Turkish Journal of Agriculture - Food Science and Technology, 9(10): 1925-1930, 2021
DOI: https://doi.org/10.24925/turjaf.v9i10.1925-1930.4514

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Comparative Study of the Antioxidant Activity of *Holarrhena Floribunda* and *Picralima Nitida*

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ARTICLE INFO

Research Article

Received : 13/06/2021
Accepted : 12/07/2021

Keywords:
Picralima nitida
Holarrhena floribunda
Antioxidant activity,
Côte d'Ivoire
Decoction

ABSTRACT

Picralima nitida (Stapf) T.Durand & H.Durand and *Holarrhena floribunda* (G.Don) T.Durand & Schinz are West and Central African plant species belonging to the Apocynaceae family. These two plants are used in traditional Ivorian medicine to treat hypertension, urinary tract infections, diarrhea, gonorrhea, malaria and diabetes. Phytochemical screening and evaluation of antioxidant activity of *P. nitida* fruit and *H. floribunda* leaf extracts of each of these two species have already been performed. In the present study, a comparative evaluation of the antioxidant activity of fruit (*P. nitida)* and leaf (*H. floribunda)* decoctions was carried out. The decoctions of *P. nitida* fruits and *H. floribunda* leaves were rich in secondary metabolites, especially polyphenols which have good antioxidant activity. Quantification of total phenols and flavonoids gave respective values of QP = 15235.632 ± 622 µg GAE / g dry matter and FP = 2.387 ± 0.387% for *P. nitida* and QH = 68597.701 ± 3171 µg GAE / g dry matter and FH = 17.581 ± 0.379% for *H. floribunda*. *P. nitida* showed antioxidant activity against DPPH radical (IC50 = 104.30 ± 3.17 µg / mL) and ferric ion Fe3 + (261.4 ± 36. 87 µmoL Eq Trolox / g extract). *H. floribunda* showed antioxidant activity against DPPH radical (IC50 = 41.73 ± 0.29 µg / mL) and ferric ion Fe3 + (365 ± 20.36 µmol Eq Trolox / g ExS).

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Introduction

For many years, man has used various plant resources to satisfy his medical and food needs (Athamena, 2009; Sevindik et al., 2017). The Ivorian flora presents a great floristic diversity, in which medicinal plants occupy an important place. The originality of these plants lies in their capacity to produce very diverse active natural substances such as: carbohydrates, proteins, lipids and nucleic acids. In addition to these primary metabolites, plants provide secondary metabolites whose physiological function represents an important source of molecules that can be used in the fields of pharmacology and food (Macheix et al., 2005; Mohammed et al., 2018; Pehlivan et al., 2018). Much of the current scientific research interest focuses on the study of natural antioxidant molecules that act as free radical scavengers (Pan et al., 2008; Sevindik, 2021). These are highly reactive compounds with a single electron and are necessary for vital mechanisms. Antioxidant compounds, in addition to their importance in the treatment of certain pathologies, are also used for the preservation of edibles in the food industry (e.g., to prevent lipid oxidation) (Pan et al., 2008; Mohammed et al., 2019).

In addition, the consumption of a diet rich in antioxidants is associated with the prevention of many degenerative diseases in our modern societies characterized by stress, smoking, sedentary lifestyle and overeating. Increasing dietary intake of antioxidants will therefore have the primary objective of preventing these

diseases (Koechlin, 2006; Mohammed et al., 2020; Mohammed et al., 2021).

In recent years, the commercial antioxidants used are synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propylgallate (PG). On the other hand, some authors have suspected that these molecules have some toxicity and could be responsible for liver damage and carcinogenesis (Pan et al., 2008; Atmani et al., 2009). Thus, we devoted our study to *P. nitida* and *H. floribunda*, two West and Central African plant species of potential antioxidant interest. The objective of the present study was to perform a comparative evaluation of the antioxidant activity of *P. nitida* fruit and *H. floribunda* leaf decoctions.

Material and Methods

Materials

Plant material

The plant material consisted of the fruits of *P. nitida* and the leaves of *H. floribunda* harvested in March 2019 in Agboville in Côte d'Ivoire.

The *P. nitida* sample was authenticated at the National Floristic Center of the Félix HOUPHOUËT-BOIGNY University of Abidjan. The herbarium was kept there under the name 1227 AKE ASSI.

The *H. floribunda* sample was authenticated at the National Floristic Center of Félix HOUPHOUËT-BOIGNY University of Abidjan. The herbarium was kept there under the name 281B E. Adjanohoun. The leaves were taken and dried at room temperature for two weeks at the Laboratory of Pharmacognosy, Botany, Plant Biology and Cryptogamy of the Faculty of Pharmaceutical and Biological Sciences of the Félix HOUPHOUËT-BOIGNY University of Abidjan (UFHB). They were then crushed to obtain a fine powder.

Equipment

A mechanical grinder (RESCHT GM 300) was used for the extraction, a precision balance (Denver Instrument Si-602) for the weighing, a heating flask for the decoctions. For the study of the anti-free radical activity, a spectrophotometer (JENWAY 7315), was used.

Methods

Preparation of the Fruits Decoction of Picralima Nitida

600g of fruits were introduced into 1000 mL of distilled water (m / v). The mixture was boiled at 100°C for 15 minutes. After cooling, the homogenate was filtered through whattman n°1 paper and placed in an oven at 50°C until a dry extract of weight Pn was obtained.

Preparation of the leaves decoction of Holarrhena floribunda

10g of powder from the leaves of *H. floribunda* were introduced into 100 mL of distilled water (m / V). The mixture was boiled at 100°C for 15 min. After cooling, the solution was filtered through whattman No. 1 paper and the resulting filtrate was the leaves decoction. This leaves decoction was evaporated in an oven at 50°C until a dry extract of weight Pf.

Determination of Total Polyphenols

The total polyphenol contents are determined according to the Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Heilerová et al., 2003) modified (Konan, 2010). Added to 1 mL of each extract diluted to 1/10 with distilled water, 1.5 mL of Na_2CO_3 (17%, m / v) and 0.5 mL of Folin-Ciocalteu reagent (0.5N). The whole mixture was incubated at 37°C for 30 min and the absorbance was read at 760 nm against a blank without extract taken as reference. The quantification of the total polyphenols was determined using a linear calibration line (y = 0.0227x-0.0126) carried out by a standard extract (gallic acid) at different concentrations (0 to 1000 µg / mL) under the same conditions as the sample. The results are expressed as micrograms of gallic acid equivalent per gram of dry matter (µg GAE/ g DM) of the powdered plant.

The total polyphenols content (Q in µg GAE / g DM) was calculated using equation X:

$$Q=\frac{V \times C \times d}{m} \quad (1)$$

V = final volume of the extract (mL)
C = concentration of the extract (µg / mL)
d = dilution
m: mass of dry matter of hydrolyzed plant material (g)

Determination of Total Flavonoids

The determination of the total flavonoids was carried out according to the method of Hariri et al. (1991) modified. 2 mL of each extract were diluted 1 / 10th and mixed with 100 µL of Neu's reagent. The absorbance was read at 404 nm and compared to that of quercetol used as a standard (0.05 mg / mL) and undergoing the same treatment as the extract. The percentage of total flavonoids is calculated in quercetol equivalent according to the following formula (equation 2):

$$F\ (\%)=\frac{0{,}05 \times Aext}{Aq} \times \frac{100 \times d}{Cext} \quad (2)$$

Aext = Absorption of the extract
Aq = Absorption of quercetol
Cext = Concentration of the extract (mg / mL)
d = dilution

Method for Antioxidant Activity Assay

DPPH radical-scavenging activity (DPPH test)

Principle

DPPH is characterized by its ability to produce stable free radicals. This stability is due to the delocalization of free electrons within the molecule. The presence of these DPPH radicals gives rise to a dark purple color in the solution. DPPH, a stable purple free radical, is reduced to a yellow-colored compound in the presence of anti-oxidant compounds. Reduction of DPPH radicals by an antioxidant leads to discoloration of the solution (Molyneux, 2004) The color change can be monitored spectrophotometrically at 517 nm and in this way the antioxidant potential of a substance or a plant extract can be determined (Molyneux, 2004; Popovici, 2010).

Procedure

The *in vitro* anti-free radical activity of the extracts was measured by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) test according to the method of Parejo (Parejo et al., 2002) with some modifications. For the preparation, 2 mL of a methanolic solution of DPPH (100 µM) was mixed with 1.5 mL of different dilutions of the extracts (from 0 to 400 µg / mL). A range of concentrations from 0 to 200 µg / mL for vitamin C was used as a reference. The resulting mixture was then stored in the dark and at room temperature for 30 minutes. Then, the absorbance was measured with a spectrophotometer at 517 nm against a control composed of 2 mL of the DPPH solution and 1.5 mL of the methanolic solution. The Percentage of inhibition (% PI) was calculated according to the formula below:

$$PI = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

PI = Percentage of inhibition (%)

A0 = absorbance of the DPPH solution in the absence of the extract (blank)

A1 = absorbance of the DPPH solution in the presence of the extract (test)

Determination in vitro of the total antioxidant activity by FRAP method (FRAP test)

Principle

The FRAP method is based on the reduction of the ferric ion (Fe3 +) to ferrous ion (Fe2 +). This method evaluates the reducing power of compounds (Ou et al., 2001). The presence of reducing agents (HA) in plant extracts results in the reduction of the Fe^3 + / ferricyanide complex to ferrous form. Therefore, Fe^2 + can be evaluated by measuring and monitoring the increase in density of the cyan blue color in the reaction at 593 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) in acidic pH.

Procedure

The FRAP (Reducing Power of Iron) test was carried out according to the method described by Pulido (Pulido et al., 2000). A fresh solution of the FRAP reagent (10 mM) was prepared by mixing 2.5 mL of the TPTZ solution (10 mM 2,4,6-Tri (2-pyridyl) -1,3,5-triazine in 40 mM of HCl) with 2.5mL of $FeCl_3.6H_2O$ (20 mM) and 25mL of acetate buffer (300 mM of sodium acetate, pH led to 3.6 by acetic acid). Then, 3500 µL of the FRAP reagent was added to 140 µL of the test compounds dissolved in a methanolic solution. After 30 min of incubation in the dark, the absorbance was read at 593 nm. The Trolox was used as a dosage control. The reducing power values obtained for the extract tested at various decreasing concentrations of Trolox (1, 0.5, 0.25, 0.125, 0.0625, 0.031 mg / mL) made it possible to draw a histogram.

Results and Discussion

Any substance with the ability to capture or neutralize free radicals is called an antioxidant (Dehpour et al., 2009). The antioxidant activity of the dry extract of the fruit decoction of *P. nitida* and the dried leaves of *H. floribunda* was evaluated by two methods of evaluation of the antioxidant power (DPPH and FRAP).

Antioxidant Activity by DPPH Method

The decoction has IC_{50} anti-free radical activity. The IC_{50} is the concentration of extracts or vitamin C (reference substance) responsible for 50% inhibition of DPPH radicals. It is determined from the calibration curve for vitamin C.

The IC_{50} of fruit decoction of *P. nitida* was higher than that of *H. floribunda* leaf decoction with values of 104.30 ± 3.17 µg / mL and 41.73 ± 0.29 µg / mL, respectively (Figure 1). On the other hand, these values nevertheless remained lower than that of the reference substance (8.06 ± 0.09 µg / mL). The results are shown in Table 1.

Table 1. Antiradical activity of fruits decoction of P. *nitida* and leaves decoction of H. *floribunda* by the DPPH method

	P. nitida	H. floribunda	Vitamine C
CI_{50} (µg/mL)	104.30 ± 3.17	41.73 ± 0.29	8.06 ± 0.09

An extract is more antioxidant if its IC_{50} is lower (Kafui et al., 2018). Thus, the results obtained with the DPPH test in our study showed that *H. floribunda* is more antioxidant than *P. nitida*, since *P. nitida* has a higher IC_{50} than that of *H. floribunda*, but that these plants are less antioxidant than the reference substance vitamin C.

Our results are in agreement with the work of several authors. The results of the anti-free radical activity of methanolic extracts (of ten medicinal plants from the Ivorian pharmacopoeia) and of quercetin (reference molecule whose IC_{50} = 2.00 ± 0.288 µg / mL) presented by Bidié (2011) showed two categories of plants. The first group comprises of plants with strong anti-free radical activity, in which their IC50s are between 2 and 10.5 µg / mL. They are: *Mitragyna ciliata* Aubrév. & Pellegr. (IC_{50} = 10.5 ± 0.288 µg / mL), *Trichilia prieuriana* A.Juss. (IC_{50} = 7.5 ± 0.288 µg / mL), *Distemonanthus benthamianus* Baill. (IC_{50} = 4.50 ± 0.288 µg / mL) and *Chrysophyllum perpulchrum* Mildbr. ex Hutch. & Dalziel (IC_{50} = 4.00 ± 0.288 µg / mL). The second category comprises of plants exhibiting weak anti-free radical activity. This is the case for: *Millettia zechiana* Harms (IC_{50} = 96 ± 0.577 µg / mL), *Ageratum conyzoides* (L.) L. (IC50 = 76 ± 0.577 µg / mL), *Parquetina nigrescens* (Afzel.) Bullock (IC_{50} = 75.5 ± 0.166 µg / mL) and *Sherbournia bignoniiflora* (Welw.) Hua (IC_{50} = 49.5 ± 0.288 µg / mL). Compounds with an IC_{50} greater than 100 µg / mL do not have a significant anti-free radical activity. They are: *Desmodium gangeticum* (L.) DC. (IC_{50} = 10,000 ± 288.7 µg / mL) and *Blighia sapida* K.D.Koenig (IC_{50} = 17,000 ± 577.4 µg / mL). Going by this hypothesis, the antioxidant activity of *P. nitida* would therefore be low because its IC_{50} was 104.30 ± 3.17 µg / mL.

Koffi et al. (2018) showed, during their study on the antioxidant activity of some plants used in the region of Tiassalé (Côte d'Ivoire) in maintaining skin health, that out of a total of 30 extracts evaluated for their antioxidant power with DPPH anti-free radical test, seven including five methanolic extracts and two aqueous showed a percentage of inhibition superimposed on that of vitamin C (95.79 ± 10.53%). However, the methanolic extracts of

Cajanus cajan (L.) Millsp. (95.71 ± 9.67%), of *Cyathula prostrata* (L.) Blume (94.91 ± 7.04%), of *Eleusine indica* (L.) Gaertn. (93.54 ± 10.21%) gave the strongest activities. *Elaeis guineensis* Jacq. (89.02 ± 12.76%), and *Anchomanes difformis* (Blume) Engl. (87.19 ± 10.54%) had intermediate activities. The IC50s are between 27.20 and 60 µg / mL. These extracts are therefore less active than Vitamin C (8.34 µg / mL). These results suggest that the aqueous and methanolic extracts concentrate the most chemical constituents and possess the ability to donate hydrogen to a free radical. This trapping helps to sweep away the potential damage caused by free radicals (Wong et al., 2006; Chang et al., 2012). They are in fact a complex mixture of organic acids, amino acids and sugars which can also contribute to the sequestration of electrically charged free radicals (Wong et al., 2006; Chang et al., 2012).

Table 2. Antiradical activity of the fruit's decoction of *P. nitida* and the leaves decoction of *H. floribunda* by the FRAP method

	P. nitida	H. floribunda
µMol Eq trolox/g ES	261,4 ± 36,87	365 ± 20,36

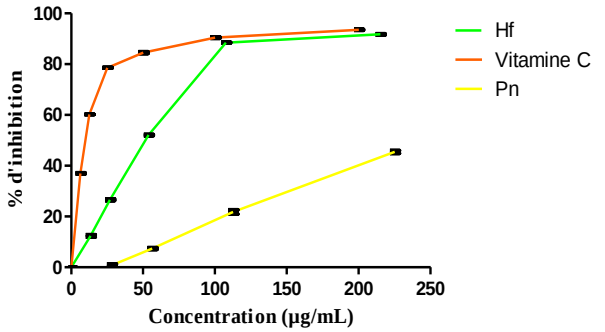

Figure 1. Percentage inhibition of the DPPH radical of the fruit decoction of P. *nitida* and the leaf decoction of *H. floribunda* compared to vitamin C

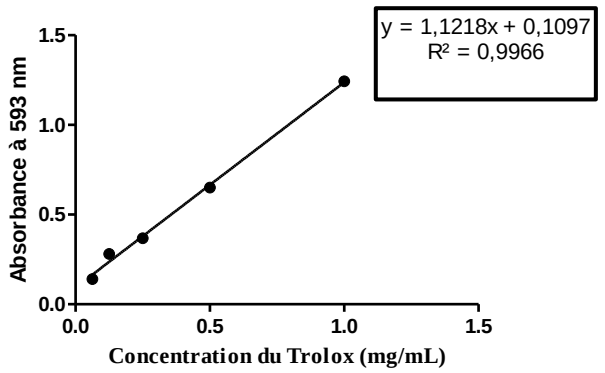

Figure 2. Calibration line for Trolox (*P. nitida* and *H. floribunda*)

According to the results obtained by Bougandoura and Bendimerad (2012) during their study on the evaluation of the antioxidant activity of aqueous and methanolic extracts of *Satureja calamintha* (L.) Scheele, the aqueous and methanolic extracts are endowed with moderate antioxidant power, their respective IC_{50} being 1.876 and 2.075. However, this IC_{50} was relatively lower than that of ascorbic acid used as a standard, which was in the order of 0.134 mg / mL. Antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins have been shown to reduce and decolorize DPPH due to their ability to release hydrogen (De Pooter and Schamp, 1986). The polyphenols contained in extracts of *S. calamintha* are probably responsible for these authors, for the antioxidant activity of these extracts.

Hatano et al. (1989), Duh et al. (1999) and N'guessan et al. (2007) showed the existence of a correlation between total phenol content and anti-free radical activity. According to Zhi and Ho (1995), the functional groups present in phenolic compounds in general can easily give up an electron or a proton to neutralize free radicals. The antioxidant activity of the two plants is therefore linked to their high total phenol content of 15,235.632 ± 622 µg GAE / g dry matter and 68,597.701 ± 3171 µg GAE / g dry matter respectively for *P. nitida* and *H. floribunda*. Konan (2010) also demonstrated that plants which have good antioxidant activity contain high levels of phenolic groups. However, in addition to their high total phenol content, *P. nitida* and *H. floribunda* also contain total flavonoids according to phytochemical screening at values of 2.387 ± 0.387% and 17.581 ± 0.379% for *P. nitida* and *H. floribunda* respectively, which flavonoids are metabolites which express good antioxidant activity (N'guessan et al., 2007; Syamsudin et al., 2008).

Antioxidant Activity Evaluation by FRAP Method

The FRAP method was used in order to confirm the results obtained by the DPPH method. The linear regression curve of the reference antioxidant (vitamin C) used as a standard is also shown in Figure 2. The fruits decoction of *P. nitida* showed an antioxidant activity value of 261.4 ± 36.87. µMol Eq trolox / g ES, value lower than that of the decoction of the leaves of *H. floribunda* whose antioxidant activity value was 365 ± 20.36 µMol Eq trolox / g ES (Table II).

The FRAP method is a method for measuring the capacity of analyzed samples, to reduce the ferric ion Fe^{3+} to the ferrous ion Fe^{2+} which is one of the antioxidant mechanisms. It is an easy, fast and reproducible technique (Karagôzler et al., 2008).

The reducing power, evaluated by the FRAP method in our study, showed that the antioxidant activity of *H. floribunda* was higher compared to that of *P. nitida*. This result confirms the presence of the antioxidant activity that we observed for the DPPH method.

Also, many researchers found that the reducing capacity of a compound can be considered as a significant indicator of its potential antioxidant activity (Bentabet et al., 2014). There is a link between the phenolic compounds content and the reducing power (Yildirim et al., 2001). The presence of polyphenols in the fruit and leaf decoctions of the two plants could therefore explain their antioxidant activity potential. However, the antioxidant activity of the decoction of *P. nitida* would be less compared to that of the decoction of *H. floribunda*, since the phenolic compound contents was respectively 39.19 ± 4.37 mg GAE / dry extract and 105.7 ± 0.89 mg GAE/ dry extract for each of the two plants.

Our results are in agreement with those of the literature. Indeed, the work carried out by Kafui Kpegba et al. (2018) reported that the *H. floribunda* plant possesses antioxidant activity with a value of 1009.9 ± 0.6 µmol Fe2 + equivalent / g.

Bakchiche and Gherib (2014) worked on the determination of the antioxidant activities of polyphenols extracted from medicinal plants and they evaluated this antioxidant activity *in vitro* in three different ways: the DPPH test, the ABTS cation radical discoloration technique and the reducing power measurement. For this last test, the results obtained showed that the extracts exhibited high absorbances compared to the negative control. The two extracts of *Arbutus unedo* L. (0.001 ± 0.006 mg / mL) and *Ziziphus lotus* (L.) Lam. (0.001 ± 0.006 mg / mL) are the most active among the extracts tested followed respectively by *Anthemis arvensis* L. extracts (0.017 ± 0.006 mg / mL), *Haloxylon scoparium* Pomel (0.002 ± 0.006 mg / mL) and *Thymus algeriensis* Boiss. & Reut. (0.025 ± 0.006 mg / mL); however, these activities remained significantly identical to that of ascorbic acid used as a positive control.

The antioxidant activity of methanolic and aqueous extracts of *S. calamintha* was evaluated by Bougandoura and Bendimerad (2012) using the FRAP method. They found that the reducing power of plant extracts is dose dependent (concentration dependent). At a concentration of 2.5 mg / mL, the reducing power of the methanolic extract of *S. calamintha* (at 700 nm) is much greater (Optical Density = 0.484) compared to the aqueous extract (Optical Density = 0.097), but significantly lower than that of ascorbic acid (Optical Density = 11.98). The reducing power of the *S. calamintha* species is probably due to the presence of hydroxyl group in phenolic compounds which can serve as electron donor. Therefore, antioxidants are considered to be reducers and inactivators of oxidants (Iddhuraju and Becker, 2007). Some previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its antioxidant activity potential (Jeong et al., 2004; Kumaran and Karunakaran, 2007**).**

Plants used in the treatment of chronic pathologies generally have good antioxidant activity combating the oxidative stress at the origin of these pathologies (Pan et al., 2008). There is also a strong correlation between antioxidant activity and the nature and content of the chemical groups present in the extract.

The presence of polyphenols in the fruits and leaves decoctions of these two plants could be responsible for this activity. Our results are in agreement with those of the literature. Indeed, the work carried out by Erharuyi et al. (2014) indicated that the plant has several pharmacological activities including antimalarial, anti-inflammatory, analgesic, antidiabetic, antimicrobial, antioxidant and antiulcer activities.

Conclusion

The present study proposed to carry out a comparative study of the antioxidant activity of the decoction of the fruits of *P. nitida* and the leaves of *H. floribunda*, two plants used in the traditional Ivorian medicine. The results indicated that the antioxidant activity of *H. floribunda* is higher than that of *P. nitida*. This study also confirms a certain correlation between phenolic compounds content and anti-free radical activity and between phenolic compounds and the reducing power of the ferric ion (Fe3 +) to ferrous ion (Fe2 +). The results of various scientific researchers have so far revealed that the antioxidant potential of these plants could be used in the treatment and prevention of metabolic diseases. However, further studies on pure extracts and compounds of these species are needed to characterize the molecules responsible for this antioxidant activity.

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