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Optimizing Mitogenic Potential of HFF Cells by Ethanol Extracts of Leaves of *Bersama abyssinica* Fresen. (Melianthaceae) and *Harrisonia abyssinica* Oliv. (Simaroubaceae)

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Abstract : ***The aim of our study was to optimize the mitogenic potential of HFF cells, in order to help the body defends itself more effectively against pathogens during skin infection and fight against skin aging. Therefore using MTT colorimetric assay, with 70% ethanol extracts of the leaves of Bersama abyssinica and Harrisonia abyssinica to examine the plants. The results showed that the 70% ethanolic plant extracts are not cytotoxic but have a mitogenic potential. EE70% of Bersama abyssinica contain a growth factor to promote cell proliferation and the mitogenic effect of H. abyssinica is due to mitochondrial succinate dehydrogenase. The mitogenic potential of HFF cells has been optimized. This study opens a path into the exploration as to the research on natural FGF growth factors and from Bersama abyssinica.***

Keywords: mitogenic potential, HFF cells, ethanol extracts, *Bersama abyssinica, Harrisonia abyssinica*, growth factor.

1. Introduction

Fibroblasts are involved in the anti-infectious and anti-viral defense through the secretion of chemotactic factors and interferon β. When skin infection occurs as a result of a pathogen, an increase in fibroblasts would allow the skin to defend itself more effectively against the pathogen. Growth factors are polypeptide molecules producedin man and in many animal species. They perform multiple physiological effects, including growth factor, differentiation and cell metabolism. There are several families of growth factors, depending on the physiological actionor cell. They are mainly IGF (Insulin-like growth factors), EGF (epidermal growth factors) and β-TGF (Transforming growth factors β) [1]. The better known growth factors and the most studied belong to the family of epidermal growth factor (EGF) [2].

In view of numerous assaults and multiple antibiotics resistance pathogens, it is more than necessary to find new bioactive molecules capable of stimulating fibroblasts for a more effective fight against dermal pathogens and to fight against aging skin.

Thus, through an ethnobotanical investigation conducted in the Region of Transua (District of Zanzan, Ivory Coast) we have identified several medicinal plants that are well used by traditional healers, for the treatment of skin diseases and scalp, especially the superficial mycoses. Among these plants, *Bersama abyssinica* Fresen. (Melianthaceae) and *Harrisania abyssinica* Oliv. (Simaroubaceae) are highly recommended.

Bersama abyssinica Fresen. is a shrub up to 6 m in height, often twisted trunk. Theleaves, imparipinnately compound, measuring up to 60 cm long, contain from six tonine pairs of glabrescent leaflets; the spine is winged [3]. It is presented as aplant having multiple therapeutic activity. Indeed, several previous studies have shown that *Bersama abyssinica* is used to treat various diseases: infections, cancer, spasms, male infertility, diabetes [4], diarrhea, cholera, intestinal worms, amebiasis and dysentery, syphilis, gonorrhea [5], malaria and general fatigue [6] [7].

Harrisonia abyssinica Oliv. is a shrub or small tree, evergreen, strongly branched, sometimes climbing, up to 6 (-13) m tall; barrel and large branches trimmed spines up to 2 cm long and conical corky growths; Pale brown bark to Grey; long and supple branches [8]. Through this study we want to optimize the mitogenic potential of HFF cells using the colorimetric MTT test by ethanol extracts.

2. Materials and Methods

2.1 Plant Material

The plant material is composed of leaves of *Bersama abyssinica* Fresen. and *Harrisonia abyssinica* Oliv. The leaves were harvested in the Region of Transua (District of Zanzan, Ivory Coast). They were identified at the National Floristic Centre (NFC).

2.2 HFF Cells

The biological material used consisted of a cell line HFF (Human Foreskin Fibroblast). It was provided by the Laboratory of Adaptation and Pathogenesis Microorganisms (LAPM) of Grenoble in France. They have the peculiarity of forming acell carpetafter several days of culture (96 hours), then we say that they are confluent, they stop dividing by contact inhibition. When these cells are cultured for only 24 hours, they are in a state of mitosis (or dividing cells). These cells are used to assess the cytotoxicity of several types of molecules.

2.3 Preparation of 70% Ethanolic extracts [9]

The leaves of *B. abyssinica* and *H. abyssinica* collected were rinsed with water and dried under shade. These dried plant parts were then reduced to a fine powder with an electric grinder-MAG IKA RTC. Greenish powder was obtained. For each plant powder, hundred grams (100 g) of powdered are homogenized in 1 liter of distilled water in a blender (mixer) at room temperature. The greenish homogenate obtained was first squeezed into a square of clean white cloth and then filtered successively on cotton wool, and finally on Whatman paper (3 mm). The extraction solvent was removed with the aid of an oven set at 50°C. The dry evaporate was recovered in powder form that is the total aqueous extract.

Ethanol / water separation

Ten grams (10 g) of the total aqueous extract of each plant are dissolved in 200 ml of 70% ethanol solution and then homogenized in a blender. After settling ina funnel, we obtained a liquid phase with a solid residue precipitate as insoluble in the alcohol-water mixture 70-30. The supernatant was collected, filtered on cotton to get rid of any residual and dried in an oven (50°C). The powder obtained was the 70% ethanolic extract (EE70%).

2.4 Optimization of mitogenic potential of HFF cells

This method is determined using the colorimetric MTT assay [10] [11] [12]. It consisted of measuring the viability of cells in culture when they are placed in the presence ofethanolic extracts (EE70%) of *B. abyssinica* and *H. abyssinica*.

All HFF cells were grown in D10 medium (Dulbecco's Minimum Essential Medium, Gibco, added with 10% fetal bovine serum; 1% glutamine; Penicillin 50U.ml^{-1} and streptomycin 50 µg/µl). These cells are maintained at 37°C in an atmosphere of 5% CO_2 for 24 hours (cell division) and/or 96 hours (confluent cells).

To measure the activity of the EE70%, the HFF cells (Human Foreskin Fibroblasts) were Seeded in 96-well plates (CellStar) at 3000 to 5000 cells per well in 100 µl of D10 media. These cells are maintained in culture for 24 hours (dividing cells) or 96 hours (confluent cells). Subsequently they were exposed for 24 hours at different concentrations (125, 250, 500 and 1000 µg/ml) of extract of plant solubilized in PBS buffer. Wells containing no cells were used as control.

This was done in triplicate.

Mitochondria of viable cells contain a reductase enzyme, succinate dehydrogenase (SDH), for reducing a tetrazolium salt, 3-[4,5- (dimethylthiazol-2yl)] - 2,5-diphenyltetrazolium bromide or MTT (yellow color) to formazan (purple) that precipitated (Figure 1). The intensity of the purple color is proportional to the number of living cells and their metabolic activities. In each well, the MTT was added at aconcentration of 500 µg/ml and incubated for 3h at 37°C. Formazan crystals are solubilized in dimethylsulfoxide (DMSO) 10 mM.

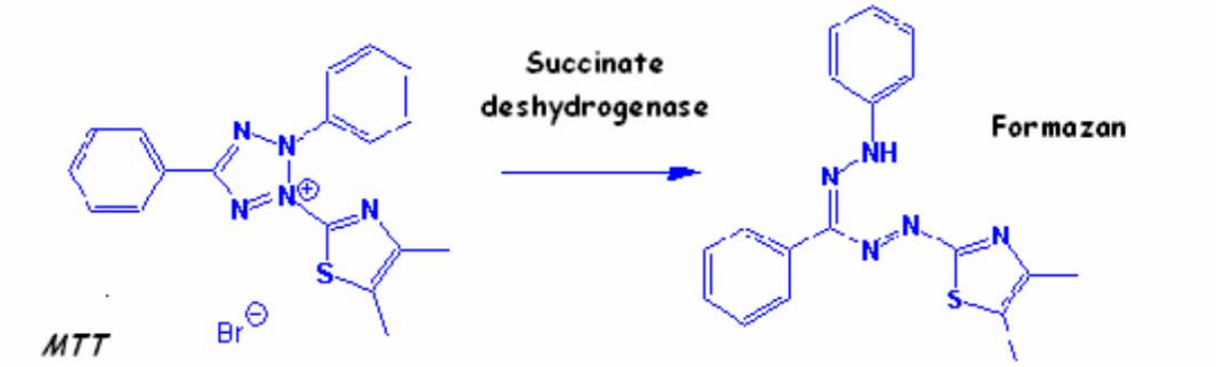

Figure 1: Principle of MTT colorimetric method [12]

The optical density was measured at 544 nm using a spectrophotometer Safir (Tecan); this measurement of absorbance was used to determine the relative amount of living cells and metabolically active. The results were expressed as percentage of viability compared to control where there is no plant extract, according to the following formula:

$$\text{Cells Viability (\%)} = \frac{\text{Abs544 nm extract x 100}}{\text{Abs544 nm Control}}$$

2.5 The ethanol extract action model

- Cells Viability >100%, the extract has a mitogenic potential,
- 50 < Cells Viability ≤ 100%, the extract is not toxic,
- Cells viability ≤ 50%, the extract is toxic.

3. Results

3.1 Ethanolic extract of *B. abyssinica*

Figure 2 shows the percentage of viability of HFF cells cultured in the presence of 70% ethanol extract of *B. abyssinica* of concentrations 125-1000 µg/ml.

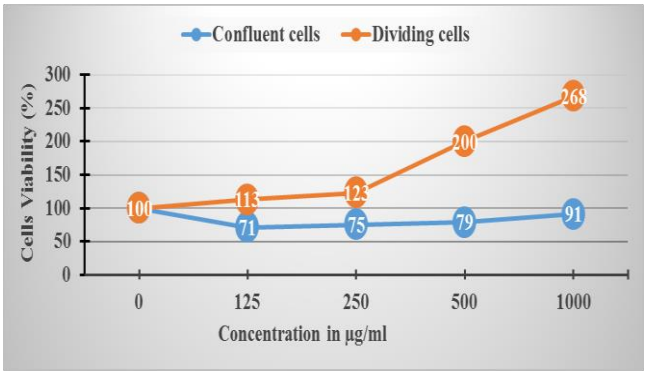

Figure 2: HFF cells viability percentage in the presence of 70% ethanol extract of *Bersama abyssinica*

3.2 Ethanol extract of *H. abyssinica*

Figure 3 shows the viability percentage of HFF cells cultured in the presence of 70% ethanol extract of *H. abyssinica* at concentrations of 125-1000 µg/ml.

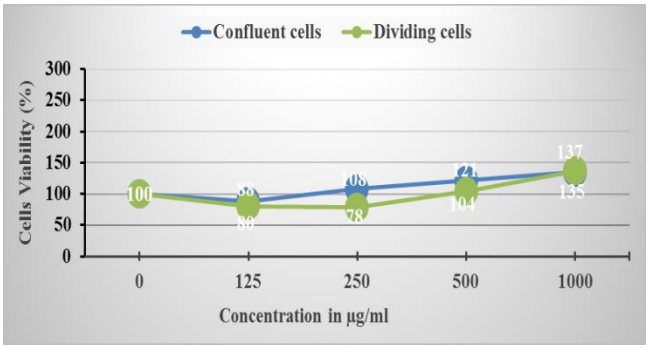

Figure 3: HFF cells viability percentage in the presence of 70% ethanol extract of *Harrisonia abyssinica*

4. Discussion

4.1 Mitogenic potential of extracts

The results of the mitogenic activity of 70% ethanol extracts of *Bersama abyssinica* and *Harrisonia abyssinica* are expressed in percentage of living HFF cells compared to the control (100% viability). The analysis thereof showed a dose effect more or less remarkable.

Bersama abyssinica

The number of cells increases significantly compared to the control, as the concentration of the 70% ethanol extract of *Bersama abyssinica* increases. It is noted that in the case of dividing cells, the viability rate increases with the concentration of plant extract; there is an effect of the ethanol extract on cell proliferation. On the other hand, where the cells no longer divide (cells confluent), no plant extract effect was observed. If this viability increases was due to activation of succinate dehydrogenase used to perform the MTT assay, we would have the same percentage both in confluent cells and dividing cells.

Probably there could be in the ethanolic extract of the leaves of *B. abyssinica* a growth factor which increases cell proliferation with the concentrations studied.

The phytochemical screening of the leaves, stem bark and root bark of *B. abyssinica* showed the presence of 24 chemical compounds in the leaves, one of which Gibberellic acid (diterpene pentacyclic in nature) [13], which allows growth and elongation of cells [14]. This would justify the increased proliferation of cells division. The EE70% *B. abyssinica* is mitogenic.

Harrisonia abyssinica

The slight weak viability rate at low concentrations in plant extracts (125 µg/ml and 250 µg/ml) could be due to the presence in the extract of a compound which would have a lower toxic effect. At high concentrations, there would be a retro-inhibiting compound; orthe harmful effect may be masked by the stimulating effect of other molecules. When the cells are not in division (confluent cells), the effect of the extract on the viability is lower compared to dividing cells.

At the highest concentration of 1000µg/ml, an increased viability rate was observed in the two conditions: dividing cells and non-dividing cells. In the MTT assay, where a viability rate is high it suggests that either the succinate dehydrogenase (SDH) is activated or a growth factor has boosted the mitosis. In our case specifically, it was assumed that it was the SDH that was activated; in fact, when the cells stop dividing this increase in viability was observed. SDH is an enzyme which is involved in the mitochondrial respiration in a cell, when the SDH is very active, this implies that the cell is metabolically very active. It shows that the leaves of *H. abyssinica* are nontoxic to the cells tested but promotes their proliferation. This extract is mitogenic, by the activation of the mitochondrial succinate dehydrogenase. It therefore does not contain growth factor. Several phytochemical studies [15] [16] revealed the presence of chemical compounds including terpenes which could explain its action on the SDH.

4.2 Comparative study of the mitogenic potential

At a concentration of 1000 µg/ml, viability rate of 268% and 137% was recorded for dividing cells in the presence of ethanol extracts of *B. abyssinica* and *H. abyssinica* respectively. The mitogenic potential of *B. abyssinica* is higher than that of *H. abyssinica*. Also, the mitogenic potential of *Harrisonia abyssinica* is due to the action of mitochondrial succinate dehydrogenase while that of *Bersama abyssinica* is due to growth factor.

5. Conclusion

This study has enable us highlight the mitogenic potential of leaves of *Bersama abyssinica* and *Harrisonia abyssinica,* two commonly used medicinal plants in traditional medicine. The results showed that the leaves of the two species of Plants are mitogenic and therefore not cytotoxic on the cell tested. This shows that the use of such plants in traditional medicine does not have any risk in terms of toxicity. At a concentration of 1000µg/ml, viability rate of 268% and 137% for dividing cells in the presence of ethanol extracts of *B. abyssinica* and *H. abyssinica* respectively. The mitogenic potential of *B. abyssinica* is higher than *H. abyssinica*. This mitogenic effect could be due to a chemical compound said to be a growth factor in the 70% ethanol extract of *B. abyssinica*. The mitogenic potential of HFF cells was therefore optimized.

Further study could help to determine and then concentrate the phytochemicals responsible for the mitogenic effect of these plants. This study is an eye opener in the research on natural FGF growth factors (fibroblast growth factors) and from *Bersama abyssinica*.

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