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

Summary:

Objective: to establish the scientific basis for the use of the fruits of *Solanum torvum* Swartz (Solanaceae), the fruit of a medicinal plant used in traditional medicine against several diseases. Methodology and Results: The 70% ethanolic extract from this plant was tested in vitro on divisional HFF cells. The result revealed that this extract has cytotoxic activity on the tested HFF cells. At 800 µg / mL, the survival rate of HFF cells dropped 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 toxic for HFF cells. It is therefore necessary to be careful in the use fruits of *solanum torvum* in traditional medicine

Key words: HFF cell, Cytotoxic, Extracts, *Solanum torvum*

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 and contains important drugs. In West and Central Africa, it is grown locally in gardens for cooking [2]. The fruits are widely used in several treatments in Cameroon. 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 [3]. Ripe fruits are used in the preparation of tonic and hemopoietic agents and also for the treatment of pain [4]. It has antioxidant properties [5]. The ethnobotanical survey carried out in the Haut-Sassandra Region also showed that all parts of *Solanum torvum* are intensively used in the traditional environment, particularly the leaves and fruits in the treatment of dermatoses and other diseases. However no study has been undertaken to our knowledge on the cytotoxic activity of fruits. The purpose of this paper is to make an ethnopharmacological study of the fruits of this plant used in the region of Haut-Sassandra (Ivory Coast). Specifically, a botanical description of this plant will be done, then a

phytochemical screening will be carried out followed by a study of fruit cytotoxicity on HFF (Human Foreskin Fibroblasts) cells.

2-Material and methods

2-1-Material

2-1-1-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)

2-1-2-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).

2-2-Methods

2-2-1-Botanical study

The botanical study took into account: botanical classification, botanical description, geographical distribution and therapeutic use [6-9].

2-2-2-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.

2-2-3-Preparation of total aqueous extract (TAE)

The preparation of this extracts was performed using the method described by [10] which consists in macerating 100 g of plant powder of species in 1L of sterile distilled water using a blender Blinder type 7 SEVEN STAR. The homogenate was filtered over hydrophilic cotton and then on Whatman filter paper. 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).

2-2-4-Preparation of 70 % ethanolic extract (70 % FE)

The extract was obtained by dissolving 5 g of TAE in 100 mL of a ethanol 70% solution and then homogenized. After decantation and filtration of the alcoholic fraction on hydrophilic cotton and on Whatman filter paper, the filtrate collected is evaporated in an oven at 50 °C. The powder obtained constitutes the 70 % ethanolic extract (70 % FE) [11].

2-2-5-Phytochimical sorting

The identification of different chemical compounds in the extracts was done by tubes characterization reactions. This method consists in detecting the different families of chemical compounds that may exist in plant extracts on the basis of characteristic colorations or precipitation reactions [12].

- **Alkaloids characterization**

The characterization of alkaloids was made from Bouchard (iodo-iodide) and Dragendorff (tetraiodo potassium bismuthate) reagent. 6 mL of each plant 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 Dragendorffreagent 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 (FeCl3). Thus, one drop of alcoholic solution of 2% ferric chloride 2 mL of solution of each plant extract was added. The formation of blue-black or green colouring more or less dark testifies 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 each plant extract were evaporated to the dry sand bath. The residue thus obtained was taken up in 5 mL dilute hydrochloric alcohol 2 times. The mixture was collected in a test tube, in which 3 meta chip of magnesium were added pink-orange or violet. 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 each plant 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 FeCl3 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 each plant 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 coumarins, 2 mg of each plant 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 each plant extract was introduced in the test tubes. Each tube was strongly stirred in a vertical position for 15 seconds, and thenleft to set 15 minutes. The height of persistent foam is higher than 1 cm, testifying the presence of saponins.

2-2-6-Cytotoxicity test

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) to solubilized plant extract 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 succinate dehydrogenase mitochondrial 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 3h 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 determine the relative amount of living and metabolically active cells [13]. Viability rate = (Abs544 nm extract / Abs544 nm control) × 100

3-RESULTS

3-1-Botanical classification

Kingdom: Plantae

Devision: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus: Solanum L.

Species: *Solanum torvum* sw

3-1-1-Botanical description

Leaves : Leaves simple, alternate, broadly ovate elliptic, variable in size, 10-15 cm long, 8-10 cm wide, margins with broad lobes, deeply cut in juvenile phases, shallow in mature leaves, apex acute to obtuse, base somewhat sagittate to auriculate, equal or oblique, petioles 2-5 cm long.

Flowers *:* The small, white flowers occur in large clusters, with simple, mostly glandular hairs on axes; corolla bright white, to 2.5 cm (1 in) across, lobed about 1/3 of its length; lobes not recurved; stamens with prominent anthers.

Fruits : The fruits are berries that are yellow when fully ripe. They are thin-fleshed and contain numerous flat, round, brown seeds.

Seeds : Seeds numerous, drab brownish, flattened, discoid, 1.5-2 mm long slightly reticulate, Self-compatible.

Odour - Pepper-like

Taste : Bitter and acrid

Parts used : Plant, leaves, fruits and root.

3-1-2-Distribution

Native to West Indies, India, Myanmar, Thailand, Philippines, Malaysia, China and tropical America. Widely naturalised in South and South East Asia

3-1-3-Traditional Medicinal Uses

In Sierra Leone, a fruit decoction is administered to children as a cough medicine, while in Senegal the plant is used to treat throat pain and stomach upset. In Ivory Coast fruit is used to treat anemia, scabies and blood pressure.

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 I). They are the polyphenols, tannins, flavonoids, saponins, and alkaloids in both 70% ethanol extract.

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

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

- :negative reaction ; + : positive reaction

EE 70 % : 70 % ethanolic extract

Sap : saponines ; **Flav** : flavonoïdes ; **Terp / Ster** : Terpènes / Stérols ; **Gall** : gallique ;

Cathé : cathéchique ; **Coum** : coumarines ; **Alc** : alcaloïdes ; **Poly** : polyphénol

3-3-Cytotoxicity test

Figure 2 gives the percentage of viability of the HFF cells cultured in the presence of concentrations of 100 to 800 µg / mL for the 70% ethanolic extract of the fruits of *Solanum torvum* compared to the control without plant extract. The number of cells decreases considerably as the concentration of the 70% ethanol extract of the fruits of *Solanum torvum* increases. At 800 µg / mL the number of dividing cells is 4%.

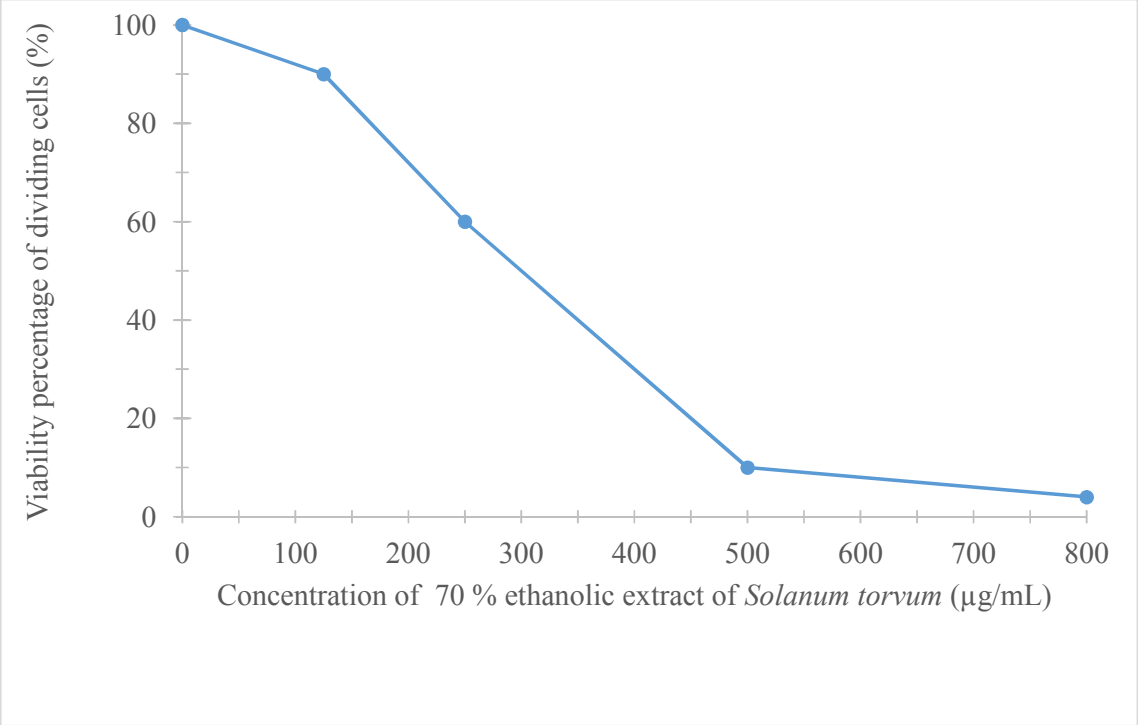

Figure 2: Cytotoxicity test of 70% ethanolic extract of fruits of *Solanum torvum* on HFF dividing cells

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 to treat anemia, bacterial infections and several other diseases. 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 [14]. 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 [15]. 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 [16]. 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 fruits could also be explained by the concentration of certain groups of chemical compounds which increases with the dose of the extract [17]. Our results on in vitro toxicity corroborate those [17] who worked on the same family of plants. Indeed according to the work of [17] the fruits of *Solanum nigrum* L. (Solanaceae) another Solanaceae rich in glucoalcaloids and saponins are toxic in internal and external uses on an organism. In addition to external use, the fruit of *Solanum torvum* is also used internally. 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 [18].

5-Conclusion

Solanum torvum is a pharmaceutically important medicinal plant of the Solanaceae family. This species is included among the ingredients of various native medicinal plants to treat a number of diseases. It should therefore be used with great caution because of its cytotoxic effect revealed in this study. As a perspective we plan to complete this study with in vivo toxicity tests to better justify the use of the fruits of this plant.

References

1. Adjanohoun J, Aboubakar N, Dramane K, Ebot E, Ekpere A, Enoworock G et al. Traditional medicine and pharmacopeia-contribution to ethnobotanical and floristic studies in Cameroon. In: CNPMS. Porto-Novo, Benin, 1996, pp. 50–52.

2.Etienne J. Solanacées médicinales et philatélie. Bull. Soc. Pharm. Bordeaux, 2005 ; 144, 311-332

3.Siemonsma J, Piluek K. Plant Resources of South-East Asia 8 (PROSEA), Bogor, Indonesia, 1994, pp. 412.

4.Kala C P. Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of Indian, J Ethno and Ethnomed, 2005 ; 1: 1-8.

5.Sivapriya M, Srinivas L. Isolation and purification of a novel antioxidant protein From the water extract of Sundakai (*Solanum torvum*) seeds, Food Chemistry, 2007 ; 104: 510 - 517.

6.Nasir, J.Y. Solanaceae. In: Flora of Pakistan. (Eds.): S.I. Ali and E. Nasir. Fascicle 85. Pakistan Agricultural Research Council, Islamabad. 1985 ; pp. 1-61.

7.Jennifer M.E., James A.C. Black nightshades, Solanum nigrum L., and related species. International Plant Genetic Resources Institute IPGRI, Italy, 1997; pp 1-113

8.APG III. The Angiosperm Phylogeny Group, « An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III », Botanical Journal of the Linnean Society, 2009**,** 161(2): 105-121.

9.Forest S, Kim S, Lloyd L. *Solanum torvum*, United States Geological Survey- Biological Resources Division Haleakala Field Station, Maui, Hawai'i, 2003, pp. 1-4.

10.Zirihi GN, Kra A, Dadié ET**.** Etude botanique et évaluation des activités antifongiques de *Mitracarpus villosus* (MV) (Rubiaceae) et *Spermacoce verticillota* (SV) (Rubiaceae) sur la croissance *in vitro* de *A. fumigatus*. Rev de Méd et de Pharma Afr, 2007 ; 20 : 9-17.

11.Zirihi GN, Kra AKM. Evaluation de l'activité antifongique de *Microglossa pyrifolia* (Larmarck) O. Kuntze (Asteraceae) « PYMI » sur la croissance in vitro de *Candida albicans*. Rev Med Pharmacol Afr. 2003; 17 : 11-8

12.Harborne J B**.** A guide to modern techniques of plant analysis. Springer, 3rd Edn, India (New Delhi), 1998, pp 5-32.

13.Mosman, T. Rapid colorimetric assay for cellular growth and survival : application to proliferation and cytotoxicityassay. Journal of immunological Methods, 1983 ; 65, 55-63

14.N'Guessan K, Kouadio K, Kouamé N'Guessan F, Traoré D, Aké-Assi L. Etude botanique des plantes emménagogues utilisées en médecine traditionnelle par les Abbey et Krobou d'Agboville (Côte-d'Ivoire). Rev Med Pharm Afr, 2008 ; 21: 43–60

15.Irie-N'guessan A. G, KABLAN B. J., Kouakou-Siransy N. G., Leblais V., Champy P. Evaluation de la toxicité de cinq plantes antiasthmatiques de la médecine traditionnelle ivoirienne Int. J. Biol. Chem. Sci. 2011; 5(3): 1316-1319.

16.Coulerie P. Etude 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, 2012 ; 296 p.

17.Busser C. Baies, fruits et pseudo-fruits toxiques utilises en médecine populaire ou en phytothérapie Phytothérapie, 2007 ; 1: 31–36

18.Zubaida Y, Ying W, Elias B. Phytochemistry and Pharmacological Studies on *Solanum torvum* Swartz Journal of Applied Pharmaceutical Science, 2013 ; 3 (4), pp. 152-160,