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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTICANCER EFFECT OF EXTRACTS *ENTANDROPHRAGMA ANGOLENSE* A MEDICINAL PLANT USED IN THE TREATMENT OF OBSTETRIC FISTULA IN IVORY COAST

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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 *in vitro* anticancer effect of extracts aqueous and ethanol of *Entandrophragma angolense* bark on the murin mastocytoma cancer cell line (P815) and the phytochemical screening of the extract as well. *In vitro* anticancer effect was evaluated by the cellular cytotoxicity against the murin mastocytoma cell line (P815). Cellular cytotoxicity was determined by the MTT assayand the phytochemical screening was based on differential staining and precipitation reactions. The *in vitro* anticancer effect showed a dose dependant cytotoxic effect. It was observed that the ethanolic extract has the best cytotoxic effect with an IC50 of 44.30 µg/mL against 2.5 µg/mL for methotrexate. While the aqueous extract has a weak cytotoxic effect with an IC_{50} of 200 µg/mL. Phytochemical screening 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 possess a best *in vitro* anticancer effect with the ethanolic extract and a weak effect with aqueous extract against P815 tumor cell line.

Key words: *Entandrophragma angolense*, *In vitro* anticancer effect, P815 cancer cell line.

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INTRODUCTION

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Obstetric fistula is one of the tragic consequences of childbirth without medical assistance. This is an abnormal communication between the genitals and urinary tract (Kouye et *al.*, 2006). It affects 50 000 to 100 000 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 150 000 in Asia. In West Africa, 5000 new cases of obstetric fistula are reported annually (Zeinab *et al.,* 2003).Although difficult delivery is the leading cause of vesico-vaginal fistula, other factors may be involved. Indeed, cancer, especially that of the genital tract, can be at the origin of a fistula (Tarrerias 2013). 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 (Kouye et *al.,* 2006). However, a recent study was conducted in Côte d'Ivoire on the level of knowledge of the disease and the plants traditionally used in its treatment (Lagou *et al.,* 2016). This study revealed that in the Abidjan district, out of 560 women interviewed, 25.71% reported having information on this condition. Obstetric fistula can be cured (76.39% of respondents) and modern medicine is the most appropriate method according to 81.73% of women. Some women (18.27%) prefer traditional medicine. A list of 13 plant species (13 genera and 8 families) has been reported as traditionally used in the treatment of obstetric fistula (Lagou *et al.,* 2016). In this survey, the families Lamiaceae, Meliaceae, Malvaceae and Euphorbiaceae were the most representative. These plants may play a role in the management of obstetric fistula (Lagou *et al.,* 2016). A previous study showed that *Entandrophragma angolense* has a good antioxidant activity (Lagou *et al.,* 2016a). This work is to achieve a phytochemical screening and evaluating *in vitro* anticancer effect of extracts (aqueous and ethanol) of *E. angolense* stem bark on the murin mastocytoma cancer cell line (P815) of the stem bark *E. angolense.*

MATERIALS AND METHODS

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.

Preparation of extracts

Extraction using increasing polarity solvent: distilled water, ethanol was carried out according to the method of Lébri *et al.,* 2015

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).

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).

Phytochemical screening

This is a qualitative test based on color reactions and / or precipitation (Lébri *et al.,,* 2015)

In vitro anticancer effect of extracts *E. Angolense*

In vitro anticancer effect of aqueous extract of The bark of *Entandrophragma Angolense* was conducted in the Laboratory of biological engineering, Faculty of Sciences and Techniques , Sultan Moulay Slimane University of Beni-Mellal (Morocco).

Tumor cell line and culture

The mastocytoma tumor cell line, were grown in RPMI1640 (Sigma-Aldrich) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS) (Sigma-Aldrich), 1% penicillin-streptomycin, and 0.2% sodium bicarbonate (Sigma-Aldrich), under a fully humidified atmosphere of 95% air and 5% CO_2 at 37 ºC.

Cytotoxicity assay

Cellular cytotoxicity was determined by the MTT reduction assay. This Colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically (Mosmann *et al.,* 1983). Growing concentrations of the tested extract (solubilised in Dimethyl sulfoxide (DMSO): 1.56; 3.12; 6.25 ; 12.5; 25; 50 ; 100; 200 and 400 µg/mL) were applied to the wells of a 96 well plate containing the confluent cell monolayer (10^6 cells per well) in duplicate. Methotrexate as positive control drug was added in the same concentrations and conditions. After 48 h of incubation, 20 µL of the MTT solution [5 mg/mL in Phosphate buffered saline (PBS)] was added. After incubation in the same conditions for 4 h, the plates were treated with a mixture of HCl / Isopropanol (24:1) to dissolve the blue intracellular formazan product. One hour later, the plates were read on a Micro ELISA reader using two wavelengths (540 and 630 nm). DMSO was used as negative control. The median inhibitory concentration (IC50) was calculated as the concentration of the sample that leads to 50% of cell lysis comparatively to the negative (positive) control.

The relative inhibition of cell proliferation was calculated by the formula:

% inhibition =100 x[1- (A / A_0)],

where A_0 and A, are the absorbencies of negative control and extracts (aqueous and ethanol) or methotrexate treated cells, respectively.

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

Phytochimical screening

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 1).

***In vitro* anticancer effect**

The *In vitro* anticancer activity of extracts (aqueous and ethanolic) *Entandrophragma angolense* was evaluated at 3.12; 6.25; 12.5; 25; 50 ; 100; 200 and 400 µg/mL.) against P815 tumor cell. The result is summarized in figure 1 and 2.The cytotoxic effect of the aqueous extract shows that this extract has a weak inhibitory effect on the growth of tumor cells. It is less than 20% at a concentration of 200 µg / mL (table 2). The inhibitory concentration (IC_{50}) of the extract of the aqueous extract is 320 µg / mL. The ethanolic extract has an inhibitory effect which increases with concentration, this effect is similar to that of Methotrexate used as a reference (table 2). It was also observed, that the maximum effect of the extract at the highest concentrations tested (400µg/mL) was 86.06 % (± 3,63) and (200µg/mL) was 63.54% (±4,28) of lysis. Further, on lower doses of 3.12; 6.25; 12.15; 25 and 50 µg/mL percent growth inhibition observed by the extract was between 5.86 % (±5,93) and 44.31% (± 5.02) (table 2).

The IC_{50} of the ethanol extract is 44.3µg/mL, this IC_{50} is lower than that of the aqueous extract. The IC_{50} of aqueous and ethanolic extracts are more higher compared with the Methotrexate (2.5µg/mL) used as positive control which.

Table1. Screening phytochemical results

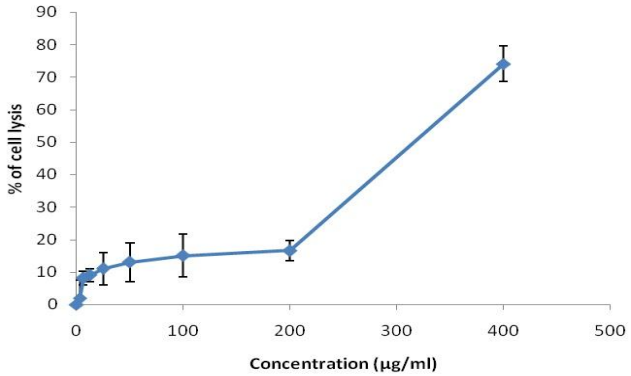
Chemical compounds	Different extracts of *E. angolense*	
	Aqueous extract	**Ethanolic extract**
Alkaloids	+++	+
Tannins	+++	+++
Flavonoids	+++ (flavones)	+ (flavonols)
Coumarins	-	-
Saponins	+++	-
Sterols and triterpenes	+++	+++

+: presence; +++: intense presence; -: absence

Table 2. Percentage of lysis of cancer cell line according to the concentration (aqueous extract, ethanol extract and méthotrexate)

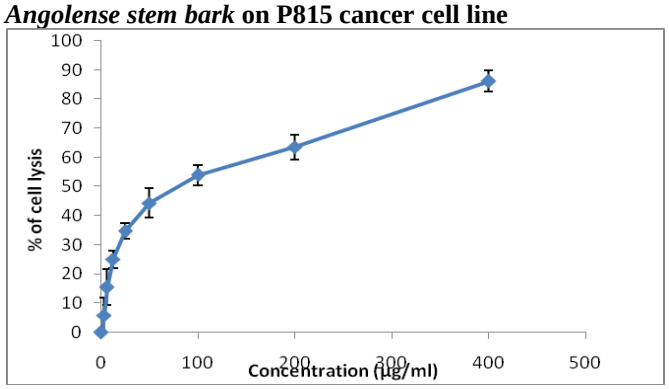
Concentration (µg/mL)	400	200	100	50	25	12.5	6.25	3.12
% of cell lysis (aqueous extract)	74.12± 5.37	16.56±3. 04	15±6.63	13±5.98	11±4.95	8.02±2.07	9±2,12	2±5.37
% of cell lysis (ethanolic extract)	86.06 ± 3.63	63.54±4. 28	53.93±3,47	44.31± 5.02	34.70±2.75	25.09±30	15.47±6.26	5.86±5.93
% of cell lysis (méthotrexate)	79.47± 4.45	70.93±3. 15	53.93±5.64	44.31± 6	34.70± 4.95	25.09±3.7	15.47± 2.7	5.86± 5.67

Fig 1. *in vitro* anticancer effect of aqueous extract of *E. Angolense stem bark* on P815 cancer cell line



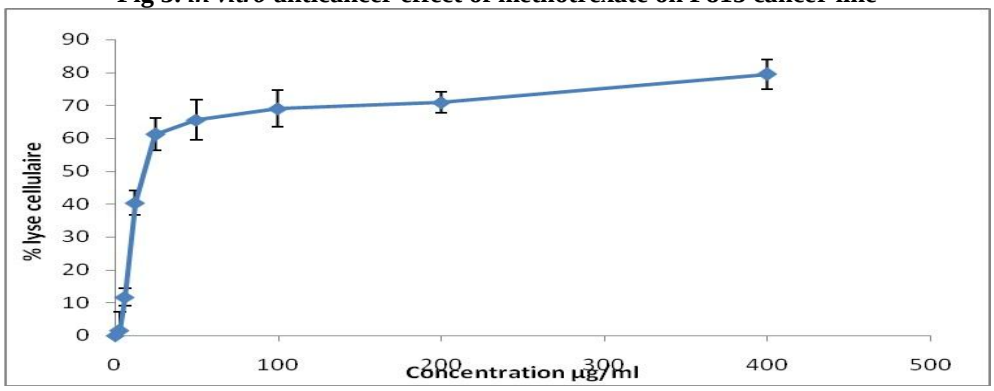
Dose - response curve of aqueous extract of *E. Angolense stem bark* on P815 cancer cell line

Fig 2. *in vitro* anticancer effect of ethanolic extract of *E. Angolense stem bark* on P815 cancer cell line



Dose - response curve of ethanolic extract of *E. Angolense stem bark* on P815 cancer cell line

Fig 3. *in vitro* anticancer effect of methotrexate on P815 cancer line



Dose-response curve of methotrexate on P815 cancer cell line

DISCUSSION

Phytochemical characterization tests have shown that the main chemical compounds found in *Entandrophragma angolense* extracts are saponosides, flavonoids, tannins, alkaloids, coumarins, sterols and terpenes (Yenon *et al.,* 2014). Several studies have shown that some of these compounds including alkaloids, flavonoids and saponosides possess antitumor properties(Sparg *et al.,* 2004; Fiot *et al.,* 2006)indeed, phenolic compounds (flavonoids) could prevent the occurrence of cancer by antioxidant action (Yang *et al.,* 2000).Thus, thanks to their cytotoxic properties, tannins, flavonoids, saponosides (Diame, 1998) can play a role in the prevention and treatment of cancer. This study evaluates the *in vitro* anticancer activity of extracts of *E. Angolense* stem bark (aqueous and ethanolic) against murin mastocytoma cells (P815). In this study, the extracts were evaluated as promising anticancer solution using MTT assays. The concentrations of extracts of *E. Angolense* stem bark leading to 50% lysis is 320 and 44.3 µg / mL respectively for the aqueous and ethanolic extract. Our results are in accordance with the results of Lébri *et al* ., 2015, that found aqueous extracts of *Abrus precatorius* leaf exhibits *in vitro* anticancer activity against murin mastocytoma cells (P815), with IC_{50} = 200 µg/mL. The richness in phenolic compounds, alkaloids, saponosides and tannins responsible for the antiradical activity (Lagou *et al.,* 2016) and cytotoxic compounds justifies the use of *Entandrophragma angolense*, to treat obstetric fistulas.

CONCLUSION

This present study showed that extract of *E. angolense* stem bark could be potentially useful for the development of therapeutic agents against cancer. The ethanolic extract of *Entandrophragma angolense* showed the best cytotoxic effect. Furthermore, the study, the in vivo anticancer activity and the molecular mechanisms involved in such activity are needed to facilitate integration of *E angolense* as an anticancer herbal medicine. Thus, this would justify the traditional use of The *E. angolense* in the treatment of obstetrical fistula.

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CONFLICT OF INTREST

No interest.

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