



Original Research Article

doi: <https://doi.org/10.20546/ijcrbp.2018.504.007>

Comparative Study of the Cytotoxicity of 70% Ethanolic Extracts of *Pitadeniastrum africanum* Hook (Fabaceae) and *Hunteria eburnea* Pichon (Apocynaceae) on HFF Cells (Human Foreskin Fibroblasts)

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Article Info

Date of Acceptance:
31 March 2018

Date of Publication:
06 April 2018

Keywords

Cytotoxicity
Ethanolic extracts
Medicinal plant
Skin diseases

ABSTRACT

Pitadeniastrum africanum and *Hunteria eburnea* are two plants that are recurrently mentioned in the treatment of several skin diseases in the Upper Sassandra Region. In order to better understand the mode of action of these two plants on skin cells, a comparative study of the cytotoxicity of ethanolic extracts *in vitro* was performed on HFF (Human Foreskin Fibroblasts) cells. The Mosman method was used to evaluate the cytotoxic activity of the ethanolic extracts of both plants. The cytotoxicity test performed *in vitro* on HFF cells revealed not cytotoxic effects of both plants on human cells at concentrations of 125 to 1000 µg / mL. The different viability rates of the two ethanolic extracts were strictly greater than 30%. All these results demonstrate a safety of use of these two plants in the traditional treatment of various skin diseases.

Introduction

Infectious diseases account for 1/3 of global mortality and 45% of deaths in developing countries because of the multi-resistance phenomenon (Simporé et al., 2006). In Ivory Coast, this rate is between 50 and 60% (OMS, 2007). The excessive and uncontrolled use of antibiotics has resulted in an increasing resistance of germs to the synthetic

molecules currently available. This has made it even more difficult to care for people with infectious diseases such as suppuration, chronic wounds and others (Yao, 2013). Faced with this threat, the search for new molecules has become a necessity for the scientific world. In this quest, medicinal plants remain the main reservoir that is used by more than 80% of populations in developing countries for their health care (OMS, 2003). It is in this perspective that

this study aims to evaluate and compare the cytotoxic activity of two medicinal plants *Piptadeniastrum africanum* and *Hunteria eburnea* on HFF cells. These two plants were selected in an ethnobotanical survey carried out in the Haut-Sassandra Region (Ivory Coast) for their frequency of use in cutaneous pathologies.

Materials and methods

Plant material

It consists of the stem barks of *Piptadeniastrum africanum* Hook. (Fabaceae) and *Hunteria eburnea* Pichon (Apocynaceae) (Figs. 1 and 2).



A: Leafy branch



B: Buttresses

Fig. 1: *Piptadeniastrum africanum* Hook (Fabaceae).



A: Leafy twig



B: Bark of the trunk

Fig. 2: *Hunteria eburnea* Pichon (Apocynaceae).

Cellular material

The cellular support consisted of human HFF (Human Foreskin Fibroblasts) cells. These are human cells that testify to the toxic activity of an extract. They have the particularity of forming a cell layer after several days of culture (96 hours - 4 days), it is said that they are confluent, they stop dividing by contact inhibition. When these cells are in culture for only 24 hours, they are in a state of mitosis (or dividing cells). These HFF cells are

cultured at 37°C under 5% CO₂ in D10 medium (Gibco, supplemented with fetal calf serum 10%, glutamine 1%, penicillin 50 U.ml⁻¹ and streptomycin 50 ug/uL).

Collection of barks of plants

The barks of *Piptadeniastrum africanum* and *Hunteria eburnea* were harvested in August 2015 in the Upper Sassandra Region (Ivory Coast). The samples of these two plants have been identified at

the National Floristic Center of the UFR Biosciences of Félix Houphouët Boigny University.

Preparation of plant extracts

After harvest, the barks were cleared of impurities, dried in the shade and in an air-conditioned laboratory for one week and then pulverized with an electric grinder. The fine powders obtained were stored in glass jars to prevent mold.

Total Aqueous Extract (ETA)

The dried barks of each plant were pulverized by a Moulinex type electric grinder. The powder obtained was used to prepare the various extracts. Thus ETA was obtained according to the following method (Zirihi et al., 2003, Bagré et al., 2006): One hundred grams of plant powder are extracted with distilled water by grinding in a mixer (Blender) three times three minutes at room temperature. The homogenate obtained is drained in a square of fabric and then filtered successively four times on hydrophilic cotton and then on Wattman paper (3 mm). The filtrate is evaporated at 50°C using a venticell® type oven. The dry evaporate obtained constitutes the aqueous total extract (ETA).

Ethanol extract 70% (EE70%)

The 70% ethanolic extract comes from the total aqueous extract, according to the following method (Zirihi et al., 2003): Ten grams (10 g) of ETA are dissolved in 100 ml of an ethanol solution 70% then homogenized in a Blender. After decantation in a separatory funnel, the supernatant is collected, filtered through cotton to remove any residue and dried in an oven (50°C). The powder obtained constitutes 70% ethanolic extract (EE70%).

Cytotoxicity test

The toxicity tests were carried out at LAPM (Laboratory Adaptation and Pathogenesis of Microorganisms) in Grenoble, France. To measure the toxicity of the ethanolic extract, the Human Foreskin Fibroblasts (HFF) cells were seeded in 96-

well plates (CellStar) at 3000 to 5000 cells per well in 100 µl of D10 medium. These cells are kept in culture for 24 hours (dividing cells) or 96 hours (confluent cells). Subsequently they were exposed for 24 hours at different concentrations (0-1000 Yg / ml) plant extract solubilized in PBS buffer. This was done in triplicate.

Viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The tetrazolium ring it contains is reduced in formazan by the mitochondrial succinate dehydrogenase of metabolically active cells, which precipitates and gives a violet color. The amount of precipitate formed is proportional to the number of living cells. In each well, MTT is added at a concentration of 500 µg / ml and incubated for 3 hours at 37°C. The formazan crystals are solubilized in dimethyl sulfoxide (DMSO) 10 mM. The measurement of the optical density at 544 nm was made using a Safir Spectrophotometer (Tecan); this measurement of absorbance will determine the relative amount of living and metabolically active cells (Mosman, 1983).

$$\text{Viability rate} = (\text{Abs}_{544 \text{ nm extract}} / \text{Abs}_{544 \text{ nm control}}) \times 100$$

Results

Cytotoxicity test of 70% ethanolic extract of *Hunteria eburnea*

The ethanolic extract of *Hunteria eburnea* did not have any toxic effect on the cells. On the other hand, when the cells are in division, as light decrease in the viability rate has been noted. At 1000 µg / mL, the percentage of viability was 101% for confluent cells and dividing cells (Fig. 3).

Cytotoxicity test of 70% ethanolic extract of *Piptadeniastrum africanum*

The analysis of the results showed that the percentage of viability of human cells increase progressively as a function of the concentrations of plant extracts. Statistical analysis of variance shows that there is a highly significant difference

($p < 0.001$) between confluent cells and dividing cells. At 1000 $\mu\text{g} / \text{mL}$, the percentage of viability is 193% for confluent cells and 215% for dividing

cells (Fig. 4). This shows that *Piptadeniastrum africanum* is not toxic, but accelerates cell division (mitogenic effect).

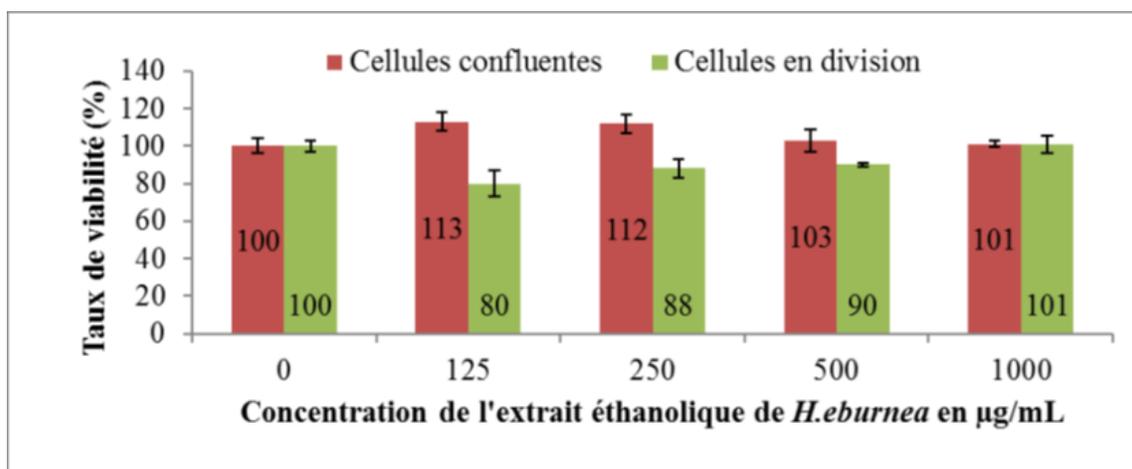


Fig. 3: Viability of HFF cells in the presence of ethanolic extract of *Hunteria eburnea*.

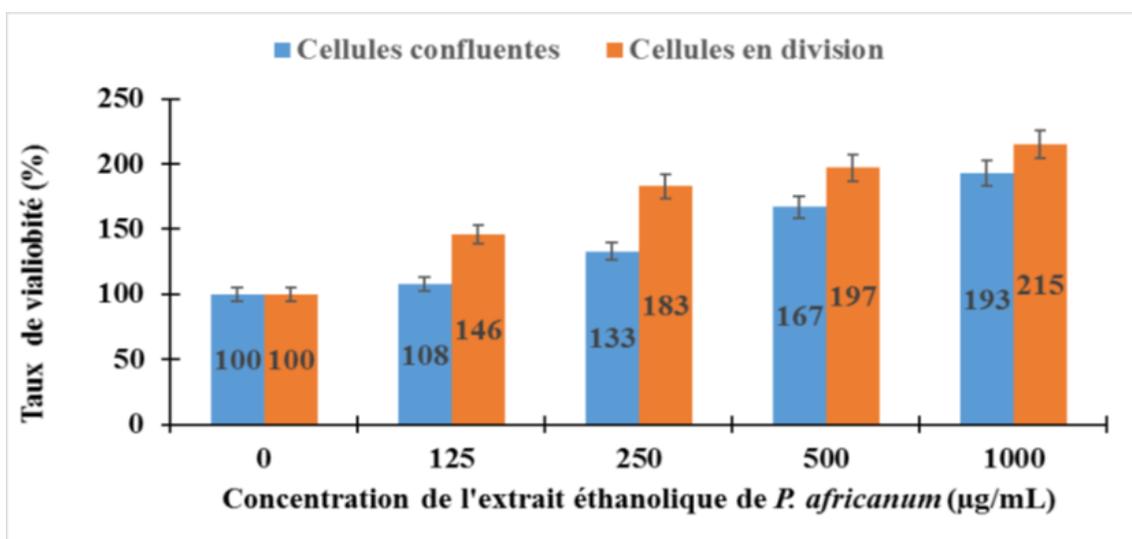


Fig. 4: Viability of HFF cells in the presence of ethanolic extract of *Piptadeniastrum africanum*

Discussion

The results of the cytotoxic tests carried out on the HFF cells have shown that the ethanolic extracts of *Piptadeniastrum africanum* and *Hunteria eburnea* are not toxic on the human HFF cells. Indeed, according to Bené (2017), when the viability of an extract is strictly greater than 30% the extract is declared not cytotoxic. But the ethanolic extract of *Piptadeniastrum africanum* showed a mitogenic effect on dividing HFF cells. This mitogenic effect is thought to be due to a chemical compound that

acts as a growth factor favoring the multiplication of HFF cells (Camara et al., 2016). Our results are consistent with those of Yapó et al. (2016) who also showed that the leaves of *Mallotus oppositifolius* (Geiseler) Müller. Arg. (Euphorbiaceae) used in traditional medium in the Great Bridges Region (Ivory Coast) had no cytotoxic effects on HFF cells. The results of the cytotoxicity of the 70% ethanolic extract of *Hunteria eburnea* compared to those of Bourobou et al. (2015) carried out on two plant species of the family Apocynaceae confirm the results of this study. Indeed, this author has shown

that *Alstonia boonei* and *Alstonia congensis* have very low toxicity on human embryonic cells (MRC-5). That said, the use of the bark of these two plants in traditional medicine poses no risk in terms of skin cell toxicity. This could justify the use of the stem bark of these plants in traditional environment.

Conclusion

The comparative study of the cytotoxic effect of the ethanolic extract of *Hunteria eburnea* and the aqueous and ethanolic extract of *Piptadeniastrum africanum* revealed that the extracts of both plants are not toxic to human HFF cells. These results may partly explain the safe use of extracts from these two plants in a traditional environment.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

The authors thank the Laboratory Adaptation and Pathogenesis of Microorganisms (LAPM) of Grenoble in France, where cytotoxicity studies were carried out and the traditional health practitioners of the Haut-Sassandra Region.

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How to cite this article:

Kanga, Y., Camara, D., Coulibaly, K., Bene, K., Zirihi, G. N., 2018. Comparative study of the cytotoxicity of 70% ethanolic extracts of *Pitadeniastrum africanum* Hook (Fabaceae) and *Hunteria eburnea* Pichon (Apocynaceae) on HFF Cells (Human Foreskin Fibroblasts). Int. J. Curr. Res. Biosci. Plant Biol. 5(4), 47-52. doi: <https://doi.org/10.20546/ijcrbp.2018.504.007>