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*Full Length Research Paper*

# Evaluation of the antioxidant activity of the leaves, stem-barks extracts and fractions of *Ochna schweinfurthiana* F.Hoffm (Ochnaceae)

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**The present study evaluates the *in vitro* antioxidant activity of the leaves and stem-barks extracts and fractions of *Ochna schweinfurthiana*. To this effect, the different extracts were obtained by maceration in four solvents namely ethyl acetate, methanol, acetone and water- ethanol mixture (20-80). The methanol extract which exhibited the best antioxidant activity was partitioned in hexane and ethyl acetate. The ethyl acetate fraction was fractionated by column chromatography with the aid of methyl dichloride/methanol ($CH_2Cl_2$/ MeOH) solvent system at different polarities. The antioxidant activity of the extracts and fractions was assessed by the 2,2-diphenyl-1-picrilhidrazil (DPPH), ferric reducing antioxidant power assay (FRAP) and the total polyphenol content was evaluated using the Folin-Ciocalteu reagent. The results were analyzed using SPSS 20 presented as mean $\pm$ standard deviation. The results phytochemical screening confirmed the abundance of flavonoids and catechic tannins in the methanol and water-ethanol extracts whose content vary between 37.83 ± 1.6 mg ascorbic acid equivalent (EAA)/g dry weight (dw) and 96.4 ±2.33 mg EAA/g dw. The leaves methanol extract possess the best antiradical power (AP) of 0.00114 ± 0.00001 g/mg and the best ferric reducing antioxidant power (542.33±16.51 mg EAA/g dw). The F3 fraction obtained using $CH_2Cl_2$/MeOH 5/1 elution system possess the best AP of 0.00125 ± 0.00001 g/mg identical to that of ascorbic acid (AP = 0.00125 ± 0.00002 g/mg) and the strongest ferric reducing antioxidant power (508.66 ± 18 mg EAA/g dw). A positive correlation between the two antioxidant tests and the polyphenols content was obtained. Thus, *Ochna schweinfurthiana* could be used by the population to prevent some diseases caused by oxidative stress, due to its high antioxidant effect.**

**Key words:** *Ochna schweinfurthiana*, extracts, fractions, antioxidant activity.

## INTRODUCTION

Nowadays, the scientific world is putting into evidence the tragic role of the uncontrollable role of the oxidative

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process induced by reactive oxygen species (ROS). Oxidative stress is defined as the disequilibrium between the biochemical processes of free radical (FR) production and those of antioxidant defenses in favour of free radical production (Sayre et al., 2008). These free radicals could react with a series of biological substrates such as DNA, proteins, lipids and carbohydrates. They are directly related to a number of diseases such as early ageing, cataract, acute respiratory distress syndrome, pulmonary oedema (Favier, 2003). An aggravation of the initial process of free radicals production causes more severe illnesses such as cardiovascular diseases, some type of cancer, diabetes, Alzheimer, rheumatism (Sas et al., 2007). Based on this reality, a reawakening of phytotherapy which produces an important quantity of bioactive molecules and which have the capacity to trap these free radicals is a largely exploited domain. In effect, natural antioxidants are involved in several research and a new approach towards the exploitation of secondary metabolites in general and polyphenols in particular in health as well as in the agro-food industry (Prior et al., 2005). Flavonoids which constitute an important class of these compounds are widely researched for their biological properties: Antioxidant, anti-inflammatory (Rahman et al., 2006), antiallergic and anticancerous agents (Viana et al., 2003). *O. schweinfurthiana* F.Hoffm is a tree or shrup which can be up to 4 m long is found in the tropical forests of Africa, America and Asia. In Africa, it is found in Guinea up to the North and South of Nigeria, Central Africa to Sudan, Uganda, Zimbabwe and Mozambique (Abdullahi et al., 2010). Besides being used as a decorative plant because of its multi-colored flowers (Burkill, 1997), it is used in traditional medicine in the North of Cameroon to treat malaria, erethism and typhoid fever. Likewise, in the North of Nigeria, *O. schweinfurthiana* is used to treat measles, typhoid fever and skin fungal infections. Literature review on this plant revealed that the antimicrobial effect of the methanol and acetone extracts of the leaves of this plant on some selected pathogens has been carried out (Abdullahi et al., 2010). But no previous antioxidant investigation on the extract and fraction of the plant has been reported**.** To this effect, the aims of this study were to search for new natural antioxidant molecules by evaluating the antioxidant properties of the extracts and fractions of *O. schweinfurthiana.*

## MATERIALS AND METHODS

The botanical material commonly known in Cameroon as Sa'aboule in fulfulde, is made up of leaves and stem-barks. It was collected in January 2014 in Ngaoundere and identified by Mr. Nana Victor of the National Herbarium of Cameroon under the identification code: 40171HNC.

### Methods of extraction of phenolic compounds

Within the framework of this study, the stem-barks and leaves were subjected to cold maceration (Prakash et al., 2005). The solvents were used in order of increasing polarity: ethyl acetate, acetone methanol, ethanol-water (80-20 v/v). To obtain the extracts, 200 g of the powdered stem-barks and leaves were soaked separately in 650 mL of pure ethyl acetate. After 48 h of maceration, the mixture was filtered. The filtrate was concentrated using a rotar vapor of the model Janke. To obtain the ethyl acetate extract, the maceration was repeated twice in order to maximize the yield. The residue obtained after the ethyl acetate maceration was dried for 24 h then used in the next extraction. The same procedure was repeated to obtain the acetone, methanol and ethanol-water extracts. The leaves methanol extract that exhibited the strongest antioxidant activity was partitioned using hexane and ethyl acetate. The ethyl acetate fraction was fractionated on column chromatography with methyl dichloride/methanol elution system at different polarities (Lhuillier et al., 2007). At the end of the fractionation, four major fractions were grouped and denoted F1, F2, F3 and F4 depending on the speed of the spots on the chromatographic plates.

### Phytochemical screening

Phytochemical screening of the extracts to identify different families of bioactive compounds found in the extracts was carried out as described by Harbone (1998) and Sofowora (1993).

### 2,2-diphenyl-1-picrilhidrazil (DPPH) antiradical test

To prepare a standard solution of 2,2-diphenyl-1-picrilhidrazil (DPPH°), 10 mg of DPPH was dissolved in 25 mL of methanol (Brand-Williams et al., 1995). From this solution, 5 mL was taken and mixed with 45 mL of methanol. After preparing the different solutions, 1950 µL of the DPPH solution was pipetted into test tubes and 50 µL of each extract at different concentrations was then added to each test tube to a final volume of 2 mL per tube. All tests were carried out in triplicate in a dark room. The optical density was measured at a wave length of 515 nm using a spectrophotometer of the brand Jenway 6305, Germany after 120 min of incubation.

### Test of the ferric reducing antioxidant power assay: FRAP

The ferric reducing antioxidant power assay (FRAP) is based on the reduction of the tripyridyltriazine ferric complex ($Fe^{3+}$-TPTZ) to the tripyridyltriazine ferrous complex ($Fe^{2+}$-TPTZ) in the presence of an antioxidant, 1950 µL of FRAP solution was pipetted into different test tubes, follow by 50 µL of extracts or fraction at different concentrations (Benzie and Strain, 1999). The tests were done in triplicate, and the mixture was incubated for 30 min in darkness. The optical density was measured at 593 nm using a spectrophotometer of the brand Jenway 6305, Germany. The FRAP solution was prepared as follows: 14.1 mg of TPTZ was diluted in 9 mL HCL at 40 mM then ferric chloride ($FeCl_2$) at 20 mM and acetate buffer 300 mM at $P^H$ 3.6 mixed in the ratio of 1: 1: 10 respectively to form the FRAP solution.

### Titration of the total polyphenol content by the Folin-Ciocalteu test

The total polyphenols was evaluated by spectrophotometry using Folin-Ciocalteu reagent as described by Chew et al. (2009). A volume of 1817 µL of distilled water was introduced in a test tube, 115 µL of Folin-Ciocalteu diluted at 1/10 and 345 µL of sodium carbonate($Na_2CO_3$) at 15% were added. The tubes were well vortexed, incubated for 2 h and the absorbance read at 765 nm

**Table 1.** Summary of the phytochemical screening of the leaves and stem barks extracts of *Ochna schweinfurthiana.*

| Tests | Ethyl acetate Ex. | | Methanolic Ex. | | Ethanol/water Ex. | | Acetone Ex. | |
|---|---|---|---|---|---|---|---|---|
| | Stem-barks | Leaves | Stem-barks | Leaves | Stem-barks | Leaves | Stem-barks | Leaves |
| Flavonoids | - | + | ++ | ++ | + | + | + | + |
| C. Tannins | - | + | ++ | ++ | ++ | + | + | + |
| Steroids | - | ++ | - | + | - | - | - | + |
| Saponins | + | - | ++ | ++ | ++ | + | + | + |
| Alkaloids | - | + | + | + | + | + | + | + |
| Triterpenes | + | - | ++ | - | ++ | + | ++ | - |

Presence (+); Absence (-); Abundance (++).

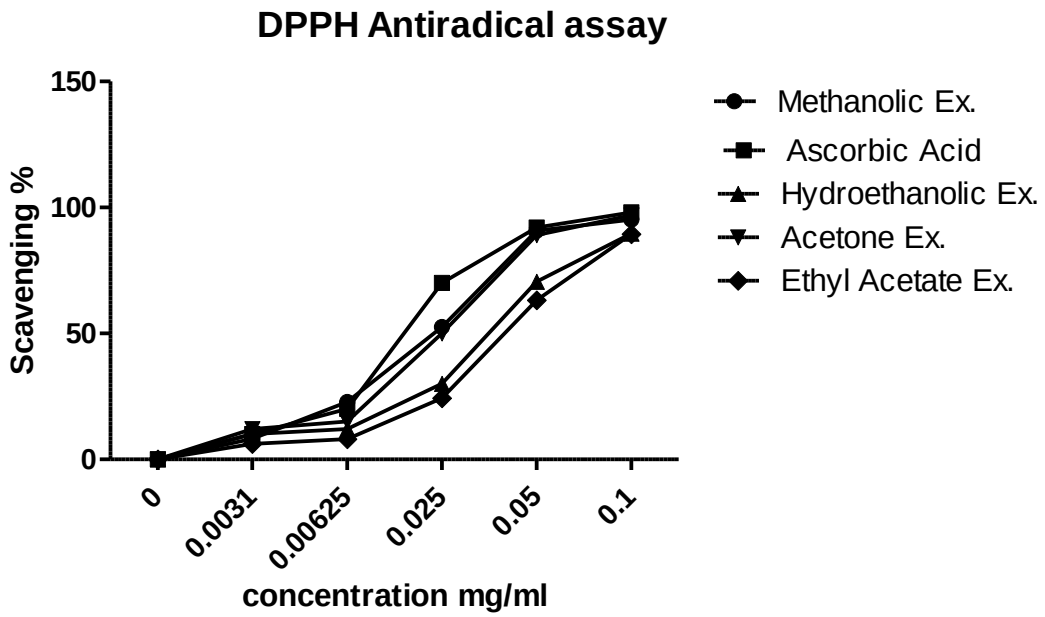


**Figure 1.** Variation curve of the DPPH scavenging percentage versus concentration of stem-barks extracts of *O. schweinfurthiana.*

using a spectrophotometer of the brand Jenway 6305, Germany. The standard solution was prepared using a freshly prepared aqueous solution of ascorbic acid.

## RESULTS AND DISCUSSION

### Phytochemical screening

Result of the phytochemical tests of the different extracts is shown in Table 1. The results indicated the abundance of flavonoids, saponins and catechic tannins in the methanolic extracts; the presence of alkaloids and triterpenes in other extracts.

### Evaluation of the *in vitro* antiradical power by the DPPH test

Figures 1 to 3 show a general increase in the scavenging percentage of DPPH free radicals in all the extracts. On a general basis, the methanol extract of the leaves shows the greatest scavenging activity, followed by the hydro-ethanol extract of the leaves; the fraction F3 shows a high scavenging activity followed by the F4 fraction. All these active extracts and fractions have a better hyperbolic curve than that those of the extracts and fractions exhibiting low scavenging activity.

Results of the antiradical DPPH test carried out on *O. schweinfurthiana* extracts and fractions are shown in Table 2. The methanolic extract of leaves and fraction F3 show the greatest antiradical power (AP) of 0.00114 ± 0.00001 g/mg and 0.00125 ± 0.00001 g/mg, respectively.

### Evaluation of the *in vitro* reducing power by the FRAP test

Figures 4 to 6 show the variation of the different extracts

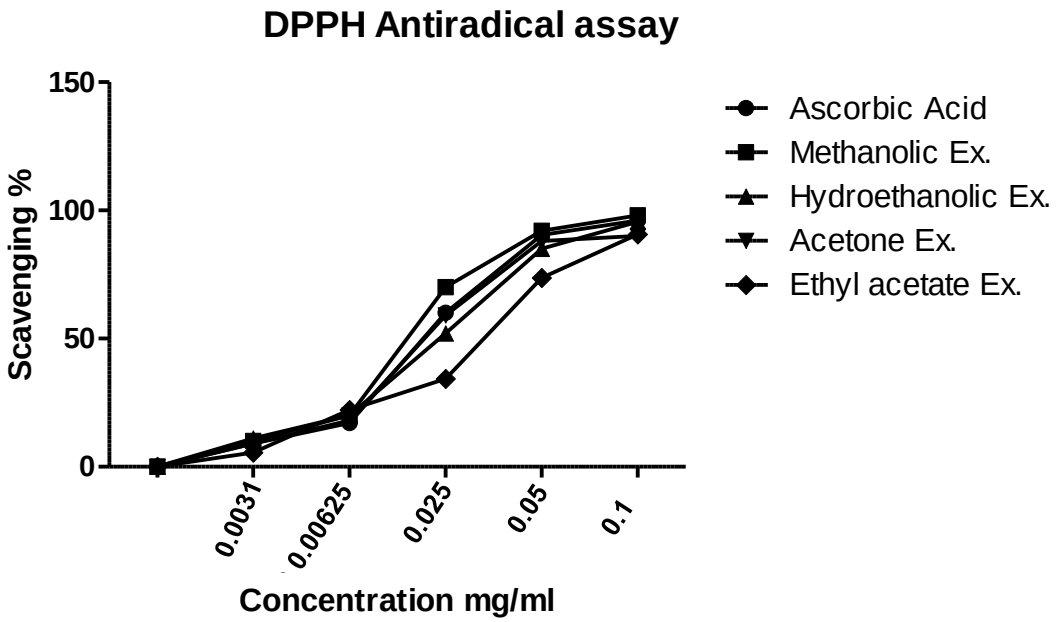


**Figure 2.** Variation curve of the DPPH scavenging percentage versus concentration of leaves extracts of *O. schweinfurthiana*.

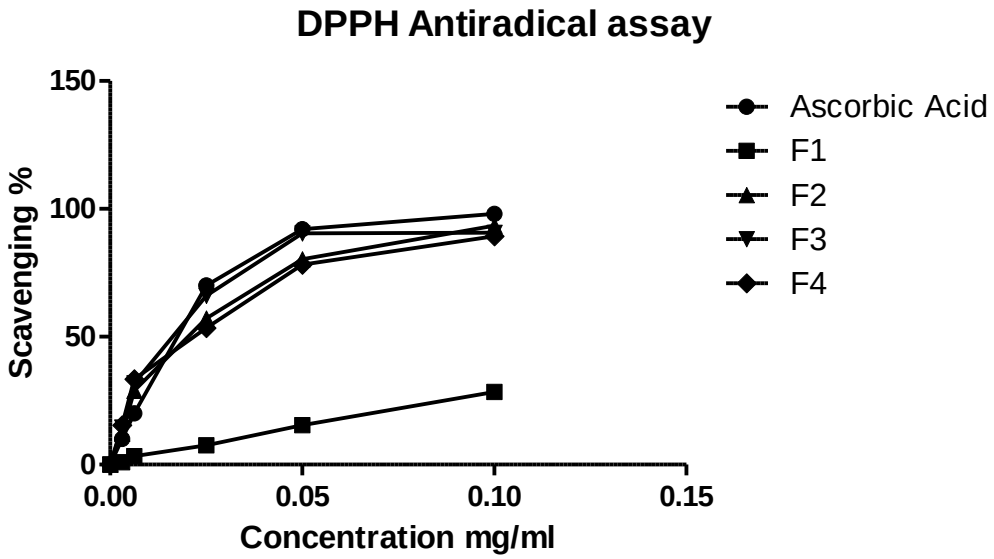


**Figure 3.** Variation curve of DPPH scavenging percentage versus the fractions concentration of *O. schweinfurthiana*.

in FRAP. The reducing power in mg ascorbic acid equivalent (EAA)/g dry weight (dw) was evaluated using the regression line of the optical density variation versus concentration of the extracts, fractions and reference molecule. In Table 3, it is seen that methanol extract of leaves, hydroethanolic stem-bark extract, fraction F3 and fraction F4 have the highest capacity to reduce ferric ions.

**Titration of the total polyphenols and correlation with the antioxidant activity of the extracts and fraction**

Table 4 summarizes the antiradical power, the reducing power and the total polyphenols content of the different extracts and fractions. The results of the titration of the total polyphenols show that the extracts and fractions exhibiting the strongest antioxidant activity contain high

**Table 2.** Summary of the antiradical activity assessment of the leaves and stem barks extracts of *Ochna schweinfurthiana.*

| Tested substances | $SC_{50}$ (g/l) | $CE_{50}$ (mg Ex/g of DPPH) | AP (g/mg) |
|---|---|---|---|
| Ascorbic acid | 0.032 ± 0.001 | 800 ±15 | $0.00125\pm0.00002^{e}$ |
| Methanol Leaves Ex. | 0.035 ± 0.001 | 875 ± 14 | $0.00114\pm0.00001^{de}$ |
| Acetone leaves | 0.037 ± 0.002 | 925 ± 16 | $0.00108\pm0.00001^{d}$ |
| Ethanol-water leaves Ex. | 0.0371± 0.002 | 928 ± 15 | $0.00107\pm0.0001^{d}$ |
| Ethyl acetate leaves Ex | 0451 ± 0.0019 | 1127.5 ± 14.3 | $8.86x10^{-4}\pm0.0000^{c}$ |
| MeOH stem-barks Ex. | 0.036 ± 0.001 | 900 ± 17 | $0.00111\pm0.00001^{de}$ |
| Acetone stem-barks Ex. | 0.036 ± 0.001 | 900 ± 17 | $0.0011\pm0.0001^{d}$ |
| Ethanol-water stem-barks Ex. | 0.038 ± 0.001 | 950 ± 15 | $0.00105\pm0.00001^{d}$ |
| Ethyl acetate Stem-barks Ex. | 0.0504 ± 0.002 | 1600 ± 16 | $7.93x10^{-4}\pm0.0000^{c}$ |
| Fraction F1 | 0.175 ± 0.007 | 4375 ± 19 | $2.28x10^{-4}\pm0.0000^{b}$ |
| Fraction F2 | 0.0359 ± 0.0001 | 897.5 ± 12.5 | $0.001\pm0.000^{d}$ |
| Fraction F3 | 0.031 ± 0.001 | 799.87 ± 6.84 | $0.00125\pm0.00001^{e}$ |
| Fraction F4 | 0.036 ± 0.002 | 900 ± 8 | $0.0011\pm0.0001^{d}$ |

Values with the same letters are statistically identical meanwhile those with different letters are statistically different with a threshold value of $P < 0.05$.

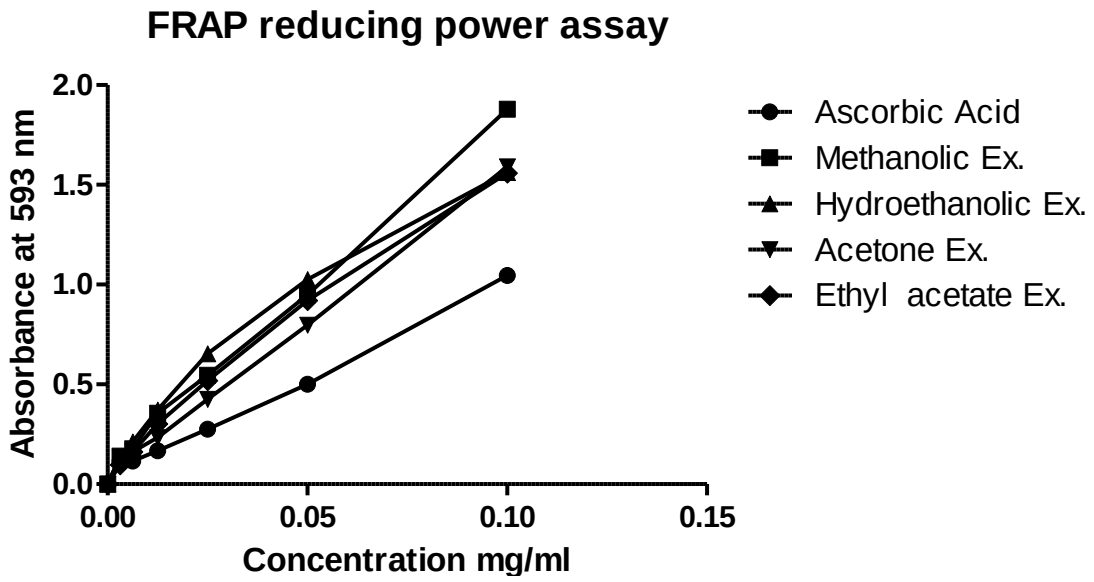


**Figure 4.** Absorbance variation curve of leaves extracts of *O. schweinfurthiana* and ascorbic acid versus concentration.

amounts of polyphenols.

In Figures 7 and 8, the correlation between the scavenging activity (DPPH), the antioxidant activity (FRAP) and the polyphenol content is positive ($R^2 = 0.95$; $R^2 = 0.91$ respectively) revealing that antioxidant activity depends on the polyphenol content.

## DISCUSSION

The phytochemical screening carried out on the extracts of *O. schweinfurthiana* indicates the presence of catechic tannins, triterpenes, alkaloids, saponins and flavonoids. All this group of compounds has been reported by Abdullahi et al. (2010) in acetone and methanol extracts of *O. schweinfurthiana*.

To the best of our mind, the antioxidant activity of *O. schweinfurthiana* has not been evaluated. However, results of the DPPH antiradical assay confirm the phytochemical screening carried out on the different extracts. In effect, the antiradical power of the different extracts and fractions was determined by the DPPH assay. It is noticed that the extracts that exhibit a weak antiradical activity with respect to ascorbic acid (AP of

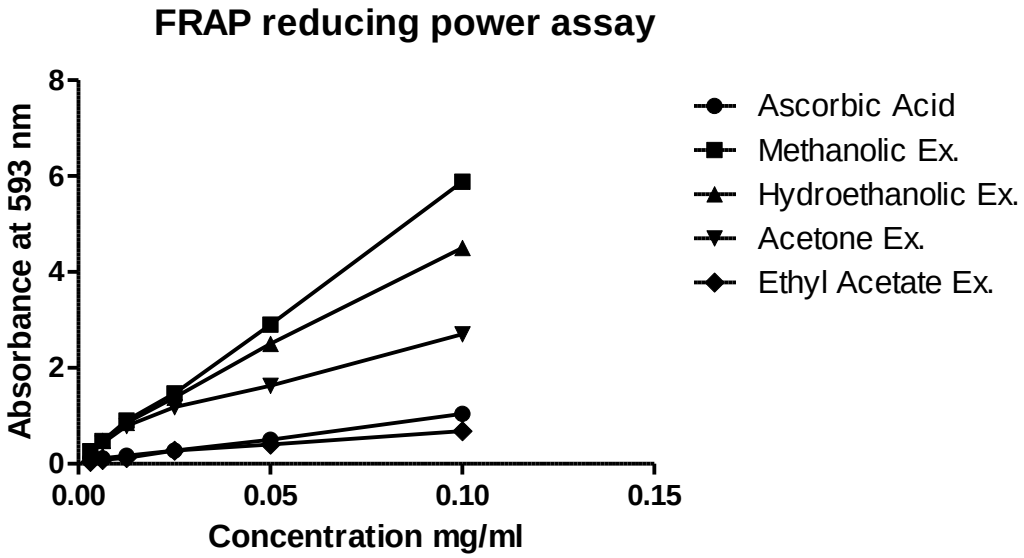


**Figure 5.** Absorbance variation curve of stem-barks extracts of *O. schweinfurthiana* and ascorbic acid versus concentration.

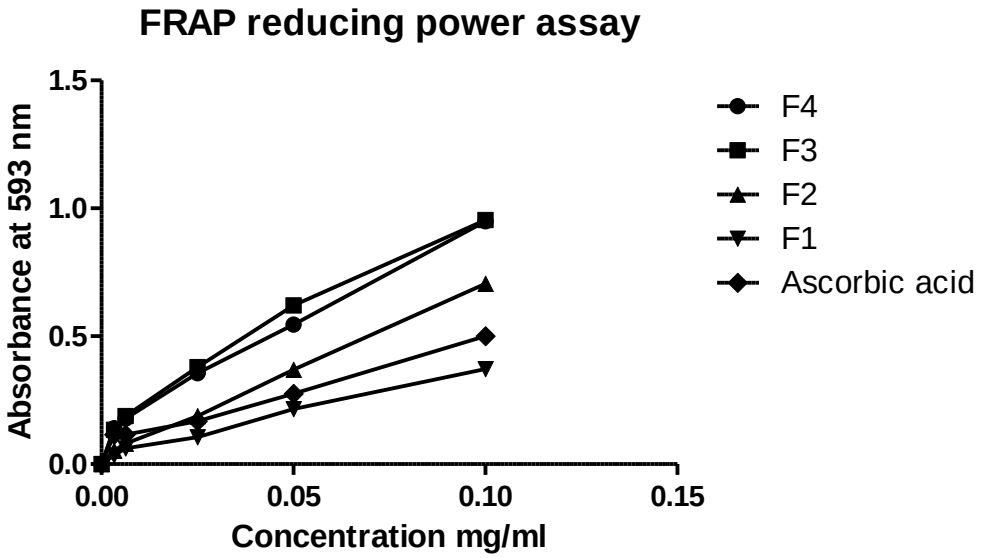


**Figure 6.** Absorbance variation curve of the fractions of *O. schweinfurthiana* and ascorbic acid versus concentration.

0.00125 ± 0.0002 g/mg) are those obtained using fairly polar extraction solvents. They are the leaves and stem-barks ethyl acetate extract, which have an AP of $8.86x10^{-4}$ ± 0.0000 g/mg and AP of $7.93x10^{-4}$± 0.0000 g/mg respectively. This could be explained by the fact that the extracts are made up of less polar compounds which have a weak antioxidant activity (Koffi et al., 2010). Acetone and ethanol-water extracts statistically have the same antiradical activity. Their antiradical power vary between AP of 0.00105 ± 0.00001 g/mg and AP of 0.00107 ± 0.00001 g/mg. Methanol leaves and stem-barks extracts exhibited the highest AP of 0.00111± 0.00001 and AP of 0.00114 ± 0.000001 g/mg respectively. Nevertheless, they possess an antiradical activity weaker than that of ascorbic acid AP of 0.00125 ± 0.00002 g/mg. This result is confirmed by the phytochemical screening which revealed that the methanol extracts contained much polyphenols and flavonoids which by themselves possess high inherent antiradical activity (Crozier et al., 2006).

**Table 3.** Summary results of the FRAP assay

| Tested substances | Reducing power (mg EAA/g dw) |
|---|---|
| Methanol leaf Ex. | $542.33\pm16.51^{h}$ |
| Ethanol-water leaf Ex. | $242.66\pm4.52^{f}$ |
| Acetone leaf Ex. | $238.33\pm3.64^{e}$ |
| Ethyl acetate leaf Ex. | $236\pm6^{f}$ |
| MeOH stem-barks Ex. | $513\pm14^{g}$ |
| Ethanol-water stem-barks Ex. | $529.33\pm15.01^{g}$ |
| Acetone stem-barks Ex. | $243\pm2^{f}$ |
| Ethyl acetate stem-barks Ex. | $67.16\pm1.8^{c}$ |
| Fraction F1 | $50.96\pm3.4^{b}$ |
| Fraction F2 | $89.51\pm2.5^{d}$ |
| Fraction F3 | $508.66\pm18^{g}$ |
| Fraction F4 | $502.33\pm10.41^{g}$ |

Values with the same letters are statistically identical meanwhile those with different letters are statistically different with a threshold value of $P < 0.05$.

**Table 4.** Antiradical power, reducing power and quantity of total polyphenols of *Ochna schweinfurthiana.*

| Tested substances | Antiradical power (g/mg) | Reducing power (mg EAA/g dw) | $Q_{polyphenols}$ (mg EAA/g dw) |
|---|---|---|---|
| Methanol leaf Ex. | $0.00114\pm0.00001^{de}$ | $542.33\pm 16.51^{h}$ | $96.4\pm2.33^{i}$ |
| Ethanol-water leaf Ex. | $0.00108 \pm 0.00001^{d}$ | $242.66\pm 4.52^{f}$ | $3783\pm 1.6^{cde}$ |
| Acetone leaf Ex. | $0.00108 \pm 0.00001^{d}$ | $238.33\pm 3.64^{e}$ | $48\pm 2^{bcd}$ |
| Ethyl acetate leaf Ex. | $8.86x10^{-4} \pm 0.0000^{c}$ | $236\pm 6^{f}$ | $28.98\pm 1.1^{bcd}$ |
| MeOH stem-barks Ex. | $0.00111\pm0.00001^{de}$ | $513\pm14^{g}$ | $62.66\pm 6.25^{efg}$ |
| Ethanol-water stem-barks Ex. | $0.00105 \pm 0.00001^{d}$ | $529.33\pm 15.01^{g}$ | $75.9\pm 4.1^{hi}$ |
| Acetone stem-barks Ex. | $0.0011 \pm 0.0001^{d}$ | $243\pm 2^{f}$ | $75.9\pm 4.1^{hi}$ |
| Ethyl acetate stem-barks Ex. | $7.93x10^{-4} \pm 0.0000^{c}$ | $67.16\pm 1.8^{c}$ | $28.93\pm 1.3^{bcd}$ |
| Fraction F1 | $2.28x10^{-4} \pm 0.0000^{b}$ | $50.96\pm 3.4^{b}$ | $22.6\pm 3.5^{abc}$ |
| Fraction F2 | $0.001\pm 0.000^{d}$ | $89.51\pm 2.5^{d}$ | $24.46\pm 2.7^{abc}$ |
| Fraction F3 | $0.00125\pm 0.00001^{e}$ | $508.66\pm 18^{g}$ | $73.43\pm 1.9^{ghi}$ |
| Fraction F4 | $0.0011\pm 0.0001^{d}$ | $502.33\pm 10.41^{g}$ | $53.4\pm 6.8^{defg}$ |

Values with the same letters are statistically identical meanwhile those with different letters are statistically different with a threshold value of $P < 0.05$.

Similarly, this study reveals that the antiradical activity of the extracts and fractions is due to the polarity of their constituting compounds. Faction F1 and F2 obtained using weak polar solvents ($CH_2Cl_2$/MeOH 50/1 to $CH_2Cl_2$/MeOH 35/1) exhibited weak AP of $2.28x10^{-4}$ ± 0.0000 g/mg and AP of 0.001 ± 0.000 g/mg respectively. Fraction F3 was obtained using the $CH_2Cl_2$/MeOH 5/1 solvent system and shows an AP of 0.00125 ± 0.00001 g/mg equivalent to that of ascorbic acid while fraction F4 obtained from the $CH_2Cl_2$/MeOH 1/1 solvent system gave an AP of 0.0011 ± 0.0001 g/mg lower than that of fraction F3. This could be explained by the fact that the antiradical activity is strongly associated to the chemical structure of the compounds responsible for such an activity and the synergistic or antagonistic effects of the different compounds present in the fraction which could increase or decrease its antiradical activity (Frankel, 1998).

It is equally noticed that, the reducing power of the extracts and fractions is strongly related to the polar nature of the compounds that make it up. In effect, the ethyl acetate extracts have low reducing powers. Their ascorbic acid equivalent concentration is between 67.16 ± 1.8 and 236 ± 3 mg EAA/g dw for the leaves and stem-barks respectively. The acetone, ethanol-water and methanol extracts have a strong reducing power and their ascorbic acid equivalent concentration is comprised between 238.33 ± 3.64 and 542.33 ± 16.51 mg EAA/g dw, respectively. These results are in harmony with those found in literature. In effect it has been demonstrated that

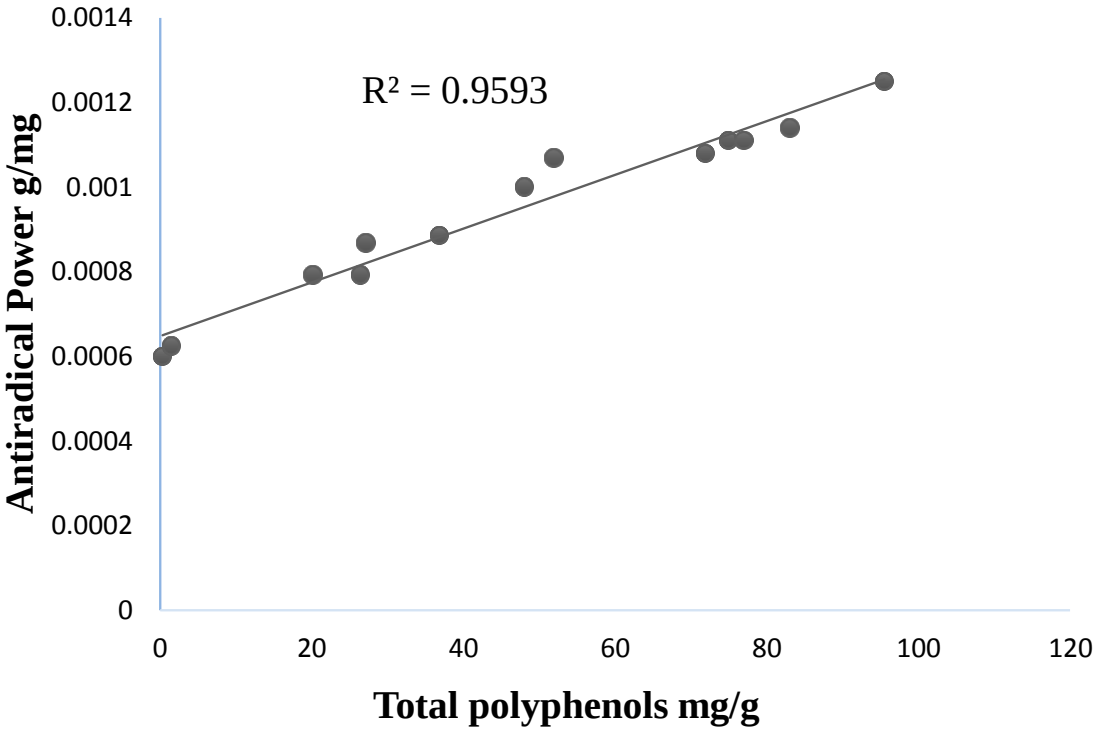


**Figure 7.** Correlation between antiradical activity of the extracts and fractions of *Ochna schweinfurthiana* and total polyphenols content.

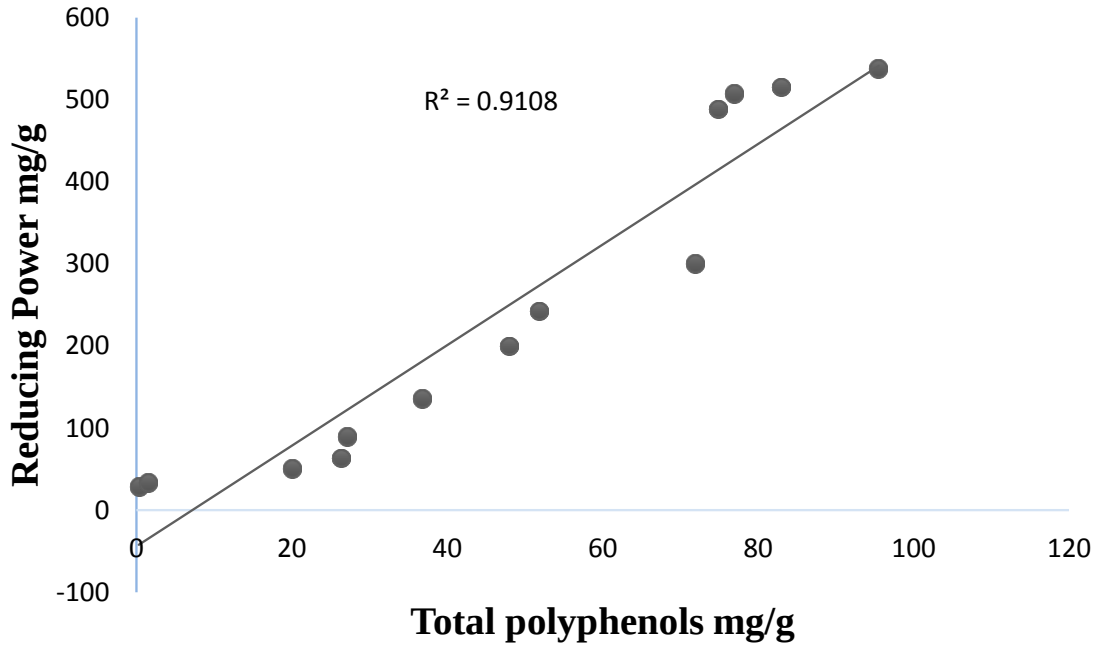


**Figure 8.** Correlation between the reducing power of the extracts and fractions of *Ochna schweinfurthiana* and total polyphenols content.

phenolic compounds most especially flavonoids by virtue of their chemical structure possess a strong reducing power (Crozier et al., 2006). The reducing power of the fractions varies between 50.96 ± 3.4 and 508.66 ± 18 mg EAA/g dw for fractions F1 and F3, respectively.

Titration of polyphenols in the different extracts and

fractions ascertain their antioxidant activity. In effect, the least active extracts and fractions are those with low polyphenol content (stem-barks ethyl acetate extract and fraction F1) equal to 28.93 ± 1.3 and 22.6 ± 3.5 mg EAA/g dw, respectively, meanwhile the most active extracts and fractions were the leaves methanol extracts and fraction F3 with have a polyphenol content of 96.4 ± 2.33 and 73.43 ± 1.9 mg EAA/g dw, respectively. The correlation between the antiradical activity, the reducing capacity of the extracts and fractions with the polyphenol content is very high. Thus, the correlation coefficient between the antiradical activity and the polyphenol content is $R^2$ = 0.95 and that between the reducing capacity and polyphenol content is $R^2$= 0.91.

## Conclusion

At the end of this study which aimed at searching for new sources of natural antioxidants by assessing the antioxidant properties of the extracts and fractions of the leaves and stem-barks of *O. schweinfurthiana*, the study reveals that this plant possesses a good antioxidant activity. *O. schweinfurthiana* is very rich in polyphenols such as flavonoids and catechic tannins. The leaves methanol extract and fraction F3 which was obtained using $CH_2Cl_2$/MeOH (5/1) solvent system gave the strongest antioxidant activity. The antioxidant activity is due to the presence of polyphenol compounds. The correlation coefficient between the antiradical activity and the total polyphenol content is $R^2$=0.95 and that between the reducing power and the total polyphenols is $R^2$= 0.91. The results of this study rationalize the ethno-medicinal use of *O. schweinfurthiana*.

## Conflict of interests

The authors have not declared any conflict of interest.

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