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RESEARCH ARTICLE

PHYTOCHEMISTRY AND ANTIOXIDANT POTENTIAL OF *PIPER CUBEBA* L. FROM CÔTE D'IVOIRE

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ABSTRACT

Piper cubeba is a species of pepper with tail, known for its many medicinal properties. The present study first focuses on the qualitative and quantitative phytochemical screening, then on the antioxidant potential measured by spectrophotometry in relation to the DPPH of four (4) accessions of this species collected in the Ivorian orchard. Qualitatively and quantitatively, the results showed the presence of several phytocompounds (polyphenols, flavonoids, alkaloids, coumarins, sterols and terpenes) in variable proportions. The DPPH test performed at different concentrations of the accessions this, compared to quercetin (the reference antioxidant), revealed a perceptible antioxidant potential.

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INTRODUCTION

Plants are a major source of medicine for humans. Among these, pepper trees constitute a family of medicinal plants widely valued in traditional medicine. *Piper cubeba* (Piperaceae) commonly called cubeb, native to Southeast Asia (Jean Guillaume, 2010) is a spice used for the treatment of coughs, respiratory diseases, intestinal diseases and rheumatism (Sumathykutty *et al*., 1999). Information from the literature indicates that *Piper cubeba* has been extensively studied both chemically (Macheix, 2005; Beta *et al*., 2005) and biologically (Hussein *et al*., 2000; Ibn Baitar, 2003; Desouza, 2005).

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However, the wild species of Côte d'Ivoire has not yet been studied. The present work is therefore an investigation devoted to the qualitative phytochemical analysis by thin layer chromatography (TLC) and quantitative analysis by spectrophotometry, as well as to the estimation of the antiradical potential against DPPH of extracts from four (4) accessions of the said species.

MATERIALS AND METHODS

Plant material: The plant material used consists of seeds from four (4) *Piper cubeba* accessions (AB, AC1, AC2 and BH), harvested respectively in Abengourou, region of Indénié-Djuablin (6°43'47" N and 3°29'47" W), in Zouan-Hounien, Tonpki region (6°55'00" N and 8°13'00" W) and in Bin-Houyé, Tonpki region (6°46'57" N and 8°18'59" W).

The accessions have been identified at the Centre National de Floristique (CNF) of the Félix Houphouët-Boigny University (Abidjan-Cocody) and at the Botany and Phytotherapy Laboratory of the Nangui Abrogoua University (Abidjan). The seeds were cleaned under running water, dried at room temperature away from light for 15 days, then kept in a 45°C oven for 3 days. The dried seeds were ground to powder with an electric grinder and stored in hermetically sealed glass jars.

Preparation of extracts: 15 g of powder from each accession was macerated in 30 ml of methanol (80 %) for 24 h. After vacuum filtration, the macerates were placed in a refrigerator (4°C) for 24h, decanted and concentrated in the rotary evaporator (BÜCHI). The concentrates AC1, AC2, BH and AB were dried for 24 h at 40 °C in an oven for quantitative analysis and measurement of antioxidant activity. For the qualitative analysis by TLC, the selective extracts obtained by liquid-liquid extraction from concentrates were used: hexanic extracts ($AC1^{I}$, $AC2^{I}$, BH^{I} and AB^{I}), chloroformic extracts ($AC1^{II}$, $AC2^{II}$, BH^{II} and AB^{II}), ethyl acetate ($AC1^{III}$, $AC2^{III}$, BH^{III} and AB^{III}) and n-butanolic extracts ($AC1^{IV}$, $AC2^{IV}$, BH^{IV} and AB^{IV}).

Qualitative analysis: Tests for the identification of families of secondary metabolites were performed by TLC on chromatoplaques according to Ladiguina *et al*. 1983; Dohou *et al*.2003; Békro *et al*., 2007; N'Gaman 2013.

Quantitative analysis: Spectrophotometric quantification of total phenols (Singleton *et al*., 1999; Heilerova *et al*., 2003), total flavonoids (N'Guessan *et al*., 2011; Dif *et al*., 2015), total tannins (Dif *et al*., 2015), and flavonicaglycones and anthocyanins (Lebreton & Jay, 1967) has been carried out.

Measurement of antioxidant activity: The measurement of antioxidant activity was performed by the DPPH spectrophotometric test with a UV-visible spectrophotometer (Jasco V500) at wavelength 517 nm according to Espin*et al*. (2000); Slandjana *et al*. (2012). Absolute ethanol was the dilution solvent. The stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich) was used as the reference oxidant (0.03 mg/ml). Quercetin (Sigma Aldrich) was the reference antioxidant used. The different concentration ranges of each analyte used were 0.07 mg/ml, 0.036 mg/ml, 0.0179 mg/ml, 0.00893 mg/ml, 0.004464 mg/ml and 0.0022321 mg/ml). The percentage reduction in DPPH (R %) was calculated according to equation (1):

$$R(\%) = \left(1 - \left(\frac{e.}{c_{\cdot}\quad\quad a}\right)\right) \times 100 \qquad (1)$$

The EC50 efficiency index (median effective concentration) at different extract times is calculated according to equation (2):

$$C_{5}(m\quad 'e.\quad\quad /m\quad\quad) = \frac{C_{5,t}}{in}$$

Where, CR50, this the median DPPH reduction concentration determined graphically.

RESULTS AND DISCUSSION

Qualitative phytochemical composition of the accessions

The TLCs of the selective hexanic ($AC1^{I}$, $AC2^{I}$, BH^{I} and AB^{I}), chloroformic ($AC1^{II}$, $AC2^{II}$, BH^{II} and AB^{II}), ethyl acetate ($AC1^{III}$, $AC2^{III}$, BH I^{II} and AB^{III}) and n-butanolic ($AC1^{IV}$, $AC2^{IV}$, BH^{IV} and AB^{IV}) extracts showed their preliminary phytochemical composition (Table 1). Sterols, terpenes and triterpenes of the lupan type were identified by Liebermann-Bürchard's reagent in the hexanic extracts. Anthocyanins, coumarins and flavonoids were detected in the chloroform extracts. Also, these secondary metabolites were detected in the ethyl acetate extracts. Alkaloids are present in $AC2^{II}$, BH^{II} and AB^{II} with abundance in AB^{II} extract. The chromatographic profile of the n-butanol extracts also revealed the coexistence of anthocyanins, coumarins and flavonoids. The chromatographic profiles of the selective extracts revealed the unbalanced distribution of phytocompounds in the different accessions. This seems to be due to the action of bio-aggressors and pedoclimatic factors. Indeed, the high temperatures, high sun exposure and soil salinity would promote the biosynthesis of phytophenols (Gelhin *et al*., 2006; Falleh *et al*., 2008).

The presence of alkaloids in extracts of *P. cubeba* has been proven, and its use as an antidepressant and anticonvulsant has been reported (Lee *et al*., 2007; Pei 1983; Addai-Mensah *et al*., (1997)). In plants, secondary metabolites are chemical phytoconstituents with no specific function, resulting from various metabolic processes. However, it is accepted, through several works carried out, that their presence governs the known biological and pharmacological virtues of plants. The existence of anthocyanins, coumarins, flavonoids, sterols and terpenes in the different accessions of *P. cubeba* would justify the wide spectrum of their antibacterial, antifungal, antimicrobial, antiseptic properties (Lepengue *et al*., 2019; Djiwonou *et al*., 2019), and their use in culinary preparation (Rastogi *et al*., 1999), perfumery and cough treatment (Lacau, 1993; Rastogi *et al*., 1999). The presence of flavonoids in particular could be linked to the action of biotic agents (Gelhin *et al*., 2006).

Quantitative phytochemical composition of the accessions

Total phenol and flavonoid contents: Total phenols (TP) and total flavonoids (TF) are contained in *Piper cubeba* in varying amounts (Figure 1). The contents of total phenols contained in *P. cubeba'*shy dromethanol concentrates were evaluated from the calibration line equation established with the different concentration ranges of gallic acid (from 3.115 to 25 µg/ml). They were expressed in micrograms equivalent of gallic acid per gram of extract (µg EAG/g). The FT content is expressed in micrograms per gram of extract in catechin equivalent (µg EC/g extract). Figure 1 shows a more significant PT content in AC1 (85.296 µgEAG/g) and AB (82.689 µgEAG/g) than in AC2 and BH. AB seeds are richer in FT (62.946 µgEC/g) than other accessions of P. cubeba 40.841 µgEC/g (BH), 37.523 µgEC/g (AC1) and 26.588 µgEC/g (AC2). The same evolution is observed for the contents of anthocyanins and flavonicaglycones expressed in mg/g (Figure 1). The levels of flavonicaglycones are relatively significant in *P. cubeba* accessions with the exception of BH, which has levels below 1 mg/g. Anthocyanin contents are essentially identical in the accessions, except for AB (0.079 mg/g) which shows a slight increase. The presence of flavonicaglycones in the accessions would explain the hepatoprotective, diuretic, antibacterial, anti-inflammatory, antidiabetic, and anti-tumour activities (Hussein *et al*., 2000; Kumar *et al*., 2007) recognized in pepper. PT and FT levels would be related to pedoclimatic conditions. Indeed, high temperatures and a prolonged explosion in the sun would stimulate the biosynthesis of polyphenols (Falleh *et al*., 2008).

Table 1. Phytocompounds identified in selective extracts

Extract		Rf (Color), phytocompoundidentified
Hexanic	AC1^{I}	0,28(Y), St/Tr ; 0,31(Y), St ; 0,81(G), St ; 0,14(Or), Tlp ; 0,20(Or), Tlp ; 0,35(Or), Tlp ; 0,49(Or), Tlp ; 0,90(Or); 0,20(P, Tr) ; 0,28(Or, Tr) ; 0,34(P, Tr) ; 0,44(YOr, Tr) ; 0,51(Or, Tr) : 0,61(P, Tr) ; 0,68(P, Tr) ; 0,78(VVi, Tr) ; 0,89(Or, Tr)
	AC2^{I}	0,19(P^{ab}) Tp ; 0,28(Or^{ab}) Tp ; 0,36(YOr^{ab}) Tp ; 0,41(G^{ab}) St ; 0,68(G) ; 0,53(P^{ab}) Tp ; 0,56(YP^{ab}) Tp ; 0,58(Or^{ab}) St ; 0,66(YGr^{ab}) Tp ; 0,73(BG^{ab}) St ; 0,78(Or^{ab}) Tp ; 0,81(Or^{a}) Tlp ; 0,83(YP^{ab}) Tp ; 0,88(P^{ab}) Tp ; 0,90(Or^{ab}-Or^{a}) Tp/Tlp
	BH^{I}	0,16(Or^{ab}) Tp ; 0,23(Or^{ab}) Tp ; 0,28(Or^{ab}) Tp ; 0,34(YOr^{ab}) Tp ; 0,41(G^{ab}) St ; 0,50(GOr^{ab}) St ; 0,55(GOr^{ab}-YP^{a}) Tp/Tlp ; 0,66(G^{ab}) St ; 0,70(P^{ab}) Tp ; 0,80(Or^{ab}-Or^{a}) Tp/Tlp ; 0,89(Or^{ab}-P^{a}) Tp/Tlp
	AB^{I}	0,51(YOr^{ab}) Tp ; 0,66(YOr^{ab}-V^{ab}) Tp/St ; 0,75(P^{ab}-Or^{a}) Tp/Tlp ; 0,80(Or^{ab}-Or^{a}) Tp/Tlp ; 0,83(YP^{ab}) Tp ; 0,86(P^{ab}) Tp ; 0,88(Or^{ab}-Or^{a}) Tp/Tlp
Chloroformic	AC1^{II}	0,04(Y^{d}) Fl ; 0,07(Y^{c}) Fl ; 0,09(Y^{d}) Fl ; 0,35(G^{c}) Fl ; 0,50(B^{c}) Fl ; 0,53(Y-Or^{d}) Fl ; 0,59(Y^{b}) Fl ; 0,66(Y^{d}) Fl ; 0,86(Y^{d}) Fl ; 0,41(B^{d}) Ant ; 0,45(B^{d}) Ant ; 0,79(B^{d}) Ant ; 0,69(B^{g}) Cou
	AC2^{II}	0,29(G^{d}) Fl ; 0,34(YG^{c}) Fl ; 0,38(G^{d}) Fl ; 0,44(BP^{c}-BG^{g}) Fl/Cou ; 0,49(B-P^{c}-G^{d}-B^{g}) Fl/Cou ; 0,54(BP^{c}-Y^{d}) Fl ; 0,64(G^{c}) Fl ; 0,71(Y^{c}-G^{g}) Fl/Cou ; 0,78(YP^{c}-G^{d}) Fl ; 0,89(Y^{b}-Or^{f}) Fl/Alc ; 0,58(B^{d}-B^{g}) Ant/Cou ; 0,66(B^{d}) Ant ; 0,80(B^{d}) Ant ; 0,21(B^{g}) Cou ; 0,40(B^{g}) Cou ; 0,69(Or^{f}) Alc ; 0,80(Or^{f}) Alc
	BH^{II}	0,05(Y^{c}) Fl ; 0,21(BP^{c}) Fl ; 0,31(BP^{c}-Y^{d}) Fl ; 0,38(G^{d}) Fl ; 0,48(BP^{c}) Fl ; 0,51(BP^{c}-Y^{d}) Fl ; 0,59(Y^{c}) Fl ; 0,64(G^{d}-G^{g}) Fl/Cou ; 0,73(YOr^{d}-G^{g}) Fl/Cou ; 0,48(B^{d}-B^{g}) Ant/Cou ; 0,80(B^{d}-Or^{f}) Ant/Alc ; 0,19(B^{g}) Cou ; 0,38(B^{g}) Cou ; 0,53(B^{g}) Cou ; 0,66(Or^{f}) Alc
	AB^{II}	0,05(Y^{c}) Fl ; 0,07(Y^{c}) Fl ; 0,23(B^{c}) Fl ; 0,31(B^{c}-G^{d}) Fl ; 0,41(G^{d}-Or^{f}) Fl/Alc ; 0,48(B^{c}-G^{d}) Fl ; 0,64(YOr^{c}-G^{d}-BY^{g}) Fl/Cou ; 0,89(Y^{d}) Fl ; 0,70(B^{d}) Ant ; 0,75(B^{d}) Ant ; 0,80(B^{d}-B^{g}-Or^{f}) Ant/Cou/Alc ; 0,83(BY^{d}) Ant ; 0,19(B^{g}) Cou ; 0,43(B^{g}) Cou ; 0,58(B^{g}-G^{d}) Cou/Fl ; 0,68(B^{g}) Cou ; 0,73(B^{g}-Or^{f}) Cou/Alc ; 0,10(Or^{f}) Alc ; 0,27(Or^{f}) Alc ; 0,51(Or^{f}) Alc, 0,55(Or^{f}) Alc ; 0,61(Or^{f}) Alc ; 0,66(Or^{f}) Alc
Ethyl acétate	AC1^{III}	0,03(Y^{cd}) Fl ; 0,06(Y^{cd}-Y^{g}) Fl/Cou ; 0,09(G^{c}) Fl ; 0,13(Y^{c}) Fl ; 0,16(Y^{d}-Y^{g}) Fl/Cou ; 0,25(Y^{d}-Y^{e}) Fl/Cou ; 0,31(Y^{cd}-Y^{e}) Fl/Cou ; 0,33(Y^{e}) Cou ; 0,39(Y^{c}) Fl ; 0,43(Y^{d}) Fl ; 0,49(Y^{c}) Fl ; 0,58(Y^{c}-Y^{g}) Fl/Cou
	AC2^{III}	0,01(Y^{d}) Fl ; 0,05(Y^{c}-Y^{g}) Fl/Cou ; 0,09(Y^{c}) Fl ; 0,11(Y^{cd}-B^{g}) Fl/Cou ; 0,15(Y^{e}) Cou ; 0,18(B^{c}) Fl ; 0,24(Y^{cd}) Fl ; 0,30(Y^{d}) Fl ; 0,40(Y^{c}) Fl ; 0,46(B.Flu) Cou ; 0,60(B.Flu^{c}-P^{d}) Fl/Ant ; 0,63(B^{d}) Cou ; 0,73(Y^{d}) Fl ; 0,78(Y^{d}-Y^{g}) Fl/Cou ; 0,81(Y^{c}) Fl ; 0,84(G^{d}-B^{g}) Fl/Cou ; 0,86(B^{c}) Fl
	BH^{III}	0,03(Y^{c}) Fl ; 0,06(B^{c}-G^{g}) Fl/Cou ; 0,09(Y^{c}-B^{g}) Fl/Cou ; 0,14(Y^{e}) Cou ; 0,19(Y^{c}-Y^{g}) Fl/Cou ; 0,31(Y^{d}) Fl ; 0,41(Y^{c}) Fl ; 0,45(Y^{d}-G^{g}) Fl/Cou ; 0,53(B.Flu) Cou ; 0,56(Y^{c}) Fl ; 0,64(B^{d}-Y^{e}) Ant/Cou ; 0,80(Y^{d}-Y^{g}) Fl/Cou ; 0,85(B^{d}) Ant ; 0,88(B^{e}) Cou
	AB^{III}	0,03(Y^{c}-G^{g}) Fl/Cou ; 0,06(Y^{cd}) Fl ; 0,09(Y^{c}) Fl ; 0,13(Y^{d}) Fl ; 0,18(Y^{c}-Y^{g}) Fl/Cou ; 0,28(Y^{e}) Cou ; 0,31(Y^{cd}) Fl ; 0,38(G^{e}) Cou ; 0,44(Y^{c}) Fl ; 0,50(Y^{e}) Cou ; 0,54(Y^{c}) Fl ; 0,58(B^{c}) Fl ; 0,61(P^{d}) Ant ; 0,68(Y^{d}) Fl ; 0,78(Y^{c}-Y^{g}) Fl/Cou ; 0,84(Y^{d}) Fl ; 0,86(B^{e}) Cou
n-Butanolic	AC1^{IV}	0,05(Y^{c}-Y^{d}-Y^{g}-B^{d}) Fl/Cou ; 0,10(Y^{d}) Fl ; 0,13(Y^{e}-G^{d}) Cou ; 0,15(Y^{c}-Y^{d}) Fl ; 0,19(B^{c}-B^{g}) Fl/Cou ; 0,24(G^{e}) Cou ; 0,26(B^{e}) Cou°; 0,30(Y^{d}) Fl ; 0,40(Y^{c}) Fl ; 0,84(P^{d}) Ant
	AC2^{IV}	0,06(G^{c}-Y^{d}) Fl ; 0,08(Y^{e}) Cou ; 0,10(Y^{d}) Fl ; 0,14(Y^{d}-G^{g}) Fl/Cou ; 0,18(B^{c}-B^{d}-B^{d}) Fl/Ant/Cou
	BH^{IV}	0,06(Y^{c}-YB^{d}-G^{g}-Y^{d}) Fl/Cou ; 0,08(Y^{d}) Fl ; 0,13(Y^{d}) Fl ; 0,15(B^{d}-B^{e}) Ant/Cou ; 0,20(B^{c}-B^{d}) Fl/Cou ; 0,83(B^{e}) Cou ; 0,84(P^{d}) Ant ; 0,88(B^{d}) Ant
	AB^{IV}	0,05(YB^{c}-Y^{d}-Y^{g}-YB^{d}) Fl/Cou ; 0,09(B^{c}-Y^{d}-B^{g}-B^{d}) Fl/Cou ; 0,20(B^{d}) Ant ; 0,21(B^{e}) Cou ; 0,23(B^{c}-B^{d}) Fl/Cou

Y: Yellow; B: Blue; V: Green; BV: Blue-Bert; BJ: Blue-Yellow; B.Flu: Fluorescent Blue; Gold: Orange; VOr: Green-Orange ; Vi: Purple ; Gold: Orange; VVi: Green-Violet; JOr: Yellow-Orange; JVi: Yellow-Violet; JB: Yellow-Blue; BVi: Blue-Violet; a: Compounds revealed with Liebermann burchard reagent; b: Compounds revealed with vanillin sulfuric solution; c: Compounds revealed with 1% $AlCl_3$ solution; d: Compounds revealed with ammonia vapours; e: compounds revealed with KOH solution; f: compounds revealed with Dragendorff reagent; g: compounds revealed with basic lead acetate; Tp: Terpene; St: Sterol; Tlp: Triterpene-Lupane; Fl: Flavonoids; Neck: Coumarins; Ant: Anthocyans; Alc: Alkaloids.

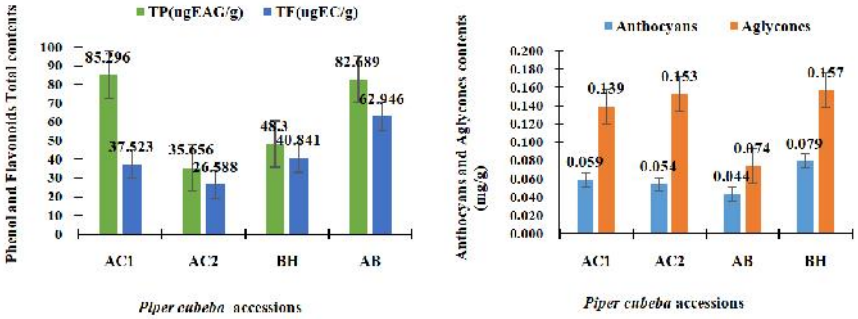

Figure 1. Levels of Total Phenols (TP), Total Flavonoids (TF), Anthocyanins and Flavonic Aglycones

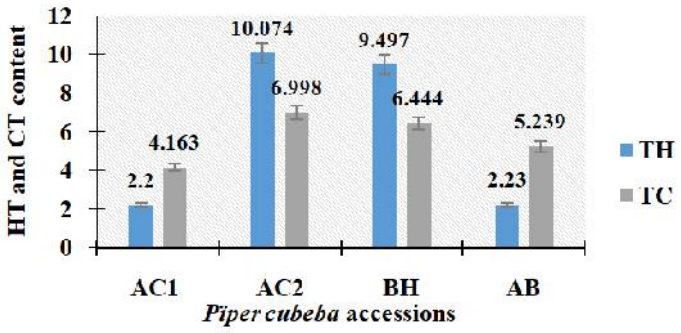

Figure 2. Hydrolyzable (HT) and condensed (CT) tannins contents (expressed in %)

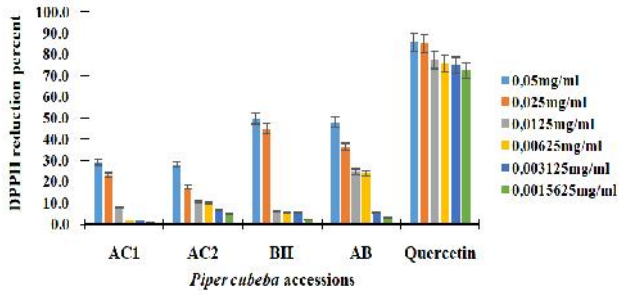

Figure 3.Antioxidant activity of accessions and quercetin

This difference in composition could be related to cultural practices, maturity at harvest and seed storage conditions (Podsedek, 2007).

Total tannin content: Total tannins were dosed into the accessions concentrates. Figure 2 shows the results obtained. It appears that the rate of hydrolyzable tannins (HT) is higher than that of condensed tannins (CT) in the AC2 (10.074 %) and BH (9.497 %) extracts. The lowest levels of HT and CTwere recorded in AC1 and AB accessions. On the other hand, the tannin contents of AC2 and BH are higher than those of LG and MK accessions of P. nigrum obtained under the same conditions by Djiwonou *et al*., (2019). HT and CT are chemical defenses that the plant uses against pathogenic microbes and herbivores.

Antioxidant profile: The results of the evaluation of antioxidant activity in relation to the DPPH radical (Figure 3) show an average reduction of the latter to 0.05mg/ml per BH (49.719 %) and AB (48.132 %). However, at low concentrations the extracts show a small reduction in DPPH. On the other hand, AC1 (29.049 %) and AC2 (27.980 %) show a lower percentage reduction of DPPH at 0.05mg/ml. However, the reduction percentages of these extracts are lower than those of quercetin. The percentages of reduction in *P. cubeba* accessions studied are slightly lower than those of *P. nigrum* accessions (LG 64.983 % and MK 55.022 %) obtained under the same experimental conditions by Djiwonou *et al*., (2019). The presence of phenolic compounds would thus explain the antioxidant aptitude of the accessions. Phytophenols are the means of defence that plants use to respond to environmental attacks and also to fight against various viral and bacterial infestations (Garnero *et al*., 2000).

Conclusion

Piper cubeba from Côte d'Ivoire is used in traditional medicine for its various therapeutic virtues. The antioxidant test DPPH compared to quercetin revealed a low antioxidant capacity. The establishment of the preliminary phytochemical composition of the analyzed accessories, showed an unbalanced distribution of the identified secondary metabolites in the plant, which would govern its medicinal aptitudes; which would attest to its use in traditional medical practice.

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