



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(4): 198-204
Received: 25-05-2016
Accepted: 26-06-2016

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Phytochemical screening and evaluation of the antioxidant activity of the polar extracts *Picalima nitida* Stapf. (Apocynaceae) family

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Abstract

Picalima nitida Stapf., synonymous *Tabernaemontana nitida* known under the vernacular name of "Ngongabé" is a medicinal plant of the family of Apocynaceae, widely used in Central African traditional medicine. This study is a contribution to the phytochemical screening and evaluation of the antioxidant activity of this species. Preliminary phytochemical tests conducted on various crude extracts of *P. nitida* have revealed the presence of some chemical groups: alkaloids, flavonoids, tannins, saponins, triterpenes and sterols. The estimate of the content of total phenols and flavonoids by calorimetric methods showed that the extracts methanolic, hydro-methanolic and aqueous of bark of trunk and root of *Picalima nitida* Stapf., showed that the extracts are rich in these compounds. The evaluation of the antiradical capability realized by the DPPH test on TLC plate on the one hand, and by the method of trapping of the free radical DPPH on microplate 96 wells, revealed that the various extracts of *P. nitida* respectively have a moderated reduction ($2,867 \pm 0,002$ mg/mL, $3,161 \pm 0,016$ mg/mL and $2,693 \pm 0,004$ mg/mL) for the extracts methanolic, hydro-méthanolic and aqueous, but relatively low compared to the molecule of reference, the ascorbic acid ($0,064 \pm 0,000$ mg/mL).

Keywords: *Picalima nitida* Stapf, phytochemical screening, total polyphenols, flavonoids, antioxidant activity

1. Introduction

In developing, the majority of the people living in the rural zones use traditional medicine in the treatment of all kinds of pathologies such as: fever, diarrhoea, jaundice, convulsion, hypertension etc... The use of local medicinal plants, to cure health problems of health can be seen like are an alternative to conventional drugs (Arab K *et al.*, 2013) [3].

In Central Africa, plants are very important in traditional medicine. The remedies using plants to care oneself are cheap and without adverse effects; because this medicine is economically and geographically accessible because those plants often have a real effectiveness.

Picalima nitida Stapf., belongs to the family Apocynaceae of synonymous *Tabernaemontana nitida* or *Picalima klaineana* Pierre or *Picalima macrocarpa* A. Chev (Omino, 2002) [30]. This plant is widely used in African traditional medicine for its therapeutic properties. It is a shrub or deciduous tree, reaching 35 meters height, having white latex in all its parts; the cylindrical trunk measuring approximately 60 cm of diameter. The bark of the trunk is hard and fragile, grayish brown or black color, smooth with slightly rough or finely striped. The fruits are ovoid and yellowish with maturity (Adjanooun *et al.*, 1996) [2]. Each fruit contains three flattened seeds covered in the pulp (Keay *et al.*, 1964; Adjanooun *et al.*, 1984; 1996; Shmelzer *et al.*, 2008) [16, 1, 2, 21]. *Picalima nitida* Stapf, is present in the forest areas of Africa, from the Ivory Coast as far as to Uganda and in the South of Gabon, in Central Africa, in DR. Congo and in Congo.

Ethnobotanic studies revealed that *Picalima nitida* is widely used in the treatment of many diseases like: paludism, the typhoid fever, anaemia, convulsion, jaundice, hypertension, gastro-intestinal disorders, hernia, etc (Burkill *et al.*, 1985; Kapadia *et al.*, 1993; Etukudo I, 2003; Kouitcheu *et al.*, 2008; Jiofack *et al.*, 2009) [7, 15, 9, 21, 13].

In spite of a bibliography available and interesting, the phytochemical study and the evaluation of the antioxidant activity of the Central African species were never carried out to our knowledge. For this reason this work aims at contributing to the valorization of this Central African medicinal plant and identifying the trunk bark and the root of *Picalima nitida* the

secondary metabolites via the tube reactions and by thin layer chromatography, and to evaluate the antioxidant activity, total polyphenols of the crude extracts of *Picralima nitida* Stapf., to understand their antioxidant capability.

2. Materials and Methods

2.1 plant material

The bark of the trunk and the root of *Picralima nitida* Stapf were collected at the Yamboro village located at 18°23'6" East longitude and 4°19'23" South East latitude in the region Ombella M'poko, 30 km south of Bangui in the Central African Republic, after the botanical identification at the Laboratory of Vegetable Biology of the Faculty of Science of the University of Bangui. These bodies were dried out of the sun and moisture, grounded into fine powder by crushing for the later analyses.

2.2 Phytochemical studies

2.2.1. Phytochemical screening

The screening was carried out by two methods mainly the traditional methods described by (Bouquet A., 1969; Jonville MC., 2011) [6, 14] for the tube reactions which are based on the colorings and precipitations reactions with the specific reagents and by the thin layer chromatography method (TLC), described by (Wagner and Bladt, 1996; Jonville Mc., 2011) [42, 14]. The plates were eluted in the systems of binary solvents or not according to the chemical groups needed:

- EtOAc/MeOH/NH₄OH (9/1/1; V/V/V) for alkaloids, revealing reagent of Dragendorff;
- Toluene/ EtOAc (7/3; V/V) for triterpenes and sterols, revealing anisaldehyde;
- EtOAc/AF/Eau (8/1/1; V/V/V) for the flavonoids, revealing the NEU;
- EtOAc / AF/Eau (8/1/1; V/V/V) for the tannins, revealing Fast Blue Salt B.

2.2.2. Quantitative analysis

2.2.2.1. Preparation of crude extracts

2 grams of powder of each body were coldly steeped in 40 mL of methanol, hydro-méthanolic (v/v) and water under magnetic agitation for 24 hours. After filtration, the different filtrates obtained are coldly preserved for different analysis.

2.2.2.2 Dose of total phenols

The total content of phenols in raw extracts were obtained by spectrophotometry according to the method with the reagent Folin-Ciocalteu (Singleton *et al.*, 1999; Mc Donald *et al.*, 2001) [36, 23] with a light modification. The absorbance is measured at 725 nm using a spectrophotometer Al 800/Spectro Direct UV/Visible against a methanol solution used like white. The results obtained, were expressed in equivalent microgram of gallic acid per gram of dry substance ($\mu\text{g EAG/g ms}$).

2.2.2.3 Dose of total flavonoids

The content of flavonoids of the extracts was determined by the colorimetric method of aluminum trichloride of (Zhishen *et al.*, 1999; Kim *et al.*, 2003) [43, 7]. The content in flavonoids were given by using like standard Rutin, and the results were expressed in equivalent microgram of Rutin per gram of dry matter ($\mu\text{gEqRut/g ms}$). The absorbance was measured at 510

nm using a spectrophotometer Al 800/Spectro Direct UV/Visible for a solution of methanol used like white.

2.3. Evaluation of the antioxidant activity

2.3.1 Chromatography method on thin layer

For the evaluation of the antioxidant activity, we used the protocol described by (Takao *et al.*, 1994; Ekoumou, 2003) [38, 8]. Five microliters (5 μL) of each extract was chromatographed on a Silicagel plate 60 F₂₅₄ Merck, with an aluminum support. The plates were placed in a chromatographic tank containing the following mobile phase: EtOAc/AF/Eau (8:1:1; V/V/V). After migration, the chromatograms were dried, and found to 0.1% DPPH in methanol. The components of each extract presenting an antioxidant activity appear as light yellow color spot on purple (purple).

2.3.2 DPPH method on 96-well microplate

This method is based on the use of a free radical: the DPPH describes by (Wood, 1958; Kim *et al.*, 2003; Molyneux P., 2004) [7, 25] with a light modification. Indeed, the reduction of the DPPH is followed by of the purple color to the yellow color of the solution measured by spectrophotometry at 525 nm. There is then antioxidant activity (Sanchez-Moreno, 2002) [34]. The intensity of the color, measured with the spectrophotometer is inversely proportional to the antioxidant activity of the extracts that we wish to determine the activity.

For our extracts, we prepared a solution of DPPH to a concentration of 7mg/20 ml in methanol. This solution was diluted 10 times and stored in the refrigerator up to two days. From the first experiment of each extract (*MeOH*, *MeOH-Water and aqueous*), four dilutions were prepared with various concentrations (60, 150, 240 and 300 mg/L). In each well of microplates well, we deposited using a micropipette pastor 20 μL of each extract and 180 μL of DPPH. For the white, we used 20 μL of DMSO and 180 μL of MeOH. The interpretation is done at 524 nm after 25 minutes of incubation in the darkness. Ascorbic acid has been used as standard prepared under the same conditions as the extracts. All the extracts are reproduced at least 4 times in order to minimize the errors. The results were expressed as a percentage of inhibition (%I).

The antioxidant activity of the extract was expressed as IC₅₀ which defined the concentration of the extract which 50% free radicals (DPPH) (Prior *et al.*, 2005) [33].

The IC₅₀ values were calculated graphically by the method of linear regressions tested graphs, percentage of inhibition according to the various concentrations of crude extracts.

3 Results and Discussion

3.1 screening phytochemical extracts

3.1.1. Reactions colorful tubes

Chart1 shows the presence of six (6) chemical groups highlighted: alkaloids, flavonoids, saponins, tannins and terpenoids. We noted the presence of reducing sugar in the barks of root of *Picralima nitida*. On the other hand, we noted the absence of the anthocyanins and quinones in the two bodies of *P. nitida*

The presence of these secondary metabolites in the barks of trunk and the root of *Picralima nitida* matches the leaves of the species of the variety of Nigeria, Cameroun and also in the

barks of trunk. In addition, in seeds of the species of the Ivory Coast certain we notice the presence of alkaloids and

terpenoids (Ilodigwe EE *et al.*, 2012; Teugwa *et al.*, 2013; Kouassi *et al.*, 2015) [12, 40, 19].

Table 1: Results of the chemical screening of the extracts of the bark of the trunk and root of *Picalima nitida*

Chemical groups	<i>Picalima nitida</i>	
	Bark of the trunk	Bark of root
Alkaloids	+++	+++
Flavonoids	++	++
Quinones	-	-
Tannins catechic	++	+++
Tannins gallic	+++	++
Saponins	++	+
Terpenoids	+++	++
Anthocyanins	-	-
Reducing sugar	-	+++

+++ : Very abundant ++: presence +: trace - Negative

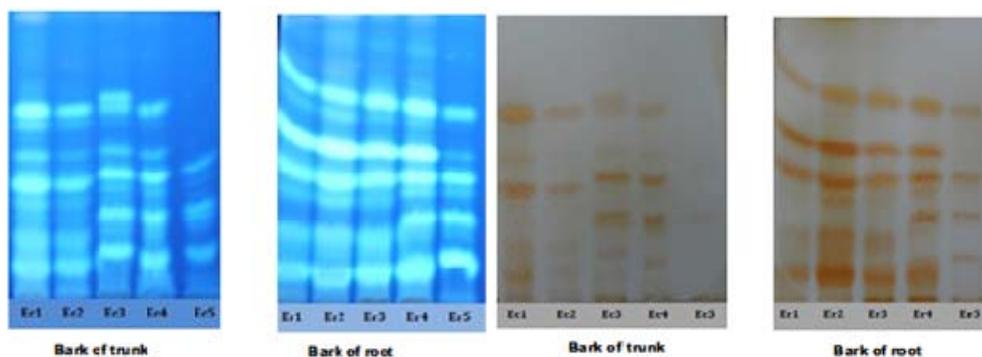
3.1.2 Method by Thin layer chromatography CCM

3.1.2.1 Alkaloids research

The chromatograms n°1 show the presence in the polar extracts of barks of the trunk and the root of *P. nitida*, of the fluorescent spots of blue color and blue-green with UV at 365

nm as well as the visible yellow spot oranges observed after pulverization with the reagent of Dragendorff. The fluorescent spots or yellow spot orange prove the presence of alkaloids in the two bodies of this species (Wagner *et al.*, 1996) [42].

Chromatogram n°1: Results TLC of the polar extracts of bark of the trunk and the root of *Picalima nitida*



Before revelation, UV at 366 nm
Revelation with Dragendorff
Ec1=Er1: MeOH; Ec2=Er2: EtOH; Ec3=Er3: EtOH-Water; Ec4=Er4: MeOH-Water; Ec5=Er5: Aqueous

3.1.2.2 Terpenoids research

The revealed chromatograms n°2 with sulphuric anisaldehyde followed by heating at 105 °C, show the presence of the spots violets, greenish in the barks of trunk and the root of *P. nitida* characteristics of triterpenes and sterols according to (Wagner and Bladt; 1996) [42].

Chromatogram n°2: Results TLC of the terpenoids of the extracts CHCl₃ and CH₂Cl₂ of bark of trunk and of the root of *P. nitida*

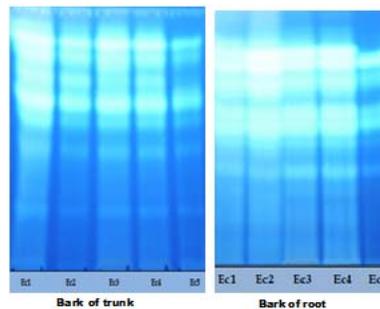


A Revelation anisaldehyde + heating at 100 °C
Ec1 = CHCl₃ = Er1; Ec2 = CH₂Cl₂ = Er2

3.1.2.3 Flavonoids research

After revelation with the NEU and observation with UV at 365 nm, the chromatograms n°3, present several blue spots and white bluish in the polar extracts of the plant studied, characterizing the presence of the phenolic mixture (acid phenolic, flavonoids and or probably coumarins).

Chromatogram n°3: Results TLC flavonoids polar extracts of bark of the trunk and the root of *Picalima nitida*

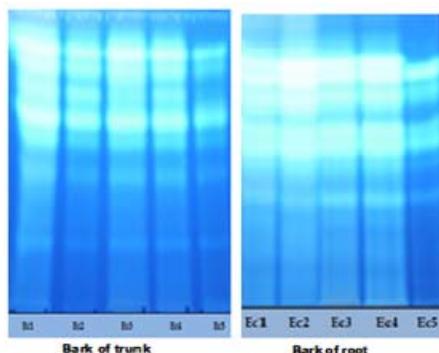


Neu + UV at 366 nm
Ec1=Er1: MeOH; Ec2=Er2: EtOH; Ec3=Er3: EtOH-Water; Ec4=Er4: MeOH-Water; Ec5=Er5: Aqueous

3.1.2.4 Tannins research

The revelation of the two chromatograms n°4 of the polar extracts of the species of *P. nitida* confirms the presence of the tannins in this species with appearance of the many blue spots.

Chromatogram n°4: Results CCM tannins of the polar extracts stem bark and root of *Picralima nitida*



Revelation Fast Blue Salt B + UV at 366 Nm

Ec1: MeOH = Er1; Ec2: EtOH = Er2; Ec3: MeOH-Water = Er3; Ec4: EtOH-Water = Er4

The results of the chromatography on light layer confirm the presence of certain chemical families observed in the reactions out of tubes, in particular the alkaloids, flavonoids, tannins and terpenoids.

Indeed the former work carried out on the various bodies of *P. nitida* had shown: the presence of alkaloids, the molecules isolated from the family of alkaloid of the type indols (Henry *et al.*, 1972) of triterpenes in the species of the variety of Nigeria, of Cameroun, Ghana like that of the Ivory Coast (Menziés *et al.*, 1998; Tane *et al.*, 2002; Ilodigwe EE *et al.*, 2012; Teugwa *et al.*, 2013; Osayemwenré *et al.*, 2014; Kouassi *et al.*, 2015) [24, 39, 12, 40, 31, 19].

3.2. Contents of total polyphenols and flavonoids

The determination of the contents of total phenols and flavonoids in the various extracts of *P. nitida* was made separately by using the colorimetric methods (Folin-Ciocalteu reagent aluminium and chloride). The contents in total phenols and flavonoids in the crude extracts of *P. nitida* were determined from the equations of the linear regression each calibration curve expressed successively in μg gallic acid equivalent of and μg equivalent of rutin per gram of the dry matter (Figures 1, and 2).

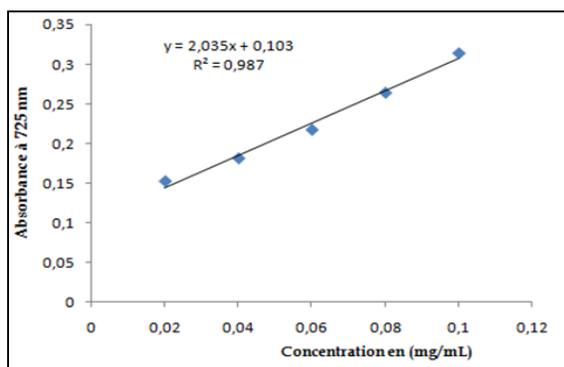


Fig 1: Calibration curve of the gallic acid for the determination of total phenols.

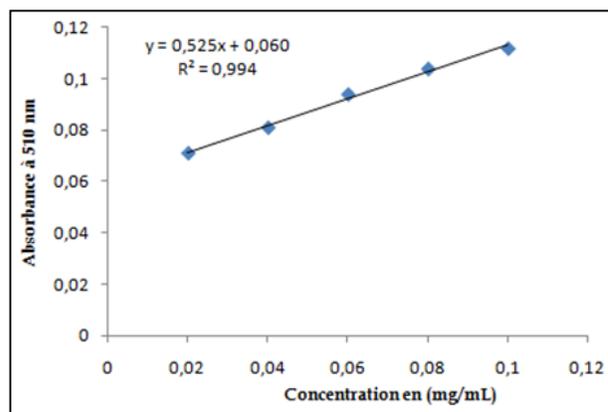


Fig 2: Calibration curve of rutin for the determination of flavonoids total.

The results obtained (chart2) show that the average contents total phenols vary from $249,63 \pm 3,8281$ to $276,6 \pm 6,21 \mu\text{g Eq AG/g ms}$ respectively in the barks of the trunk and those of the root of *P. nitida*. On the other hand those of the flavonoids vary from $1003,59 \pm 12,09$ to $1520,21 \pm 16,05 \mu\text{g Eq Rut/g ms}$. We found that this plant has the high levels of flavonoids compared to total polyphenols, so much in the barks of the trunk than in the roots.

Furthermore these results shown in Chart2 enable us to underline what follows: in the barks of the trunk; the aqueous extract on the other hand presents a higher content polyphenols at the level of the barks of roots the highest content is obtained in the extracts methanolic. The aqueous fraction of the barks of roots presents the content more bases out of polyphenols of about $167,24 \mu\text{g Eq AG/g ms}$. The highest content of flavonoids is obtained with the extract methanolic in the two bodies of the plant ($2333,96 \pm 11,86$ and $1167,61 \pm 3,22 \mu\text{g Eq AG/g ms}$).

These results confirm the great wealth of phenolic substances (flavonoids, tannins) present in the species *P. nitida* highlighted in tube and TLC. The work of (Kouam *et al.*, 2011) [18] had confirmed the presence of many molecules polyphenols isolated in the roots of this plant.

Moreover we noted that, this plant has high percentages of flavonoids compared to total polyphenols as well in the barks of the trunk as in the roots. The total polyphenol rate in the extract methanolic of the barks of trunk is close to that found by (Teugwa *et al.*, 2013) [40]. ($215,31 \pm 15,10 \mu\text{g Eq Cat/g ms}$ for the species of Cameroun. This variability in the results could be related to the climatic conditions of biotic species, the physiological state or the various methods followed during the extraction.

Table 2: Contents of total polyphenols and flavonoids extracts of *P. nitida* Stapf.

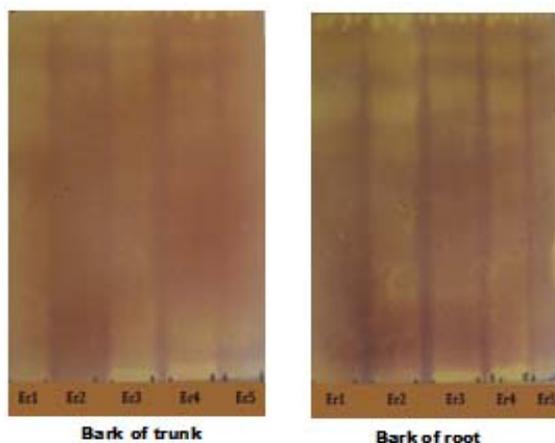
bodies	Extracts	Content total Polyphenols ($\mu\text{g Eq AG/g MS}$)	Content total Flavonoids ($\mu\text{g Eq Rut/g Ms}$)
Bark of trunk	Methanolic	217,03 \pm 3,81	1167,61 \pm 3,22
	Hydro-methanolic	235,87 \pm 2,04	935,23 \pm 14,26
	Aqueous	295,98 \pm 5,63	907,93 \pm 18,80
General average		249,63 \pm 3,82	1003,59 \pm 12,09
Bark of root	Methanolic	412,28 \pm 6,13	2333,96 \pm 11,86
	Hydro-methanolic	250,28 \pm 10,51	1170,81 \pm 10,45
	Aqueous	167,24 \pm 1,99	1055,87 \pm 25,84
General average		276,6 \pm 6,21	1520,21 \pm 16,05

3.3. Evaluation of antioxidant activity

3.3.1 Method by thin layer chromatography

The chromatogram n°5 of the various polar extracts of barks of the trunk and the root of *P. nitida* revealed by a solution of DPPH 0,1% in methanol present many tasks of yellow color white on purple bottom; what testifies to the significant antioxidant activity of these compounds in these polar extracts (N'gaman *et al.*, 2009; Gabriel *et al.*, 2007) [27, 11].

Chromatogram n°5: Chromatographic profiles of the antioxidant activity of the various polar extracts of *Picralima nitida* Stapf.



Ec1=Er1: MeOH; Ec2=Er2: EtOH; Ec3=Er3: EtOH-Water; Ec4=Er4: MeOH-Water; Ec5=Er5: Aqueous

3.3.2 Method of 2, 2-diphényl-1-picrylhydrazyl (DPPH) on 96-well microplate

The antioxidant activity of the polar extracts of barks of trunk and of the root of *Picralima nitida* Stapf., and of the mixture of reference (ascorbic acid) with respect to free radical DPPH was evaluated using a reader of plates *MULTISKAN FC version 100-79*. The reduction of the DPPH is followed by its passage by the purple color (DPPH⁻) to the yellow color (DPPH-H) measurable at 524 nm. This reduction in capacity is determined by a decrease in absorbance induced by the anti-radical substances.

The results of the reducing activity of the polar extracts (methanolic, hydro-methanolic and aqueous) of the studied plant are represented on figure 3. We note that the reduction is proportional to the increase in the concentration.

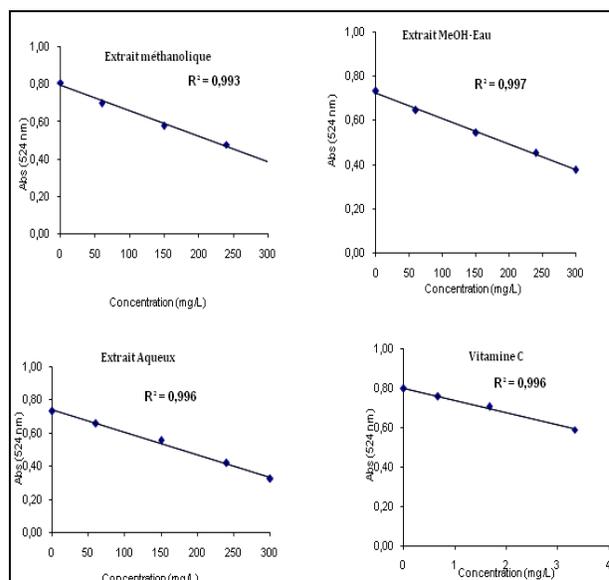


Fig 3: Reduction of the polar extracts of the plant *P. nitida* and the ascorbic acid.

The values of the percentage of inhibition IC_{50} (table 3) obtained starting from the graphs (fig.2), show that all the polar extracts present the values of high IC_{50} of 2,693 \pm 0,004 mg/mL; 2,867 \pm 0,002 mg/mL and 3,161 \pm 0,016 mg/mL, respectively for the aqueous extracts, methanolic and hydro-methanolic compared to the vitamin C (0,064 \pm 0,000 mg/mL). We note that it is the fraction of the aqueous extract (2,693 \pm 0,004 mg/mL) which presents a significant antioxidant activity comparable to that of the vitamin C (0,064 \pm 0,000 mg/mL). Also, the difference of the values of IC_{50} obtained in each type of extract could be due to the presence and the structural nature of the phenolic compounds in these extracts. The reduction of these polar extracts confirms the results found by the thin layer chromatographic method; it is probably at the relatively high rates of the flavonoids in these extracts. On the other hand former work revealed the values of IC_{50} significant and significant (0,053 \pm 0,001 to 1,00 \pm 0,06 mg/mL) respectively in the extracts methanolic of the sheets and for bark of trunk of *Picralima nitida* Stapf. (Teugwa *et al.*, 2013; Osayemwenre *et al.*, 2012) [40].

Table 3: Values of the concentration of IC_{50} of the polar extracts of bark of trunk of *P. nitida*.

Extracts	MeOH	MeOH-Water	Aqueous	Vitamin C
Average IC_{50} (mg/mL)	2,867 \pm 0,002	3,161 \pm 0,016	2,693 \pm 0,004	0,064 \pm 0,000

4. Conclusion

This study allowed to highlight the following families: alkaloids, flavonoids, tannins, triterpenes and sterols, and of saponins in the species *Picralima nitida* Stapf. The rates of the lowest phenolic mixtures low obtained amount to 249, 63 ± 3,82 µg Eq AG/g ms in the barks of trunk and 276,6 ± 6,21 µg Eq AG/g ms in the barks of roots for polyphenols. On the other hand the rates of flavonoids are higher (1003, 59 ± 12, 09 and 1520,21 ± 16,05(µg Eq Rut/g ms) that those of phenols. The polar extracts showed a reduction, rather significant with the aqueous extract (2,693 ± 0,004), which enables us to say that the species *P. nitida* has antioxidant properties which, probably would be due to the phenolic mixtures.

The species *Picralima nitida* Stapf, thus appears to be a plant rich in secondary metabolites, widely used in traditional medicine in the region of Ombella M'poko, in the south of Bangui in Central African Republic for treatment of various ailments. An exploitation of its pharmacological properties implies a more thorough search for its active ingredients, currently in on going.

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