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Evaluation of the Antioxidant Activity and Dosage of Polyphenols in Aqueous, Hydroethanolic and Hexane Extracts of the Bark of *Spathodea campanulata* P. Beauv. (Bignoniaceae)

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A B S T R A C T

The present study focused on the evaluation of the antioxidant properties and the determination of total phenols and flavonoids *Spathodea campanulata* P. Beauv by spectrophotometry. Quantitative analyzes of total phenols and flavonoids were determined from the linear regression equation of the calibration curve, that is plotted using gallic acid and quercetin respectively as standard. The highest content of phenols was measured in the hydro-ethanolic extract, with a value equal to 10.17 mgGAE/g DE. Likewise, the determination of the flavonoids revealed that the hydroethanolic extract contains a maximum of flavonoids, with a level of 22.43 mg EQ / g DE. The antioxidant properties were evaluated by two tests, namely the diphenyl-picryl-hydrazyl radical scavenging test (DPPH) and the reducing power test (FRAP) in comparison with ascorbic acid and BHT respectively used as reference molecules. The results obtained show that the antioxidant activity of the hydroethanolic extract is greater with the two tests than those of the aqueous and hexane extracts.

Introduction

The use of synthetic antioxidant molecules is currently being questioned because of the potential toxicological risks they present. Today, new molecules of plant origin (natural antioxidant) are being sought (Suhaj, 2006). The study of plant chemistry is still relevant today because the plant kingdom today represents an important and unavoidable

source in the discovery of a huge variety of new bioactive molecules (Harrar, 2012). Several scientific studies have shown that different secondary metabolites of these plants have been isolated and are used as therapeutic molecules (Huang *et al.,* 2005). Among these metabolites are the phenolic compounds which are gaining in importance thanks to their beneficial effects on health. They are also participating in the protection of plants against various attacks (Bruneton, 1999). The Ivorian flora is characterized by numerous medicinal plants which is the single most used element, especially in rural areas, to solve public health problems. According to the National Program for the Promotion of Traditional Medicine of Côte d'Ivoire, 1421 species of medicinal plants involved in traditional medicine and allowing patient care, have been identified to date by Ivorian researchers.

Also *Spathodea campanulata*, a plant with multiple therapeutic virtues is used in traditional medicine. Stem bark preparations are used for enemas, to treat fungal skin diseases, herpes, stomach aches, diarrhea; antimalarial activities have also been observed in stem bark extracts (Jardim *et al.,* 2003; Niyonzima *et al.,* 1999; Rangasamy Dhanabalan *et al.,* 2008). Several phytochemical studies have been performed with different parts of *Spathodea campanulata*, namely the bark of the stem, the leaves, the flowers and the fruits (Amusan *et al.,* 1996).

The leaves provided spathodol, caffeic acid, other phenolic acids and flavonoids showed the presence of anthocyanins in the flowers of *Spathodeae campanulata* (El-Hela, 2001a; El-Hela, 2001b; Banerjee et DE 2001). This work is concerned with the evaluation of the antioxidant activity and the quantitative dosage of the phenolic compounds of the aqueous, hydroethanolic and hexane extracts of the bark of *Spathodea campanulata*.

Materials and Methods

Plant Material

The plant material is composed of the bark of *Spathodea campanulata*. These barks were cut up and dried out of direct sunlight at room temperature (25-30 ° C) for four weeks, before being reduced to a fine powder by grinding using a mechanical grinder. Then stored in glass bottles protected from light and humidity for their use.

Chemical material

In order to extract the compounds, we used 70°ethanol and distilled water. For evaluating the antioxidant properties, Folin-Ciocalteur reagent, methanol, sodium carbonate solution, gallic acid, potassium acetate, sodium phosphate, potassium ferric cyanide, trichloroacetic acid, Ferric chloride, aluminum chloride and 2,2-diphenyl-picrylhydrazyl (DPPH) and butylhydroxytoluene (BHT) were used.

Methods of preparing extracts

The preparation of the total aqueous, hydroethanolic and hexane extracts was carried out according to the method of Zirihi *et al.,* (2003), by homogenization using a mixer. The extracts were obtained from 100 g of powder and 1 L of solvent which was homogenized in a mixer. After 15 min of homogenization, the obtained homogenate was collected in a square of white (clean) tissue and pressed by hand using strong pressure applications. The collected solution was filtered twice through cotton wool and then through Whatman 3 mm filter paper.

Aliquots of the filtrate were placed in a dryer at 40 ° C for 48 hours for the aqueous extract and at 50 ° C for 24 hours for the hydroethanolic and hexane extract.

Evaluation of the content of phenolic compounds

Determination of total phenols

The total phenol content was determined in the extracts of *Spathodea campanulata* according to the method described by Mc Donald *et al.*, (2001) using the Folin-ciocalteu reagent. To 0.5 mL of the extract, (0.1 g / mL), 5 mL of the Folin-ciocalteu reagent which is diluted to 1 / 10th with distilled water and 4 mL of sodium carbonate (1M) are respectively added.

After 15 min of incubation at room temperature, the optical density is measured with a spectrophotometer at 765 nm. Gallic acid prepared in a (50/50, v / v) methanol / water solvent mixture is used as a standard at concentrations ranging from 0 to 250 mg / L. The total phenol content of the various extracts is expressed in terms of gallic acid equivalents per gram of extract (mg GAE / g).

Dosage of total flavonoids

The quantification of flavonoids was done by a colorimetric method based on the formation between aluminum chloride and the oxygen atoms present on the 4 and 5 carbons of the flavonoids (Lagnika, 2005). The protocol used is based on that described by (Meda *et al.*, 2005) with some modifications. 0.5 mL of distilled water, 0.5 mL of aluminum chloride, 0.5 mL of potassium acetate and 2 mL of distilled water are successively added to a volume of 0.5 mL of methanolic extracts.

The resulting solution is left to stand for 30 min in the dark and the optical density is read at 415 nm against the blank. A calibration range is carried out under the same conditions as the test using a quercetin stock solution at 0.01 mg / mL to determine the flavonoid content of the sample. The flavonoid contents of the extracts are expressed in mg quercetin equivalents per gram of extract (mg EQ / g of extract).

In Vitro evaluation of the antioxidant activity of aqueous, hydroethanolic and hexane extracts of *Spathodea campanulata*

Free Radical Scavenging Activity

Hydrogen atom or electron donating abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picryl hydrazyl (DPPH). This spectrophotometric assay uses the stable free radical, DPPH as a reagent (Parejo *et al.*, 2000).

Different concentrations of each extract were added, at an equal volume, to methanolic solution of DPPH (100 µL). After 30 min at room temperature, the absorbance was recorded at 517 nm. Test was repeated for three times. Vitamin C was used as standard control. The DPPH radical scavenging effect was calculated as inhibition of percentage (I%) using the following formula: I% = (A Blank-A Sample / A Blank); A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

The values of inhibition were calculated for concentrations of the extract. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Chemicals Reagents All chemicals used were of analytical grade. Methanol, aluminum chloride, potassium acetate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferrous chloride, ferrozine, potassium ferricyanide, Folin-ciocalteu reagent, standards such as Ascorbic acid, ethylene diaminetetra acetic acid (EDTA), gallic acid, quercetin all from Sigma Chemicals Co. (St. Louis, MO, USA).

Reducing power measurement (FRAP)

Principle

The FRAP method is based on the reduction of ferric ion (Fe3 +) to ferrous ion (Fe2 +). This method evaluates the reducing power of the compounds. The presence of reducing agents (HA) in plant extracts causes the reduction of Fe3 + / ferricyanide complex to the ferrous form.

Therefore, Fe2 + can be evaluated by measuring and monitoring the increase in the density of the cyan blue color in the reaction medium at 700 nm (Chung *et al.,* 2002).This method measures the reducing power of antioxidants present in a mixture by their ability to reduce ferric tripyridyltriazine (Fe3 + -TPTZ) to ferrous ion (Fe2 + -TPTZ) at acidic pH.

According to the method described by Yildirim *et al.,* (2001). 2 mL of extracts were mixed with 2.5 mL of sodium phosphate buffer solution (0.2 M; pH 6.6) and 2.5 mL of potassium ferric cyanide (1%).

The mixture was incubated at 50 ° C for 30 min, then 2.5 mL of trichloroacetic acid (10%) was added to the mixture and the resulting solution was centrifuged at 2000g for 10 min. To a volume of 2.5 mL of the supernatant is added respectively 2.5 mL of deionized water and 0.5 mL of ferric chloride, then the absorbance was measured with a spectrophotometer at 700 nm.

Statistical Analysis

The statistical analysis was performed by Graph Pad Prism 6 statistical software. Results are expressed as mean ± SD and analyzed by ANOVA and Tukey tests with univariate rate determination of significance with $P \leq 0.001$ considered statistically significant.

Results and Discussion

Determination of total phenolic compounds present in aqueous, hydroethanolic and hexane extracts of *Spathodea campanulata*

The quantitative analyzes of total phenols and total flavonoids were determined from the equation of the linear regression of the calibration curve y = 8.1544x +00; R2 = 0.9977 and y = 1.357x-0.0181; R2 = 0.9976, plotted using gallic acid and quercetin as standard respectively. The values obtained are expressed in mg GAE / g DE and in mg EA / g DE (Figures 1 and 2).

For this study, three solvents were used with the powder and the bark of *Spathodea campanulata*, namely distilled water, hydro-ethanol and hexane, which made it possible to obtain three extracts: the aqueous extract, the hydroethanolic extract and the hexane extract.

First of all, as for the determination of total phenols, it can be observed a blue color after adding Folin-Ciocalteu reagent, which confirms the presence of total phenols in the various extracts of *S. campanulata*. The highest content of phenols was found in the hydro-ethanolic extract, with a value equal to 10.17 mg GAE / g DE, followed by the hexane and aqueous extracts respectively 7.64 and 4.44 mg GAE / g DE. To our knowledge, no quantitative studies have been carried out on extracts from the bark of the plant, however Umenwa *et al.,* (2017) in Nigeria, have noted the presence of polyphenols in the methanolic fractions of the leaves of S. campanulata. This is because the contents of phenolic compounds vary qualitatively and quantitatively in the same plant as well as from one plant to another, and this can be explained by the origin of the plant, by the method of extraction (Djeridane *et al.,* 2013). The polyphenolic profile of plant extracts can vary under the influence of several factors,

among which are the variety, the geographical location, the climatic conditions of growth, and the stage of maturity of the plant according to Anusuya et Manian, (2013), likewise, the different diseases that can affect the plant (Perron et Brumaghim, 2009).

The highest content of phenols was measured in the hydro-ethanolic extract, hexane and aqueous extracts. The current results are in agreement with those of Conde *et al.,* (2009)who have shown that the polyphenolic profile of extracts from the same plant can vary according to the type and polarity of the extraction solvent as well as the analysis techniques and the substrate employed(Zhao *et al.,* 2007). According to Alonso *et al.,* (2007), light increases the biosynthesis of phenolic compounds that accumulate in plant cells. In addition, the extraction time factor is very important, since a long time increases the possibility of oxidation of phenolic compounds, according to (Naczk et Shahbi, 2004). In addition, undesirable reactions such as enzymatic oxidation and polymerization could be fostered by a prolonged extraction time.

Furthermore, the quantitative estimate of total flavonoids by the aluminum trichloride method shows that the hydroethanolic extract contains a maximum of flavonoids, with a rate of 22.43 mg EQ / g DE compared to the hexane and aqueous extracts. Results for total flavonoid content vary widely amongst different extracts.

The concentration of flavonoids in plant extracts depends on the polarity of the solvents used in the preparation of the extracts (Pedneault *et al.,* 2001). Recent studies have shown that extrinsic factors (such as geographical and climatic factors), genetic factors, but also the degree of maturation of the plant and the shelf life have a strong influence on the content of polyphenols and flavonoids (Fiorucci, 2006). In addition, Katalinic *et al.,* (2010)work confirms our results by indicating that ethanol allows a better extraction of total polyphenols. This largely explains the richness of the hydro-ethanolic extract of the bark of *S. campanulata* in polyphenols compared to other extracts. It is the same for the content of flavonoids.

In addition, the antioxidant activity of aqueous, hydro-ethanolic and hexane extracts of the bark of *Spathodea campanulata* was also studied using two methods based on the ability of compounds to scavenge synthetic free radicals (DPPH), generating free radicals, and to test the reducing power of different extracts. In fact, the DPPH test is one of the most widely used tests to determine the anti-free radical activity of plant extracts (Laguerre *et al.,* 2007). The results of anti-free radical activity revealed that aqueous, hydro-ethanolic and hexane extracts inhibited DPPH by 40%, 77% and 60%, respectively. The hydro-ethanolic extract has good anti-free radical activity with an IC50 of 30.20 ± 0.40 (µg / mL) close to that of vitamin C and clearly superior to those of Umenwa *et al.,* 2017who found an IC50 of 178, 46 µg / mL with the hexane fraction of the leaves of *S. campanulata*. Similarly, by observing the results of the iron reducing power test of our various extracts, it is clear that the hydroethanolic extract also has the highest reducing power, this is explained by the high content of phenolic compounds in *S. campanulata*. According to Singh *et al.,* (2006) reductones are compounds with strong reducing power and are able to reduce ferric iron (Fe3 +), give up electrons and transform active free radicals into stable products. The antioxidant activity of *S. campanulata* extracts. According to the reducing power test, it is revealed that the latter have an important activity. The evaluation of antioxidant activity in vitro has shown that our extracts are able to reduce DPPH free radicals and iron.

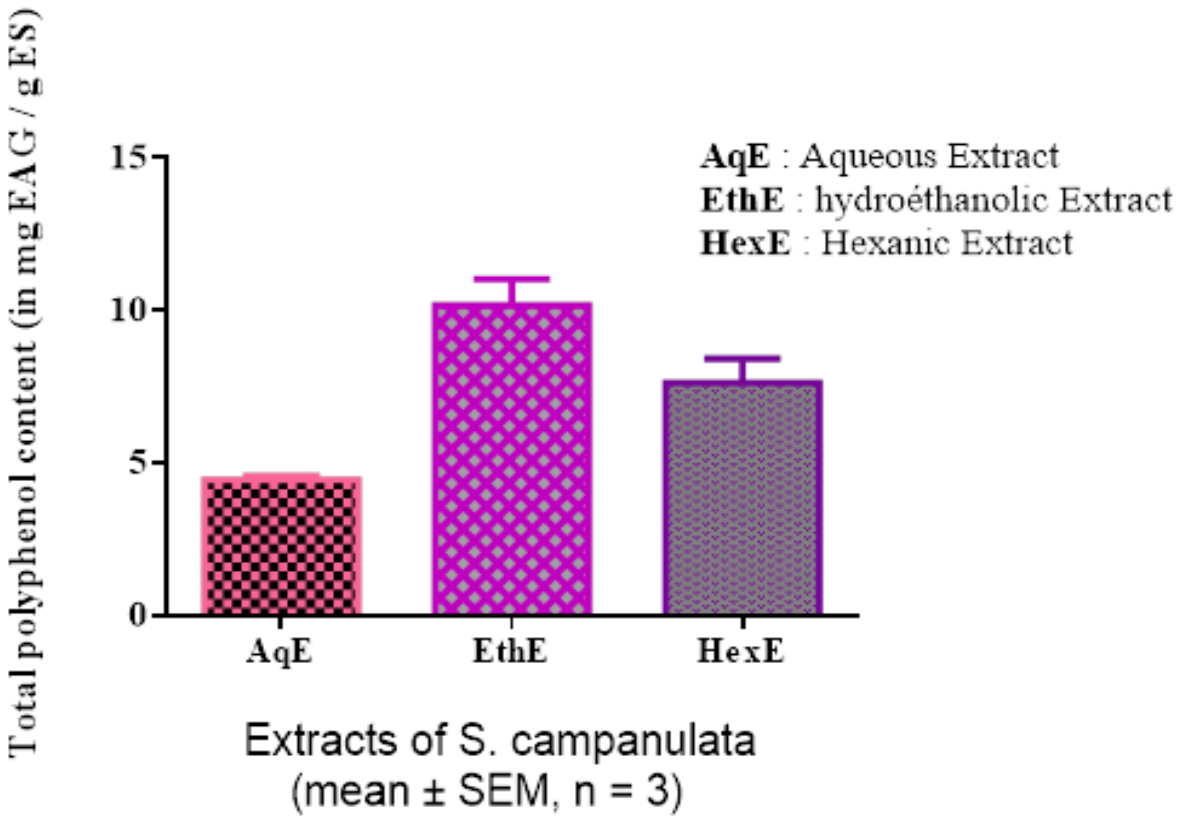
Table.1 Content values of total phenols and flavonoids present in extracts of *S. campanulata.*

Extracts	Total Phénols (mg/EAG/g d'ES)	Total Flavonids (mg EQ/g d'ES)
EAq	4,44±0,07	7,23±0,13
EEth	10,17±0,49	22,43±0,55
EHex	7,64±0,45	8,52±0,62

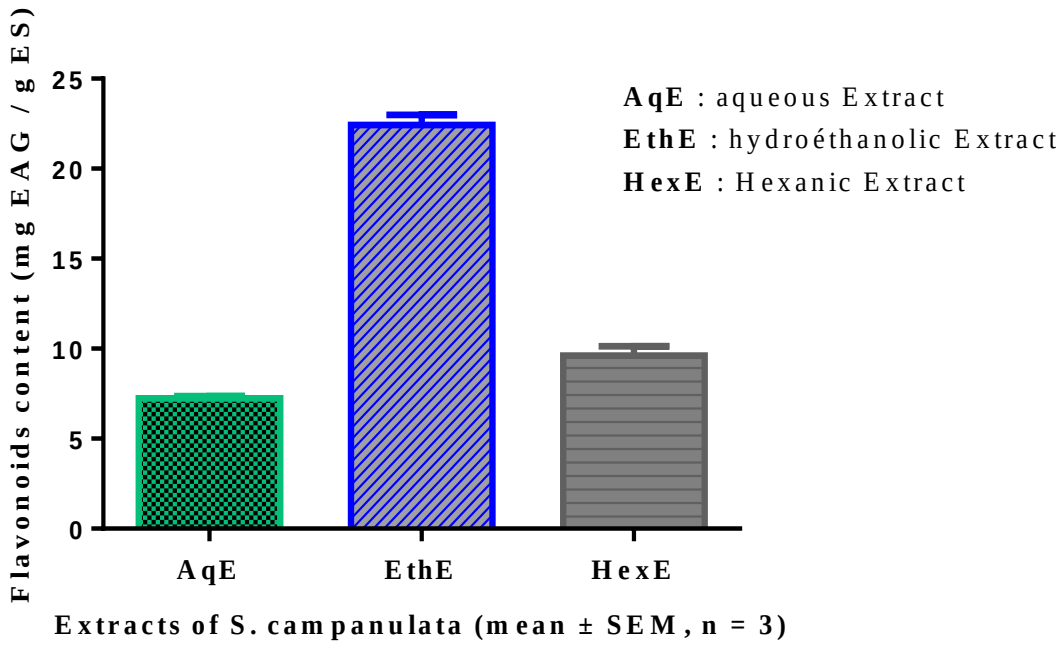
EAq: aqueous extract; **EEth**: hydroethanolic extract**; EHex**: hexane extract; **mg / EAG / g ES:** milligram of gallic acid equivalence per gram of dry extract**; mg EG / g ES:** milligram of quercetin equivalence per gram of dry extract**.**

NB: Each value corresponds to the mean ± Standard deviation. (Student's test: *** p <0.001)

Graph.1 Total phenol content of extracts of *S. campanulata* bark (Mean ± SD of three trials)



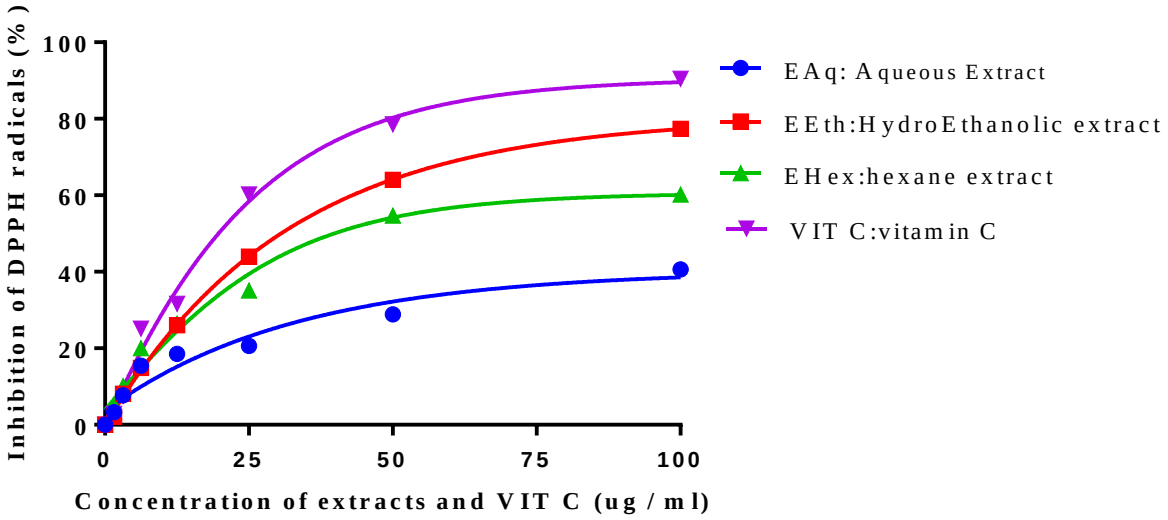
Graph.2 Flavonoids content of extracts of *S. campanulata* bark (Mean ± SD of three trials)



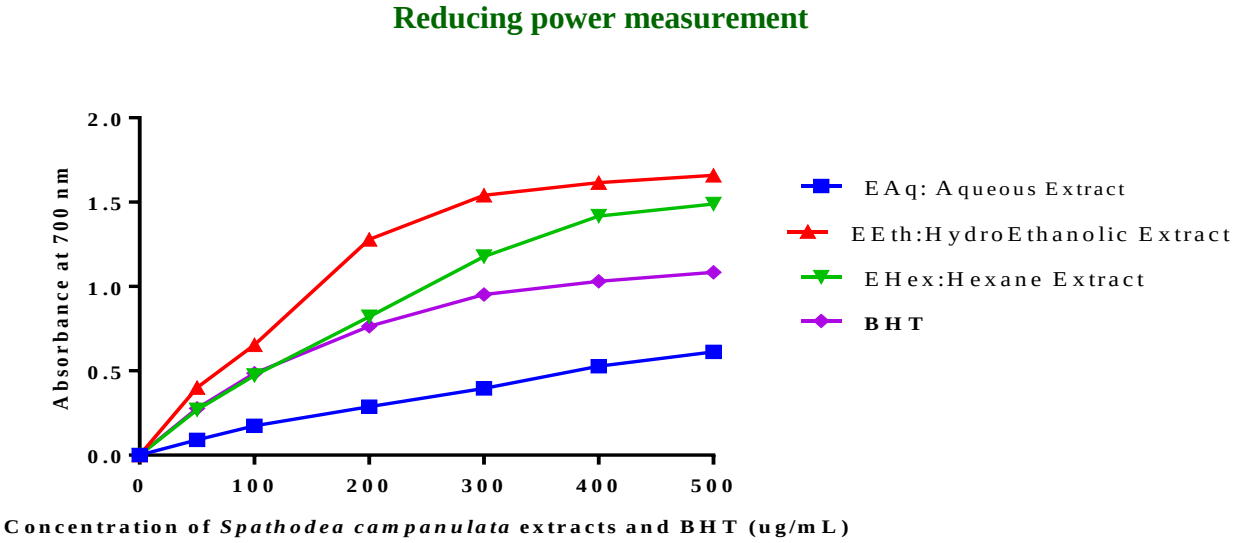
Evaluation of the antioxidant activity of aqueous and ethanolic extracts of *Spathodeae campanulata* in vitro

Graph.3 Evolution of the anti-free radical activity of extracts of *Spathodea campanulata* and vitamin C according to the concentration

Measurement of anti-radical activity



Graph.4 Evolution of the reducing power of extracts of *Spathodea campanulata* and BHT according to the concentration



This reducing and anti-free radical power is based on the content of phenolic compounds in the bark of *S. campanulata*. Indeed, polyphenols are compounds possessing an antioxidant power due to their redox properties(Zeng et Wang, 2001). These properties make it possible to neutralize free radicals by donation of electrons or protons(Chen et Ho, 1995), to block the reaction chain of free radicals by transfer of hydrogen atoms (Meir *et al.*, 1995). This antioxidant activity of polyphenols is often exploited in order to prevent and treat diseases linked to oxidative stress.

The study of the antioxidant activity of bark extracts from the species *Spathodea campanulata* according to the method of trapping the free radical DPPH and that of the reducing power of the ferric ion showed that the hydroethanolic extract has good antioxidant activity than aqueous and hexane extracts. However, this activity remains significantly lower than that of ascorbic acid and BHT used as reference molecules, but these are crude extracts containing a large number of different compounds. It is therefore very likely that they contain compounds which, once purified, may exhibit an activity comparable to that of the reference molecules. Likewise, the quantitative determination of the phenolic compounds by spectrophotometry of the dry extracts of the bark of *S. campanulata* showed the relative richness in phenolic constituents. These results have enabled us to deduce the recurrent use of this medicinal plant.

Conflict of interests

The authors claim that there is no conflict of interest.

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