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### Evaluation of the anti-radical activity of two plant extracts: *Ficus thonningii* and *Acacia nilotica*, used by the korhogo population in the north of the Ivory Coast

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#### Date Published: ABSTRACT

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#### Keywords:

Anti-Radical Activity,  
Aqueous Extract,  
Ethanol Extract,  
*Ficus Thonningii*  
*Acacia*  
*Nilotica*.

The objective of this work was to evaluate the anti-radical power of ethanolic and aqueous extracts of *Ficus thonningii* and *Acacia nilotica*, two plants widely used by the population of Korhogo, in the north of Ivory Coast. Phytochemical screening was carried out by the tube staining and precipitation method. As for the determination of total polyphenols, it was done using the colorimetric technique of Folin –Ciocalteu then using a calibration line  $Y = ax + b$ , while that of total flavonoids and tannins was determined through a spectrophotometer with absorbance read respectively at 404 nm with quercetin as standard and 500 nm (catechin as standard). Finally, the antioxidant activity carried out by the DPPH method. The results obtained revealed the presence of several secondary metabolites including polyphenols, flavonoids, anthocyanins and tannins. The 50% free radical inhibitory concentrations (IC<sub>50</sub>) of the different *Acacia nilotica* extracts were 0.0044 mg/mL for the ethanolic extract and 0.0047 mg/mL for the aqueous extract. This result is very close to that of vitamin C (0.0036 mg/mL) taken as the reference molecule, while the IC<sub>50</sub> of *Ficus thonningii* are respectively 0.0194 mg/mL and 0.083 mg/mL for the ethanolic extract. and watery. We note that the extracts have good anti-radical power but that the best remains the ethanolic and aqueous extracts of *Acacia nilotica*.

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**INTRODUCTION**

The use of plants is of particular interest in both the food and medical fields (Iffat A. et al., 2020). In the medical field, plants are considered a source of essential raw materials for the discovery of new molecules necessary for the manufacture of drugs (Doukani et al., 2017). Indeed, the active principle of medicinal plants is linked to their secondary metabolites, which are used in therapy as anti-inflammatory, antimicrobial, antiseptic, anticoagulant and antioxidant agents. Antioxidants are defenders of oxidative stress. Indeed, oxidative stress is characterized by an imbalance in the body between the antioxidant and pro-oxidant in favor of pro-oxidants, which consequently leads to the appearance of cellular damage (Morel et al., 1998; Delattre et al., 2005). Thus, the body, to protect itself against this toxic effect of oxygen, develops defense systems which make it possible to control the production of Reactive Oxygen Species (ROS). Among the regulatory agents, we can distinguish superoxide dismutases (SOD), catalase and glutathione peroxidase. In addition, protection against ROS can be achieved through exogenous intake which supports endogenous systems. This is indeed the case for medications based on vitamin C, vitamin E, and food supplements. Likewise, polyphenols, which are very widespread in the plant kingdom, are good scavengers of ROS and very excellent chelators of transition metals such as iron and copper (Aleg et al., 2007). It is for this reason that our study was mainly directed towards the quantitative research of the antioxidant activity of the leaves of *Ficus thonningii* and the grains of

*Acacia nilotica*, two plants widely used by the populations of the northern region of the Coast. Ivory. This work consists of evaluating the anti-radical power of these two plants and comparing it with that of vitamin C and deducing its interest in their use in the northern Ivorian region

**Materials and methods****Plant material**

The plant material consisting of grains of *Acacia nilotica* and leaves of *Ficus thonningii* (Moraceae) were collected between July 2024 in Korhogo, then dried for two to four weeks protected from the sun. The identification was made at the National Floristry Center of the Félix Houphouët Boigny University of Cocody. Subsequently, these plant organs were ground in a Blender (Silver Crest, Germany) to obtain powders, which were packaged in kraft envelopes and stored away from light and humidity.

**Technical equipment**

The technical equipment consists of a blinder mixer for the extractions of the active ingredients as well as an oven (Hemmert, Germany) for drying the different extracts. A "vortex mixer" (China) was used to homogenize the different substances, an electronic balance to carry out weighings, etc.

**Methods****Preparing the extracts**

200 grams of powder (leaves and seeds) were macerated in 2 liters of distilled water for 72 hours. After filtration of the mixture obtained, the solvent

is evaporated under reduced pressure at 30°C using a rotary evaporator (Buchi, Switzerland). The extract was collected dry and stored in the freezer for analyses. The production of ethanolic extracts follows the same principle, only that 96% ethanol is used instead of distilled water.

### Calculation of returns

The different yields (R) were calculated for each type of extracts studied according to the following formula: (1)

$$R = (\text{Mass of dry extract}) / (\text{Mass of vegetable powder}) \times 100 \quad (1)$$

### Phytochemical screening of plant species

The major chemical groups of the plant organs of plants used were determined according to the method of solubility, coloring and precipitation tests followed by ultraviolet ray examinations (Khaldi et al., 2012)

#### Characterization in tubes

Tests for the detection of large groups of chemical compounds focused on plant extracts. We used the analytical techniques described in the work

previous ones. The tests are carried out according to Tona et al. (1998) and Longanga et al. (2000) and Sofowara, A., 2008.

### Polyphenol detection

Polyphenols are revealed by the reaction with ferric chloride (FeCl<sub>3</sub>). To 2 mL of each solution, a drop of 2% alcoholic ferric chloride solution is added. The presence of a blue-blackish or green color, more or less dark, characterizes polyphenols.

### Detection of flavonoids

Flavonoids are detected using the sodium hydroxide reaction. To 1 mL of each solution, two to three drops of a basic sodium hydroxide solution (10% NaOH) are added. The appearance

#### Anthocyanin detection

Anthocyanins are revealed by the reaction with sulfuric acid. To 2 mL of each solution, 2 mL of 2N sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) are added. The appearance of a pink-red color, which turns purplish blue by the addition of ammonia, indicates the presence of anthocyanins.

### Detection of sterols and polyterpenes

Sterols and polyterpenes were revealed by the Liebermann reaction. An aliquot of residue is dissolved hot in 1 mL of acetic anhydride in a capsule, then taken up in a test tube into which 0.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> is poured. The appearance

a purple color that turns blue then green indicates a positive reaction.

### Tannin detection

#### Catechic tannins (condensed tannins)

Catechic tannins are revealed using the Stiasny reaction. To 2 mL of each plant extract, 6 mL of Stiasny reagent (30% formalin/concentrated HCl) are added. The mixture is brought to a water bath at 80°C for 30 minutes then cooled. The observation of large light brown flakes characterizes the presence of catechic tannins.

#### Gallic tannins (hydrolyzable tannins)

To detect gallic tannins, the solution used to detect gallic tannins is filtered to eliminate large flakes. Subsequently, the filtrate is saturated with sodium acetate (CH<sub>3</sub>COONa), then a drop of 2% alcoholic ferric chloride solution is added. The positive reaction results in the appearance of an intense blue-black color, which characterizes gallic tannins.

### Detection of saponosides

To detect saponosides, 10 mL of hot water was added to each plant residue contained in a Petri dish, after evaporation. The solutions collected in test tubes are shaken vigorously for 15 seconds then left to stand for 15 minutes. The appearance of

persistent foam, greater than 1 cm in height, indicates the presence of saponosides.

### Coumarin detection

To 2 mL of each extract divided into 2 test tubes, 1.5 mL of 10% NaOH are added to one of the tubes then the tubes are heated in a water bath until boiling. After cooling, 4 mL of distilled water is added to each test tube. The reaction is positive when the tube where the alkaline solution was added is transparent or more transparent compared to the control test tube (without alkaline solution).

To confirm the presence of coumarins, the extract solutions to be investigated were prepared by dissolving 10 mg of extract in 4 mL of hot distilled water. The solution obtained is transferred into two test tubes. The first served as a control while 0.5 mL of 10% titrated ammonia solution was added to the second tube. The latter are observed under the ultraviolet (UV) lamp at 365 nm (Ciulei, 1982). The appearance of blue or green fluorescence indicates the presence of coumarins.

### Alkaloid detection

A volume of 9 mL of each plant extract collected in a Petri dish is evaporated to dryness. The residue is then taken up in 9 mL of 60% alcohol and the alcoholic solution thus obtained is divided into two test tubes. In the first tube, two drops of Dragendorff reagent (potassium iodobismutate reagent) are added. The appearance of a precipitate or an orange color indicates the presence of alkaloids. The second tube served as a control.

### Methods for determining total polyphenols from the extracts under study

The quantity of total polyphenols was determined following the Folin-Ciocalteu colorimetric method reported by Singleton et al. (1999), taken up and modified by N'gaman (2013).

To 1 mL of extract diluted 1/10 with distilled water, 1.5 mL of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) (17%, m/v) and 0.5 mL of Folin reagent were added. -Ciocalter

(0.5N). The reaction mass was incubated at 37°C for 30 min; the absorbance was read at 760 nm against a blank without extract, taken as a reference. The quantification of total polyphenols was carried out according to a linear calibration line ( $y = ax + b$ ) carried out with a standard solution, gallic acid at different concentrations (0 to 1000  $\mu\text{g/mL}$ ) in the same conditions as the sample. The results were expressed in microgram of gallic acid equivalent per gram of dry matter ( $\mu\text{g EAG/g DM}$ ) of the powdered organ. The total polyphenol content (Q) is calculated according to the following formula (2):

$$Q (\mu \text{ EAG/ g dry matter g}) = V \times C \times d / m \quad (2)$$

C: extract concentration ( $\mu\text{g/mL}$ ); V: volume of the mixture analyzed (mL), d: dilution factor, m: mass of the dry matter of the analysis sample (g).

Dosage of total flavonoids of the extracts of the extracts under study

The determination of total flavonoids was carried out according to the modified method of Hariri et al. (1991). Two mL of each organ powder extract was diluted 1:10 and mixed with 100  $\mu\text{L}$  of sodium nitrate reagent. The absorbance was read at 404 nm with quercetol taken as standard (0.05  $\text{mg/mL}$ ), diluted under the same conditions and treated with the same quantity of reagent. The percentage of total flavonoids was calculated in quercetol equivalent according to the following formula (3):

$$F (\%) = (0.05 \times A_{\text{ext}} / A_{\text{q}}) / C_{\text{ext}} \times d \times 100 \quad (3)$$

A ext: absorbance of the sample; Aq: absorbance of quercetin; d: dilution factor; C ext: sample concentration ( $\text{mg/mL}$ ).

### Dosage of condensed tannins from the extracts studied

The determination of condensed tannins in the different extracts was carried out according to the method described by Heimler et al. (2006). For 400  $\mu\text{L}$  of each sample or standard, 3 mL of a 4% methanolic vanillin solution and 1.5 mL of concentrated hydrochloric acid are added. The

mixture is incubated for 15 min and the absorbance is read at 500 nm. The concentrations of condensed tannins are deduced from the calibration ranges established with catechin and are expressed in mg of catechin equivalent per g of dry matter (mgEC/g DM), formula (4)

### Evaluation of the antioxidant activity of the extracts under study

This test aims to highlight the in vitro antioxidant effects of extracts from selected plant species by determining its anti-radical properties in the presence of 2,2-diphenyl 1-picrylhydrazyl (DPPH). The evaluation of the antioxidant potential of the substances was carried out following the method of Blois (1958) and modified by Dosseh et al., (2014). DPPH was solubilized in absolute ethanol to obtain a solution with a concentration of 0.3 mg/mL. Different concentration ranges (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL) of the aqueous extracts of the selected plant species were prepared in absolute ethanol. 2.5 mL of extract and 1 mL of ethanolic DPPH solution were introduced into dry and sterile tubes. After shaking, the tubes were placed away from light for 30 min. The absorbance of the mixture was then measured at 517 nm against a blank consisting of 2.5 mL of pure ethanol and 1 mL of DPPH solution. The reference positive control is ascorbic acid (vitamin C) (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL ).

The percentage of DPPH inhibition was calculated according to the following formula (5):

$$\% \text{ inhibition of DPPH} = 1 - (100 \times (\text{Absorbance extract})/(\text{Absorbance control})) \quad (5)$$

The 50% inhibitory concentrations (IC<sub>50</sub>) of the aqueous extracts and vitamin C were determined by the graphic method. This experiment was repeated 3 times.

The antioxidant capacity of the extracts, expressed in mg of Trolox equivalent per gram of dry matter, (mg of TE. g-1 of DM) was determined according to formula (6) proposed by Wangcharoen and Morasuk (2007):

$$\text{DPPH value (mg of TE.g-1 of dry matter)} = [((\text{Absorbance extract} - \text{Absorbance control})/\text{Slope}) \times (V/v) / (m) \times (1000)] \times 1 \quad (6)$$

Slope = slope of the equation line expressing the absorbance values as a function of trolox concentrations.

V = Total volume of extract prepared to obtain the stock solution.

v = Volume of extract for the test.

m = mass of sample taken to prepare the stock solution.

r = yield: TE = Trolox equivalent

### Results

#### 1. Yield of the extracts produced

Plants Yield %	Aqueous Extract	Ethanol Extract
<i>Ficus thonningii</i>	15.80±1.36	10.20±1.26
<i>Acacia nolitica</i>	16.30±1.04	12.30±1.42

## 2 . Qualitative phytochemistry

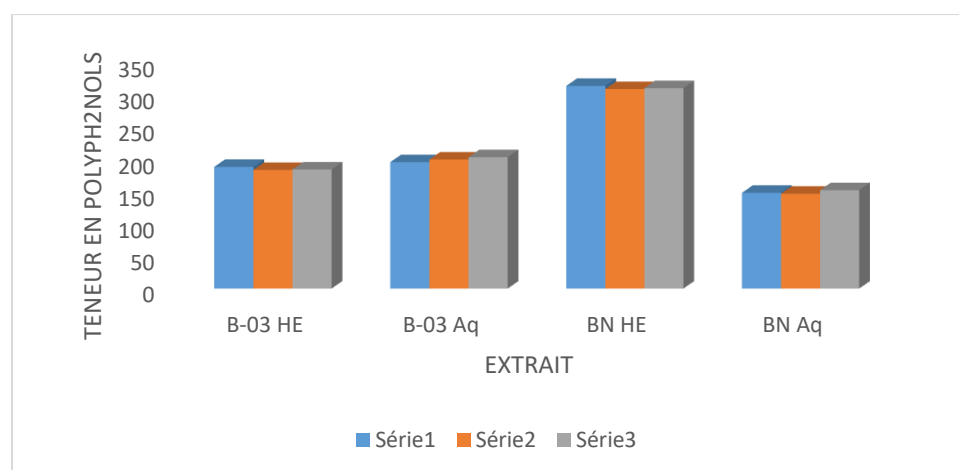
Métabolites secondaires	Ficus thonningii (Bn)		Acacia nolítica (B03)	
	Extrait Aqueux (Aq)	Extrait Ethanologique	Extrait Aqueux	Extrait Ethanologique (HE)
<b>Polyphénols</b>	+	+	+	+
<b>Flavonoïdes</b>	+	+	+	+
<b>Tanin C</b>	–	–	+	+
<b>Tanin G</b>	+	+	+	+
<b>Stérols&amp;Terpenoides</b>	+	+	+	+
<b>Quinolones</b>	+	+	+	+
<b>alcaloides</b>	+	+	+	+
<b>Coumarine</b>	+	+	+	+
<b>Saponosides</b>	+	–	+	–
<b>Anthocyanes</b>	+	+	+	+

Absence (–) ; Presence (+)

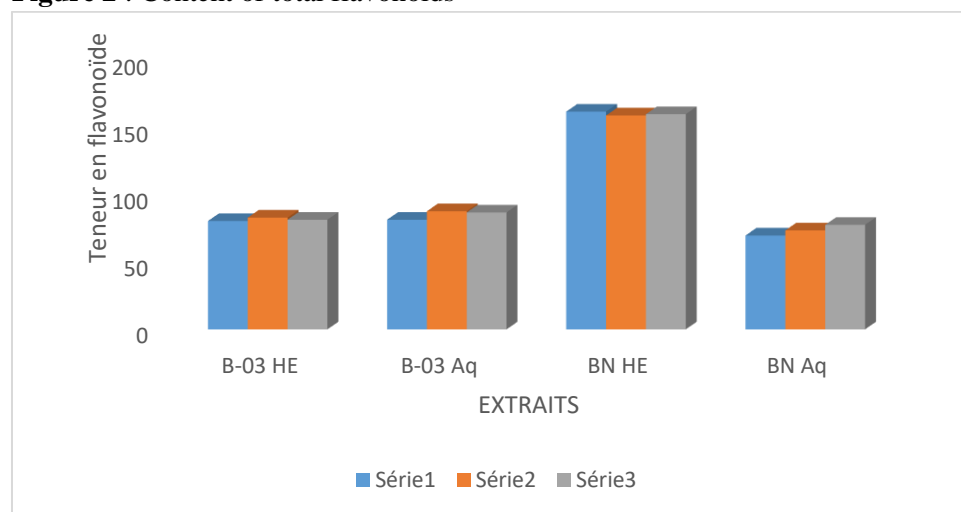
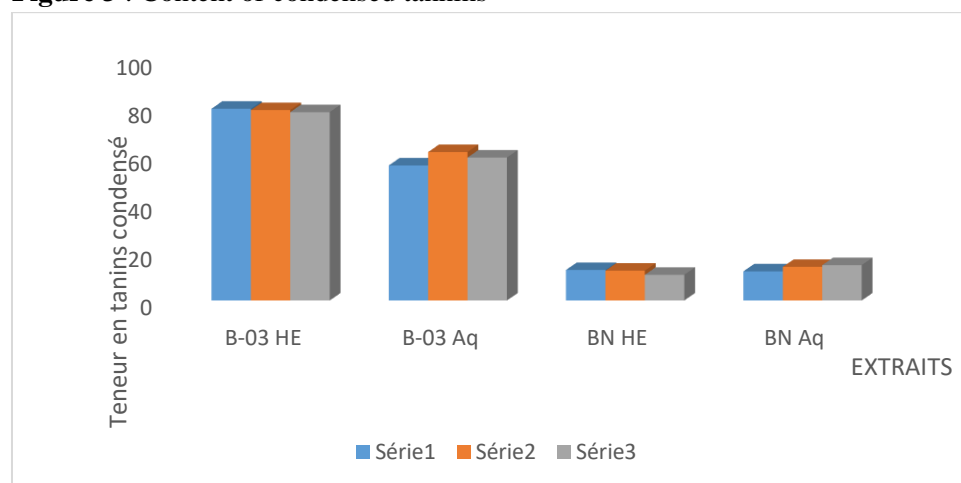
## 3. Phytochimie quantitative

Teneurs en moyennes				
Extraits	Organe	Polyphénols ± ET (mg EGA/gde MS)	Flavonoïdes ± ET (mg EGA/gde MS)	Tanins ± ET (mg EGA/gde MS)
<b>B-03 HE</b>	<b>Graines</b>	185,77±2,57	81,68±1,28	79,40±0,72
<b>B-03 aqueux</b>		199,98±3,86	85,37±3,41	59,35±2,81
<b>Bn HE</b>		311,53±2,55	160,24±1,45	11,98±1,09
<b>Bn aqueux</b>	<b>Feuilles</b>	149,43±2,86	73,66±3,96	13,66±1,33

**Figure 1** : Content of total polyphenols



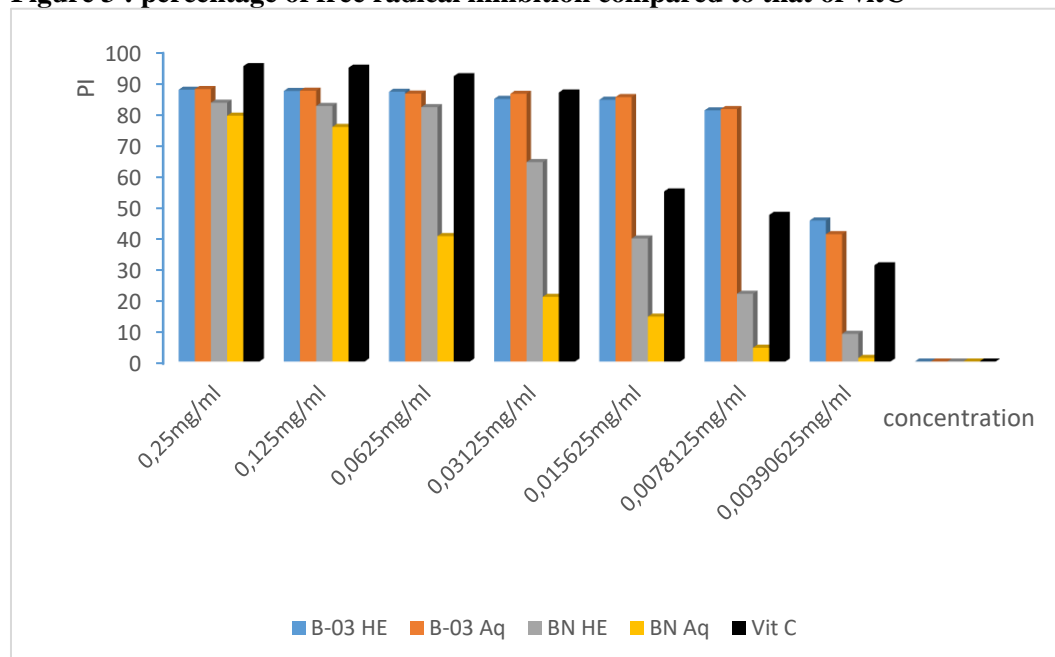


**Figure 2 :** Content of total flavonoids**Figure 3 :** Content of condensed tannins

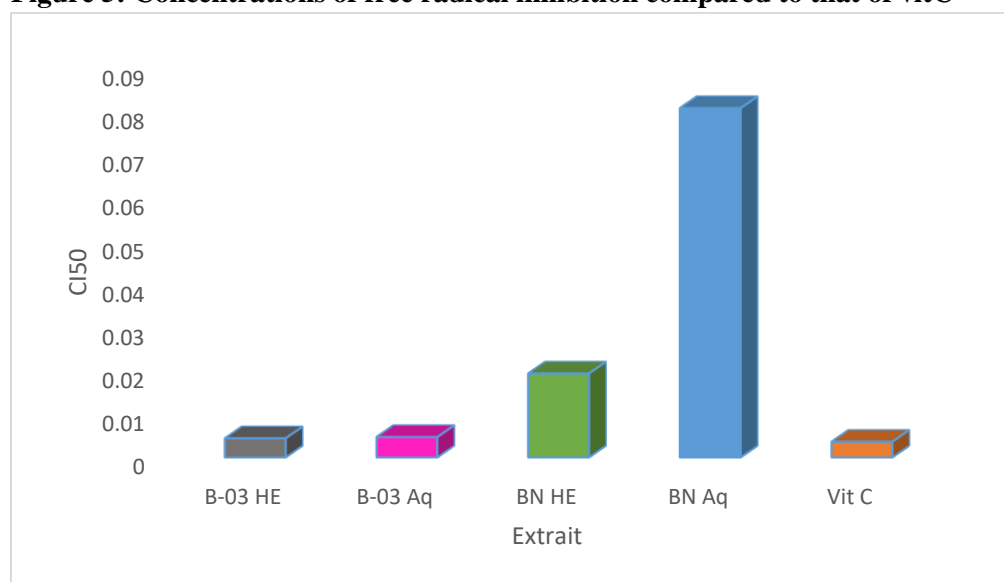
#### 4. inhibition of free radicals

E spèces végétales utilisées et composés de références	Organe	Extraits	% d'inhibition du radical DPPH	CI <sub>50</sub> (mg/mL)	R <sup>2</sup>
Vitamine C	—	—	71.53±26.58	0,0036	0,9289
	Graines	B-03	79.73±15.33	0,0044	1
	Graines	HE			
	Feuilles	B-03 Aq	79.19±16.99	0,0047	1
	Feuilles	Bn HE	54.53±31.10	0,0194	0,9903
		Bn Aq	31.68±33.86	0,083	0,9794

**Figure 3 : percentage of free radical inhibition compared to that of vitC**



**Figure 5: Concentrations of free radical inhibition compared to that of vitC**



The yields of the aqueous extracts of the two plants used are higher than those of the ethanolic extracts. Thus, we obtain  $15.80 \pm 1.36$  and  $16.30 \pm 1.04$  for the aqueous extracts of *Ficus thonningii* and *Acacia nilotica* respectively. While we obtain respectively for the ethanolic extract of *Ficus thonningii*  $10.20 \pm 1.26$  and  $12.30 \pm 1.42$  for *Acacia nilotica* (Table 1).

Qualitative phytochemistry revealed the presence of polyphenols, favonoids, steroids and terpenoids, quinolones, alkaloids, coumarins, and anthocyanins both in the aqueous and ethanolic extracts of the plants with the exception of cathectic tannins in the aqueous and ethanolic extract of *Ficus thonningii*. Also the saponosides were not present in the ethanolic extract of *Ficus thonningii*, nor in the



ethanolic extract of *Acacia nilotica*. But present watery aqueous of the two plant extracts (Table 2).

As for quantitative phytochemistry, the dosage of total polyphenols revealed that the ethanolic extract of *Ficus thonningii* contains a large quantity of total phenols with  $311.53 \pm 2.55$  mg EGA/ g of dry matter (DM), unlike the ethanolic extract of *Acacia nilotica* which is  $185.77 \pm 2.57$  mg EGA/g of dry matter. However, the aqueous extract of *Acacia nilotica* contains a significant quantity of total phenols than the aqueous extract of the leaves of *Ficus thonningii* respectively  $199.98 \pm 3.86$  and  $149.43 \pm 2.68$  mg EGA/g of Material dry (Table 3). Regarding total flavonoids, the quantities are similar to those of the total phenols of the different ethanolic extracts of the leaves of *Ficus thonningii* containing the largest quantity with  $160; 24 \pm 1.45$  mg EGA / mg DM. While the ethanolic extract of *Acacia nilotica* grains contains only  $81.68 \pm 1.28$  mg EGA / mg of DM. As for the aqueous extracts of the two organs, that of *Acacia nilotica* concentrates the most flavonoids with  $85.37 \pm 3.41$  mg EGA / mg of DM compared to  $73.66 \pm 3.96$  mg EGA / mg of DM (Table 3).

The tannins are more concentrated in the ethanolic extracts of *Acacia nilotica* ( $79.40 \pm 0.72$  mg EGA / mg of DM) unlike the extracts of *Ficus thonningii* which contains a small quantity ( $11.66 \pm 1.33$  mg EGA / mg of DM). The aqueous extracts of both plants contain small quantities with respectively  $13.66 \pm 1.33$  mg EGA / mg of DM.

And  $59.35 \pm 2.81$  mg EGA/mg DM for *Ficus thonningii* and *Acacia nilotica* (Table 3). The dosage of the DPPH radical showed a higher IC<sub>50</sub> (Concentration for 50% inhibition of free radicals) for *Acacia nilotica* extracts which is 0.0044 mg/ml and 0.0047 mg/ml respectively for the ethanolic and aqueous extract. The extracts of *Ficus nilotica* recorded 0.0194 mg/ mL and 0.083 MG/ mL (Table 4), while the reference molecule, vitamin C, recorded 0.0036 mg/ mL.

## Discussion

The extraction yields obtained differ from each other. As for the ethanolic extract, it is the grains of *Acacia nilotica* which give the best yield, unlike the leaf extracts of *Ficus thonningii*. This high yield of extracts means that the ethanolic extract best concentrates the active ingredients of this plant. In other words, the secondary metabolites would be more soluble in

ethanol than in water. We could also say that this difference would undoubtedly be linked to the nature of the organ of the plant used.

Furthermore, the different extracts of the two plants studied showed their richness in secondary metabolites at different proportions. Thus, through the literature, we note that these metabolites are real catalysts or agents for maintaining the functional balance of a human organism (Alhazmi et al., 2021). This is what would justify the use of certain plants as medicine by h Of the two plants used, only the ethanolic extract of *Acacia nilotica* grains gave an IC<sub>50</sub> (Inhibition concentration for 50% of free radicals) which is close to that of the reference molecule which is vitamin C. indeed the reducing power of this ethanolic extract of *Acacia nilotica* grains would probably be due to the presence of hydroxyl groups of the phenolic compounds found there and which can serve as an electron donor (Al farsi et al., 2005). The anti-radical activity of the ethanolic extract of *Acacia nilotica* could be due to the anthocyanins which act by preventing the polymerization of deoxyhemoglobin S molecules into tactoids (Gbolo et al., 2022).

In addition to the polyphenols, the Flavonoids present could play a significant role in the expression of the antioxidant power of the ethanolic extract of *Acacia nilotica*. Indeed, Flavonoids are characterized by their affinity for trapping nucleic acids and metal ions, as well as their electron transfer capacity to trap free radicals (Kapepala, 2017).

## Conclusion

Considering the richness of the extracts studied in secondary metabolites and the anti-radical power of the two plants studied, the populations of Korhogo, a town located in the north of Ivory Coast have good reasons to use it in their diet, under form of sauce or infusion as a pick-me-up. However, the ethanolic extract of *Acacia nilotica* presented the best antioxidant power unlike *Ficus thonningii*. Thus, it would be interesting to carry out a molecular characterization study on this extract and deduce the structures of the phytomolecules responsible for the anti-radical activity.

## Data availability.

On reasonable request, the datasets will be made available to the interested party.

## Conflicts of interest.

The authors declare no conflicts of interest before and during the research.

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