Phytochemical screening and evaluation of the cytotoxicity of fruits of *Solanum torvum* Swartz (Solanaceae) on HFF cells (Human Foreskin Fibroblasts).

Abstract:

Objective: The aim of this work is to evaluate the cytotoxic activity of the 70% ethanolic extract of the fruits of *Solanum torvum* Swartz (Solanaceae) on HFF (Human Foreskin Fibroblast) cells in *in vitro* culture and to determine its phytochemical composition.

Study plan: An ethnobotanical survey was conducted in August 2015 in the Haut-Sassandra Region (Ivory Coast), on medicinal plants with multiple uses, and at the end of this survey the fruits of *Solanum torvum* have been selected then harvested in the Sub-prefecture of Bédiala (Ivory Coast). Then after drying, the ethanolic extract of the fruits was prepared and sent to France in February 2016 at the Laboratory Adaptation and Pathogenesis of Microorganisms (LAPM) of Grenoble for cytotoxic tests. Phytochemical screening was carried out at the Faculty of Biological Sciences of Félix Houphouët Boigny University (Côte d'Ivoire).

Methods: From an ethnobotanical survey, the fruits of *Solanum torvum* were harvested. The 70% ethanolic extract prepared from the fruits of this plant was tested *in vitro* on divisional HFF cells after phytochemical screening.

Results: The result revealed that this extract has cytotoxic activity on the tested HFF cells. At 800 µg/mL, the survival rate of HFF cells increased from 100% to 4% of living cells. Phytochemical screening revealed the presence of compounds such as alkaloids, tannins, polyphenols, saponins and flavonoids.

Conclusion: This extract is cytotoxic on HFF cells. It is therefore necessary to continue studies on toxicity and to be cautious in the use of *solanum torvum* fruits in traditional medicine.

Key words: HFF cell, Cytotoxic, Extracts, ethnobotanical survey, *Solanum torvum*, Phytochemicals

1. Introduction

Solanum torvum is native to Central and South America, from Mexico to Brazil and Peru, it has spread widely in the Caribbean [1]. *Solanum torvum* belongs to the family Solanaceae. In West and Central Africa, it is grown locally in gardens for cooking [2]. Fruits are widely used

in the treatment of shingles in Cameroon [3]. They are also used as a vegetable and considered an essential ingredient in the diet of the South Indian population [1]. A fruit decoction is used in Ghana for the treatment of cough, liver disease and spleen [4]. Antioxidant rich ripe fruits are used in the preparation of tonic and hemopoietic agents and also for the treatment of pain throughout the body [5, 6]. Previous work has revealed that the fruits of *Solanum torvum* are used in the treatment of various conditions: microbial infections [7], hypertention [8], kidney disease [9] diabetes [10]. Phytochemical studies of Pérez-Amador *et al*. [11] have demonstrated the presence of glycoalkaloids in the fruit of *Solanum torvum*. However in 2013 an Egyptian experiment in arthritic rats showed that very low doses of glycoalkaloids in some Solanaceae are toxic [12,13] . In view of the interest of *Solanum torvum* fruits in the traditional environment and to help people gain a real benefit from the use of *Solanum torvum* fruits, we have undertaken to evaluate scientifically the cytotoxicity and phytochemical screening of the fruits of *Solanum torvum*. An ethnobotanical survey was conducted in August 2015 in the Haut-Sassandra Region (Ivory Coast). This survey showed that all parts of *Solanum torvum* are intensively used, especially leaves and fruits in the treatment of dermatosis and anemia. The aim of this work is to perform a phytochemical screening and a cytotoxicity study of *Solanum torvum* fruits on Human Foreskin Fibroblasts (HFF) cells.

2. Material and methods

2.1 Plant material

The fruits of *Solanum torvum* (Fig 1) were harvested in Biadiala in the Department of Daloa (Ivory Coast).

Fig 1: Leafy and fruiting twig of *Solanum torvum* (Solanaceae)

2.3 Cellular material

The cellular support consists of human HFF (Human Foreskin Fibroblasts) cells. These are human cells that testify to the toxic activity of an extract. When these cells are in culture for only 24 hours, they are in a state of mitosis (or dividing cells).

2.4 Preparation of plant extracts

After harvest, the fruits were freed of impurities, dried in the shade for a week and then pulverized with an electric grinder. The fine powders obtained were stored in glass jars to prevent mold.

2.5 Preparation of total aqueous extract (TAE)

The preparation of this extracts was performed using the method described by Zirihi *et al* [14] which involves macerating 100 g of plant powder of species in 1L of sterile distilled water using a Blinder type 7 SEVEN STAR. The homogenate was filtered over hydrophilic cotton and then on Whatman filter paper (n° 3). The aqueous filtrate thus obtained is evaporated in an oven of Med Center Venticell type at 50°C to obtain powders that constitute the total aqueous extract (TAE).

2.6 Preparation of 70 % ethanolic extract (70 % FE)

The extract was obtained by dissolving 5 g of TAE in 100 mL of ethanol 70% (v ;v) solution and then homogenized. After decantation and filtration of the alcoholic fraction on hydrophilic cotton and on Whatman filter paper (n° 3), the filtrate collected is evaporated in an oven at 50 °C. The powder obtained constitutes 70 % ethanolic extract (70 % EE) [15].

2.7 Yield calculation

The yield is the quantity of extract obtained from the plant powder. It is expressed as a percentage. In practice, it has been determined by the ratio of weight of the solids content after evaporation on the weight of the dry plant material powder used for the extraction, multiplied by 100. This results is indicated by the following formula:

Yd = (m x 100)/M

Yd : Extraction yield in percentage

m : mass in grams of the dry extract

M : mass in grams of the drug powder.

2.8 Phytochimical screening

The identification of different chemical compounds in 70 % ethanolic was done by tubes characterization reactions. This method consists of detecting the different families of chemical compounds that may exist in plant extracts on the basis of characteristic colorations or precipitation reactions [16].

- **Alkaloids characterization**

The characterization of alkaloids was made using Bouchard (iodo-iodide) and Dragendorff (tetraiodo potassium bismuthate) reagent. 6 mL of 70 % ethanolic extract solution was evaporated to dryness. The residue was taken up in 6 mL of alcohol at 60 °C. The filtrate thus obtained was divided into two test tubes. In the first tube, two drops Dragendorff reagent were added. The presence of alkaloids was characterized by observing orange-coloured precipitates. In the second tube, two drops of Bouchard reagents was added. The appearance of a reddish-brown color indicates the presence of alkaloids. A control test was made with quinine.

- **Characterization of polyphenols**

The polyphenols colorimetry forms colored precipitates with a solution of ferric chloride ($FeCl_3$). Thus, one drop of alcoholic solution of 2% ferric chloride and 2 mL of solution of 70 % ethanolic extract was added. The formation of blue-black or green colouring more or less dark confirms to the presence of polyphenols. A control test was performed with a solution of phenol.

- **Characterization of flavonoids**

Flavonoids have been characterized by the reaction to cyanidin. Thus, 2 mL of 70 % ethanolic extract were evaporated to the dry sand bath. The residue thus obtained was mixed with 5 mL dilute hydrochloric acid 2 times. The mixture was collected in a test tube, in which pink-orange or violet colouration will appear. The addition of 3 drops of isoamyl alcohol intensifies this coloring and confirms presence of flavonoids. An alcoholic solution of quercetin was used as a control.

- **Tannins characterization**

The Stiasny reagent (Formalin 30%, concentrated HCl 1/0.5) helped to distinguish the catechin tannins (by precipitation) of gallic tannins (by saturation). Tannins cathéchiques: to 10 mg of 70 % ethanolic extract, were added 10 mL of Stiasny reagent. The mixture was heated in a water bath at 80 °C for 30 minutes. After cooling in a stream of water, observation of precipitate in the form of clear-brown flakes characterizes catechin tannins. An alcoholic solution of catechin was used as a control. Gallic tannins: For this test, the filtrate obtained from the reaction of catechol tannins characterization was saturated with sodium acetate. To this mixture was added a few drops of a dilute aqueous solution of $FeCl_3$ at 1% (approximately 1 mL). The appearance of an intense blue-black coloration indicates the presence of gallic tannins not precipitated by Stiasny reagent. An alcoholic solution of gallic acid was used as a control.

- **Terpenes characterization**

Sterols and terpenes characterizationwas made by the Liebermann-Burchard reaction. To 0.2 g of 70 % ethanolic extract, were added 5 mL of ethyl ether, then the mixture was macerated for 30 minutes. The solution obtained after the maceration was filtered and then evaporated to dryness. The residue was then dissolved in 0.5 mL of acetic anhydride. Using a pipette, 2 mL of concentrated sulfuric acid were laid down at the bottom of the test tube without stirring. The appearance of brownish red or purple ring reflects the two liquid contact zone. The upper liquid turns green or purple to green or purple indicating the presence of sterols and terpenes. A control test was performed with progesterone.

- **Coumarins characterization**

For the detection of coumarins, 2 mg of 70 % ethanolic extract was added to 2 mL of warm water and then homogenized. The homogenate thus obtained was divided into two test tubes. Thereafter, 0.5 mL of diluted ammonia at 25% was added to the contents of one of the tubes. After observation under UV 365 nm, the presence of fluorescence in the tube where ammoniac was added indicates the presence of coumarins.

- **Saponins characterization**

For the detection of saponins, 10 mL of 70 % ethanolic extract was introduced in the test tubes. Each tube was strongly stirred in a vertical position for 15 seconds, and then left to set 15 minutes. The height of persistent foam is higher than 1 cm, testifying the presence of saponins.

2.9 Cytotoxicity test

To measure the toxicity of the ethanolic extract, the Human Foreskin Fibroblasts (HFF) cells were inoculated in 96-well plates (CellStar) at the rate of 3000 to 5000 cells per well in 100 µl of D10 medium. These cells are kept in culture for 24 hours (dividing cells). Subsequently they were exposed for 24 hours at different concentrations (125-800 µg /mL) in plant extract solubilized in PBS buffer. This was done in triplicate, also for control control without plant extract. 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 purple 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 10 mM dimethylsulfoxide (DMSO). The measurement of the optical density at 544 nm was made using a Safir spectrophotometer (Tecan); this measurement of absorbance will make it possible to determine the relative quantity of living and metabolically active cells [17]. The results were expressed as a percentage of viability compared to control without plant extract. Viability rate = (Abs544 nm extract / Abs544 nm control) × 100

3. RESULTS

3.1 Yield of different extracts of fruits of solanum torvum

We obtained from 200 g of powder, 20 g of total aqueous extract a yield of 10 % and from 5 g of total aqueous extract, we got 2 g of 70 % ethanolic extract or a yield of 40 %.

3.2 Phytochimical sorting

The phytochemical sorting performed with the extracts of fruits of *Solanum torvum* allowed to detect the presence of various chemical groups (Table 1). They are the polyphenols, tannins, flavonoids, saponins, and alkaloids in 70% ethanol extract.

Table 1 **:** Chemical compounds in the fruits of *Solanum torvum*

Species	Extract	Chimical compounds							
		Sap	Flav	Terp/ster	Tanins		Coum	Alc	Poly
					Gall	Cathé			
Solanum torvum	EE 70 %	+	+++	-	++	++	-	+	+

177 - :negative reaction ; + : positive reaction

178 **EE 70 % :** 70 % ethanolic extract

179 **Sap:** saponins**; Flav:** flavonoids**; Terp / Ster:** terpenes / sterols**; Gall:** gallic**;**

180 **Cathé:** cathechic**; Coum:** coumarines**; Alc:** alkaloids**; Poly:** polyphenol

181 **3.3 Cytotoxicity test**

182 Figure 2 gives the percentage of viability of the HFF cells cultured in the presence of
183 concentrations of 100 to 800 µg / mL for the 70% ethanolic extract of the fruits of *Solanum*
184 *torvum* compared to the control without plant extract. The number of cells decreases
185 considerably as the concentration of the 70% ethanol extract of the fruits of *Solanum torvum*
186 increases. At 800 µg / mL the number of dividing cells is 4%. The averages with the same
187 superscript superscript letters are not different at 5% according to the turkey test.

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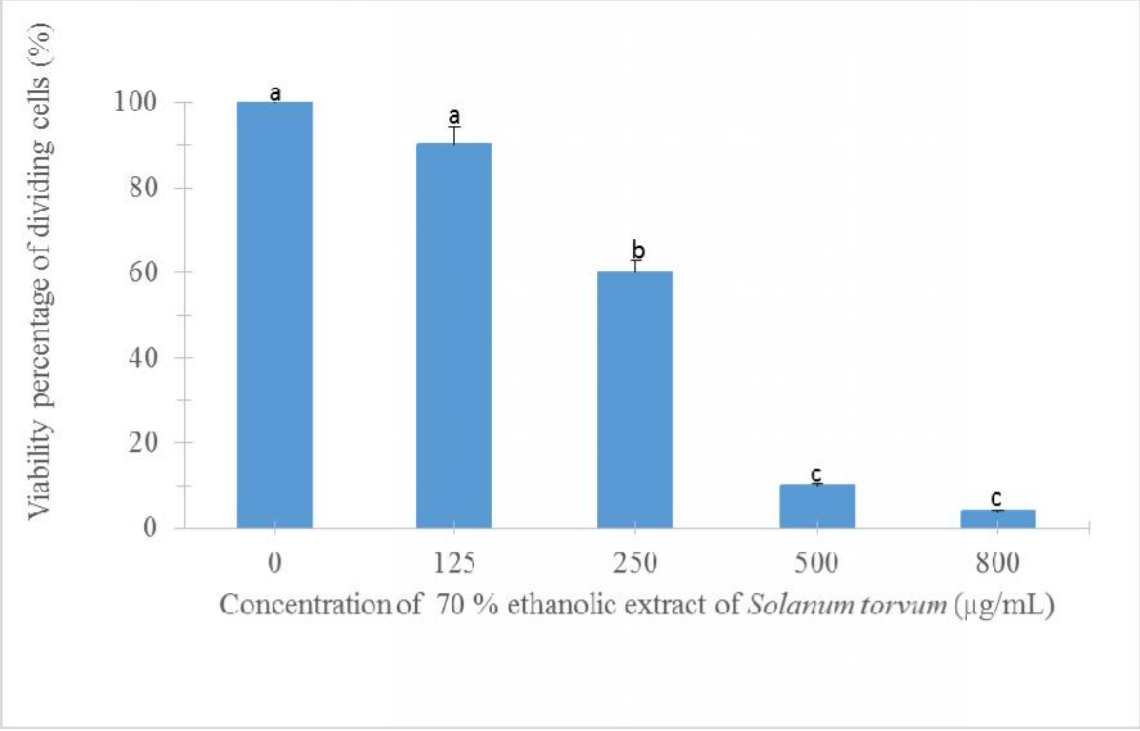

189
190

191 Figure 2: Cytotoxicity test of 70% ethanolic extract of fruits of *Solanum torvum* on HFF
192 dividing cells

193

4. Discussion

Medicinal plants play a central role in traditional medicine. Ethnobotanical surveys conducted among traditional health practitioners have made it possible to harvest the fruits of *Solanum torvum*, which are used in the treatment of anemia, bacterial infections and several other diseases [18]. The recipes obtained from the fruits of this plant are monospecific, which is an advantage for the patients, because the associations of wrongly mixed plants, are sometimes dangerous for the health [19]. Phytochemical screening revealed the presence of chemical compounds such as alkaloids, tannins, polyphenols, saponins and flavonoids. The presence of these chemical compounds could justify the multiple activities of the fruits of this plant [20]. The cytotoxic essay performed on HFF cells showed a gradual decrease in purple staining in each well. Since the dye penetrates only in living cells, the coloring is weaker as the plant extract is cytotoxic by inhibition of HFF cells [21]. The sharp decrease in the relative amount of the dividing HFF cells could be explained by the fact that the HFF cells would be killed by the 70% ethanol extract of *Solanum torvum.* Indeed, extracts resulting in a cell death greater than 30% could be considered as cytotoxic [22]. This extract could therefore contain a chemical compound that inactivates succinate dehydrogenase, an enzyme important for mitochondrial respiration, the blockage of which would lead to cell death. This result demonstrates the cytotoxic effect of 70% ethanolic extract of *Solanum torvum*, a Solanaceae from the Ivorian pharmacopoeia on the cell line tested. Which means that the external use of the fruits of this plant would probably be dangerous for human health. This toxicity of fruit could also be explained by the presence of certain groups of chemical compounds such as glycoalcaloides which are toxic in some Solanaceae [13]. Our results on *in vitro* toxicity corroborate those [23] who worked on the same family of plants. Indeed according to the work of Busser and Baies [23] the fruits of *Solanum nigrum* L. (Solanaceae) another Solanaceae rich in glucoalcaloids and saponins are toxic in internal and external uses on an organism.

5. Conclusion

Solanum torvum is an important medicinal plant of the family of Solanaceae. From the evaluation of the biological activity, it appears that 70% ethanolic extract of the fruits of *Solanum torvum* is cytotoxic on the HFF cells. We therefore plan to continue the acute and subacute toxicity studies to compare the results with those obtained *in vitro* to confirm or refute the toxicity of the fruits in this study.

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