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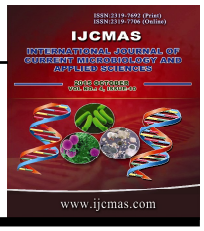

Original Research Article

Cytotoxic and Antiviral Activity of Methanolic, Ethanolic and Acetate Extracts of Six Varieties of Capsicum

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Keywords

Capsicum, Cytotoxic, Antiviral

A B S T R A C T

The ethanolic extracts, methanolic and acetate extracts of *Capsicum* species were tested on the survival of VERO cells, L20B, on herpes virus 1 and poliovirus 1. Acetate extract was more cytotoxic to the cells at all concentrations (0.5; 0.25; 0.12; 0.06; 0.03 mg/mL). On the other hand, the methanolic and ethanolic extracts have proven to be non-toxic as from the concentration of 0.25 mg/mL. The test was carried out in 96-well plates. The experiment performed show that no extract had an antiviral effect on the *in vitro* propagation of the poliomyelitis virus 1. However the methanolic and ethanolic extracts had an antiviral effect on the multiplication of the herpes simplex virus type 1 from the concentration of 0.25 to 0.06 mg/mL. These extracts protected VERO cells against infection of herpes simplex virus. Varieties of *Capsicum annuum* and *Capsicum chinense* were most effective on herpes virus 1 with an effective concentration (EC50) of 0.09 mg/mL (methanol extract) and 0.1 mg/mL (ethanol extract).

Introduction

Large-scale epidemics were due to enterovirus precisely enterovirus 71 affected countries such as Taiwan and Japan. They have affected nearly 60,000 and 300,000 children causing 55 deaths due to encephalitis. Despite this, enteroviruses are often perceived as minor pathogen in humans, with the exception of the poliomyelitis virus whose eradication is fast becoming a reality, thanks to the considerable efforts made by the scientific community (Delpeyroux and Crainic, 1998).

In Côted'Ivoire, the presence of enteroviruses in the environment had already been described by Gershy-Damet *et al.* (1987) and by Bini *et al.* (2006). Their disease areas are very extensive because they are capable of causing damage to the nervous system, gastrointestinal tract, respiratory tract, muscles, skin and eyes. Polio and meningitis are the most serious diseases caused by enteroviruses. These viruses also involve in myalgia, arthritis in chronic heart disease, Guillain- Barre syndrome, haemorrhagic conjunctivitis and type I diabetes (Health Canada, 2004). Some viral infections can also spread through the consumption of water and food contaminated by faeces. Thus, enterovirus and other viruses such as adenoviruses were identified in the hospital environment as a marker of viral pollution of surface water (Mansotte and Jestin, 2000).

In Côted'Ivoire, the recent massive exodus has resulted in a precarious living conditions and poor hygiene in some cities. Thus, foodborne disease is among the diseases that affect the greatest number of the population and cause most deaths (Panicet *et al.*, 2003). In addition, nationally, 54% of our population do not have proper sanitation and would be exposed to waterborne diseases (WHO, 2007). These viruses are circulating widely in the human and animal environments. They multiply in the throat and digestive tract and are found in the stool. Their principal transmission is through faecal-oral directly or indirectly, to a lesser extent by air (Bally *et al.*, 1999).

Similar for Other viral infections transmitted by *herpes virus* that is transmitted from person to person causing serious health problems worldwide. This is one of the most studied viruses in the world due to its antiviral resistance (Bestman-Smith, 2004). Virus diseases can have significant impacts on health, but also on the economy. There is more and more evidence about the emergence of virus resistance to antivirals currently in use. The discovery of new active molecule is very important in order to develop new antiviral molecules and fight against resistance phenomena. In African countries, plants are the main medical resources for public health care practices (Békro *et al.,* 2007). Among these plants are the pepper plants whose leaves, fruits and roots are used not only in the preparation of sauces (FAO, 2006) but also in traditional medicine (Kouassi *et al.,* 2012). Previous studies have shown that the different varieties of peppers have antimicrobial activity in pathogenic bacterial strains (*Escherichia coli, Vibrio cholerae, Staphylococcus aureus* and *Pseudomonas aeruginosa*) and fungal strains. The present study was conducted to evaluate the cytopathic and antiviral activity of three species of peppers consumed in Côte d'Ivoire on the poliovirus 1 and herpes virus 1.

Material and Methods

Plant material

The study was carried out on commercial pepper samples from fresh whole fruit of six varieties, *Capsicum annuum* variety antillais, *Capsicum frutescens* variety soudanais, *Capsicum frutescens* variety attié *Capsicum frutescens* variety doux *Capsicum frutescens* variety oiseau and *Capsicum chinense* variety pendulum at maturity stage of full fruit size. Plant materials were obtained from five local wholesale markets (Abobo, Yopugon, Adjamé, Treichville and Port Bouët) in Abidjan, Côte d'Ivoire. These fruits were surveyed and selected to compile a representative list of these Bell pepper fruits mostly used by traditional healers of Côte d'Ivoire for their availability, accessibility and wide utilization in food. These *Capsicum* fruit varieties were

identified by the national floristic center of the University of Félix Houphët Boigny, Abidjan, Côte d'Ivoire. *Capsicum annuum* antillais and *Capsicum chinense* pendulum selected were fresh, ripened and firm. *Capsicum frutescens* varieties selected were dried because used in this state.

Cell culture and viruses

Two viruses were used: Poliovirus Sabin I [VP1, CDC (Atlanta)], RNA non-enveloped and Herpes simplex virus type 1 (HSV1) isolated from a patient in the hospital of the CHU Treichville, (Abidjan Côte d'Ivoire) virus DNA having an envelope. These strains were provided by the virology laboratory of the Pasteur Institute of Côte d'Ivoire. The cell lines used are: L20B cells (used as the host cell for growing poliovirus 1) and VERO cells (used for culturing the *Herpes simplex virus 1*). These are cells commonly used in virology laboratory of the Pasteur Institute in Côte D'Ivoire. The two cell lines were grown in Eagle's minimal essential medium (MEM) (Mediatech Cellgro, VA) supplemented with 10% fetal bovine serum, PBS, penicillin (100 IU) and streptomycin (100 mg/mL) (Mediatech Cellgro®). Cells were cultured in a humidified atmosphere at 36°C in 5% CO_2.

Preparation of extracts

The extracts were prepared according to two methods to know, the method in series and the direct method from the chili powder.

Method in series was used according to Guédé-Guina, 1993. Crude aqueous, hydro-alcoholic and Ethylacetate extracts were prepared from the powder of fruit from each pepper variety. Thus, 50 grams were extracted by homogenization in one liter (1 L) of distilled water. The mixture was filtered through Whatman paper No.2 (Whatman International, Maidstone, England) then oven evaporated at 60°C until completely dry. The powders obtained constitute the crude aqueous extract coded Etaq. 25 grams of the crude aqueous extract were dissolved in 500 mL of ethanol (70%). Solution per homogenization during 24 hours with room temperature (25–30°C). The mixture was filtered through Whatman paper N° 2 and the filtrate was concentrated in a rota vapor at 50°C. The powders obtained constitute the ethanol 70% coded Eeth 70%. 10 grams of ethanolic 70% extract are dissolved in500mL of a solution composed of a mixture of ethyl acetate and distilled water (v/v). The mixture was mixed for 24 hours using a magnetic stirrer. The homogenate was filtered through Whatman paper No. 2 and the filtrate was concentrated in a rotavapor at 50°C. The dried extracted product constituted the acetatic 1 coded Eace 1.

The direct method from the fruit powder was used according to Kouassi and Koffi-nevry *et al.* (2012). 25 grams of chili powder were added to 100ml of solvent (ethanol, methanol or ethylacetate) by homogenization in a blender. The homogenate thus obtained was filtered on paper Whatman No. 2. The result obtained was homogenized in 100 ml of solvent then filtered on paper Whatman No. 2. This process was repeated three times, the filtrates collected, in each case were evaporated in a rotavapor at 50°C. The power obtained respectively constitutes the crude extract ethanolic coded Eeth 100%, crude extract methanolic coded Emeth and crude extract acetatic coded Eace 2.

Preparation of cell suspension

Cell suspensions were prepared according to WHO recommendations (2004). L20B and the VERO cells used in this study are adherent cells, sterile, cells may continue growing indefinitely and have a high

multiplicative potential. All these cells were cultured in MEM. After 24 hours incubation at 36°C under 5% CO_2, cell confluent monolayer was obtained. At the time of use of the cells, the medium containing the cells will be replaced by a Fetal Bovine Serum Medium FBSM at 2%.

Preparation of stock virus

The trypsinized cells in 75 cm^2 flasks are taken up by 30 mL of MEM 2% FBS containing 500 µL of virus suspension. After three days of incubation, the flask is subjected to two successive cycles of freezing and thawing for complete disruption of the cells therefore releasing intracellular virions. The viral suspension was subjected to centrifugation (Eppendorf 5810R, Germany) for 10 minutes. The viral suspension was distributed into cryotubes at a rate of 2 mL and frozen at -80 until use. Before analysis, the viral title was determined on a 96-wells Microtiter.

Cytotoxicity Test (Ojo *et al.*, 2009)

Fruit extracts were distributed in 6 tubes numbered from C_1 to C_6 with concentrations ranging from 1 mg/mL to 0.03 mg/mL binding by a geometrical reason of 2. In the tube C1, 10mg of each extract used were dissolved in 10 mL of MEM medium containing 0% then homogenized and filtered through a millipore membrane (0.2 mm) in order to have a concentration of 1mg/mL. In the other 5 remaining tubes, 5 mL of MEM medium were distributed. The technique consisted of transferring then to homogenizea volume of 5mL of the tube C_1 into C_2 tube containing 5 mL of MEM. This process was repeated until the tube C_6 whose excess has been rejected. These different concentrations of extracts were distributed into 96-well plates. A plate for each extract and a plate for each type of cell were used. To each well were added, 100 µL of the extract and 100 µL of the cell suspension of 2.10^5 cells/mL. We constituted three types of witnesses. A first control (negative) containing the extracts, a second control containing the 2% MEM medium and cells (L20B or VERO) and a third control containing only the MEM medium. Twelve wells were used for each concentration of the test sample. Each plate was incubated for 5 days at 36°C in of 5% CO_2. Cytological changes (lysis, granulation and changes in size) in cells were evaluated by microscopic observation after 24, 48 and 72 hours of incubation.

Evaluation of antiviral activity

The method used was that of Kim *et al.* (1997) and Obi *et al.* (2005). After evaluation of the cytotoxicity of extracts of *Capsicum* on L20B and VERO cells, non toxic concentrations in the cells (L20B and VERO) were used for antiviral tests on (Poliovirus 1 and Herpes virus 1). Thus, we used the following extract concentrations: 0.25; 0.12; 0.6 and 0.3 mg/mL. The antivirus tests were performed using 96-well plates fitted with cover. In all, three tests were carried out:

The first test was to put together the different concentrations of extracts selected with the viral suspension for 1 hour at 36°C. This reaction mixture (50 µL of extract + 50 µL of virus) was then inoculated with viable cells (100 µL of L20B and VERO cells). Three types of controls were used. A cell control column consisted of 100 µL of MEM 2% medium and 100 µL of viable cells, and then a sample virus column composed of 100 µL of cell suspension, 50 µL of virus suspension and 50 µL of MEM 2% medium and finally a third control containing 100 µL of extract + 100 µL of cells were performed.

For the second test, cells were allowed to

come in touch with the viral suspension for 1 hour (100 µL of viable cells + 50 µL of virus) prior to the addition of different concentrations of extract (50 µL).

The third test consisted of putting together the extracts and cells for 1 hour (100 µL of viable cells + 50 µL of extract) before the addition of the viral suspension (50 µL). The plates were then incubated at 36°C in an atmosphere rich in 5% CO_2 for 7 days and observed regularly under an inverted microscope. A neutralized reaction was observed by the absence of cytopatogenic effect CPE in the cell column after seven days of incubation. The viral cytopathic effects are noted by comparing control wells with test wells. Wavelength reading of each plate was read with spectrometer at 450 and 620 nm. The effective concentration 50% (EC50) of the antiviral activity was determined.

Statistical analysis

The different results obtained have been subjected to an analysis of variance (ANOVA) with the Statistica 7.1 software. In case of significant difference between the studied parameters, the ranking of means was done according to the Newman-Keuls test. The significance threshold value is $\alpha = 0.05$. Statistical differences with a probability value of less than 0.05 ($P<0.05$) are considered significant. When the probability is greater than 0.05 statistical differences are not significant.

Results and Discussion

Toxic concentrations of the extracts on L20B and VERO cells

The cytotoxicity tests results of *Capsicum* extract obtained are given in tables 1–4. All extracts of *Capsicum* varieties exhibit toxicity at 1 mg/mL and 0.5 mg/mL regardless of variety on L20B cells. On VERO cells, the extracts were toxic at 1 mg/mL but at 0.5 mg/mL, only acetate extract was toxic. However, at a concentration of 0.25 mg/mL no extract was toxic to these two cells (L20B and VERO). The acetate extract is the most toxic extract to the VERO cells.

L20B Cell survival Rate

The survival of L20B cells in the presence of crude extract methanolic, crude extract ethanolic and crude extract acetatic of *Capsicum* based on diluted concentrations is shown in table 5. The cell survival rate increases gradually as the extract concentration decreases. This rate varies depending on the type of extracts, varieties and different concentrations of extracts of *Capsicum*. There is no significant difference ($P> 0.05$) in all the extracts at concentration level of 0.25 to 0.12 mg/mL. However, there is a significant difference ($P<0.05$) in the extract between concentration 0.25; 0.12 mg/mL and concentrations 0.06; 0.03 mg mL. The methanolic extract of all varieties of *Capsicum* induced the largest rate of survival in all dilutions followed by crude extract ethanolic which had also large rate of survival. In the presence of crude extract methanolic and crude extract ethanolic of *Capsicum annuum antillais* and *Capsicum chinense pendulum*, the percentage of survival of L20B cells is high.

VERO cells Survival rate

Table 6 shows the survival rate of VERO cell in the presence of extracts of *Capsicum*. This rate varies depending on the variety, type of extract and concentration. There is a significant difference ($P<0.05$) in the survival rate of various extracts of *Capsicum annuum* from a concentration of 0.25 to 0.06

mg/mL. However, there is no significant difference between the survival rates with *Capsicum frutescens* species. The highest survival rate (77%, 76% and 75.6%; 70.3%) was obtained with crude extract methanolic and crude extract ethanolic of the *pendulum* and *antillais* varieties at concentrations between 0.06 and 0.03 mg/mL. The lowest survival rates were obtained at concentrations 0.25 mg/mL with acetate extract. VERO cells have a survival rate of more than 50% at all concentrations in the presence of methanolic extracts of *Capsicum annuu*m *antillai* and *Capsicum chinense pendulum*.

Antiviral activity

Different extracts of *Capsicum* had no activity on the *poliovirus 1*. In the absence of antiviral activity of extracts of *Capsicum* on poliovirus 1, only Herpes viruses 1was used for subsequent work.

The results are shown in table 7. The extracts showed varying responses against the virus. Concentrations from 0.25 to 0.06 mg/mL of crude methanolic extract and crude ethanolic extract of *Capsicum annuum antillais* and *Capsicum chinense* pendulum inhibit *in vitro* growth of Herpes virus. Herpes virus was more sensitive to methanolic and ethanolic extracts of these two varieties of *Capsicum*, in cases where the virus was in contact with the extract in 1 hour before adding the cells and also in the case where the extract was put in contact with the cell in 1 hour before adding the Herpès virus. However the virus are less sensitive to extracts in the case where the virus were in contact with the cell 1 hour before the addition of extracts then cells before the addition of the extracts.

Kinetics of cell destruction

Figures 1 and 2 illustrate the curves of cell destruction kinetics due to Herpes virus 1 according to the concentration of the extract used. The curves for the activity of crude methanolic extract and crude ethanolic extract have a decreasing pace with more or less steep slopes up to the concentration 0.12 mg/mL. The curve representing the activity of the acetate has a linear shape and remains constant regardless of the concentration of the extract. The higher the percentage of cell destruction increases, the lower the concentration of the extract.

Effective Concentration (EC50)

The values of the effective concentration of 50% cellular inhibition (EC50) are determined graphically on figure 1 and 2. The EC50 values of methanolic extracts of *Capsicum annuum antillais* and *Capsicum chinenes pendulum* are identical (0.09 mg/mL). The EC50 values of the ethanolic extracts of the same varieties are 0.1 mg/mL.

The extracts have shown toxicity to L20B and VERO cells at a concentration ≥0.5 mg/mL. The crude acetatique extract was more toxic on VERO and L20B cells at all concentrations. This difference in toxicity between the two types of cells could be explained by Kouamé *et al.* (2009) on the cytotoxic properties of the leaves of Galle in HeLa cells. In fact, these authors demonstrated that the cytotoxic activity of a compound can vary depending on cell line and experimental conditions. Indeed, L20B cells are derived from the transgenic mouse cell line while VERO cells derived from monkey kidney epithelial cells. Cell survival depends on the concentration of extract used. Thus, the cell survival rate increases when the extract concentration decreased. The different extracts had no activity on the poliovirus 1. This means that poliovirus 1 has resisted the action of extracts of *Capsicum*.

Table.1 Toxic concentrations of crude aqueous, ethanolic 70% and acetate 1 extracts on L20B cells

V. *Capsicum*	Extracts	L20B Cells					
		Different concentrations (mg/mL)					
		1	0.5	0.25	0.12	0.06	0.03
C. annuum antillais	Crude aqueous	+	-	-	-	-	-
	Ethanolic70%	+	+	-	-	-	-
	Acetatic	+	+	-	-	-	-
C. frutescens soudanais	Crude aqueous	+	-	-	-	-	-
	Ethanolic70%	+	+	-	-	-	-
	Acetatic	+	+	-	-	-	-
*C.frutescens*attié	Crude aqueous	+	-	-	-	-	-
	Ethanolic70%	+	+	-	-	-	-
	Acetatic	+	+	-	-	-	-
C.frutescens doux	Crude aqueous	+	-	-	-	-	-
	Ethanolic70%	+	+	-	-	-	-
	Acetatic	+	+	-	-	-	-
C. frutescens oiseau	Crude aqueous	+	-	-	-	-	-
	Ethanolic70%	+	+	-	-	-	-
	Acetatic	+	+	-	-	-	-
C.chinense pendulum	Crude aqueous	+	-	-	-	-	-
	Ethanolic70%	+	+	-	-	-	-
	Acetatic	+	+	-	-	-	-
Control (+)	Cells L20B	-	-	-	-	-	-

C.: *Capsicum*; (+): extract toxic; (-): extract non toxic

Table.2 Toxic concentrations of crude aqueous, ethanolic 70% and acetate extracts on VERO cells

V. *Capsicum*	Extracts	VEROCells					
		Different concentrations (mg/mL)					
		1	0,5	0,25	0,12	0,06	0,03
C. annuum antillais	Crude aqueous	+	-	-	-	-	-
	Ethanolic 70%	+	-	-	-	-	-
	Acetatic	+	+	-	-	-	-
C. frutescens soudanais	Crude aqueous	+	-	-	-	-	-
	Ethanolic 70%	+	-	-	-	-	-
	Acetatic	+	+	-	-	-	-
*C.frutescens*attié	Crude aqueous	+	-	-	-	-	-
	Ethanolic 70%	+	-	-	-	-	-
	Acetatic	+	+	-	-	-	-
C.frutescens doux	Crude aqueous	+	-	-	-	-	-
	Ethanolic 70%	+	-	-	-	-	-
	Acetatic	+	+	-	-	-	-
C. frutescens oiseau	Crude aqueous	+	-	-	-	-	-
	Ethanolic70%	+	-	-	-	-	-
	Acétate 1	+	+	-	-	-	-
*C.chinense*pendulum	Crudea queous	+	-	-	-	-	-
	Ethanolic70%	+	-	-	-	-	-
	Acétate 1	+	+	-	-	-	-
Control (+)	Cells VERO	-	-	-	-	-	-

C.: *Capsicum*; (+): extract toxic; (-): extract non toxic

Table.3 Toxic concentrations of crude extract methanolic, crude extract ethanolic and crude extract acetatic on L20B cells

		L20BCells					
V. *Capsicum*	Extracts	**Different concentrations (mg/mL)**					
		1	0,5	0,25	0,12	0,06	0,03
C. annuum antillais	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C. frutescens soudanais	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C.frutescen sattié	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C.frutescens doux	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C. frutescens oiseau	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C.chinense pendulum	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
Control (+)	Cells L20B	-	-	-	-	-	-

C.: *Capsicum*; +: Extract toxic; -: extract non toxic

Table.4 Toxic concentrations of crude extract methanolic, crude extract ethanolic and crude extract acetatic on VERO cells

		VERO Cells					
V. *Capsicum*	Extracts	**Different concentrations (mg/mL)**					
		1	0,5	0,25	0,12	0,06	0,03
C. annuum antillais	Crude methanolic	+	-	-	-	-	-
	Crude ethanolic	+	-	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C. frutescens soudanais	Crude methanolic	+	-	-	-	-	-
	Crude ethanolic	+	-	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C. frutescens attié	Crude methanolic	+	-	-	-	-	-
	Crude ethanolic	+	-	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C. frutescens doux	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
C. frutescens oiseau	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
*C. chinense*pendulum	Crude methanolic	+	+	-	-	-	-
	Crude ethanolic	+	+	-	-	-	-
	Crude acetatic	+	+	-	-	-	-
Control (+)	Cells	-	-	-	-	-	-

C.:*Capsicum*; +: Extract toxic; -: extract non toxic

Table.5 L20B Cell survival rate in the presence of methanolic, ethanolic 100% and acetate extract

	Survival rate (%)				
		Concentrations (mg/mL)			
Variéies *Capsicum*	Extract	0,25	0,12	0,06	0,03
C. annuum antillais	Crude methanolic	35,6±5[a]	44±4[a]	56,3±1,5[b]	63,3±4,1[c]
	Crude thanolic	34,3±,32[a]	42,3±2,5[a]	56±2,6[b]	62±2,6[c]
	Crude acetatic	32,6±2[a]	40±1[a]	43,3±3[b]	60,6±2[c]
C. frutescens soudanais	Crude methanolic	32±1[a]	40,6±3[a]	55,3±1,5[b]	61±1[c]
	Crude ethanolic	31,3±1,5[a]	40±1[a]	50,3±1,5[b]	59±1[c]
	Crude acetatic	31±3,6[a]	37±2[a]	43,6±1,5[b]	54±3[c]
C. frutescens attié	Crude ethanolic	31±2,6[a]	42±3[a]	53,3±3[b]	62,3±3[c]
	Crude ethanolic	30,6±2[a]	35±2[a]	50,5±2,5[b]	55,3±2,5[c]
	Crude acetatic	28±2,6[a]	35±3[a]	39,3±2,5[a]	48,3±1,5[a]
C. frutescens doux	Methanolic	35,3±2,3[a]	39±1[a]	52,3±3[b]	59±3,6[b]
	Crude ethanolic	31,6±1[a]	36,6±1,5[a]	51,2±1,5[b]	57,6±2,5[b]
	Crude acetatic	27,6±2,5[a]	32,6±2,5[a]	37,3±2,5[a]	55±2[b]
C. frutescens oiseau	Crude methanolic	34,3±3,7[a]	39±1[a]	51±3,6[b]	58±4,5[b]
	Crude ethanolic	32,3±2,5[a]	37,6±2,5[a]	50,3±2[b]	52,6±3,2[b]
	Crude acetate	28±2,6[a]	33±2[a]	35,3±3,5[a]	44±4,5[b]
C. chinense pendulum	Crude ethanolic	37,3±1,5[a]	47,3±2[a]	63,3±1,1[b]	78±2,6[d]
	Crude ethanolic	34,6±2,5[a]	40,3±1[a]	62,3±3,7[b]	71±2,6[d]
	Crude acetate	30,3±1,5[a]	38,3±3,5[a]	52,6±2,5[b]	67±2[c]

For each concentration of extract, in row and column, values with the same letters are not significantly different from the threshold value at 5% according to the tests of NeuwmannKul; *C.: Capsicum*.

Table.6 Survival rate of VERO cells in the presence of crude extract methanolic, crude extract ethanolic and crude extract acetatic

		Survival rate (%)			
		Concentrations (mg/mL)			
Variétés *Capsicum*	Extracts	0,25	0,12	0,06	0,03
C. annuum antillais	Crude methanolic	57±3,7[b]	57,5±2[b]	64,3±3[b]	76±2[c]
	Crude ethanolic	52±2,1[b]	53,3±2,5[b]	61,6±4[b]	70,3±1,5[c]
	Crude acetatic	37,6±6,4[a]	43,6±4,1[a]	45±3[a]	54,6±4,5[b]
C. frutescens soudanais	Crude methanolic	40±2[a]	45±5[a]	53,6±4,7[b]	59,3±3[b]
	Crude ethanolic	36±2,6[a]	43,3±1,5[a]	46,6±1,5[a]	55,3±1,5[b]
	Crude acetatic	32,6±1,5[a]	37,3±2[a]	43±2,6[a]	46±1[a]
C. frutescens attié	Crude methanolic	46±2[a]	52,6±3,2[b]	60±4[b]	67±2,6[b]
	Crude ethanolic	42,3±2,5[a]	47±2,6[a]	54,3±4[b]	62,6±2,5[b]
	Crude acetatic	35±6[a]	40,3±1,5[a]	45,6±2[a]	52±2[b]
C. frutescens doux	Crudemethanolic	42±3[a]	47,3±3[a]	52±3[b]	63,6±5,1[b]
	Crudeethanolic	35,6±2[a]	44,6±3,5[a]	51,3±3,2[b]	57,3±2[b]
	Crude acetatic	36±2,6[a]	42±3[a]	52±3[b]	57±2[b]
C. frutescens oiseau	Crudemethanolic	37,6±1,5[a]	47±2,6[a]	57,3±2[b]	64,3±4[b]
	Crudeethanolic	37,3±2[a]	44±2[a]	49,3±4,5[b]	57,3±2[b]
	Crudeacetatic	33,6±4,1[a]	43±4,1[a]	50±2[b]	54,6±1,5[b]
C. chinense pendulum	Crude methanolic	57,3±2[b]	67,3±2[c]	72±2,6[c]	77±1[c]
	Crude ethanolic	54±4,5[b]	67,3±2[c]	70,3±1,5[c]	75,6±2[c]
	Crude acetatic	34±3,6[a]	38±1[a]	43,6±1,5[a]	54±3,6[b]

For each concentration of extract, in row and column, values with the same letters are not significantly different at the 5% threshold level according to the tests of NeuwmannKul; *C.: Capsicum*.

Table.7 Evolution of the virus according to the conditions of the extract of *Capsicum*

		Virus HSV1		
		Concentrations (mg/mL)		
		A	B	C
Variété *C.*	Extracts	0.25; 0.12; 0.06; 0.03	0.25; 0.12; 0.06; 0.03	0.25; 0.12; 0.06; 0.03
C. annuum antillais	Crude methanolic	+ + +/- -	+/- - - -	+ + - -
	Crude ethanolic	+ + - -	- - - -	+ + - -
	Crude acetate	- - - -	- - - -	- - - -
C. frutescens soudanais	Crude methanolic	+/- - - -	- - - -	+/- - - -
	Crude ethanolic	- - - -	- - - -	- - - -
	Crude acetate	- - - -	- - - -	- - - -
*C. frutescens*attié	Crude methanolic	- - - -	- - - -	- - - -
	Crude ethanolic	- - - -	- - - -	- - - -
	Crude acetate	- - - -	- - - -	- - - -
C. frutescens doux	Crude methanolic	- - - -	- - - -	- - - -
	Crude ethanolic	- - - -	- - - -	- - - -
	Crude acetate	- - - -	- - - -	- - - -
C. frutescens oiseau	Crude methanolic	- - - -	- - - -	- - - -
	Crude ethanolic	- - - -	- - - -	- - - -
	Ctude acetate	- - - -	- - - -	- - - -
*C. chinense*pendulum	Crude methanolic	+ - - -	+/- - - -	+ + - -
	Crude ethanolic	+ + - -	- - - -	+ + - -
	Crude acetate	- - - -	- - - -	- - - -
Control (+)	Cell VERO	+ + + +	+ + + +	+ + + +
Control (-)	Cell +HSV1	- - - -	- - - -	- - - -

HSV1: *herpès virus* 1; C.: *Capsicum*; A: Virus + extract (cell); B: Virus + cell (extract); C: cell + extract (virus); (+): Virus inhibition; (+/-): partial inhibition; (-): No inhibition, presence.

Figure.1 Cell destruction Kinetics of VERO cells in the presence of crude methanolic, crude ethanolic and crude acetatic of *Capsicum annuum* antillais

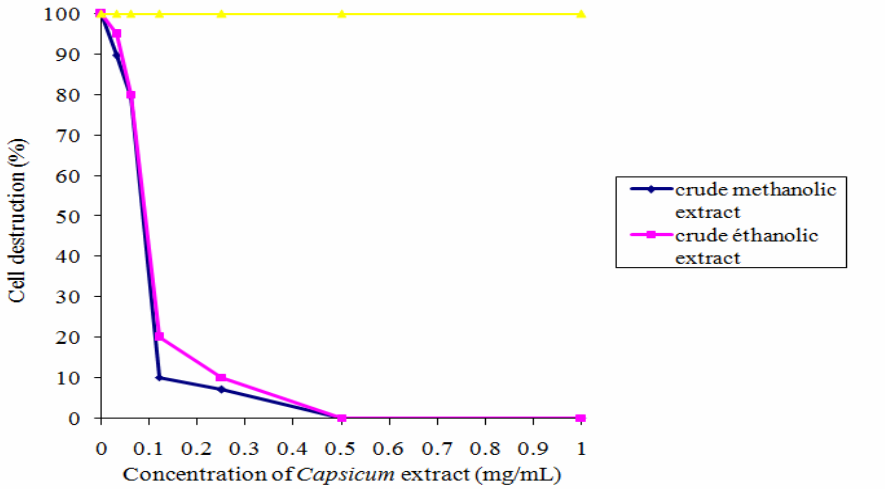
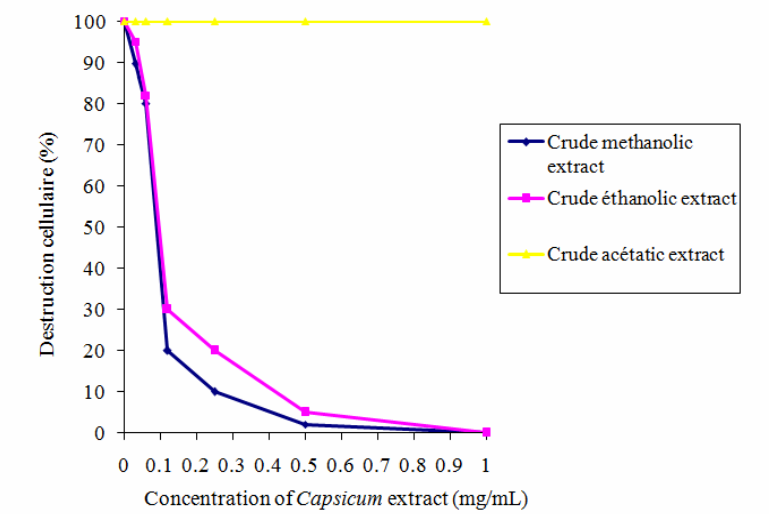


Figure.2 Cell destruction Kinetics of VERO cells in the presence of crude methanolic, crude ethanolic and crude acetatic of *Capsicum chinense* pendulum



According to Lipipun *et al.* (2003) this resistance is a characteristic of this virus. Indeed, Poliovirus 1 is a non-enveloped RNA virus which is resistant in the external environment. Thus, the absence of envelope confers resistance in chloroform and alcohol (Mesbah *et al.*, 2000). These results corroborate to that of Ojo *et al.* (2009) who showed that the poliovirus is a naked virus, it would require for its inactivation, more active products in higher concentrations.

The crud methanolic extract and crude ethanolic extract of *Capsicum annuum antillais* and *Capsicum chinense pendulum* showed activity on *herpes virus 1*. The three tests have shown the different ways to administer the *Capsicum* extracts in the presence of viruses and cells. When the virus was exposed to the extracts of *Capsicum* before the addition of cells, inhibition of viral *in vitro* growth was observed from 0.12 to 0.25 mg/mL. According to some authors such (Sulaiman *et al.* (2011) and Bakari *et al.* (2012), the virus can be inactivated by another substances when it is put in contact with the extracts directly. According to these authors, *Capsicum* extracts could interfere at the attachment points and penetration of the virion in cells. The principal act if of the extract is probably modified the cellular and viral receptors, preventing the virus from attaching to cells. The cell line grown in the presence of extracts of *Capsicum* before the addition of the virus has caused the inhibition of virus growth at concentrations from 12.5 to 0.25 mg / mL. According to Obi *et al.* (2005), if the receiving sites are modified before viral infection, the virus's ability to bind and penetrate the living cells would be greatly reduced. It could be that the crud methanolic extract and crud ethanolic extracts develop their antiviral activity by acting directly on the virus when they are in contact. It could happen that between ethanol or methanol and components of pepper, a synergy reaction acting on the herpes virus 1.

The antiviral activity of extracts of *Capsicum annuum* antillais and *Capsicum chinense* pendulum could also be due to phytochemicals, present in the extracts of *Capsicum*. Indeed, the studies of Kouassi

and Koffi-Nevry (2012) showed that *Capsicum annuum* and *Capsicum frutescens* contain bioactive compounds and vitamins such as ascorbic acid (vitamin C) and the ß - carotene (vitamin A).

The effective concentrations for 50% inhibition EC50 of cell are 0.09 mg/mL of methanolic extract of *Capsicum annuum antillais* and *Capsicum chinenes pendulum* and 1 mg/mL of the ethanolic extract of those varieties of *Capsicum*. This result could be explained by the fact that the methanol extracts and crude ethanolic extract of *Capsicum* would protect VERO cells against *herpes virus* infection at a minimum concentration of 0.12 mg/mL. Meanwhile, *Capsicum annuum* antillais is the variety that seems to be more protective of VERO cells.

In conclusion, it appears that a concentration of 0.5 mg/mL, extracts of *Capsicum* are toxic to VERO cells and L20B. These extracts had no effect on the development of *poliovirus1*. On the other hand the crud methanolic and crud ethanolic extracts of *Capsicum annuum* antillais and *Capsicum chinense pendulum* were active at concentrations of 0.25 and 0.12 mg/mL on the development of *herpes virus.*

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