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Evaluation of cytotoxicity activity of acetal and hexane extracts of *Entada mannii* (Fabaceae) on cells of the L-6 cell line (rat skeletal muscle, myoblast) *in vitro*

Doumbia Idrissa[1], Bahi Calixte[2], Dhanabal Palaniswamy[3], Ashish Wadhwani[4], Yapi Houphouet Felix[5], N'guessan Jean David[2], Djaman Allico Joseph[6]

[1, 2] Food and Bioproducts Processing Laboratory, Agronomic, Department of Forestry and Environmental Engineering, University of Man, Man, Ivory Coast

[1, 5-7] Biochemical Pharmacodynamic Laboratory, Department of Biosciences, Felix Houphouet-Boigny University, Abidjan, Ivory Coast

[3, 7] Department of Pharmacognosy and Phytochemistry, JSS College of Pharmacy-Ooty, Tamil Nadu, India

[4] Department of Pharmaceutical Biotechnology, Animal Tissue Culture Laboratory, JSS College of Pharmacy-Ooty, Tamil Nadu, India

[7] Biochemical Laboratory of Pasteur Institute of Ivory Coast, Abidjan, Ivory Coast

Abstract
The cytotoxicity activity of acetal and hexane extracts of *Entada mannii* (Fabaceae) were evaluate *in vitro*. *E. mannii* 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 IC50 of the acetal (AEEM, IC50 = 141.5115 µg / mL) and hexane extracts (HEEM = 99.2682 µg / mL) determined in this study are much higher than the respective pharmacological doses (antioxidant activity) which are (AEEM, IC50 = = 52.30 ± 2.05 µg / mL) and (HEEM, IC50 = 70.51 ± 1.84 µg / mL). The acetal and hexane extracts of *Entada mannii* thus offer interesting margins of safety which could be an additional advantage for the use of these extracts.

Keywords: *E. mannii*, L-6 cell line, cytotoxicity

1. Introduction
At a time when modern medicine is booming, Ivorian and African populations in general are increasingly using traditional medicine. The strong preponderance of these plants in the management of various diseases will increase in the coming years according to the forecasts of the various specialists. Several factors militate in favor of this trend [1, 2]. However, despite its undeniable richness, the African pharmacopoeia gives rise to some mistrust. Indeed the lack of knowledge of the bioactive principles, the doses of the extracts administered by the healers can expose the user populations to real risks of therapeutic accidents. These plants can heal or intoxicate depending on the preparation, the dosage and the use that is made of it [3, 4, 5].

In order to make a contribution in this direction, we undertook to evaluate the cytotoxicity of the acetal and hexane extracts of *Entada mannii* (Fabaceae) on the cells of the rat L6 line. *Entada mannii* is a plant from the Ivorian pharmacopoeia used by people in southeastern Côte d'Ivoire to treat several diseases among which we have diabetes. This plant has an antioxidant potential that could be an additional asset in the management of diseases related to oxidative stress such as cancer, degenerative diseases [6]. The physiopathology knowledge of several diseases makes it possible to establish a link between these pathologies and oxidative stress, so that the use of antioxidant substances can be likened to direct management of these diseases [7, 8, 9, 10, 11, 12]. This study will provide data to guide and rationalize the use of this plant.

2. Material and Methods
2.1 Plant material
The barks of *Entada mannii* (Fabaceae) 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.

2.1.1 Preparation of the acetal extract of *Entada mannii* (Fabaceae)
The harvested bark was dried at room temperature (28 ± 1 ° 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 °C. After drying, a brown powder obtained, was used to prepare the acetal extract of *Entada mannii* (AEEM).

2.1.2 Preparation of hexane extract of *Entada manni* (Fabaceae)

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 ° C. After drying, we obtain a brown powder used to prepare the hexane extract of *Entada mannii* (HEEM).

2.2 Animal material

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

2.2.1 Experimental protocol

2.2.2 *In vitro* cytotoxicity assay

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

2.2.3 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 1.0x105 cells/mL using DMEM medium containing 10% FBS. To each well of a 96 well flat bottom micro titre plate, 100µl 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 µl of different test sample concentrations prepared in maintenance media. The plates were then incubated at 37º C for 48 hours in 5% CO2 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 µl of MTT (2mg/mL) in MEM-PR (MEM without phenol red). The plates were gently shaken and incubated for 2 hours at 37ºC in 5% CO2 atmosphere. The 100 µl 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 [13, 14].

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

3. 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 according to concentrations of the plant extracts. The cytotoxic effect of the acetal and hexane extracts of *Entada mannii* is therefore dose-dependent.

The trend line in figure 1 represents the evolution of cell mortality according to the concentrations of acetal extracts of *E. mannii*. This curve made it possible to determine IC50 of acetal extract which is 141.5115 µg/mL. This IC50 is much greater than the dose necessary to express the antioxidant activity of the acetal extract which is 52.30 ± 2.05 µg / mL (AEEM, IC50 = 52.30 ± 2.05 µg / mL) [15]. The comparison of these data indicates that the acetal extract of *E. mannii* offers very interesting safety margins.

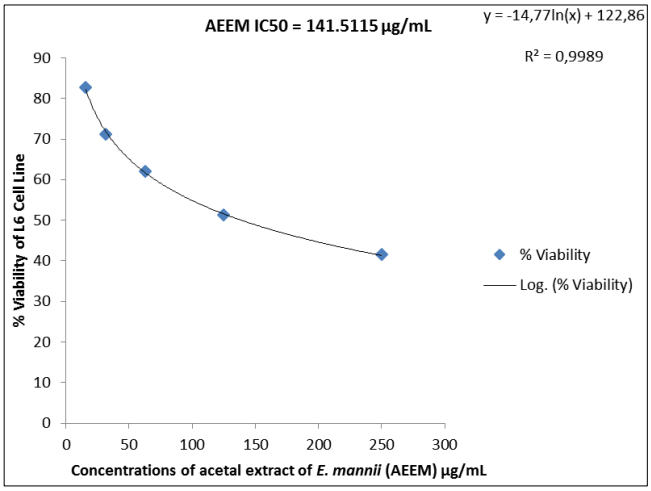

Fig 1: Evolution of L6 Cell Line viability according to the concentrations (µg / ml) of acetal extracts of *Entada mannii*.

The trend line in Figure 2 represents the evolution of cell death according to the concentrations of hexane extracts of *E. mannii*. This curve made it possible to determine the IC50 of the hexane extract which is 99.2682 µg / mL. This IC50 is higher than necessary dose to express the antioxidant activity of the hexane extract which is 70.51 ± 1.84 µg / mL (HEEM, IC50 = 70.51 ± 1.84 µg / mL) [15]. Comparison of these data indicates that the hexane extract of *E. mannii* also offers very good safety margins.

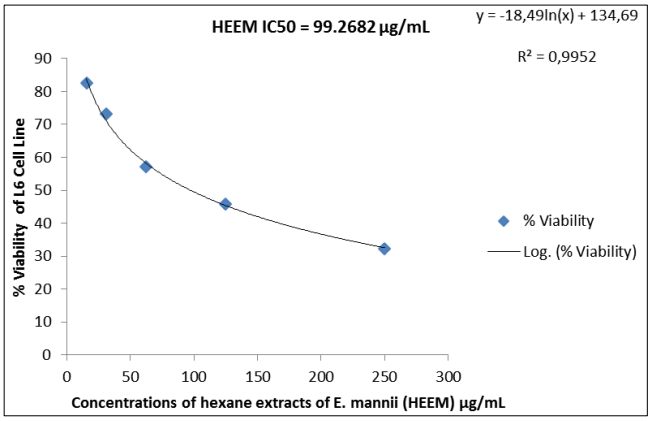

Fig 2: Evolution of L6 Cell Line viability according to the concentrations (µg / ml) of hexane extracts of *Entada mannii*.

Like many other plants from the Ivorian and African pharmacopoeia, the acetal and hexane extracts of *E. mannii* exert a cytotoxic actvity on the cells of the L6 cell line which is therefore dose-dependent [16, 17, 18, 19]. 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. mannii* 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 taxotere 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 [20, 21]. 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 [22, 23].

4. Conclusion

In conclusion, the acetal and hexane extracts of *E. mannii* 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. -prolifération.

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6. Conflict of interest

The authors claim that there is no conflict of interest

7. References

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