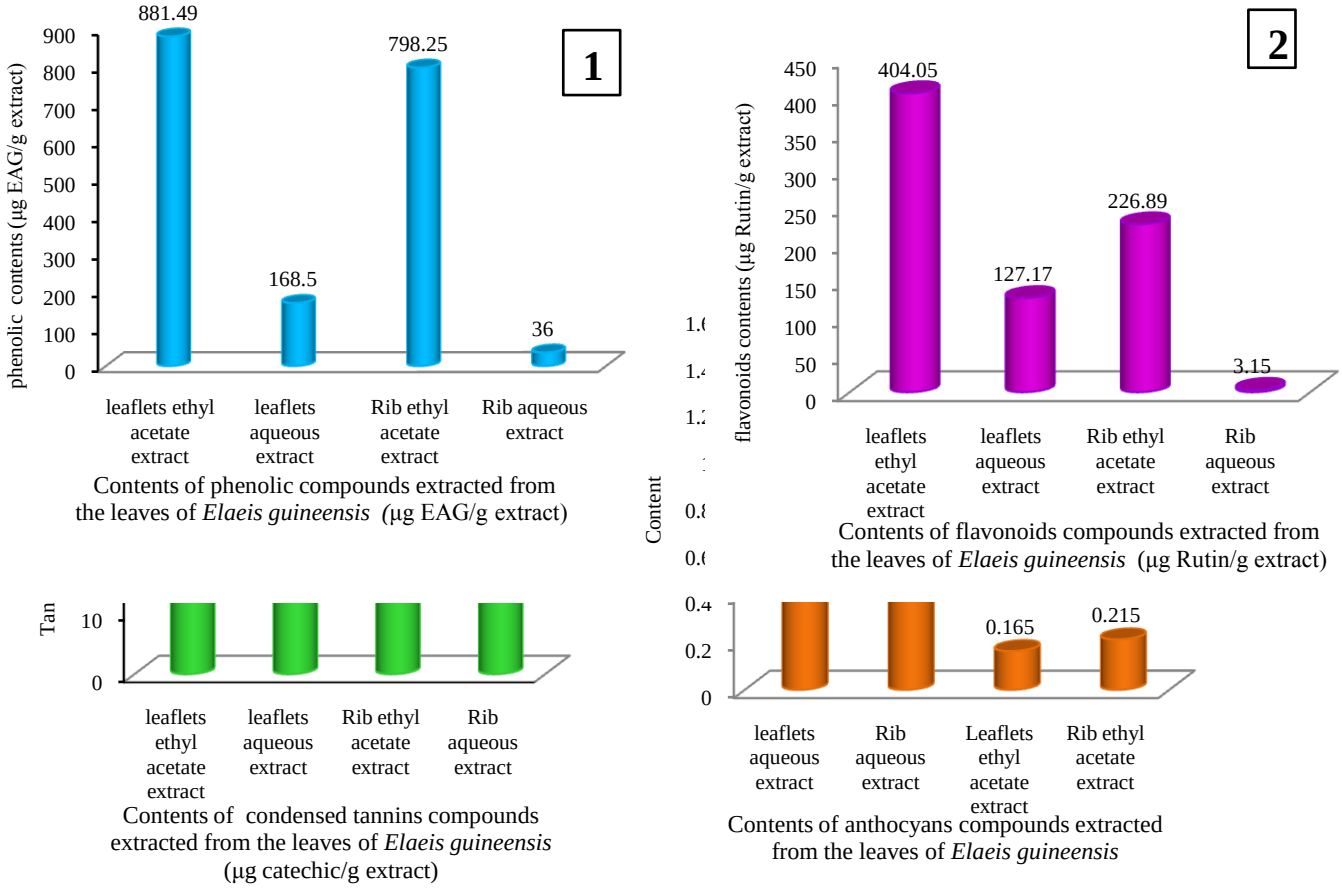

Figure 1: Contents of 1: total phenolic, 2: flavonoids, 3: condensed tannins, and 4: anthocyans extracted from the leaves of Elaeis guineensis

3.3. DPPH radical scavenging activity

Total antioxidant capacity represents both oil soluble and water soluble antioxidants that are capable of scavenging reactive oxygen species and protects from chronic diseases such as cancer, diabetics and arthritics.

By manipulating the regression equation of ascorbic acid calibration curve ($y = 0.0111x + 0.0059$, $R^2 = 0.989$), the total antioxidant capacity of each extract was calculated and expressed as ascorbic acid equivalent (EAA) to facilitate the comparison. Therefore, in this work, we calculated the total antioxidant capacity in units of mol ascorbic acid equivalents of per mg extract of extract. Results from antioxidant activity using DPPH' method revealed a wide range of total antioxidant capacity in studied. Their content ranged from 0.218 ± 0.001 to 10.344 ± 0.031 mol EAA /mg extract of extracts, with an average of 5.281 mol EAA /g extract. As shown in figure, the leaflets ethyl acetate extract had the highest antioxidant capacity (10.344 ± 0.031 mol EAA /mg extract), followed by rib ethyl acetate extract (2.046 ± 0.001 mol EAA /g extract), leaflets aqueous extract (0.758 ± 0.001 mol EAA /mg extract), and rib aqueous extract (0.218 ± 0.001 mol EAA /mg extract). Result showed significant differences ($p < 0.05$) in total antioxidant capacity among the four samples. The scavenging action of plant constituents has been found to relate to polyphenolic compounds [29, 30]. Free radicals and reactive oxygen species are involved in a variety of pathological events such as aging, inflammation, cancer, atherosclerosis, diabetes. *Elaeis guineensis* would be useful for the treatment of various diseases mediated by free radicals [31].

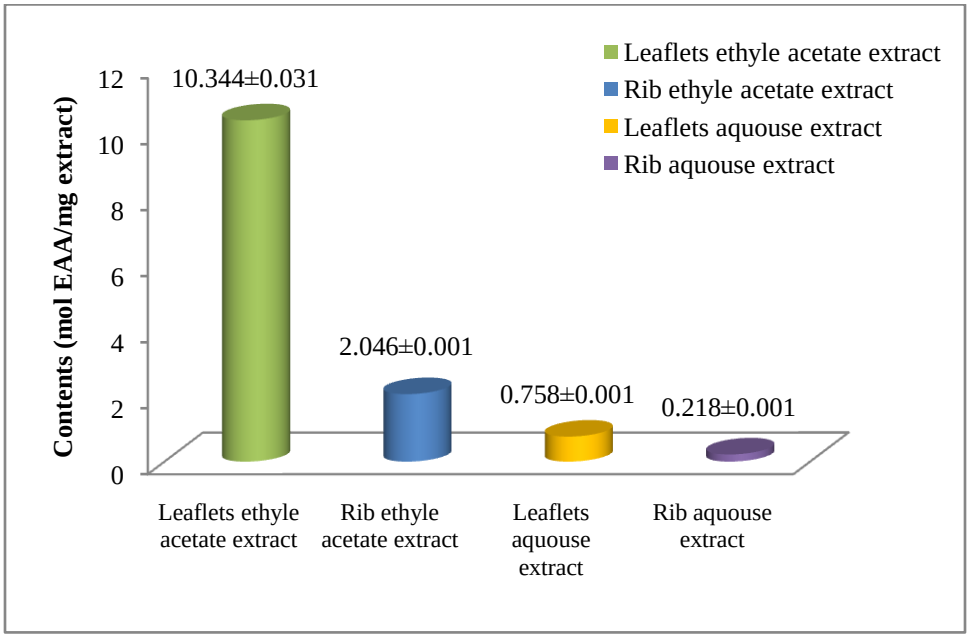

Figure 2: Total Antioxidant capacity of Elaeis guineensis

The results in this work indicate that there a modest significant linear correlation between the antioxidant capacity and the flavonoid content ($R^2 = 0.6567$; $p < 0.05$) and tannins content ($R^2 = 0.6619$) polyphenol content ($R^2 = 0.9362$; $p < 0.05$) but low linear correlation between antioxidant capacity and polyphenol content ($R^2 = 0.3824$; $p < 0.05$). There was also a poor significant linear correlation between antioxidant capacity and anthocyanin content ($R^2 = 0.0932$; $p < 0.05$). This suggests that the phenolic, flavonoids, tannins and anthocyanins compounds, involved in 38.24%, 65.67%, 66.19% and 9.32% respectively in the antioxidant capacity of different extracts of *E. guineensis*. Flavonoids and tannins are therefore at the origin of the antioxidant activity of extracts of *E. guineensis*. This is logical given that flavonoids represent the majority of polyphenol compounds. In fact, phenolic compounds, more particularly flavonoids are recognized as potentially antioxidant substances having the ability to trap the radical species and the reactive forms of oxygen, the scavenger effect of the flavonoids is attributed to their low redox potential which the makes thermodynamically able to reduce free radicals by transferring hydrogen from the hydroxyl [32].

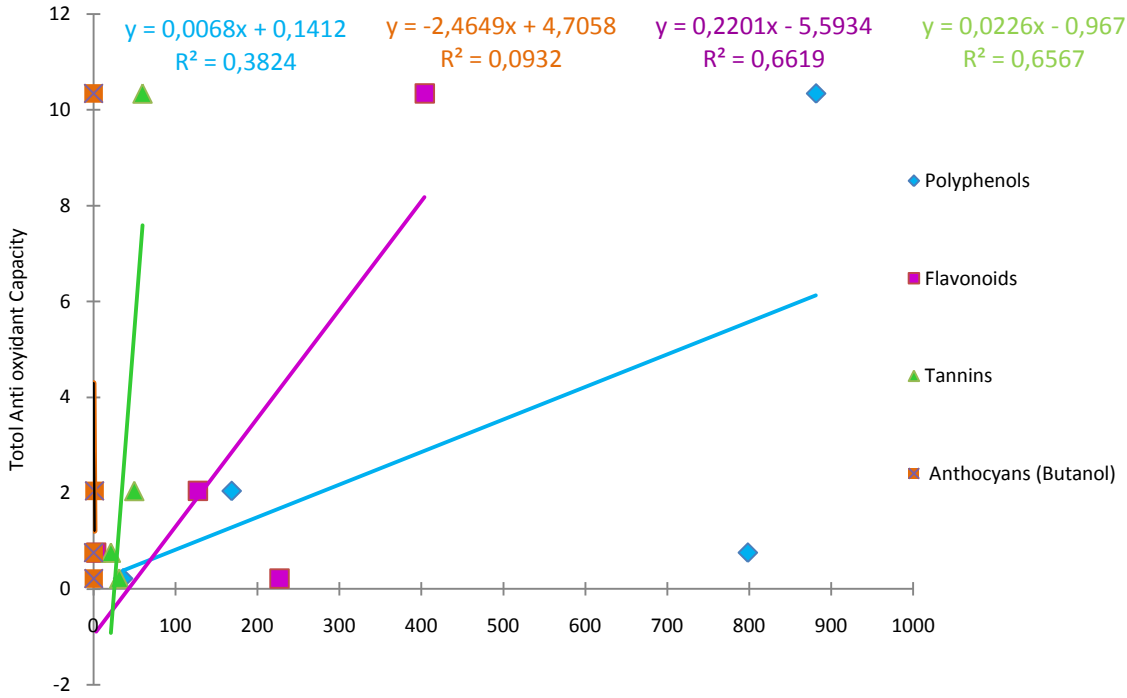

Figure 3: Correlation between Total Antioxidant Capacity and some important bioactive families compounds

4. Conclusion

This study indicates that *Elaeis guineensis* extracts have important phenolic compound, flavonoids, tannins condensed, anthocyanins and exerts significant antioxidant activity. It showed a good correlation between the flavonoids contents and antioxidant activity. However, it's important to mention that the ethyl acetate extracts gave the most interesting results. Overall, *Elaeis guineensis* would be useful as an antioxidant and free radical scavenging agent and thus help in treatment of many diseases mediated by Reactive oxygen species These findings support the traditional use of *Elaeis guineensis* leaves extracts for controlling diabetics, hypertension, antiplasmodial, and diuretic and natridiuretic proprieties.

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