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Harrisonia abyssinica Oliv. (Simaroubaceae), Plant with Multiple Therapeutic Uses: Botanical Study, Phytochemical and Antioxidant Evaluation

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ABSTRACT

In traditional medicine, *Harrisonia abyssinica* is a plant species used for multiple therapeutic uses. By the DPPH free radical scavenging method, antioxidant assay was carried out and a phytochemical analysis was done to justify the effects observed. The results showed that the six aqueous extracts and 70% ethanolic extract of *Harrisonia abyssinica* have good antioxidant activity with better activity with ethanolic extracts. These extracts could, therefore, be an alternative to certain synthetic additives. This activity is nevertheless clearly lower than that of Trolox (reference antioxidant), but these are crude extracts containing large number of bioactive compounds. It is most likely that they contain compounds which, when purified, may exhibit comparable and perhaps even better activity to that of Trolox and other synthetic antioxidants.

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INTRODUCTION

In view of the occurrence of new diseases such as those associated with oxidative stress, which is the primary cause of several diseases [1], it is, therefore, necessary to search for new antioxidants that could combat oxidative stress and its Associated pathologies.

This fact has taken us to undertake an ethnobotanical study of medicinal plants found mostly in the Region of Zanzan (Côte D'Ivoire). The survey was carried out specifically in the District of Transua where the uses of *Harrisonia abyssinica* in traditional medicine were very popular. This plant species is used in the treatment of skin diseases, anthelmintic disorders, diarrhea, dysentery, snake bites, scabies, chicken pox, stomach pain, headaches and sinusitis. Taking account of these multiple therapeutic uses, we have focused our research on the antioxidant and phytochemical properties of this plant in particular.

MATERIALS AND METHODS

Preparation of plant extracts

The plant material consisted of the leaves, stem bark and root of *Harrisonia abyssinica* (coded ZG03) harvested during the ethnobotanical survey. The plant organs were rinsed with water and dried under shade away from the sun. These dried medicinal plants were then reduced to fine powder by means of an IKA-MAG RTC electric grinder. The extraction of the active ingredients was carried out according to the method of [2] coupled with the exhaustion method.

One hundred gram (100g) of powdered drug was homogenized in one (1) liter of distilled water in a Life's Superb blender (LS-317) for three times three minutes at room temperature. The homogenate obtained was filtered successively on a square of white cloth, on hydrophilic cotton and then on Whatman paper. With the aid of an oven set at 50^0C., the extraction solvent was eliminated. The dried evaporate was recovered in the form of powder and constitutes the total aqueous extract (TAE).

Ten grams (10g) of the TAE were dissolved in 200 ml of an ethanol-water solution (70/30) and then homogenized in a blender. After decantation in a separating funnel, a liquid phase with a solid residue which precipitated was obtained, since it is insoluble in the alcohol-water mixture 70-30. The supernatant was collected, filtered on cotton to remove any residue and

dried in an oven (50^0C.). The powder obtained constituted the 70% ethanolic extract (EE70%).

Phytochemical screening

A phytochemical screening was carried out in order to detect some groups of secondary metabolites contained in the different extracts tested and responsible for possible activities. The colored reagent screening was used [3].

Antioxidant activity

The antioxidant capacity of the plant extracts was determined according to the method of [4-5]. The detection of the antioxidant activity started with the determination of the Trolox calibration curve. Small tubes are used for this test. In each tube 0.1 ml Trolox ((S) - (-) - 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a reference antioxidant and 2.9 ml of Methanolic solution of 0.004% DPPH (4mg / 10ml) was then added to each tube except that of the white tube. The white tube consisted of a mixture of DPPH and methanol (without Trolox). The concentrations varied from 100 to 6.25 µg / ml (according to a geometric sequence of ½). The mixture was allowed to incubate in the dark for 30 minutes at room temperature.

The absorbance was measured at 517 nm. The antioxidant analysis was repeated three times and the reading was reduced to an average.

1. Determination of Free radical scavenging activity of the DPPH test

This method is based on the measurement of the absorbance at 517nm when a stable free radical DPPH reacts with an antioxidant. The DPPH, a stable free radical of violet color, is reduced to a yellow color compound in the presence of anti-oxidant compounds (Figure 1). The intensity of the coloration is inversely proportional to the antioxidant activity of the extracts whose activity is to be determined.

Drug extracts (0.1ml) of *Harrisonia abyssinica* at different concentrations (6.25, 12.5, 25, 50 and 100µg/ml) were mixed with 2.9ml of 0.004% DPPH in methanol. After homogenization, the mixture was incubated at room temperature in the dark for 30 minutes. The absorbances were read at 517 nm against a negative control containing no extract. The tests were carried

out three times to ensure the reproducibility of the results. Trolox was evaluated at the same concentrations. The other extracts were evaluated at a concentration of 100µg/ml.

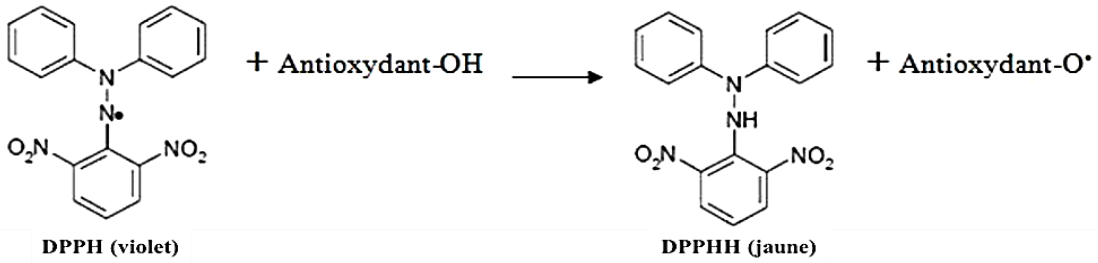


Figure 1: Reaction of an antioxidant with the DPPH radical

2. Percentage inhibition and effective concentration 50% (EC_{50})

The calculation of the percentage inhibition of the DPPH is made according to the formula:

$$\textbf{Inhibition of DPPH (\%)} = [1 - (DO_{Test} / DO_{White})] \times 100$$

DO_{Test}: Optical density of the test sample, containing the plant extract,

DO_{White}: Optical density of white, negative control (without extract).

The EC_{50} is the effective concentration at which 50% DPPH has been inhibited, it is inversely related to the antioxidant capacity. It is determined graphically. A low EC_{50} value indicates high antioxidant activity in a sample [6]. Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) (800-25M) was used as a reference compound for calibration **[7].**

3. Quantity of antioxidant in plant extracts

The amount of antioxidant (mg equivalent standard per gram of dry weight) in ten plant extracts was calculated using the equation below [8]:

$$\textbf{Quantity of DPPH (mg/g of dry weight)} = \frac{[(DO_{Test} - DO_{White}) / (Slope)][V/v]}{m \times 1000}$$

***DO_{Test}**: Optical density of the test sample, containing the plant extract,*

***DO_{White}**: Optical density of white, negative control (without extract),*

***Slope**: from the calibration line*

***V**: total volume of extraction solvent (ml),*

***v**: volume of the extract for the DPPH test (0.1 ml),*

***M**: mass of vegetable material used for extraction (g),*

***1000**: change factor of µg in mg.*

Statistical analyses of antiradical activity

The Tukey test (ANOVA) by the software IBM SPSS 22.0 was used in the first place to compare the means ± standard deviation of the quantities of antioxidants contained in the extracts of *Harrisonia abyssinica* and then to compare the effective concentrations 50% (EC_{50}) of the most active extracts and Trolox (reference antioxidant) at threshold $\alpha = 0.05$.

RESULTS

1. Botanical study of *Harrisonia abyssinica*

Synonym: *Harrisonia occidentalis* Engl. (1895).

Phytogeography

The *Simaroubaceae* are represented in Côte d'Ivoire by six species, divided into five genus including one in Africa (*Gymnostemon*) and four multicontinental, including the genus *Harrisonia*. *Harrisonia abyssinica* is a micro phanerophyte taxon spread throughout the Guineo-Congolese region (dense humid forest) [9].

Sample comparison was done at the National Floristic Center: Grand-Lahou Road, Bandaman Forest, 02 April 1968, Aké-Assi, 12112.

External morphology of Harrisonia abyssinica

Description according to [10], *Harrisonia abyssinica* is a shrub or small evergreen tree, strongly branched, sometimes climbing, up to 6 (-13) m tall; large branches with spines up to 2 cm long on conical corky outgrowths; brown bark, pale to gray; long and flexible branches. Leaves alternate, imparipinnate with 2-7 pairs of leaflets, up to 25cm long, glabrous or hairy; Stipules absent; Petiole up to 3cm long, with 2 spurs curved at the base, petiole and winged reaches 1-3mm wide; Petioles 0-2mm long; Leaflets elliptic or broadly obovate to almost circular, asymmetric base, cuneate to rounded, apex rounded to eliminate, edges variably toothed or whole. Inflorescence: axillary or terminal panicle, erect, glabrous to hairy. Flowers

bisexual, regular, 4-5 (-6); Pedicel of varying length. Fruit: depressed globose berry 4-8-lobed, red to black at maturity, glabrous, fleshy, 4-8-seeded (Figure 2).

Ecology

Harrisonia abyssinica is found in the dry evergreen forests, in the edge of savannas forests, riparian areas and coastal areas, from sea level up to 1700m altitude. It can be found on eroded soils [10].

A.: Fruity shoot (Photo BENE, 2015)

B.: *1, Flowering branch; 2, Branch and leaf detail; 3, fruit.* **[11]**

Figure 2 (A et B): *Harrisonia abyssinica* Oliv.

2. Phytochemical Screening

Table I gives the results obtained from the phytochemical screening of *Harrisonia abyssinica*. The tests carried out revealed the presence of various secondary metabolites in the extracts tested. The ethanolic extract of the leaves contained, in varying degrees, all the chemical compounds evaluated. Polyphenols and tannins are the secondary metabolites mostly present in an accentuated quantity. Extracts from stem and root barks have a low presence or absence of several chemical compounds.

Table I: Results of phytochemical screening of *Harrisonia abyssinica* extracts

Extracts / Compounds	Total aqueous extract (TAE)			Ethanolic Extract (EE70%)		
	ZG03Le	ZG03B	ZG03R	ZG03Le	ZG03B	ZG03R
Alcaloïdes	-	++	-	++	++	+
Polyphenols	++	-	-	+++	-	-
Coumarine	+	+	-	+	-	-
Tanins	+	+	-	++	-	-
Tanins galliques	+	+	-	+++	-	-
Saponosides	+	-	++	+	+	+
Flavonoïdes	+	-	-	++	-	-
Sterols and triterpenes	-	-	+	+	-	+

ZG03: Harrisonia abyssinica

TAE: Total aqueous extract, EE: Ethanol extract,

Le: leaves, B: Stem bark, R: stem of roots,

- : Absence ; + : Weak presence ; ++ : Strong presence ; +++ : Highly accentuated presence

3. Antioxidant Activity

In order to determine the antioxidant activity of the selected plant extracts, three steps were taken: the calibration curve, percentage inhibition of DPPH, and determination of the amount of antioxidants in plant extracts.

3.1 Determination of the calibration curve

The DPPH at 4mg/10ml, analysis method was used to highlight the high antioxidant activity of the methanol extract of Trolox a reference antioxidant. Figure 3 shows the Trolox standard curve.

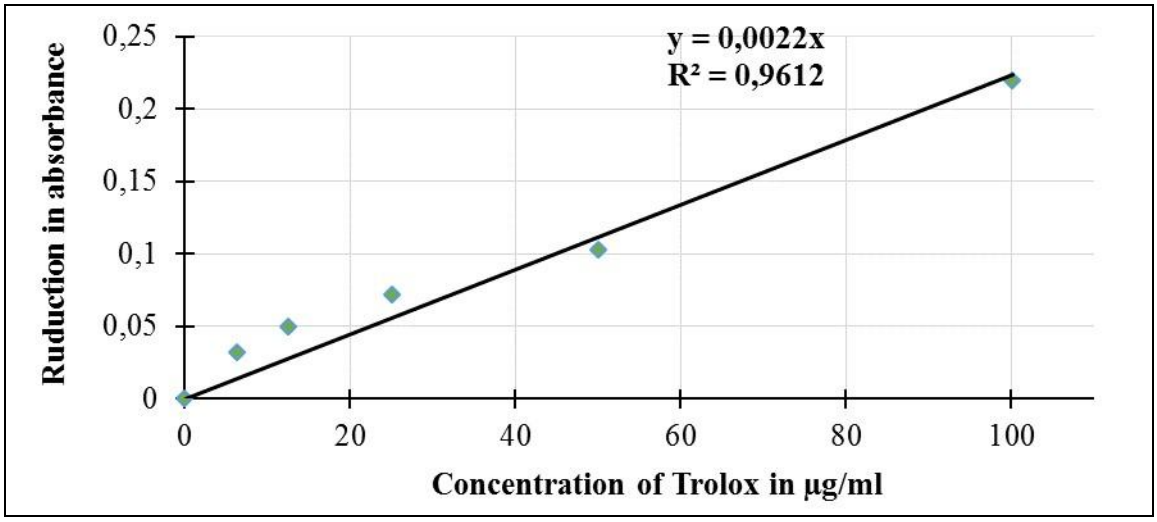


Figure 3: Standard calibration curve for Trolox equivalent concentration

3.2 Determination of the percentage inhibition of DPPH and six extracts of *Harrisonia abyssinica*

Figure 4 showed that the extracts studied have antioxidant capacity due to their DPPH inhibitory effect. All the six extracts tested showed an inhibitory rate varying from one extract to another. Two extracts (i.e. 33% of the extracts tested) have a percentage inhibition greater than 50%. The ethanolic extract of leaves of *Harrisonia abyssinica* (ZG03Le) recorded the maximum percentage inhibition of DPPH, while the aqueous extract of the root bark of *Harrisonia abyssinica* (ZG03R) showed the lowest percentage (7%) inhibition of DPPH (Figure 4).

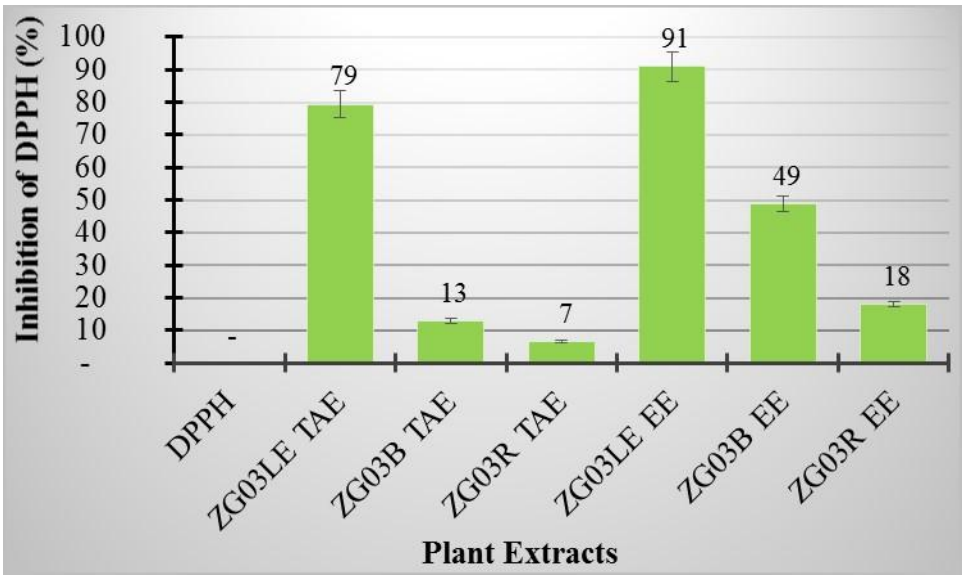
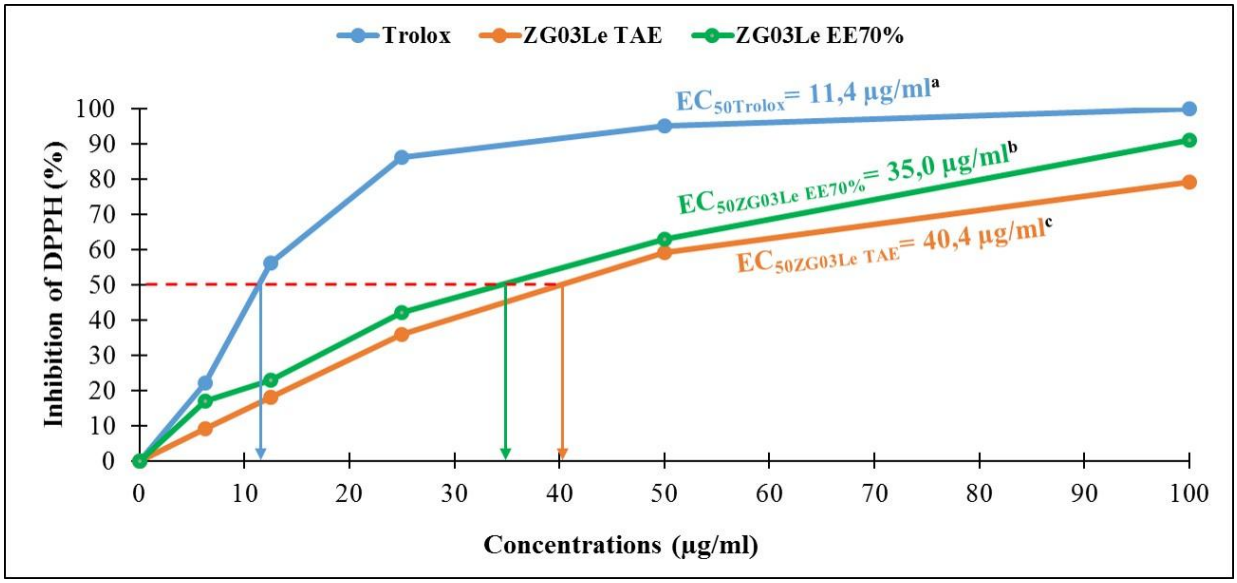


Figure 4: Percentage Inhibition of DPPH of Plant Extracts Tested

3.3 Antiradical activity of the Trolox and the two most active extracts

Figure 5 showed the results of measurement of the percentage inhibition of the DPPH radical based on the concentration of the substances tested. They indicated that the percentage of inhibition of the free radical increases with the increase in the concentration of Trolox and the most active extracts of *Harrisonia abyssinica* as well. The percentage inhibition of Trolox is observed to be higher than that of plant extracts in all concentrations. The percentage inhibition of the ethanolic extract of leaves was greater than that of its aqueous extract. The EC_{50} are 11.4; 35.0 and 40.4µg/ml respectively for Trolox, ethanolic and aqueous extracts of leaves of *Harrisonia abyssinica*. There was a significant difference between the 50% effective concentrations (EC_{50}) of the substances tested.



Values with different letters indicate that there is a significant difference between them (P <0.05)

Figure 5: Trolox anti-radical activity compared to those of the ethanolic extracts of the leaves of *Harrisonia abyssinica* (ZG03Le) based on the concentrations

3.4 Determination of the amount of antioxidants in plant extracts

Table II shows the different values of the antioxidants contained in the extracts of *Harrisonia abyssinica.* The Tukey test verified the level of significance of these antioxidants. There were no significant differences in the amounts of antioxidants contained in aqueous extracts of stem bark and plant roots at $P < 0.05$. It may be noted that the ethanolic extracts better

concentrate the antioxidants. The ethanolic extract of leaves of *Harrisonia abyssinica* contained the most antioxidants (62.35 ± 0.22mg/g).

Table II: Quantities of antioxidants (mg/g) in the plant extracts tested

Extracts	Solvent	Antioxidant Activity	ANOVA Statistical Parameter		
		Mean±SD (mg/g)	ddl	F	P
ZG03B	TAE	0,34±0,05[a]	5	9143,875	< 0,001
ZG03R	TAE	0,18±0,04[a]			
ZG03Le	TAE	9,21±0,03[b]			
ZG03R	EE70%	14,65±0,39 [c]			
ZG03B	EE70%	30,52±0,66 [d]			
ZG03Le	EE70%	62,35±0,22[e]			

The values of the extracts with different letters are significantly different (P <0.05)

ZG03: Harrisonia abyssinica

TAE: Total aqueous extract, EE: ethanol extract;

Le: leaves,

B: Stem bark,

R: Stem of Root

DISCUSSION

The DPPH radical method used in this study is a common method in which the antioxidant activity of the studied sample is determined by the degree of discoloration of the DPPH solution. This violet chromogen is easy to use, has high sensitivity, allows rapid analysis of the antioxidant activity of a large number of samples and gives reproducible results [12]. Indeed, the stable free radical, 2.2 diphenyl-1-picrylhydrazyl (DPPH) of violet color, in the presence of an antioxidant turns to a yellow compound (diphenyl-picrylhydrazine).

According to the results recorded, the aqueous and ethanolic extracts have a more or less moderate antioxidant capacity. This capacity would be justified by the amount of antioxidant contained in different proportions in the extracts.

The EC_{50} is inversely proportional to the antioxidant capacity of a compound because it expresses the amount of antioxidant required to decrease the concentration of the free radical by 50%. The EC_{50} values in µg/ml express the effective concentrations of Trolox and

antioxidant extracts necessary for trapping and reducing by 50% dissolved DPPH in methanol [6]. The smaller the EC_{50} value, the greater the antioxidant activity of the compound.

The analysis of the percentages of inhibition enables us to detect the most active between the aqueous and ethanolic extracts of *Harrisonia abyssinica* (ZG03Le). The activities of the aqueous and ethanolic extracts of leaves of *Harrisonia abyssinica* (ZG03Le) are the most pronounced. The determination of the effective concentrations 50% (EC_{50}) gave 11.4, 35.0 and 40.4µg/ml respectively for Trolox, ethanol extract and aqueous extract of *Harrisonia abyssinica* leaves. This difference, which is significant, could be explained by the fact that Trolox is a (purified) molecule of reference. The purification of the two extracts studied and especially of the ethanolic extract of the leaves of *Harrisonia abyssinica* could give results similar to that of the Trolox and perhaps even better. The difference between the effect of these two extracts could be explained by the fact that ethanol concentrates better the active ingredients. These molecules being concentrated after the partition can better react.

Several authors have demonstrated that antioxidant molecules such as Trolox, ascorbic acid, phenols, flavonoids and tannins reduce and discolor DPPH because of their ability to loose hydrogen [13], [6]. In addition, [14] found that phenols and flavonoids possess antioxidant activities.

The phytochemical screening revealed the presence of secondary metabolites such as phenols, flavonoids and tannins having antioxidant capacity, which therefore justifies the observed antiradical effects.

The reducing power of the ethanolic extract of leaves of *Harrisonia abyssinica* is dose-dependent (concentration dependent). The reducing power of this species is probably due to the presence of hydroxyl groups in the phenolic compounds which can be used as an electron donor. Therefore, antioxidants are considered oxidant reducers and inactivators **[**15]. Previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity [16], [17]. Oxidative processes are multiple and the nature of The antioxidant activity can be multiform. It can be attributed to chelating mechanisms of metal ions [18]. Therefore, any substance capable of capturing the single electron from a free radical without giving itself such a radical product is defined as a free radical scavenger.

CONCLUSION

The antioxidant tests of the extracts of *Harrisonia abyssinica* according to the method of free radical scavenging of DPPH showed that the two extracts aqueous and ethanolic 70% of leaves possess a good antioxidant activity with a better activity with the ethanolic extract (EE 70%). These extracts could, therefore, be an alternative to certain synthetic additives. This activity is nevertheless clearly lower than that of the Trolox (reference antioxidant), but they are only crude extracts containing a large number of bioactive compounds. It is therefore very likely that they contained compounds which, when purified, may exhibit comparable and perhaps even better activity to that of Trolox and other synthetic antioxidants.

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