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Comparative cytotoxicity of *Enantia polycarpa* (DC) Engl. and Diels (Annonaceae) and *Bersama abissynica* Fresen. (Melianthaceae) two Ivorian medicinal species commonly used

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ABSTRACT: In Ivory Coast, the barks of *Enantia polycarpa* and the leaves of *Bersama abissynica*, two medicinal species are usually used by populations to treat various diseases such as malaria, diarrheas and cutaneous diseases. To estimate the biological dangers connected to the frequent use of these medicinal plants, the current study was undertaken with objective to evaluate the cytotoxic activity of 70% ethanolic extract on Human Foreskin Fibroblast (HFF) cells in the *in vitro* culture. 70% ethanolic extract of plants were prepared and the colorimetric test of the MTT is used to evaluate the toxicity of these extracts. The results proved that the ethanolic extract of *Enantia polycarpa* showed the biggest yield (56,4%). The 70 % éthanlic extract of *Bersama abissynica* is not cytotoxic at 1000 µg / ml concentration, but mitogen. Our study has shown that the ethanolic extract of *Bersama abissynica* stimulates HFF cells in division growth (268%). While *Enantia polycarpa* seems cytotoxic on HFF cells at 1000 µg / ml concentration (36% of viability confluents cells and 55% of viability cells in division). Such results well support that the moderate use of these medicinal plants only represents a limited risk in terms of toxicity. However, follow-up studies must be envisaged in order to determine the chemical compounds responsible of the cytotoxic effect at *Enantia polycarpa* and those responsible for the mitogen effect at *Bersama abissynica*.

KEYWORDS: toxicity, HFF cells, *Enantia polycarpa*, *Bersama abissynica*, Ivory Coast.

1 INTRODUCTION

Enantia polycarpa and *Bersama abissynica* are shrubs of the Ivory Coast forests. The first one is a species distributed since Sierra Leone to Nigeria and the West of Cameroon. It is particularly plentiful in Ivory Coast and in Sierra Leone. It is a small tree of undergrowth of the evergreen dense forests which can reach 20 m of height and 40 cms in diameter. The outside of the bark is green in blackish color; while the inside is of bright yellow color, characteristic of its local name " African yellow wood " [1]**.** The second, *Bersama abissynica* is also a reaching shrub 6 m of height with an often tortuous trunk. The leaves, measure up to 60 cms in length. They contain from six to nine pairs of glabrescent sepals and the rachis is winged. The inflorescences, it racemes solitary persons or by small number, are axillaries near the extremity of twigs [2]. Both species have the big reputation to be variously used and intervenes in traditional treatment of various diseases in Africa [1, 3, 4, 5, 6, 7].

Previous ethnobotanic studies had showed that in Africa, the barks of *Enantia polycarpa* and the leaves of *Bersama abissynica*, are usually used by populations to treat diarrheas [7, 8]. But the misuse of these plants expose them to various accidents because, out of active ingredients, traditional drug contains other molecules of which some have toxic. All these factors make necessary the reassurance of the use of these two medicinal plants.

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The current study aims to evaluate the cytotoxic activity of 70% ethanolic extract of *Enantia polycarpa*'s barks and *Bersama abissynica*'s leaves on HFF (Human Foreskin Fibroblast) cells in the *in vitro* culture, to estimate the biological dangers connected to the frequent use of these medicinal plants.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The plant material was constituted by 70% ethanolic extracts of *Enantia polycarpa*'s barks and *Bersama abissynica*'s leaves.

2.2 BIOLOGICAL MATERIAL

The biological material was constituted by a HFF (Human Foreskin Fibroblast) cell line. It was furnished by the Laboratory Adaptation and Pathogenesis of the Microorganisms (LAPM) from Grenoble to France. They have the particularity to form a cellular mat after several days of culture (96 hours), we say they are confluents and they stop dividing by inhibition of contact. When these cells are in culture for only 24 hours, they are in a state of mitosis (or cells in division). These cells are used in several researches in laboratories to evaluate the cytotoxicity of therapeutic substances interest.

2.3 METHODS

2.3.1 COLLECTION OF THE PLANTS

The barks of *Enantia polycarpa* were collected in the South of Ivory Coast, in Yakassé-Méh (Region of Agnéby). While the leaves of *Bersama abissynica* were collected in the East, in Transua (Region of Gontougo). Their identification was made for the Centre National of Floristic (CNF). After the harvest, organs were cleared of the impurities, dried in the shade and outdoors from two to three weeks then reduced in fine powders.

2.3.2 PREPARATION OF PLANTS EXTRACTS

Obtained fine powders were extracted as follows: 100 g of powders were extracted in a liter of distilled water, by grinding in a Mixer of Moulinex type. The obtained homogenate was pressed at first then filtered then dried by evaporation in type Venticell's steam room in 50 °C. Then, powders obtained constitute the aqueous extracts (ETA). These extracts were weighed to estimate their yield.

10 g of aqueous extracts were dissolved in 200 ml of a hydroalcoholic solution (70% Ethanol + 30% distilled Water) for 10 to 15 minutes in a Mixer. After settling in a bulb to settle the hydroalcoholic phase and the deposit was separated [9, 10]. It floating was collected, filtered on some cotton and dried in the steam room (50 °C). The obtained powder constitutes the 70% ethanolic extract (EE70 %). These extracts obtained are weighed in the optics to estimate their yield.

2.3.3 CALCULATION OF THE YIELD (R)

The yield is the quantity of extract obtained from the vegetable powder. It is expressed in percentage and calculated according to the following formula: **R= m / M x 100**

R: yield on extraction; **m**: mass of the extract; **M**: mass of the fine powder.

2.3.4 *IN VITRO* CELL CULTURE

All the HFF cells were cultivated in D10 medium (Dulbecco Minimum Essential Medium, Gibco, added by fetal veal serum 10 %; 1 % glutamine; penicillin 50 U.ml-1 and Streptomycin 50 µg / µl). These cells are maintained in 37 °C in an atmosphere of 5 % CO_2 during 24 hours (cells in division) and 96 hours (confluents cells).

2.3.5 CYTOTOXICITY TEST

The cytotoxicity test consisted in measuring the viability of cells in culture when they are brought together with our plant extracts. The cytotoxicity of 70% éthanolic extracts of *Enantia polycarpa's* barks and the leaves of *Bersama abissynica* was determined by using the colorimetric test MTT [11, 12].

The HFF cells were sowed in 96-well microtitreplate. The plates were maintained at 37 °C in 5% CO_2 for 24 hours (cells in division) and 96 hours (confluents cells) at the rate of 3000 to 5000 cells/well in 100 µl of D10 medium. These cells were then exposed for 24 hours into 100 µl to different concentrations of 70% ethanolic extract of *Enantia polycarpa* and *Bersama abissynica* (125 µg / ml – 1000 µg / ml) solubilized in phosphate buffer solution (PBS). The viability was determined by addition into each well of the 96-well plate, 500 µg / ml of bromide of 3-(4, 5-diméthylthiazol-2-yl) 2, 5-diphenyl tetrazolium (MTT) and the plate was incubated for 3 hours in 37 °C. The ring of tetrazolium which it contained reduced there formazan by the succinate dehydrogenase mitochondrial of living cells metabolically active. The reduction released crystals of formazan which precipitated and gave a purple color. The quantity of the formed precipitate is proportional among living cells. The crystals of formazan were solubilized in DMSO (10 mM) then the absorbance was measured. The measure of the absorbance of each well at 544 nm in the spectrophotometer Safir (Tecan) allowed to determine the relative quantity of living and metabolically active cells. The results were expressed in percentage of viability compared with the witness of control without any plant extract according to the formula:

Percentage of viability = (Abs.544 nm Extract) / (Abs.544 nm Witness) X 100

The extracts cytotoxic effect was appreciated by the modality of natural substances action used by Zirihi [5]:

Not cytotoxic extract: 1000 µg / ml concentration leads to more than 50 % of cellular viability; Cytotoxic extract: 1000 µg / ml concentration leads to less than 50 % of cellular viability.

2.4 STATISTICAL ANALYSIS

Three separate experiments were conducted and the results obtained are expressed as mean ± ecart-type. The data from the experiments were compared with the control by anova one-way analysis of variance and Duncan's test. The means considered significant at $P < 0,05$.

3 RESULTS

3.1 EXTRACT YIELD

The extractions of the barks of *Enantia polycarpa* and the leaves of *Bersama abissynica* supplied extracts having variable tints. The values representing the average of the yield in percentage of the studied plants vary from 5 to 56,4% (Figure 1). These results show that the biggest and the lowest yield is observed with 70% ethanolic extract of *Enantia polycarpa* respectively at 56, 4% and 5%.

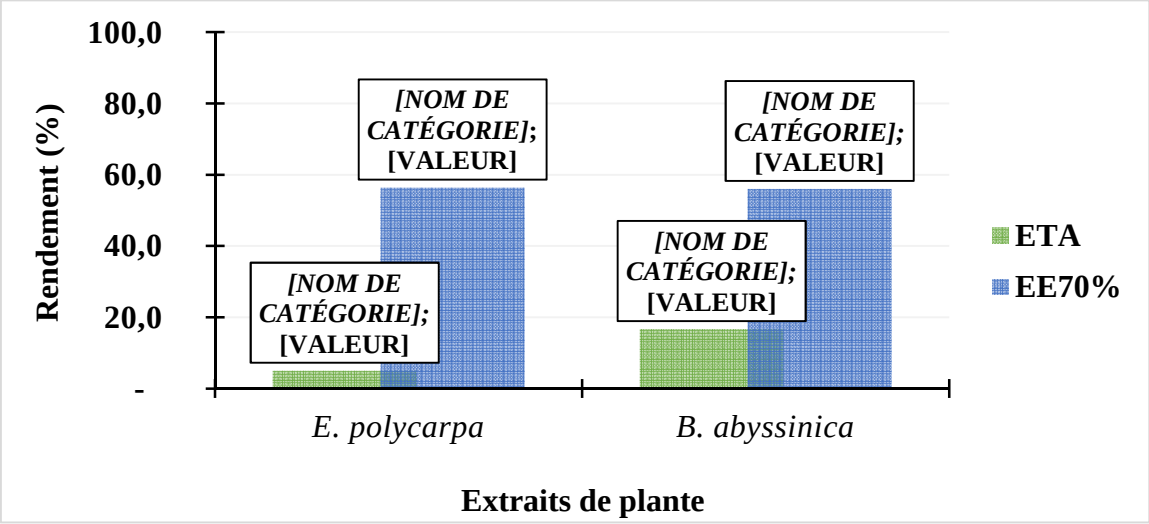

Figure 1: Yield of Enantia polycarpa and Bersama abissynica extracts

ETA: aqueous extract; EE70 %: 70 % ethanolic extract

3.2 CYTOTOXICITY OF THE EXTRACTS

The results of the cytotoxic activity of *Enantia polycarpa* and *Bersama abissynica* extracts are expressed in percentage of HFF cells alive compared with the positive control (only HFF cells without any plants extracts that is 100% of viability). Extracts are tested in four (4) different concentrations (125, 250, 500 and 1000 µg / ml) as presented on figures 2 and 3. These analysis showed a dose effect more or less remarkable. We observed a significant reduction in cellular viability when *Enantia polycarpa* 70 % ethanolic extract concentration increases. At 1000 µg / ml, the percentage of viability was 55% for cells in division and 36 % for confluents cells, what lets appear a significant toxicity ($p < 0,05$) in high concentration (figure 2). The 70 % ethanolic extract of this plant thus exercises a strong toxicity on confluents cells and a pretty medium cytotoxic activity on HFF cells in division.

The variance analysis with *Bersama abissynica* showed that there is a significantly different between confluents and division cells ($p< 0,05$). The figure 3 gives the percentage of viability of HFF cells cultivated in the presence of the concentrations from 125 to 1000 µg / ml for 70 % ethanolic extract of *Bersama abissynica*. The number of cells increases by a significant way compared with the positive control, as the concentration of 70% ethanolic extract of *Bersama abissynica* increases. At 1000 µg / ml, the percentage of viability of HFF cells was 268% for cells in division and 90% for confluents cells. The obtained histograms compared with that of the control, show that the cellular viability is superior to see widely superior to that of the control when the extract is used in 1000 µg / ml concentration for cells in division. That lets show through a significant increase ($p < 0,05$) at the level of the percentage of viability of HFF cells in division in the concentration of 1000 µg / ml. It emerges from the leaves of *Bersama abissynica* are not toxic on the tested cellular lineage but favor their proliferation. We can conclude that this extract is mitogen.

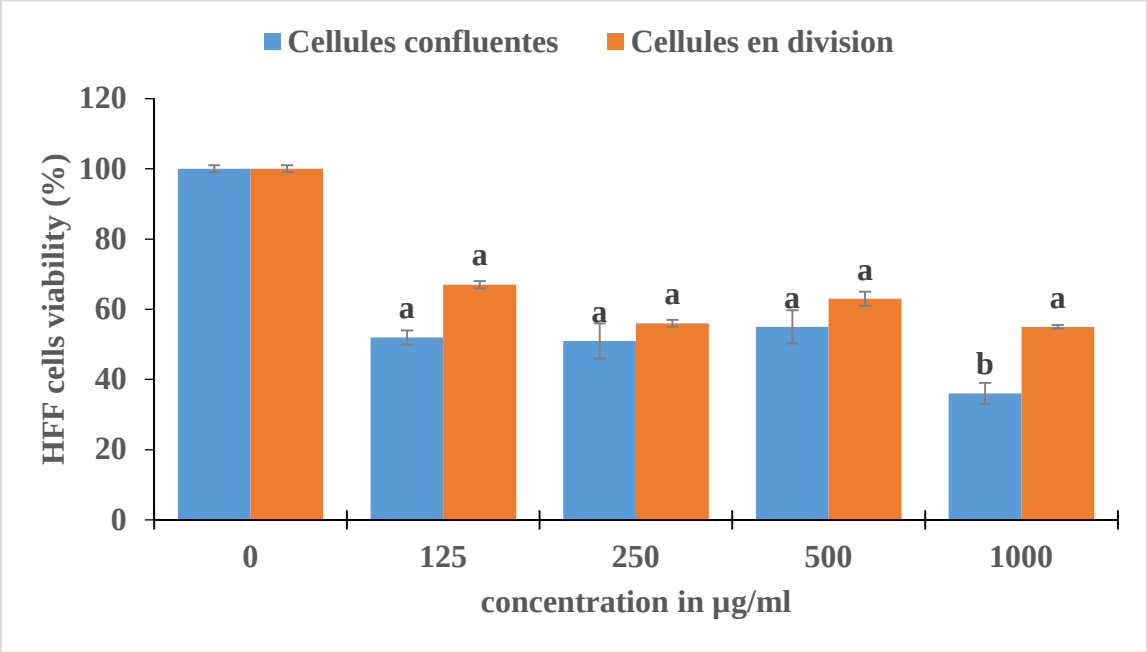

Figure 2: Percentage of HFF cells viability in the presence of 70% ethanolic extract of Enantia polycarpa. Data expressed as mean±ecart-type (n=3) Bars with same alphabets are not significantly different (p< 0,05). Control was 100%HFF.

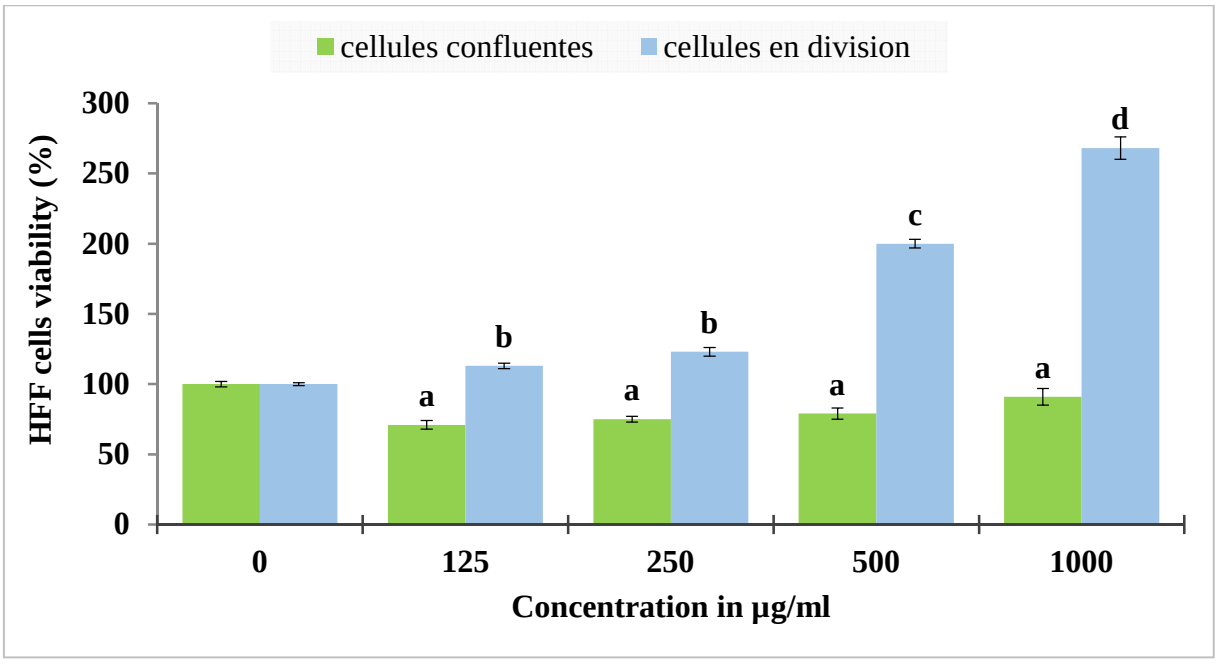

Figure 3: Percentage of HFF cells viability in the presence of the 70% ethanolic extract of Bersama abissynica. Data expressed as mean±ecart-type (n=3) Bars with same alphabets are not significantly different (p< 0,05). Control was 100%HFF.

4 DISCUSSION

Our study aimed to evaluate the cytotoxic potential of the barks of *Enantia polycarpa* and the leaves of *Bersama abissynica* two medicinal plants usually used in Ivorian traditional medicine.

The cytotoxic tests made on HFF cells showed that the leaves of *Bersama abissynica* are not cytotoxic but mitogen while those of *Enantia polycarpa* are cytotoxic on the tested cell line. The mitogen effect of *Bersama abissynica* could be due to a

chemical compound. These results allow to assert that the harmlessness of the leaves of *Bersama abissynica* could justify its frequent use in the traditional medicine in Ivory Coast. Our results corroborate those of Kuete *and al.* [6] who's found that all extracts of *Bersama abissynica* were non-toxic. But our results are opposite with those of Zirihi [5] which showed that *Bersama abissynica* is cytotoxic against Hella cell line. On the other hand, the barks of *Enantia polycarpa* would seem toxic in high concentration on HFF cells tested. The cytotoxicity would be due to a chemical compound which would inactivate the succinate dehydrogenase, an enzyme important for the mitochondrial breath, the blocking of which would drive to the cellular death. These results of the cytotoxicity confirm those of Coulerie [13] on the same plant family. This author showed that the extracts of the barks of *Meiogyne tiebaghiensis*, endemic Annonaceae of New Caledonia, are highly cytotoxic against the embryonic cells of human lung (MRC5). So suggesting certain one moderation of employment of this vegetable substance in the traditional treatments.

5 CONCLUSION

The current study allowed to highlight the cytotoxic potential of *Enantia polycarpa* and *Bersama abissynica* two medicinal plants usually used in traditional medicine. The results showed that the leaves of *Bersama abissynica* are mitogen and not cytotoxic while the barks of *Enantia polycarpa* are cytotoxic on the tested cellular lineage. It shows that the use of such plants in traditional medicine only represents a very limited risk in terms of toxicity. This study could be possibly completed by a phytochemical screening and a bio-guide of extracts to determine the chemical compounds responsible of the cytotoxic effect of *Enantia polycarpa* and those responsible of the mitogen effect of *Bersama abissynica*.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

[1] **Anosa G. N., Udegbunam R. I., Okoro J. O., Okoroafor O. N.,** *In vivo* antimalarial activities of *Enantia polycarpa* stem bark against *Plasmodium berghei berghei* in mice. Journal of Ethnopharmacology, vol. 153, pp. 531–534, **2014.**

[2] **Aké-Assi L.,** Flore de la Côte d'Ivoire: catalogue systématique, biogéographie et écologie. Conservatoire et Jardin Botanique, Génève, Switzerland, Boisiera, vol. **57,** 396 p, **2001.**

[3] **Ajali, U.,** Antibacterial activity of *Enantia polycarpa* bark. Fitoterapia, vol. 71, pp. 315–316, 2000.

[4] **Atindehou, K.K., Schmid C., Brun R., Koné,M.W.,Traoré, D.,** Antitrypanosomal and antiplasmodial activity of medicinal plants from Côte d'Ivoire. Journal of Ethnopharmacology, vol. 90, pp. 221–227, 2004.

[5] **Zirihi G. N.,** Eudes botanique, pharmacologique et phytochimique de quelques plantes médicinales anti-paludiques et/ou immunogènes utilisées chez les bété du département d'Issia, dans l'ouest de la Côte-d'Ivoire. Thèse de Doctorat d'Etat de botanique, spécialité ethnobotanique. Université de Cocody, Abidjan, Côte d'Ivoire. 199 p.**,** 2006.

[6] **Kuete V., Mbaveng A.T., Tsaffack M., Benga V.P., Etoa F.X., Nkengfack A.E., Meyer J.J.M., Lall N.,** Antitumor, antioxidant and antimicrobial activities of *Bersama engleriana* (Melianthaceae). Journal of Ethnopharmacology, vol. **115, pp.** 494–501, **2008**.

[7] **Zekeya N., Shahada F. and Chacha M.,** *In vitro* Antibacterial and Antifungal Activity of Tanzanian Bersama abyssinica. International Journal of Science and Research, vol. 3, n°7, pp. 1150-1153, 2014.

[8] **Ambé A. S.A., Ouattara D., Tiebre M.S., Vroh B.T.A., Zirihi G.N., N'guessan K. E.,** Diversité des plantes médicinales utilisées dans le traitement traditionnel de la diarrhée sur les marchés d'Abidjan (Côte d'Ivoire). Journal of Animal &Plant Sciences, vol. 26, n° 2, pp. 4081-4096, 2015.

[9] **Zirihi G. N., Kra A. M. et Guédé-Guina F.,** Évaluation de l'activité antifongique de *Microglossa pyrifolia* (Lamarck) O. Kunze (Asteraceae) <<PYMI>> sur la croissance *in vitro* de *Candida albicans*, *Revue de Médecine et Pharmacie. Africaine*, vol. 17, pp. 11-18, 2003.

[10] **Coulibaly K.,** Etudes botanique, pharmacologique et explorations phytochimiques des extraits de *Terminalia ivorensis* et *Terminalia superba*, deux espèces ligneuses commerciales, médicinales antimicrobiennes de la forêt de Mopri. Tiassalé (sud de la Côte d'Ivoire). Thèse de Doctorat de botanique, spécialité ethnobotanique. Université de Félix HOUPHOUET-BOIGNY, Abidjan, Côte d'Ivoire. 200 p., 2012.

[11] **Mossman T.,** Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. Journal of immunological Methods, vol. **65, pp.** 55-63, 1983.

[12] **Carmaux S.,** Caractérisation de la mort des cellules animales cultivées en bioréacteur. Thèse de Doctorat d'Etat en Pharmacie. Université Henri Poincaré - Nancy 1, 105 p., 2008.

[13] **Coulerie P.,** Étude phytochimique et pharmacologique de plantes de Nouvelle-Calédonie à potentialités anti-dengue. Thèse de Doctorat de chimie des substances naturelles. Université de la Nouvelle-Calédonie : École Doctorale du Pacifique, ED 469. 296 p., **2012.**