



**IN VITRO CYTOTOXICITY STUDY OF ACETAL AND HEXANE EXTRACTS OF  
ENTADROPHRAGMA ANGOLENSE (MELIACEAE) ON CELLS OF THE L-6 CELL LINE  
(RAT SKELETAL MUSCLE, MYOBLAST)**

**Doumbia Idrissa<sup>1,2\*</sup>, Yeo Dodehe<sup>2</sup>, Dhanabal Palaniswamy<sup>4</sup>, Ashish Wadhvani<sup>5</sup>, Yapi Houphouet Felix<sup>2</sup> and  
Djaman Allico Joseph<sup>2,3</sup>**

<sup>1</sup>Food and Bioproducts Processes Laboratory, Agronomic, Forestry and Environmental Engineering Department, University of Man, PO Box 20 Man, Ivory Coast.

<sup>2</sup>Biochemical Pharmacodnamy Laboratory, Biosciences Department, Felix Houphouet-Boigny University, PO Box 582, Abidjan 22, Ivory Coast.

<sup>3</sup>Biochemical Laboratory of Pasteur Institute of Ivory Coast, PO Box 490, Abidjan 01, Ivory Coast.

<sup>4</sup>Department of Pharmacognosy and Phytochemistry, JSS College of Pharmacy-Ooty, Rocklands, Post Box No 20, Udthagamandalan-643001, Tamil Nadu, India.

<sup>5</sup>Department of Pharmaceutical Biotechnology. Animal Tissue Culture Laboratory. JSS College of Pharmacy-Ooty, Rocklands, Post Box No 20, Udthagamandalan-643001, Tamil Nadu, India.

**\*Corresponding Author: Dr. Doumbia Idrissa**

Food and Bioproducts Processes Laboratory, Agronomic, Forestry and Environmental Engineering Department, University of Man, PO Box 20 Man, Ivory Coast.

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**ABSTRACT**

This study aimed to evaluate the cytotoxicity activity of the acetal and hexane extracts of *Entadrophragma angolense* (Meliaceae). *E. angolense* is a plant used in the traditional treatment of diabetes and several diseases in the south-east of Coast Ivory. In addition to this antidiabetic activity, this plant rich in polyphenolic compounds has an antioxidant potential that could be beneficial in the management of diseases related to oxidative stress. The determination of mitochondrial synthesis by assaying MTT is the principle of the cytotoxicity assay used in this study which was performed on cells of the L-6 cell line (rat skeletal muscle, myoblast). The IC<sub>50</sub> of the acetal (AEEA, IC<sub>50</sub> = 227.9824 µg / mL) and hexane extracts (HEEA = 217.2291 µg / mL) determined in this study are much higher than the respective pharmacological doses (antioxidant activity) which are (AEEA, IC<sub>50</sub> = 48.704 ± 1.295 µg / mL) and (HEEA, IC<sub>50</sub> = 62.97 ± 1.88 µg / mL). The acetal and hexane extracts of *Entadrophragma angolense* thus offer interesting margins of safety which could be an additional advantage for the use of these extracts.

**KEYWORDS:** *Entadrophragma angolense*, L-6 cell line, cytotoxicity.

**I. INTRODUCTION**

The use of plants for therapeutic purposes by man is as old as the history of mankind. In Côte d'Ivoire, in almost all the countries of the African continent this ancestral practice is experiencing a renewed interest. Among the many reasons for this rush to traditional medicine are : socio-cultural factors, socio-economic constraints, the numerous side effects of molecules derived from therapeutic chemistry and the availability of plants. These plants are part of the immediate environment of the populations. They are rightly the first therapeutic resource.

Currently it is estimated that nearly 80% of the African population uses the drugs of healers to heal.<sup>[1]</sup> However, the therapeutic use of plants is not always safe for the people who use them. These plants can heal or intoxicate

depending on the preparation, the dosage and the use that is made of it. Ignorance of bioactive doses and principles of empirically administered extracts exposes the populations that use them to real risks of therapeutic accidents that can sometimes be tragic.<sup>[2]</sup>

This situation requires that medicinal plants be the subject of further scientific studies in order to rationalize the use of these plants by the user populations. This study aims to make a contribution in this direction through the in vitro cytotoxicity study of acetal and hexane extracts of *Entadrophragma angolense* (Meliaceae) on cells of the L6 cell line. *E. angolense* is a plant used by people in southeastern Côte d'Ivoire for the treatment of various conditions including diabetes.<sup>[3]</sup> It has an antioxidant potential that raises real hopes in the management of pathologies related to oxidative stress.<sup>[4]</sup>

The link between oxidative stress and several pathologies such as cancer, arterial hypertension, cardiovascular diseases, diabetes and neurodegenerative pathologies is perfectly established so that all substances with antioxidant activity can be perceived as an upstream or indirect treatment of these pathologies.<sup>[5,6,7,8,9,10]</sup> The results of this study could help to orient and rationalize the therapeutic use of this plant.

## II. MATERIAL AND METHODS

### II.1 Plant material

The barks of *Entadrophragma angolense* (Meliaceae) from Agboville (south-east of Ivory Coast) have been identified by the National Center of Floristry at the University Felix Houphouet Boigny (Cocody-Abidjan). A specimen of the plant was deposited in the herbarium of this Center.

#### II.1.1 Preparation of the acetal extract of *Entadrophragma angolense* (Meliaceae)

The harvested bark was dried at room temperature ( $28 \pm 1^\circ\text{C}$ ) for one month out of the sun. The dried bark was ground to a fine powder. The powder (50 g) was macerated in 250 ml of ethyl acetate for 24 h at room temperature. The mixture was then filtered through the gauze and a second time on Whatman filter paper (3 MM). The evaporation of the solvent was carried out in an oven at  $50^\circ\text{C}$ . After drying, a brown powder obtained, was used to prepare the acetal extract of *Entadrophragma angolense* (AEEA).

#### II.1.2 Preparation of hexanic extract of *Entadrophragma angolense* (Meliaceae)

The dry bark powder (50 g) obtained above was macerated in 250 ml of hexane for 24 hours at room temperature. The mixture was then filtered through the gauze and a second time on Whatman filter paper (3 MM). The evaporation of the solvent was carried out in an oven at  $40^\circ\text{C}$ . After drying, we obtain a brown powder used to prepare the hexane extract of *Entadrophragma angolense* (HEEA).

### II.2 Animal material

The animal material used in this study consists of the cells of the L6 Cell line (Rat Skeletal Muscle, Myoblast).

### II.3 Experimental protocol

#### II.3.1 *In vitro* cytotoxicity assay

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays.

#### II.3.2 Determination of mitochondrial synthesis by MTT assay

This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTT to a blue formazan derivative by living cells is clearly a

very effective principle on which the assay is based. The principle involved is the cleavage of tetrazolium salt MTT (3-(4,5 dimethyl thiazole-2 yl)- 2,5-diphenyl tetrazolium bromide) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The numbers of cells were found to be proportional to the extent of formazan production by the cells used.

The cell culture was centrifuged and the cell count was adjusted to  $1.0 \times 10^5$  cells/mL using DMEM medium containing 10% FBS. To each well of a 96 well flat bottom micro titre plate, 100  $\mu\text{l}$  of the diluted cell suspension (approximately 10,000 cells/well) was added. After 24 hours, when the cell population was found adequate, the cells were centrifuged and the pellets were suspended with 100  $\mu\text{l}$  of different test sample concentrations prepared in maintenance media. The plates were then incubated at  $37^\circ\text{C}$  for 48 hours in 5%  $\text{CO}_2$  atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 48 hours, the sample solutions were centrifuged and the pellets were re-suspended with 20  $\mu\text{l}$  of MTT (2mg/mL) in MEM-PR (MEM without phenol red). The plates were gently shaken and incubated for 2 hours at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  atmosphere. The 100  $\mu\text{l}$  of DMSO was added and the plates were gently shaken to solubilise the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm. The percentage cell viability was calculated using the following formula and concentration of drug or test samples needed to inhibit cell growth by 50% values were generated from the dose-response curves.<sup>[11,12]</sup>

$$\% \text{Cell Viability} = \frac{(\text{Mean OD of individual test group}) \times 100}{\text{Mean OD of control group}}$$

## III. RESULTS AND DISCUSSION

Cytotoxicity assays performed on L6 cell lines showed a progressive decrease in cell viability as concentrations of the extracts increased. Conversely, there is an increase in the mortality of cells of the L6 line as a function of the concentrations of the plant extracts. The cytotoxic effect of the acetal and hexane extracts of *Entadrophragma angolense* is therefore dose-dependent.

The trend line in figure 1 represents cell mortality evolution according to concentrations of acetal extracts of *E angolense*. This curve made it possible to determine IC50 of acetal extract which is  $227.9824 \mu\text{g} / \text{mL}$ . This IC50 is much greater than the dose necessary to express the antioxidant activity of the acetal extract which is  $48.704 \pm 1.295 \mu\text{g} / \text{mL}$  (AEEA,  $\text{IC}_{50} = 48.704 \pm 1.295 \mu\text{g} / \text{mL}$ ).<sup>[3,4]</sup> The comparison of these data indicates that the acetal extract of *E. angolense* offers very interesting safety margins.

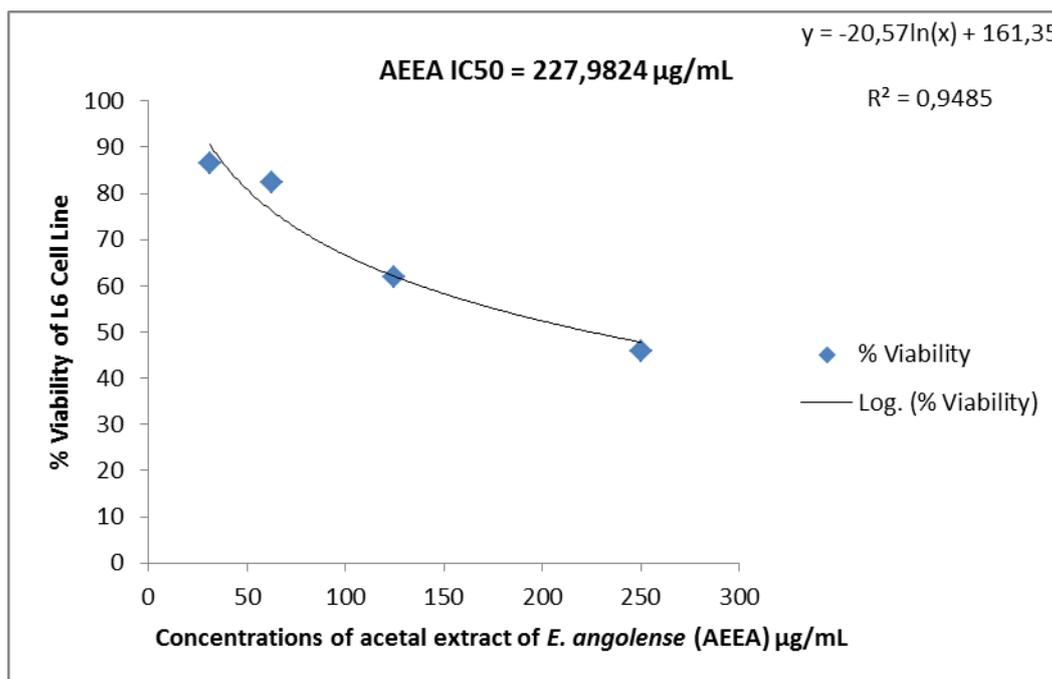


Figure 1: Evolution of L6 Cell Line viability according to the concentrations (µg / ml) of acetal extracts of *Entadrophragma angolense*.

The trend line in Figure 2 represents evolution of cell death according to concentrations of hexane extracts of *E. angolense*. This curve made it possible to determine the IC50 of the hexane extract which is 217.2291 µg / mL. This IC50 is much higher than the dose necessary to

express the antioxidant activity of the hexane extract which is  $62.97 \pm 1.88 \mu\text{g} / \text{mL}$  (HEEA, IC50 =  $62.97 \pm 1.88 \mu\text{g} / \text{mL}$ ).<sup>[3,4]</sup> Comparison of these data indicates that the hexane extract of *E. angolense* also offers very good safety margins.

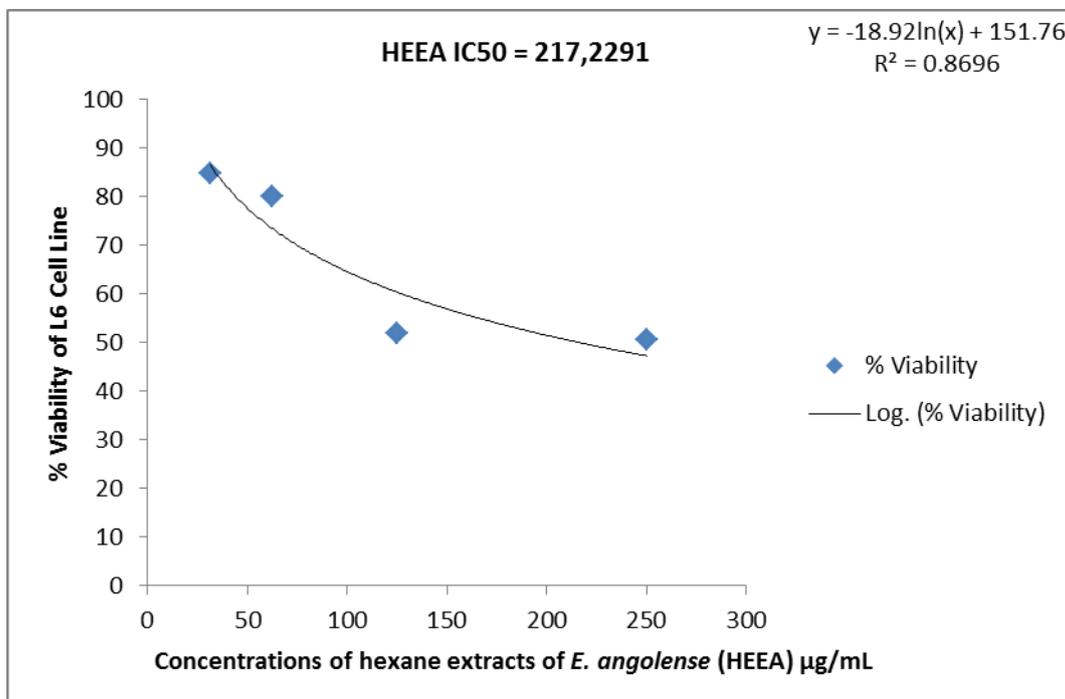


Figure 2: Evolution of L6 Cell Line viability according to the concentrations (µg / ml) of hexane extracts of *Entadrophragma angolense*.

Like many other plants from the Ivorian and African pharmacopoeia, the acetal and hexane extracts of *E. angolense* exert a cytotoxic activity on the cells of the L6

cell line which is therefore dose-dependent.<sup>[13,14,15,16]</sup> The hexane extract has a greater cytotoxic activity than the acetal extract.

Cytotoxicity is the property of a toxic agent to induce initial molecular changes or to cause functional impairment of living cells that can be described as rupture of homeostasis. This dysfunction can cause cellular damage and in the extreme case lead to cell death.

Cytotoxic doses are much higher than pharmacological doses. The acetal and hexane extracts of *E. angolense* therefore offer interesting safety margins that could facilitate their use in human therapeutics.

Moreover, seen from another angle, this cytotoxic activity, which is expressed by an antiproliferative activity of the cells of the L6 line can be perceived as a beneficial activity in the management of some pathologies such as cancers. Indeed, plant extracts with antiproliferative activity on cultured cells are considered as potential anti-cancer. This anti-cancer action could be improved on the one hand by the use of an appropriate cell line in comparison with a reference molecule such as taxol and on the other hand by a purification of the extracts in order to isolate the molecules responsible of this anti-proliferative activity.

The mechanisms evoked to explain the antiproliferative activity of chemical substances are numerous.<sup>[17,18]</sup> Cellular injury is considered as the functional and structural impairment of cells linked to a sequence of events occurring when the cell has exceeded its ability to adapt to a stimulus. Cellular lesions can be reversible, this is the case of cell degeneration, or irreversible it is the case of cell death. Cell death can occur following two deferential processes: necrosis and apoptosis.

Several cell organelles may be targets of cytotoxic substances. Cell membranes can be the site of various alterations such as lipid peroxidation, loss of selective permeability of the plasma membrane. At the mitochondria level, toxic substances inhibit oxidative phosphorylation, beta-oxidation of fatty acids, cellular respiration, and lead to ATP concentration. At the level of lysosomes, they inhibit the cell's ability to degrade. Genetic heritage may be altered by genotoxic.<sup>[19,20,21]</sup>

#### IV. CONCLUSION

In conclusion, the acetal and hexane extracts of *E. angolense* exert cytotoxic effects on the cells of the L6 cell line. The hexane extract has a greater cytotoxic activity than the acetal extract. The cytotoxicity of these two extracts is expressed at doses that are much higher than the pharmacological doses, which offers them interesting safety margins that could facilitate their use in human therapy. This cytotoxic activity, which is expressed by an antiproliferative activity of the cells of the L6 line, can be perceived as a beneficial activity in the management of certain pathologies such as cancers and degenerative pathologies.

This anti-cancer action could be improved on the one hand by the use of an appropriate cell line in comparison with a reference molecule and on the other hand by a purification of the extracts in order to isolate the molecules responsible for this anti-cancer activity. - proliférative.

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#### CONFLICT OF INTEREST

The authors claim that there is no conflict of interest.

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