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Comparative Study of Phytochemical Composition and *In vitro* Antioxidant Activities of *Pseudocedrela kotschyi* Leaf, Trunk and Root Barks

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ABSTRACT

The current work aimed to compare phytochemical composition and antioxidant activities of leaf versus trunk and root barks of *Pseudocedrela kotschyi* to discover whether the leave can be used effectively in traditional medicine instead of trunk and root in order to combat the extinction of the plant. Leave, trunk and root barks of *P. kotschyi* were successively extracted by maceration with hexane, dichloromethane and ethanol. Qualitative phytochemical composition and phytophenol contents of three ethanolic extracts were investigated. Antiradical activities and reducing power of the ethanolic extracts were evaluated to appreciate their antioxidant properties. The findings revealed that the leave has almost the same phytochemical composition compared to trunk and root barks of *P. kotschyi*, but its phenolic compound content is lower and related to its antioxidant activities. A probable increase in the concentration of leaf recipes can probably allow their effective exploitation instead of trunk and root.

Keywords: *Pseudocedrela kotschyi*, Traditional medicine, Ethanolic extracts, Phenolic compounds, Antioxidant activities.

INTRODUCTION

For a long time, medicinal plants have contributed significantly to the well-being of populations. They constitute an immense source for the discovery of new bioactive molecules to be used to treat many pathologies[1].

Despite the countless studies already carried out on plants, they still remain the most interesting and essential source for the discovery of natural molecules with bioactive potential. For this reason, medicinal plants are the primary pool of molecules whose isolation has enabled the production of highly effective pharmaceutical products. In fact, it is estimated that around 25% of all medicines sold in pharmacies worldwide are

obtained from plants[2]. It seems that most of the medicinal plants already recorded on the globe are found in tropical countries, particularly in Africa, with very impressive therapeutic virtues[3]. So far, the poor populations of Africa have managed to survive despite the severity of certain epidemic diseases, often thanks to the use of proven phytomedicines.

During their growth, the ability of plants to synthesize various molecules, some of which have therapeutic properties, is linked to the fact that they undergo biotic and abiotic stresses in their environment. Abiotic stress refers to unfavorable environmental conditions such as drought, flooding, extreme temperatures, soil salinity and nutrient deficiencies. These stresses can impact the ability of the plants to fully perform their vital functions[4]. However, biotic stress involves attacks by living organisms such as: birds, mammals, herbivores, arthropods, microorganisms and weeds which cause serious problems for plants. All these various stresses then trigger the synthesis of natural substances in plants to allow them to develop, multiply and survive, despite the attacks to which they are constantly exposed. This makes plants potential sources of storage of bioactive substances[5] which are used by humans as active ingredients in the formulation of medicinal products. Among the substances usually synthesized by plants, there is a class of biomolecules known as phenolic compounds. This is a heterogeneous group of phytoconstituents, each containing at least one phenol function in its structure. Among the phenolic compounds, flavonoids and tannins are considered to be the most studied subclasses, due to their therapeutic virtues considered to be very useful to humans. The mammalian body is also stressed daily due to the formation of free radicals, i.e. highly reactive unstable compounds containing single electrons. Unfortunately, in order to stabilize themselves, these free radicals attack certain vital biological molecules by creating other free radicals. This triggers chain reactions that have the disadvantage of degrading numerous cellular constituents, notably: DNA, lipids and proteins[6].

Indeed, clinical disorders such as: cancer, liver disease, diabetes, heart failure, arterial hypertension, atherosclerosis, Alzheimer's disease, rheumatic arthritis, hypercholesterolemia, neurodegenerative diseases, aging, immunological disorders and chronic inflammation[7,8] are the adverse consequences arising from the harmful effects of free radicals in humans.

Phenolic compounds synthesized by plants are therefore historically highly reputed for combating the harmful effects due to free radicals. In Togo, the treatment of diseases using plants is still the most accessible method for the population, especially those of predominantly rural origin. Indeed, the proportion of the Togolese population who treat themselves with medicinal plants is around 60-80%[9,10].

It is no longer a secret that the majority of molecules with therapeutic properties are produced by plants. One such plant that can be used by humans to stop or slow down the harmful effects of stress is *P. kotschyi*. In fact, previous studies have revealed that the aqueous extract of root bark and the ethanolic extract of the leaves both have impressive antioxidant properties[11,12].

Furthermore, it has been reported that *P. kotschyi* is widely used in non-conventional medicine thanks to its multiple biological activities, such as: activity[13], antioxidantactivity[11,12], antidiarrheal activity[12], antidiabetic activity[14], antimicrobial activities[15], hepatoprotective activity[16], antimalarial activity, anti-inflammatory activity, analgesic activity, antibacterial activity, anthelmintic activity, and antipyretic activity. This is confirmed through previous studies showing that the root is often used for the treatment of numerous pathologies[17]. All these properties are often attributed to the phytochemical groups contained in various plant organs. However, overuse of the trunk and especially the roots of the plant for therapeutic purposes could lead to its disappearance, as it is often the whole plant that is uprooted after recovering the roots.

In the interest of preserving the plant by using the leaves for health care instead of trunk and root, this work focused on the phytochemical composition and antioxidant activities of ethanolic extracts of *P. kotschyi* leave in comparison with trunk and root barks.

MATERIALS AND METHODS

Plant material

Leaves, trunk and root barks, used as raw materials to extract the phytoconstituents of *P. kotschyi*, were harvested during october 2021, in Haho district, not far from the city of Notse, Togo.

The plant was identified using the following code "TOGO 15976" in the LBEV laboratory of the Faculty of Sciences in Université de Lomé, Togo.

Methods

Pretreatment of plant material

After harvesting, all plant material (leave and trunk and root barks) was brought back to the laboratory, then dried for about two weeks, at room temperature (30-32°C) and away from sunlight. Then, every dry plant material was crushed by a mill (Thomas Scientific Laboratory Model 4, USA), equipped with a sieve of 1 mm pore diameter. The powders obtained were re-dried before being stored in well-labeled bottles, then preserved for later use.

Extraction of phytoconstituents contained in plant material

The extraction method applied in the present study was maceration, carried out successively with three organic solvents of increasing polarity, which are: hexane, dichloromethane and ethanol (95% vol.). The solid-liquid ratio 1:10 (m/v) was considered. In practice, 40 g of each sample of dry vegetable powder were successively extracted with 400 mL of each solvent. Indeed, in the first step, each vegetable powder was first degreased by soaking in hexane for 72 h and with manual stirring. In the second step, the residue recovered after degreasing was extracted with dichloromethane. Finally, in the third step, the new residue obtained after extraction with dichloromethane was extracted again with ethanol.

The ethanolic solution obtained for each powder sample was filtered using filter paper. The solvent contained in the filtrate was removed under vacuum using Büchi rotary evaporator system. Then, the dry ethanolic extract was collected in a tinted glass bottle before being stored in a freezer whose temperature was set at –23°C.

Qualitative phytochemical characterizations of ethanolic extracts

The different groups of biomolecules contained in the ethanolic extracts of *P. kotschyi* were highlighted by the staining and/or precipitation tests. Thus, the alkaloids were sought by the reaction with Dragendorff reagent[18] ; phenolic compounds and tannins, respectively based on the Stiasny reagent and the test with $FeCl_3$[19]; flavonoidsby reaction with cyanidins[20]; anthocyanins by the reaction with hydrochloric acid (HCl), followed by addition of ammoniac NH_3[21]; coumarins by adding 2 drops of NaOH (10% : w/v) to the extract followed by water bath heating[22], anthraquinones, with ammoniac NH_3 (10%)[23]; cardiac glycosides, by using a mixture of chloroform $CHCl_3$ and acetic anhydride HOOC-CO-COOH with concentrated sulfuric acid H_2SO_4[24]; reducing sugars with Fehling reagent test[19]; saponins, using foam test[19]; and finally, sterols and triterpenes,with Liebermann-Burchard test[22].

Determination of phenolic compounds found in ethanolic extracts of *P. kotschyi*

Total phenol contents in extracts

Total phenol contents in extracts were determined by applying the colorimetric method using UV-Visible spectrophotometer and Folin-Ciocalteu reagent[25].

According to the experimental protocol, a volume of 1,600 µL of aqueous solution of Na_2CO_3 (6%: m/v) was mixed with 2,000 µL of aqueous Folin Ciocalteu reagent (10%: v/v) in a test tube. The previous mixture was agitated with vortex and subsequently left to stand for 5 minute. Then, 400 µL of each ethanolic extract (1 mg/mL) or gallic acid GA (0-300 µg/mL) was added. The final mixture was agitated with vortex, then incubated for 30 min at room temperature (28-30°C) and away from light.

The absorbance of the resulting samples was measured with METASH UV-5200PC spectrophotometer against the blank at the wavelength of 760 nm. The calibration curve (Fig. 1) was established with gallic acid (GA) used as a standard for a concentration range of 0-300 µg/mL. The total phenol contents of the samples thus analyzed were expressed in mg of GA equivalent (Eq) per g of dry extract DE (mg GA Eq/g DE).

Total flavonoid contents in extracts

The contents of total flavonoids in extracts were measured by the colorimetric method using UV-Visible spectrophotometer[26].

Experimentally, 200 µL of each aqueous ethanolic extract (1 mg/mL) or ethanolic quercetin QC (0-800 µg/mL), used as a standard, was mixed in a test tube with 1,600 µL of distilled water. Then, 80 µL of aqueous $AlCl_3$ (10%: m/v) and 120 µL of aqueous $NaNO_2$ (5%: m/v) were successively added. The previously prepared solution was incubated

for 6 min, then added with 1 mL of aqueous NaOH solution (1 M) before being stirred with a vortex. The absorbance of the final solution was measured with METASH UV-5200PC spectrophotometer at a wavelength of 510 nm, against the blank. The total flavonoid contents of the samples were deduced based on a calibration curve (Fig. 2) established with quercetin QC (0-800 µg/mL). The results were expressed in mg of QC equivalent (Eq) per g of dry extract DE (mg QC Eq/g DE).

Total hydrolyzable tannin contents in extracts

The contents of total hydrolyzable tannins in extracts were carried out by colorimetric dosage with ferric chloride using UV-Visible spectrophotometer[27].

According to the experimental approach, 1 mL of each ethanolic extract (1 mg/mL) or ethanolic tannic acid (TA: 0-200 µg/mL) was introduced into 3.5 mL of $FeCl_3$ (0.01 M) prepared with aqueous HCl solution (0.01 M). The preceding mixture was vigorously vortexed before reading its absorbance at the wavelength of 660 nm against the blank, using METASH UV-5200PC spectrophotometer.

The total hydrolysable tannin contents were quantified in mg of TA equivalent (Eq) per g of dry extract DE (mg TA Eq/g DE), from a calibration curve (Fig. 3) constructed by TA (0-200 µg/mL).

Total condensed tannin contents in extracts

Determination of the contents of condensed tannins or proanthocyanidins or total non-hydrolyzable tannins in the three ethanolic extracts of *P. kotschyi* was obtained using the colorimetric method using UV-Visible spectrophotometer[28].

In practice, 50 µL of each ethanolic extract (1 mg/mL) or ethanolic catechin (1-400 µg/mL) were introduced into a test tube containing 1,500 µL of the ethanolic vanillin (4%: m/v). The mixture gotten was agited vigorously with vortex, and 750 µL of HCl was added. The final solution was incubated at room temperature (28-30°C). After 20 min, the absorbance of the solution was measured with METASH UV- 5200 PC UV-Visible spectrophotometer at the wavelength of 550 nm.

A calibration curve (Fig. 4) was performed with catechin CT (0-400 µg/mL), and the total condensed tannin contents were expressed in mg of catechin (CT) equivalent (Eq) per g of dry extract DE: mg CT Eq/g DE.

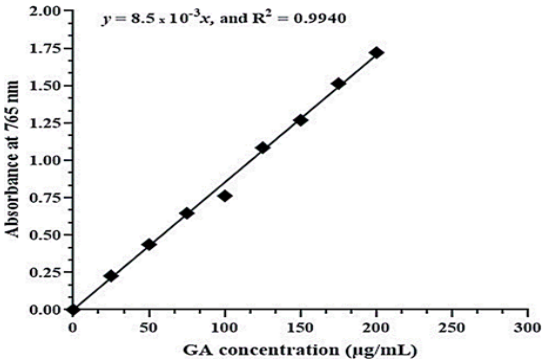

Fig. 1. Calibration curve of GA

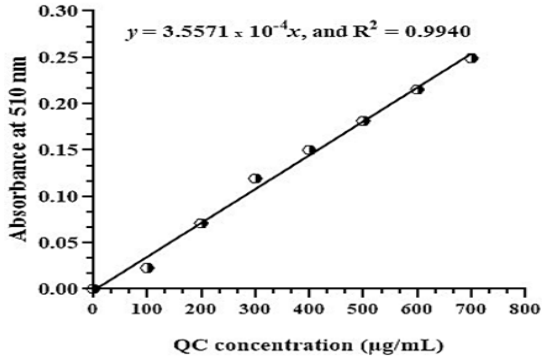

Fig. 2. Calibration curve of QC

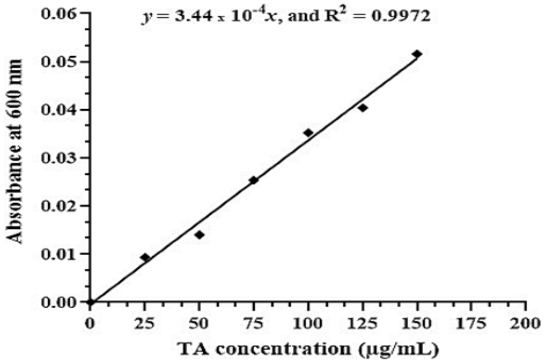

Fig. 3. Calibration curve of TA

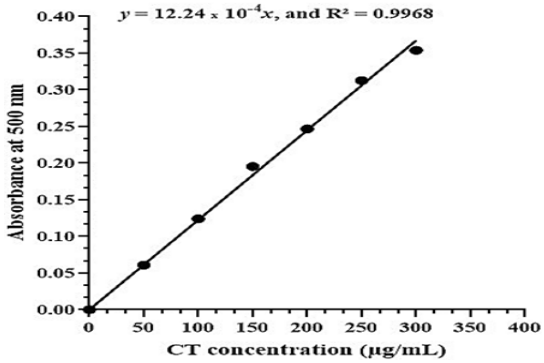

Fig. 4. Calibration curve of CT

AG = Gallic acid; QC = Quercetin; TA = Tannic acid; CT = Catechin.

Antioxidant activity evaluation

Two types of antioxidant activities were evaluated in the current work: the test carried out with DPPH• reagent, and FRAP test. These two tests are called respectively : antiradical activity and reducing power.

Antiradical activity of the extracts

The protocol used to evaluate antiradical activity of the ethanolic extracts is described as follows[29]. 100 µL of each ethanolic extract (0-80 µg/ mL) or ethanolic GA (0-80 µg/mL) were added to a 3,000 µL of ethanolic DPPH• (0.4%: m/v).

After an incubation period of 10 min at (28-30°C) and away from light, the absorbance of the solution was measured at 517 nm with METASH UV- 5200 PC UV-visible spectrophotometer against the blank. The tests were carried out in triplicate and inhibition percentage was calculated (Formula 1) for each measurement.

$$\% \text{ Inhibition} = \left[\frac{A_b - A_s}{A_b}\right] \quad (1)$$

With: A_b: Absorbance of blank, and A_s: Absorbance of sample.

The average value of the three measurements carried out for each sample was taken into account. The linear curves of inhibition of the radical DPPH• by the extracts, and by gallic acid (GA) were plotted with a concentration range of 0-80 µg/ mL (Figure 5).

The equations (Table 1) of the linear regression curves, established from Fig. 5, were used to determine the half-maximal inhibitory concentration (IC_{50}) of the samples analyzed in the current study. The squared values of the correlation coefficient (R) of these curves denoted the precision of the analysis methods.

Table 1: Equations of linear regression curves for IC_{50} determination of the samples

Samples	Equations	R^2
GA	y = 1.1477 x	0.9948
Le-Ext	y = 0.5246 x	0.9906
Tr-Ext	y = 1.0377 x	0.9952
Ro-Ext	y = 0.8869 x	0.9892

GA: Gallic acid; Le-Ext: Leaf extract; Tr-Ext: Trunk bark extract; Ro-Ext: Root bark extract; IC_{50}: Half-maximal inhibitory concentration; and R: Correlation coefficient.

Reducing power of extracts

The method applied for reducing power evaluation of the extracts is described as below[30].

FRAP reagent was prepared by mixing 160 mL of acetate buffer (300 mM, pH = 3.6), 20 mL of aqueous TPTZ-Fe^{3+} (10 mM) in HCl (40 mM), and 20 mL of aqueous $FeCl_3.6H_2O$ (20 mM), in the proportions of (8:1:1). 2,000 µL of freshly prepared FRAP reagent was added to 1,000 µL of each ethanolic extract in ethanol (0-12.5 µg/ mL) or ethanolic ascorbic acid AA(0-12.5 µg/mL).

The preceding mixture was incubated for 10 min, and the absorbance was measured at 593 nm with METASH UV-5200 PC UV-visible spectrophotometer against the blank.

The the extract reducing powers were evaluated from the established calibration curves (Fig. 6). An increase in absorbance corresponds to an increase in the reducing power of the extracts. Ascorbic acid AA was used as a reference.

The equations (Table 2) of the linear regression curves, established from Fig. 6, were used to deduce the reducing powers (RP) of the samples analyzed in the current study by using formula 2.

$$RP = S_{samp}/S_{ref} \quad (2)$$

With: S_{samp}: slope of the sample curve (expressed as mL/g); S_{ref}: slope of the reference (AA) curve (expressed as mL/mg), and RP: Reducing power, expressed as mg Eq AA /g DE of each sample thus analyzed. The squared values of the correlation coefficient (R) of these curves indicated the precision level of the measurements.

Table 2: Equations of linear regression curves for RP determination of the samples

Samples	Equations	R^2
AA	y = 0.09281 x	0.9996
Le-Ext	y = 0.04692 x	0.9940
Tr-Ext	y = 0.07297 x	0.9973
Ro-Ext	y = 0.05214 x	0.9991

AA: Ascorbic acid; Le-Ext: Leaf extract; Tr-Ext: Trunk bark extract; Ro-Ext: Root bark extract; RP: Reducing Power; and R: Correlation coefficient.

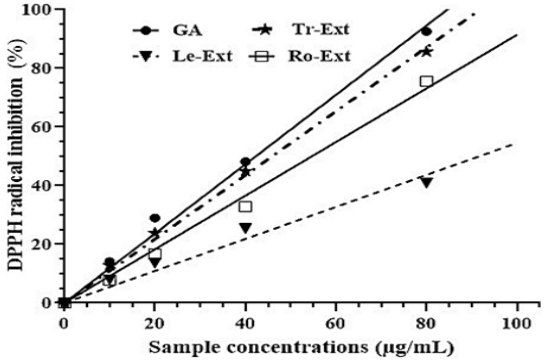

Fig. 5. Inhibition curves of DPPH· radical by the samples

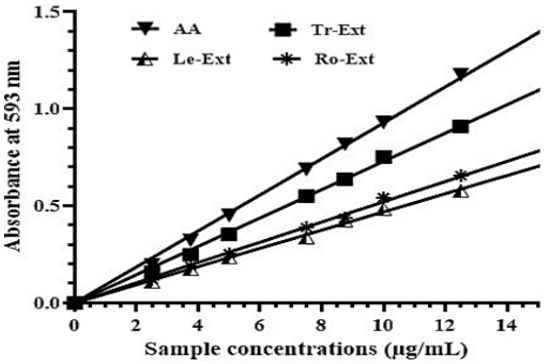

Fig. 6. Calibration curves for RP of the samples

GA: Gallic acid; AA: Ascorbic acid; Le-Ext: Leaf extract; Tr-Ext: Trunk bark extract; Ro-Ext: Root bark extract; RP: Reducing Power

Determination of correlations between phenolic compound contents and antioxidant activities highlighted with the ethanolic extracts

This correlation was established by plotting the variation curves of the phenolic compound contents as a function of its antiradical capacities evaluated by using DPPH• reagent test (Fig. 7) and as a function of their reducing powers evaluated by using FRAP test (Fig. 8) according to the method applied in literature[31,32].

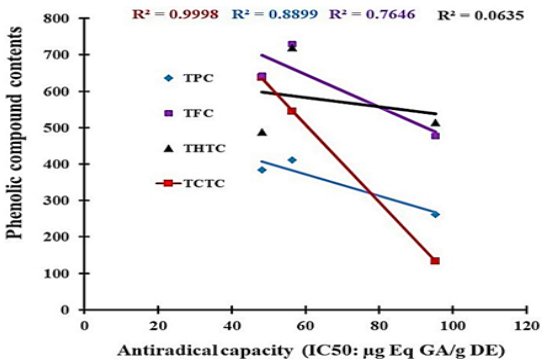

Fig. 7. Correlation between phenolic compound contents and antiradical capacities of the ethanolic extracts

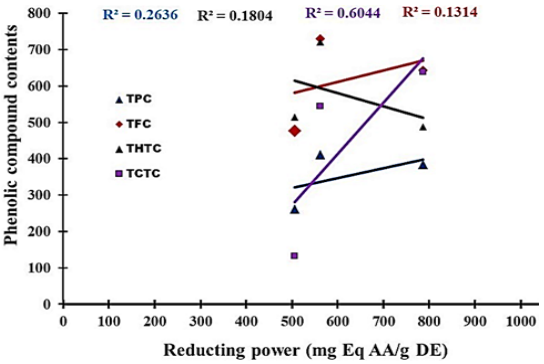

Fig. 8. Correlation between phenolic compound contents and RP of the ethanolic extracts

RESULTS

Phytochemical composition of the ethanolic extracts

Phytochemical composition of the ethanolic extracts is recorded in Table 3. Among the various phytoconstituents investigated, only triterpenes and sterols were not detected in the three ethanolic extracts of *P. kotschyi*. Anthraquinones and cardiac glycosides were only absent in the ethanolic root extract, while anthocyanins were only absent in the ethanolic leaf extract.

Table 3: Phytochemical constituents detected in ethanolic extracts of *P. kotschyi* leaf, trunk bark and root

Phytoconstituents revealed	Le-Ext	Tr-Ext	Ro-Ext
Alkaloids	+	+	+
Phenolic compounds	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Anthocyanins	-	+	+
Coumarins	+	+	+
Anthraquinones	+	+	-
Cardiac glycosides	+	+	-
Saponins	+	+	+
Reducing sugars	+	+	+
Triterpenes and sterols	-	-	-

Le-Ext: Leaf extract; Tr-Ext: Trunk bark extract; Ro-Ext: Root bark extract. (+): indicates the presence of phytochemicals; and (-): indicates the absence of phytochemicals.

Phenolic compound contents in the three ethanolic extracts

Total phenol contents in the three ethanolic extracts

The results displayed in Fig. 9 show that the total phenolic contents (mg GA Eq/g DE) of the ethanolic extracts of leaf, trunk and root barks of *P. kotschyi* are: 261.732 ± 8.315; 383.3 ± 11.202; and 410.933 ± 4.220, respectively.

Total flavonoid contents in the extracts

The total flavonoid contents in the three *P. kotschyi* extracts are reported in Fig. 10. The results presented in Fig. 10 indicate that the leaf extract has a total flavonoid content of 477.417 ± 45.305 mg QC Eq/g DE, while those of trunk and root barks are 643.33 ± 79.605 and 729.583 ± 25.165 mg QC Eq/g DE, respectively.

Total hydrolyzable tannin contents in three ethanolic extracts

The total hydrolyzable tannin contents in the three ethanolic extracts of *P. kotschy* are recorded in Fig. 11. The values obtained (expressed in mg TA Eq/g DE) for the ethanolic extracts of the leaf, trunk and root barks are: 514.517 ± 62.61; 487.678 ± 26.637 and 720.413 ± 43.585, respectively.

Total condensed tannin contents in the three ethanolic extracts

In Fig. 12 are exposed the total condensed tannin contents in the three ethanolic extracts of *P. kotschy*. The values found (expressed in mg CT Eq/g DE) are: 133.170 ± 1.593; 638.671 ± 19.581 and 544.254 ± 10.931, respectively for ethanolic extracts in leaf, trunk and root barks of *P. kotschy*.

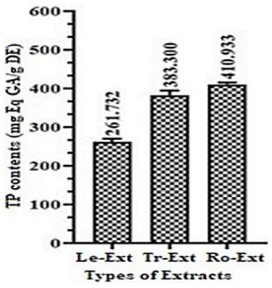

Fig. 9. TP contents of the ethanolic extracts

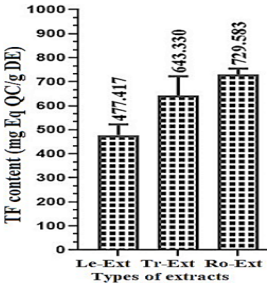

Fig. 10. TF contents of the ethanolic extracts

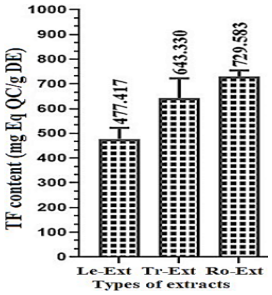

Fig. 11. THT contents of the ethanolic extracts

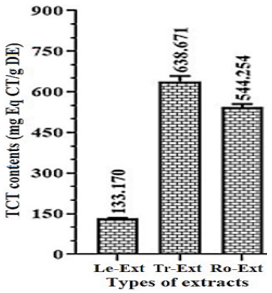

Fig. 12. TCT contents of the ethanolic extracts

TP: Total phenols; TF: Total flavonoids; THT: Total hydrolyzable tannins; TCT: Total condensed tannins; GA: Gallic acid; QC: Quercetin; TA: Tannic acid; CT: Catechin; DE: Dry Extract; Eq: Equivalent; Le-Ext: Leaf extract; Tr-Ext: Trunk bark extract; Ro-Ext: Root bark extract.

Antioxidant activities of the three ethanolic extracts

The antiradical activities of the three ethanolic extracts of *P. kotschyi* are illustrated in Fig.13. The concentration of dry extract required for 50% inhibition, noted IC_{50} (expressed in µg DE/mL) corresponding to the results of Fig. 13 are: 95.311 ± 1.126; 48.183 ± 1.521 and 56.372 ± 1.054, respectively for ethanolic extracts of leaf, trunk and root barks of *P. kotschyi*, while that of gallic acid, used as a reference, is 43.565 ± 0.970.

Reducing powers of the ethanolic extracts

The reducing powers (RP) of the ethanolic extracts of *P. kotschyi*, evaluated by means of reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) ion test, allowed to obtain results reported in Fig. 14. The values found (expressed in mg AA Eq/g DE) are: 505.5489; 786.2299 and 561.7929, respectively for the ethanolic extracts of leaf, trunk and root barks of *P. kotschyi*.

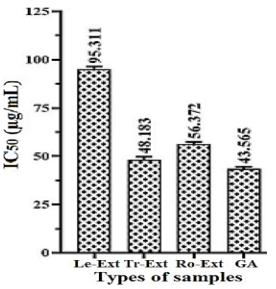

Fig. 13. Antiradical capacities of the samples

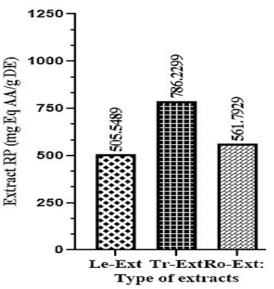

Fig. 14. Reducing powers of the samples

Le-Ext: Leaf extract; Tr-Ext: Trunk bark extract; Ro-Ext: Root bark extract.; DE: Dry Extract; IC_{50}: Half-maximal inhibitory concentration; Eq: Equivalent; AA: ascorbic acid; DE: Dry extract; AG: Gallic acid

Correlations between phenolic compound contents and antioxidant activities highlighted with ethanolic extracts

The results obtained about the correlation between the phenolic compound contents and the antioxidant powers are summarized in Table 4.

Strong correlations between the total phenol contents and the antiradical capacities of the three ethanolic extracts were observed according to the results presented in the Table 2. However, there is no effective link between the total phenol contents and the reducing capacities of the extracts, except the total condensed tannin contents.

Table 4: Correlation coefficients between phenolic compound contents and antioxidant capacities of ethanolic extracts

Antioxidants assays	Correlation coefficients (R^2) TPC	TFC	THTC	TCTC
DPPH Assay	0.8899	0.7646	0.0635	0.9998
FRAP Assay	0.2636	0.1314	0.1804	0.6044

TPC: Total phenol content, TFC: Total flavonoid content, THTC: Total hydrolyzable tannin content; TCTC: Total condensed tannin content.

DISCUSSION

The current work focuses on the effectiveness of exploitation in phytomedicine of the leaf instead of trunk and root barks of *P. kotschyi* in order to preserve this plant species, through the comparaison of phytochemical composition and the antioxidant activities of its three organs.

Phytochemical screening carried out on this plant simultaneously revealed the effective presence in the three ethanolic extracts of chemical groups such as: alkaloids, phenolic compounds, flavonoids, tannins, coumarins, saponins and reducing sugars (Table 3).

However, although anthocyanins were identified in the ethanolic extracts of trunk and root barks, on the other hand, they were absent in the leaf ethanolic extract. In addition, anthraquinones and cardiac glycosides were also reported in the leaf and trunk bark, but both were not found in the root bark of the plant. However, the search for triterpenes and sterols in all three ethanolic extracts of the plant was unsuccessful. The results presented in Table 3 are similar to those obtained for the leaf of this plant by[12]; next for the bark of the trunk by[33]; finally for the root by[34]. Except, these authors did not report the absence of sterols and triterpenes in their works. [35]firstly, and [36]secondly, reported the presence of the same phytochemical compounds, respectively in trunk and the root barks, except alkaloids. This variability in the composition of secondary metabolites of plants could be related either to the period of harvest of the plant organs, or to the chemical composition or the pedological structures of the soils, or to the climatic factors, or to the stage of development of the plant[35].

Based on the results found on the comparative phytochemical composition of the three ethanolic extracts of *P. kotschyi*, it can be deduced that, on the whole, the leaf contains almost the same phytoconstituents as trunk and root barks of the plant (Table 3). Therefore, the use of the leaf instead of the barks of the trunk and root in phytomedicine should probably lead to almost the same therapeutic efficacies. However, there is some enquiry about the degree of concentration of the phytoconstituents identically revealed in the leaf compared to the barks of the trunk and root. This aspect needs to investigate the contents of some phytoconstituents with therapeutic properties, in particular phenolic compounds.

The results of the current study revealed that root and trunk barks are respectively 1.57 times and 1.46 times richer in total phenolic compounds than the leaf.

Comparatively, these total phenolic compound contents found in the current work are all significantly higher than those found for the trunk bark of the plant by[35], then[38], which are respectively: 6.849 ± 0.326 mg GA Eq/g DE and 64.82 ± 0.99 mg GA Eq g DE. [37]also found in the leaf of the plant a total phenolic compound content of 39.97 ± 3.63 mg GA Eq/g DE. This result was very lower than our own result. Contrary to this, the total phenol content found for the root bark in this work is lower than that found by[11] which was 508.8 ± 4.5 mg GA Eq/g DE. Concerning total flavonoids, the root bark also provided the highest content of 729.583 ± 25.165 mg QC Eq/g DE, and the leaf, the lowest content of 477.417 ± 45.305 mg QC Eq/g DE; while that of the trunk bark is 643.33 ± 79.605 mg QC Eq/g DE. Some authors[11,38,39] have also reported in Burkina that total flavonoid contents of leaf, trunk and root barks were respectively: 4.86 ± 0.10; 3.00 ± 0.10, and 10.4 ± 0.10 mg QC Eq/g DE. Nevertheless, all these values are very low compared to those found in the current work.

Regarding total hydrolyzable tannins, the highest content found in the current work was also recorded for the root bark (720.413 ± 42.585 mg TA Eq/g DE) while about total condensed tannins, it was rather trunk bark which had the high value (638.671 ± 19.581 mg CT Eq/g DE). However, the trunk bark has the lowest content of total hydrolyzable tannins (487.678 ± 26.637 mg TA Eq/g DE), followed by the

leaf (514.517 ± 62.61 mg TA Eq/g DE), knowing that the contents of total condensed tannins (mg CT Eq/g DE) of the root bark and the leaf are respectively: 544.254 ± 10.931 and 133.170 ± 1.593.

In contrast, the total condensed tannin content of the trunk bark (130.977 ± 2.105 mg CT Eq/g DE) reported by[33] was approximately 4.87 times lower than that obtained in this work.

It's well known that the effectiveness of a plant organ in phytotherapy is directly linked to its biological activities among which the antioxidant activity occupies a significant place. For this reason, it is very important to evaluate antioxidant activities of ethanolic extracts of leaf, trunk and root barks of *P. kotschyi*. However, since a single test is not enough to better classify the antioxidant powers of the compounds, then the three extracts of *P. kotschyi* were evaluated in the current work by two antioxidant methods. These include the radical method, measured with the DPPH• radical reagent, and the reducing power, evaluated by FRAP test.

Indeed, for the trapping of free radicals, the results found revealed that the trunk bark has the highest antiradical activity, with IC_{50} of 48.183 ± 1.521 µg DE/mL, while the leaf has the lowest antiradical activity, with IC_{50} of 95.311 ± 1.126 µg DE/mL, then the IC_{50} of the trunk is 56.372 ± 1.054 µg DE/mL. This ranking is explained by the fact that the antiradical power is inversely proportional to IC_{50} values. Therefore, gallic acid used as the reference molecule, with IC_{50} of 43.565 ± 0.970 µg DE/mL, stands out as the compound with the greatest antiradical activity compared to our three ethanolic extracts tested.

Regarding the values of reducing power of ethanolic extracts of *P. kotschyi* evaluated by FRAP test, trunk bark still shows itself as the best, with its reducing power of 786.2299 mg AA Eq/g DE ahead of the root bark and the leaf with their reducing powers of 561.7929 mg mg AA Eq/g DE and 505.5429 mg AA Eq/g DE, respectively.

Given the results found in the current work, it appears that ethanolic extract of trunk bark of *P. kotschyi* stands out as the most antioxidant while the ethanolic extract of the leaf is the least antioxidant, taking into account the results of the two antioxidant tests carried out.

In reality, the antioxidant capacity of a plant extract is generally proportional to its concentration of phenolic compounds. In this respect, since the leaf has the lowest contents of phenolic compounds (total phenols, total flavonoids, and total condensed tannins), consequently, this justifies its low antioxidant capacity. Similarly, if we only take into account the total condensed tannins, the fact that the ethanolic extract of the trunk bark is noted as the most antioxidant is quite logical. On the other hand, concerning the contents of total phenols, total flavonoids and total hydrolyzable tannins, we can believe that it is the ethanolic extract of the root bark that should be the most antioxidant but this was not verified in the current study.

In addition, the correlation between phenolic compound contents and antioxidant activities is also important. Correlation coefficients between 0.6 and 1.0 indicate a strong relationship[31]. In this context, previous studies have shown a strong correlation between phenolic compound contents and antioxidant activity[40]. In this work, the positive correlation results were obtained between total phenol, total flavonoid, total condensed tannin and total hydrolysable tannin contents and antiradical capacities of the three extracts. This correlation means that the antiradical capacity of the extracts mostly depends of the total phenolic compound contents. The antioxidant activities of the different extracts tested could be attributed to their richness in molecules with high anti-free radical potential such as polyphenols, flavonoids and especially condensed tannins. However, the correlation is moderately weak between the total phenol, the total flavonoid and the total hydrolyzable tannin contents and the reducing powers of the extracts. The disparity between the results of the two antioxidant tests applied in the current work should be justified by the fact that the mechanisms of action in which the phyto-organic compounds are involved vary according to the nature of the reactions involved but also the nature of the structures of these types of reagents. The antioxidant activities of the three extracts of *P. kotschyi* are due to the presence of flavonoids and especially condensed tannins. Thanks to phenolic

compounds, the food and cosmetic industries have experienced a remarkable emergence[41]. Indeed, several studies have reported that polyphenols, including flavonoids and tannins, are endowed with very remarkable antioxidant activities, thus giving them biological properties such as anti-inflammatory; antitumor; antidiabetic; antihypertensive, and anticancer properties[42]. In addition, these phenolic compounds have positive effects in the treatment of cardiovascular and neurodegenerative diseases[43,44]. They also have the particularity of inhibiting the peroxidation of lipid compounds, by acting as proton donors and free radical acceptors, thus stopping the auto-oxidation mechanism of fatty substances[45,46]. The high antioxidant capacity of flavonoids and tannins is then explained by the existence in their intrinsic structures of several phenolic functions. Indeed, previous studies have shown that phenolic compounds containing trihydroxyl groups, such as gallotannins and galloylated proanthocyanidins, have a strong activity capable of neutralizing superoxide anions.$O_2^{\bullet-}$[42]. For example, gallocatechins have the property of neutralizing HO• and HOO• radicals due to their redox potential. These free radicals are known as major contributors to several clinical disorders such as cancer, liver diseases, diabetes[47,48].

CONCLUSION

At the end of current study, we noted that the leaf of *P. kotschyi* contains almost the same secondary metabolites as the barks of the trunk and root, but its contents in total phenolic compounds are lower compared to those of the barks of the trunk and root. This attests to the results obtained for the antiradical activity and the antioxidant power of the leaf. The higher antioxidant activities of trunk and root barks compared to the leaf may justify their widespread use in traditional medicine in Togo. However, in order to obtain the same therapeutic results, it may be possible to increase the doses of the recipes formulated based on the leaf. Looking ahead, further studies on the leaf's antimicrobial activities and toxicity are needed before promoting its use in herbal medicine.

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Conflicts of interest

The authors declare that no conflict of interest exists regarding the publication of this paper.

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