Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(10):192-197
(http://derpharmachemica.com/archive.html)

Phytochemical analysis and *in vitro* antioxidant activity of extracts of *Entandophragma angolense* (Welw.) C.DC. (Meliaceae) a medicinal plant used in the treatment of obstetric fistula in Ivory Coast

S. M. Lagou[1,4], M. Lébri[2,4*], M. Tilaoui[3], H. Achibat[4], W. M. Koné[1], H. F. Tra Bi[1], H. Ait Mousse[3], H. Ouchetto[4], A. Hafid[4], A. Zyad[3] and M. Khouili[4]

[1]*UFR Sciences de la Nature Université Nangui Abrogoua, BP 801 Abidjan 02, Ivory Coast*
[2]*Laboratoire de Pharmacodynamie-Biochimique, Université Félix Houphouët-Boigny, BP 582, Abidjan 22, Ivory Coast*
[3]*Laboratory of Biological Engineering, Natural Substances, Cellular and Molecular Immunopharmacology, Immunobiology of Cancer Cells Cluster, Faculty of Science and Technology, PB 523, 23000 Beni-Mellal, Morocco*
[4]*Laboratoire de Chimie Organique & Analytique, Université Sultan Moulay Slimane, Faculté des Sciences et Techniques, BP 523, 23000 Beni-Mellal, Morocco*

ABSTRACT

The study focused on extracts of Entandrophragma angolense a plant of Meliaceae family used for Ivorian traditional healers to treat obstetric fistula. The investigation was carried to evaluate antioxidant activity of different concentrations of extracts (aqueous, ethanol, ethyl acetate and hexane) of E. Angolense bark. DPPH radical scavenging assay were carried to determine antioxidant activity of extracts. All extracts exhibited marked antioxidant activity by scavenging DPPH free radical in a concentration dependent manner. It was recorded increasing IC50 values of (60 to 5 µg/ml). Ethanol extract showed an IC50 (5 µg/mL) close to vitamin C (4.5 µg/ml). Preliminary phytochemical analysis of extracts shows the presence of alkaloids, flavonoids (flavones, flavonol), tannins, coumarins, sterol, triterpenoids and saponins. Our results suggest that extracts of E. angolense bark contains several chemical groups and exhibit strong antioxidant DPPH radical scavenging activity.

Keywords: *Entandrophragma angolense*, obstetric fistula, phyochemical analysis, antioxidant activity , Ivory Caost

---

INTRODUCTION

Obstetric fistula is one of the tragic consequences of childbirth without medical assistance. This is an abnormal communication between the genitals and urinary tract **[1].** It affects 50000 to 100000 women annually worldwide and the WHO estimates that more than 2 million current number of women with obstetric fistula. That number is growing every year 50000 in Africa and 150000 in Asia. In West Africa, 5000 new cases of obstetric fistula are reported annually **[2].** In Ivory Coast, the prevalence of fistula is difficult to estimate because of the lack of proper documentation on the subject, the taboo nature of the disease, problems of accessibility of sick women to rare reference centers in the capital and the high cost of surgical treatment only means of healing offered by modern medicine **[1].** The social burden of this disease is heavy for women are often rejected for community life and deprived of all income generating activities. And traditional medicine is the only source of affordable and accessible care for poor patient's case. An ethno-botanical survey of the traditional treatment of obstetric fistula was then conducted. The choice fell on a traditional drug obtained from *Entandrophragma angolense*. This plant is known for its many uses in traditional medicine. A decoction of the bark is drunk to treat fever and the bark is also used, usually in internal applications, as an anodyne against stomach-ache and peptic ulcers, earache, and kidney, rheumatic or arthritic pains. It is also applied externally to treat ophthalmia, swellings and ulcers **[3].** Bark extracts

have been reported for moderate antiplasmodial activity; and the compounds 7α-obacunylacetate and 24-methylenecycloartenol exhibited pronounced activity against chloroquine-resistant strains of *Plasmodium falciparum* **[4]**. Methylangolensate, the major compound isolated from the methanol extract of the stem bark of *E. angolense* produced a dose-related inhibition of gastric ulceration and the activity of smooth muscles, and reduces the propulsive action of the gastrointestinal tract in mice.

The methylangolensate also demonstrated sedative activity in tests with mice and rats **[5, 6].** This work is to achieve a phytochemical screening and evaluating the antioxidant activity of the extracts of the stem bark *Entandrophragma Angolense*.

MATERIALS AND METHODS

2.1 Plant collection

The bark of *Entandrophragma Angolense* has been collected in Petit-Yapo (Agboville), south of Côte d'Ivoire, in September 2015 and was identified at National Centre Floristic of University Felix Houphouet-Boigny; deposited a herbarium specimen of the plant. The bark was dried at in air before crushing into powder.

2.1.1 Preparation of extracts

Extraction using increasing polarity solvent: distilled water, ethanol, ethyl acetate and hexane, was carried out according to the method of **[7, 8]**.

2.1.1.1 Decoction

10 g of powder taken up in 100 ml of distilled water is heated to boiling for 1h. After cooking, the decoction is filtered several times and then heated in an oven at a temperature of 55 ° C for drying for 24 h. Then the aqueous extract was obtained (ETA *Ea*).

2.1.1.2 Maceration

25 g of powder of bark was subject to maceration under magnetic agitation for 48 hours in 125 ml of ethanol. Ethanolic mixture was filtered once on cotton wool and then filtered on filter paper (whatman). The filtrate was concentrated using a rotary evaporator at 65°C (HEIDOLPH WB 2000). The concentrate was totally dried in the steam room at 55°C (SELECTA) during 24 hours. Then the ethanolic extract was obtained (EE *Ea*).

To obtain ethyl acetate extract, 25 g of powder of leaves was subject to maceration under magnetic agitation for 48 hours in 125 ml of ethyl acetate. The ethyl acetate mixture was filtered twice with cotton wool. The filtrate is left evaporate in the room temperature during 24 hours and dried totally in the steam room at 55 °C during 24 hours. Then the ethyl acetate extract was obtained (EA *Ea*).

To obtain hexanic extract, 25 g of powder of leaves was macerated under magnetic agitation for 48 hours in 125 ml of hexanic mixture. After that the extract was filtered twice with cotton wool. The filtrate is left evaporate in the laboratory temperature during 24 hours and dried totally in the stove at 55 °C during 24 hours. Then the hexanic extract was obtained (EH *Ea*).

2.1.2 Phytochemical analysis

This is a qualitative test based on color reactions and / or precipitation **[9].** Table 1 indicates different desired chemical groups and specific reagents.

Table 1: Specific reactions and phytochemical screening reagents

Chemical Groups	Specific Reagents	Characteristic Reactions
Alkaloids	Dragendorf (Potassium tetraiodobismuthate)	Orange coloring with appearance precipitate
Tannins	Stiasny reaction ($FeCl_3$)	blue-dark green or black color
Flavonoids	Cyanidin reaction	orange-pink coloring; purple or red rose
Coumarins	Bornträger reaction-UV	Intense fluorescence
Saponosides	Determination of the Foam Index (MI)	Positive test if MI>1cm, Intense foam
Sterols and Triterpenes	Libermann-Buchard (acetic anhydride -H_2SO_4)	The appearance at the interface of a purple or purple ring, turning blue to green
Reducing compound	Fehling's reaction	Brick red precipitate

2.2 Antioxidant activity of extracts by DPPH free radical scavenging assay

DPPH free radical scavenging assay was performed to determine the antioxidant activity of different concentrations of extracts and ascorbic acid (800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/ml). DPPH (0.04%) was used as free radical. An equal volume of various concentrations of DPPH and methanolic extracts were mixed in the micro-plates and were incubated at room temperature in the dark for 30 minutes. The optical density was measured at 517 nm.

Their using a micro-plate reader. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. Vitamin C was used as the positive standard and methanol as the blank. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation:

DPPH radical scavenging activity (%) = [(Abs $_{control}$ – Abs $_{sample}$)/(Abs $_{control}$)] X 100

Where Abs $_{control}$ is the absorbance of DPPH radical + methanol and Abs $_{sample}$ is absorbance DPPH radical + sample extract/standard.

IC50 value was determined from the plotted graph of scavenging activity against the different concentrations of extracts, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

2.3 Statistical Analyses

All results are expressed as mean ±SD. The significance of difference was calculated by Student's *t* test, and significant difference was accepted at $p < 0.05$ significant.

RESULTS

3.1 Phytochimical analysis

The qualitative phytochemical study revealed the presence of several chemical groups (alkaloids, tannins, flavonoids (flavones, flavonols), saponins, sterols and triterpenes) in the stem bark *Entandrophragma angolense.* However the aqueous extract of the bark contains more chemical groups than other extract obtained by maceration **(Table 2)**.

Table 2: Screening phytochemical results

	Different types of extracts			
Chemical compounds	**ETA Ea**	**EE Ea**	**EA Ea**	**EH Ea**
Alkaloïds	+++	+	+++	-
Tannins	+++	+++	+	-
Flavonoides	+++ (Flavones)	+ (Flavonol)	+++ (Flavonol)	-
Coumarins	-	-	-	-
Saponosides	+++	-	-	-
Sterols & Terpenes	+++	+++	+++	+++
Reducing compound	-	-	-	-

+: presence; +++: intense presence; -: absence; ETA: aqueous extract; EE: ethanolic extract; EA: acetic extract; EH: hexanic extract; Ea: Entandrophragma angolense

3.2 Antioxidant activity of extracts by DPPH free radical scavenging assay

DPPH is a stable free radical that can accept an electron to become a stable molecule, commonly used as a substrate to evaluate antioxidant activity; it is a stable. The reduction of DPPH radical was determined by the decrease in its absorbance at 517 nm induced by antioxidants **[10].**

Our results reveal that the extracts as well as vitamin C (Vit C) revealed dose-dependent anti-radical activity (Figure 1;2;3;4).The dose-response curve of DPPH radical scavenging activity of the ethanolic extract of the *Entandophragma angolense* had higher activity with an IC50= 5 µg/mL than acetic extract, hexanic and aqueous extracts with an IC50 of 10, 20, 25 µg/mL respectively (table 3) compared with Vit C, which used as the positive control with IC_{50} value of 4,5. Comparing IC_{50} values of Vit C and other extracts especially the ethanol extract, we could say that *Entandophragma angolense* has good potential as a source for natural antioxidants. According to the above results, the study showed that the extracts could serve as free radical inhibitor or scavengers, acting possibly as antioxidants.

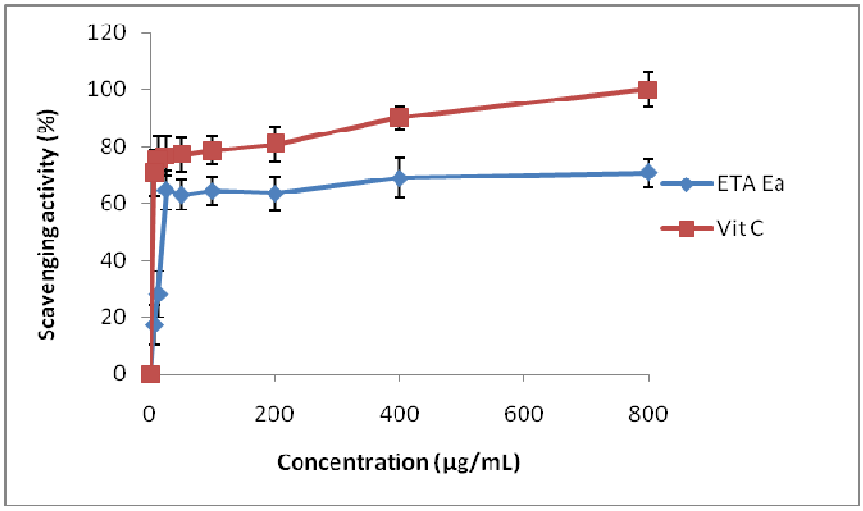

Figure 1: *in vitro* antioxidant activity of aqueous extract of *Entandrophragma angolense* (ETA *Ea*)

Dose-response curve of DPPH radical scavenging activity of ETA *Ea*
Scavenging activity (%) concentration (µg/mL)

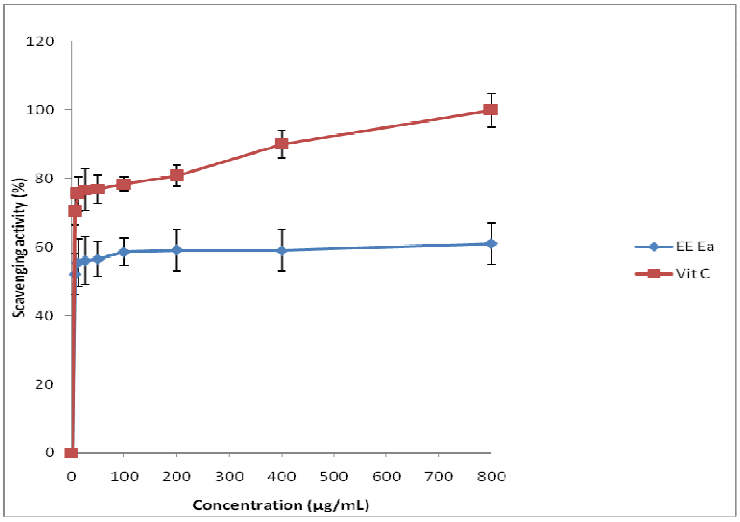

Figure 2: *in vitro* antioxidant activity of ethanolic extract of *Entandrophragma angolense* (EE *Ea*)

Dose-response curve of DPPH radical scavenging activity of EE *Ea*

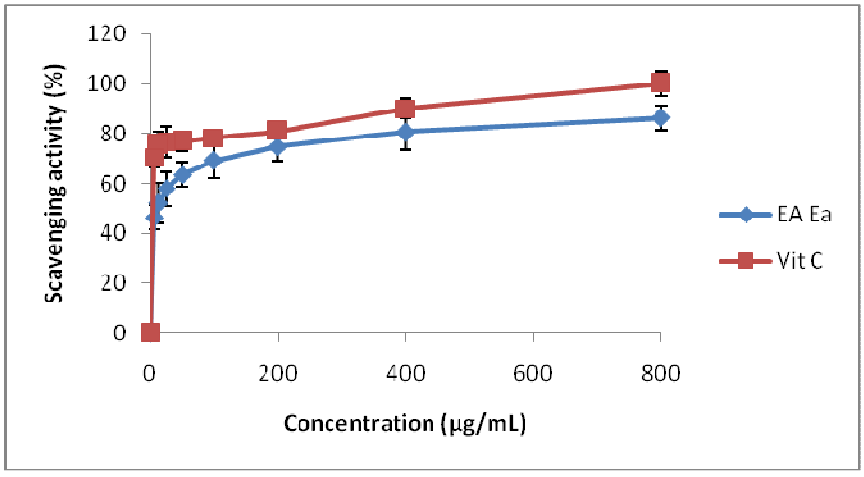

Figure 3: *in vitro* antioxidant activity of acetic extract of Entandrophragma *angolense*

(EA Ea)

Dose-response curve of DPPH radical scavenging activity of EA *Ea*

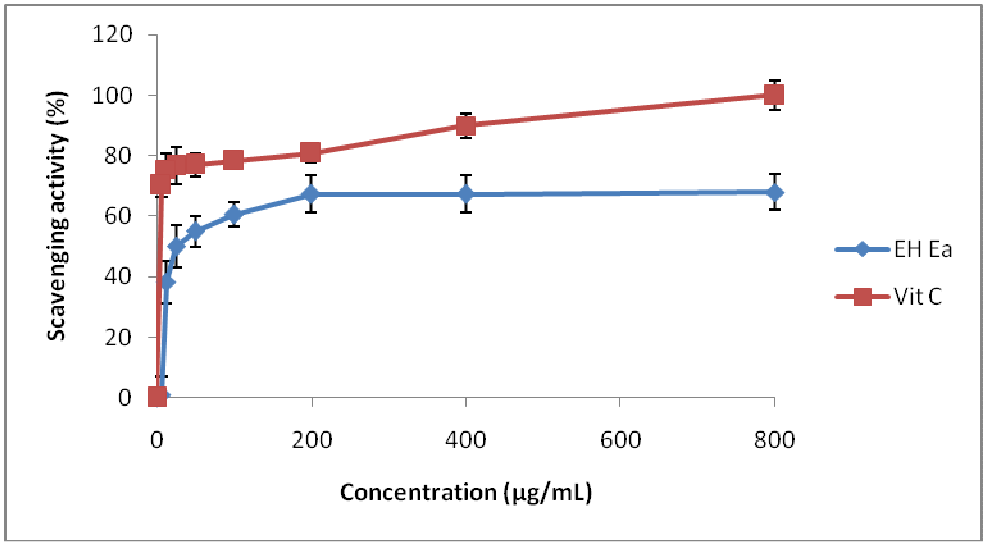

Figure 4: *in vitro* antioxidant activity of hexanic extract of Entandrophragma *angolense* (EH Ea)

Dose-response curve of DPPH radical scavenging activity of EA *Ea*

Table 3: DPPH radical scavenging activity (IC_{50}) of the different extracts of the *Entandophragma angolense*

Extracts of *Entandrophragma angolense*	Aqueous extract	Ethanolic extract	Ethyl acetate extract	Hexanic extract	Vit C
IC_{50} (µg/mL)	20	5	10	25	4,5

DISCUSSION

The qualitative phytochemical study revealed the presence of several chemical groups: alkaloids, tannins, flavonoids (flavones, flavonols), saponins, sterols and triterpenoids. This qualitative phytochemical analysis showed that chemical compounds identified in the stem bark *Entandrophragma angolense* were strongly characterized in the extract obtained by traditional preparation (decoction) **[11]**. From a qualitative point of view, the extraction method used in traditional folk medicine is effective compared to the other studied methods of extraction (Ethanolic, Ethyl acetate and Hexanic). These results are similar to those some authors whose work has characterized the same chemical groups contained in stem bark *Entandrophragma angolense* **[12].** The abundance of active principles confers to the plant the remarkable pharmacological properties.

The extracts of *Entandophragma angolense* exhibited marked antioxidant activity by scavenging DPPH free radical in a concentration dependent manner as well as methanolic extract of *Abrus pulchellus* Wall (Fabaceae) **[13]**. The important antioxidant activity of *Entandophragma angolense* extracts could be related of the presence of tannins, which are a major group of compounds that act as primary antioxidant or free radical scavengers **[14]**. Furthermore, the results strongly suggest that phenolics are important components of this plant, and some of its pharmacological activities could be attribute to the presence of these constituents. The phenolic compounds in natural products are known to have antioxidant activity due to their redox properties, allowing them to play a role as free radical scavenger, metal chelators, hydrogen donors and reducing agents **[15]**. It is well-established that free radicals are one of the causes of several diseases, such as cancer, heart disease, Parkinson's disease and coronary **[16, 17, 18]**. This study showed that *Entandophragma angolense* extracts, especially ethanolic extract, have excellent antioxidant activities.

CONCLUSION

This present study showed that *E. angolense* bark extracts contains several chemical groups and possess an *in vitro* antioxidant activity by DPPH radical scavenging assay. This plant could be potentially useful for the development of therapeutic agents against the several diseases caused by free radicals.

Acknowledgements
We express our gratitude to Department of Natural Science, Nangui Abrogoua University of Abidjan (Côte d'Ivoire) and to the Sultan Moulay Slimane University of Beni-Mellal, Faculty of Technical and Sciences (Morocco) for providing the facilities for conducting this research.

REFERENCES

[1] P. Kouye, G. Kabas, M. Gbane, C. N'Da, Etude socio-anthropologique sur les fistules obstétricales en Côte d'Ivoire. DCPNSR-PF/UNFPA. **2006,** 98 p
[2] M.Y. Zeinab, B. Karambiri, A. Bore, I. Famanta, D.O. Diombele, A. Ba, K. Traore, K. Ouattara**.** Situation des fistules obstétricales au Mali. UNFPA. **2003**, 38 p
[3], H.M. Burkill, The useful plants of West Tropical Africa. 2nd Edition. Volume 4, Families M–R. Royal Botanic Gardens, Kew, Richmond, United Kingdom. **1997,** 969 p
[4] J. Bickii, G.R.F. Tchouya, J.C. Tchouankeu & E. Tsamo, *African Journal of Traditional, Complementary and Alternative Medicines,* **2007,** 4, 135-139.
[5] V.C.O. Njar, J.K. Adesanwo, , J.M. Makinde & O.B. Taiwo, *Phytotherapy Research,* **1994**, 8, 46-48.
[6] S. Amos, A. Orisadipe, L. Binda, M. Emeje, A. Adesomoju, J. Okogun, P. Akah, C. Wambebe & K. Gamaniel, *Pharmacology and Toxicology,* **2002,** 91, 71-76.
[7] M. Lébri, C. Bahi, Y.B.N. Fofié, G. Gnahoué, S.M. Lagou, H. Achibat, A. Yapi., G.N. Zirihi, A. Coulibaly, A. Hafid, M. Khouili, *Int. J. Biol. Chem. Sci.***, 2015,** 9, 1472-1473
[8] M. Lébri, M. Tilaoui, C. Bahi, H. Achibat, S. Akhramez, Y. B. N. Fofié, G. Gnahoué,
S.M. Lagou, G. N. Zirihi, A. Coulibaly, A. Zyad, A. Hafid and M. Khouili, *Der Pharma Chemica*, **2015**, 7, 113-114
[9] P.J. Houghton, A. Raman**,** Laboratory Handbook for the Fractionation of Natural Extracts. Ed Chapman and Hall: New York, **1998**, 208.
[10] Y.V. Ramshankar, P. Vinay, P. Vijayan, *Pharmog Mag.* **2008**, 4, 330-334
[11] N.G. Konkon, D. Simaga, A.L. Adjoungova, K.E. N'Guessan, G.N. Zirihi, B.D. Kone, *Pharm. Méd. Trad. Afr,* **2006**, 15: 73-74.
[12] A. A. Yenon, H.F. Yapi, E.K. Ayébé, Yapo A.F, J.D. N'guessan, A.J. Djaman, *Asian Journal of Biomedical and Pharmaceutical Sciences,* **2014**, 4, 67-70.
[13] K.S. Vinayaka, S.P. Swarnalatha, H.R. Preethi, K.S. Surabhi, T.R. Prashith Kekuda and S.J. Sudharshan,. *African Journal of Basic & Applied Sciences,* 2009, 1, 110-116
[14] O. Polterait, *Current Org.Chem*, **1997,** 1, 415-440.
[15] J. Javanraedi, C. Stushnoff, E. Locke, J.M. Vivanco**,** *Food Chem.* **2003,** 83, 547-550.
[16] P. Cerutti, *Lancet*, **1994**, 344, 862-863.
[17] J.D Adams, I.N. Odunze , *Free Radical Biol. Med.*, **1991,** 10, 161-169.
[18] M.G.L. Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, D. Kromhout, *Lancet*, **1993,** *342*, 1007-1011.