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Phytochemical study and evaluation of the antiviral activity of aqueous extracts of three medicinal plants; *Xylopia aethiopica*, *Gliricidia sepium* and *Ocimum gratissimum* used in Cote d'Ivoire

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Abstract:

Background: The present work is part of the exploration of new antiviral molecules to combat antimicrobial resistance. In purpose, this study determined the phytochemical analysis, cytotoxicity and antiviral activity of extracts from three Ivorian medicinal plants; *Gliricidia sepium, Ocimum gratissimum* and *Xylopia aethiopica* against poliovirus 1, a non-enveloped RNA virus.
Methodology: Aqueous extract of the three plants, which were identified at the herbarium of National Floristic Center Abidjan, was done using a previously described method. The precipitation or staining technique was used to highlight the chemical groups in the three extracts while the polyphenol content of each extract was assessed by the colorimetric method. Cytotoxicity and antiviral activity tests were performed in 96-well plates. Cytotoxicity of each extract on L20B (a genetically engineered mouse cell line) was determined by observation of the cell line carpet. Antiviral activity of three extracts against poliovirus type I was determined after 72 hours using an assay that measures inhibition of the cytopathic effect on cell culture.
Results: The three plant extracts contain polyterpenes, sterols and polyphenols, flavonoids, catechetical tannins, saponosides and quinones but none of the extract contains gallic tannins. With the exception of *O. gratissimum*, alkaloids were found in extracts from the two other plants, and extract of *G. sepium* was richer in polyphenol than the other two extracts. The cell carpet of L20B after 72 hours contact period with three extracts remained intact at concentrations ranging from 2 to 1000 µg/ml. The aqueous extract of *G. sepium* showed higher antiviral activity on poliovirus 1 (74.569%) at 2µg/ml than the extracts of *O. gratissimum* (45.6112%) and *X. aethiopica* (44.5247%) after 72 hours of incubation.
Conclusion: The extract of *G. sepium* showed potent antiviral activity against poliovirus 1 than that of *O. gratissimum* and *X. aethiopica*. This was justified by its higher polyphenol content than the two extracts.

Keywords: *Gliricidia sepium*, *Ocimum gratissimum*, *Xylopia aethiopica*, Phytochemistry, Cytotoxicity, Antiviral

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Etude phytochimique et évaluation de l'activité antivirale d'extraits aqueux de trois plantes médicinales; *Xylopia aethiopica*, *Gliricidia sepium* et *Ocimum gratissimum* utilisés en Côte d'Ivoire

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Résumé:

Contexte: Le présent travail s'inscrit dans le cadre de l'exploration de nouvelles molécules antivirales pour lutter contre la résistance aux antimicrobiens. Dans le but, cette étude a déterminé l'analyse phytochimique, la cytotoxicité et l'activité antivirale d'extraits de trois plantes médicinales ivoiriennes; *Gliricidia sepium, Ocimum gratissimum* et *Xylopia aethiopica* contre le poliovirus 1, un virus à ARN non enveloppé.
Méthodologie: L'extrait aqueux des trois plantes, qui ont été identifiées à l'herbier du Centre National de Floristique d'Abidjan, a été réalisé selon une méthode précédemment décrite. La technique de précipitation ou de coloration a été utilisée pour mettre en évidence les groupements chimiques dans les trois extraits tandis que la teneur en polyphénols de chaque extrait a été évaluée par la méthode colorimétrique. Des tests de cytotoxicité et d'activité antivirale ont été réalisés dans des plaques 96 puits. La cytotoxicité de chaque extrait sur L20B (une lignée cellulaire de souris génétiquement modifiée) a été déterminée par l'observation du tapis de la lignée cellulaire. L'activité antivirale de trois extraits contre le poliovirus de type I a été déterminée après 72 heures en utilisant un test qui mesure l'inhibition de l'effet cytopathique sur la culture cellulaire.
Résultats: Les trois extraits végétaux contiennent des polyterpènes, des stérols et des polyphénols, des flavonoïdes, des tanins catéchétiques, des saponosides et des quinones mais aucun extrait ne contient de tanins galliques. A l'exception d'*O. gratissimum*, des alcaloïdes ont été trouvés dans les extraits des deux autres plantes, et l'extrait de *G. sepium* était plus riche en polyphénols que les deux autres extraits. Le tapis cellulaire de L20B après 72 heures de contact avec trois extraits est resté intact à des concentrations allant de 2 à 1000 µg/ml. L'extrait aqueux de *G. sepium* a montré une activité antivirale plus élevée sur le poliovirus 1 (74,569%) à 2 µg/ml que les extraits d'*O. gratissimum* (45,6112%) et de *X. aethiopica* (44,5247%) après 72 heures d'incubation.
Conclusion: L'extrait de *G. sepium* a montré une activité antivirale puissante contre le poliovirus 1 que celle de *O. gratissimum* et *X. aethiopica*. Ceci était justifié par sa teneur en polyphénols plus élevée que les deux extraits.

Mots clés: *Gliricidia sepium*, *Ocimum gratissimum*, *Xylopia aethiopica*, Phytochimie, Cytotoxicité, Antivirale

Introduction:

About fifty years after scientific research began on antiviral agents, viral diseases remain a major global concern. This is due on the one hand to the toxicity of the many drugs developed (1,2) and on the other hand to the rapid appearance of drug-resistant strains (3, 4,5). In order to discover new effective antiviral molecules, alternative avenue of investigation could be exploited, which is medicinal plants used for health care practices in low-income countries (6). Indeed, compounds isolated from medicinal plants such as flavonoids, tannins, proteins, polysaccharides, and alkaloids have been reported to exhibit antiviral activity (7). A report indicates that a flavone (natural flavonoid) isolated from *Agastache folium* has good antiviral activity in cell culture against most picornaviruses without showing toxicity to growing cells (8).

An excellent model for studying viral replication is the poliovirus, an RNA virus, belonging to the Picornaviridae family. The latter is responsible for poliomyelitis, a disease eradicated by many countries but which remains a threat to the whole world. Researchers (9) have reported that pathologies such as meningitis, myocarditis, encephalitis and respiratory diseases can be caused by enteroviruses, the genus to which poliovirus belongs.

Gliricidia sepium, O. gratissimum, and *X. aethiopica* are three medicinal plants used against viral diseases on the basis of an ethnobotanical survey carried out in the district of Abidjan, Côte d'Ivoire (10). *G. sepium* has been reported to have antibacterial, antifungal and antiviral properties (11). *Ocimum gratissimum* is used in ethnomedicine generally for the treatment of the upper respiratory tract (cough, pneumonia, etc.) and digestive disorders (diarrhea, dysentery, etc.), skin pathologies, fever, headaches and conjunctivitis (12,13). The extracts obtained by decoction of the dried fruits of *X. aethiopica* are used to treat various respiratory, digestive and inflammatory diseases and infections, including dysentery and malaria (14).

The general objective of this study is to investigate the phytochemical constituents and evaluate the antiviral activity of aqueous extracts of these three Ivorian medicinal plants; *G. sepium*, *O. gratissimum*, and *X. aethiopica.* This specifically will involve carrying out on the one hand, qualitative phytochemical analysis and dosage of polyphenols of the extracts, and on the other hand, the cytotoxicity and antiviral activity of the extracts respectively on L20B cells (a genetically engineered mouse cell line) and the poliovirus type 1.

Materials and method:

Plant materials

The plant material consists of the leaves of *G. sepium*, *O. gratissimum* and fruits of *X. aethiopica*. The leaves of *G. sepium* and

O. gratissimum were collected in August 2021 in the district of Abidjan while the fruits of *X. aethiopica* were bought at the Adjamé market. The three plants were identified at the National Floristic Center under the respective herbarium numbers; UCJ010419, UCJ008879 and UCJ001462.

Preparation of aqueous extracts

The different leaves were cleaned and dried in a dry place, ventilated away from light for five days except for the fruits which were dry. The preparation of the total aqueous extracts was carried out according to the method of Zirihi et al., (15). One hundred grams (100g) of vegetable powder was dissolved in 2 liters of distilled water. The mixture was then homogenized 10 times at a rate of 2 minutes per revolution using a mixer (Blender Bruon H-999A).

The homogenate obtained was drained in a square of cloth and then filtered successively three times on cotton wool and then on Wattman paper (3 mm). The filtrate was evaporated at 55°C using a Venticell® type oven for three days (72 h) to obtain a powder which is the total aqueous extract. At the end of the extractions, R = (100 x m)/M, where 'R' is yield (%), 'm' is mass of dry extract (g), and 'M' is mass of plant material used (g).

Phytochemical study: qualitative analysis and polyphenol content

The precipitation or staining technique was used to highlight the desired chemical groups of alkaloids, tannins, polyphenols, flavonoids, quinones, saponosides, sterols and polyterpenes in aqueous extracts of *O. gratissimum*, *X. aethiopica* and *G. sepium* (6,16,17, 18).

The polyphenol content of each extract was evaluated according to the Folin-Ciocalteu colorimetric method described by Bakchiche and Gherib (19) with some modifications. To 1 ml of each plant extract are added respectively 2.5 ml of Folin-Ciocalteu diluted 1/10 in distilled water and after 6 min, 2 ml of sodium carbonate (20%). The whole is incubated at room temperature and protected from light for 2 hours. Optical densities (OD) were read with a spectrophotometer at 730 nm against blank prepared in the same manner, replacing the extract with distilled water. The gallic acid which constitutes our standard was prepared under the same conditions as the extract with a mixture of ethanol/water solvent (50:50, V/V) at concentrations ranging from C1 to C6 tubes.

L20B cells and poliovirus 1

L20B cells (genetically modified mouse cell lines) were cultured 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®). The latter were maintained in a humidified atmosphere at 36° C in 5% CO_2. Sabin I poliovirus [VP1, CDC, Atlanta], non-enveloped RNA virus was provided by the Department of Epidemic Viruses of the Institut Pasteur in Côte d'Ivoire. The viral suspension (contained in a 2 ml cryotube) was frozen at -80°C before use.

Determination of the cellular toxicity of extracts on L20B cells

The cytotoxicity test was carried out according to the method described by Ojo et al.**,** (20) with some modifications. The extracts of *G. sepium, O. gratissimum* and *X. aethiopica* dissolved in 2% MEM (minimum essential medium) were distributed in 10 microtubes each. The concentrations vary respectively from 1 mg/ml to 2 µg/ml (cascade dilution). The extracts at different concentrations were placed in 96-well microplates. Each well contains 100 µl of extract and 100 µl of L20B cell suspension (0.25 x 10^6 cells/ml). The controls are of two types: a negative control consisting of cells without extract and a positive control consisting of cells and Tritton. Each microplate covered with an adhesive film is incubated for 3 days at 36°C in 5% CO_2. Cell morphology (e. g. rounding, narrowing detachment) is examined by light microscopy after 24, 48 and 72 hours of incubation.

Antiviral activity

The evaluation of the antiviral activity was carried out by the method described by Meyer et al., (1996) with modifications. 50 µl of L20B cell suspension (0.25 x 10^6 cells/ml) were incubated with the suspensions of poliovirus I in equal proportion (1:1) at 37°C for 1 hour 30 min in a 96-well plate. 50 µl of the smallest non-toxic concentration of each extract (2 µg/ml) were added (in triplicate for each extract). Two types of control, a negative control (consisting of cell suspension without extract or virus) and a positive control (consisting of cell suspension and virus) were used.

The effect of each extract on poliovirus I was evaluated by determining the optical density (OD) at 490 nm in a Multiskan FC reader as; Percentage (%) CPE inhibition = (At-Ap)/(An-Ap) x 100, where 'An' is the average absorption of negative control (cell suspension without virus or extract), 'Ap' is the average absorption of the positive control (cell suspension with virus without extract), and 'At' is the average absorption of the sample tested.

Statistical analysis of data

The experiments were performed in triplicate and the data were analyzed using ANOVA. The value of the significance threshold

was 0.05. Statistical difference with a probability value less than 0.05 ($p<0.05$) was considered significant.

Results:

Extract yield

The yields obtained at the end of the extraction were respectively 13.01% for the leaves of *G. sepium*; 10.61% for the leaves of *O. gratissimum* and 9.81% for the fruits of *X. aethiopica*.

Phytochemical study

All the plant extracts contain polyterpenes, sterols and polyphenols, flavonoids, catechetical tannins, saponosides and quinones. On the other hand, no extract contains gallic tannins. With the exception of *O. gratissimum*, alkaloids were found in extracts from other plants (Table 1).

The polyphenol contents of the plant extracts were determined from the calibration line for gallic acid (Y=5.1316x) (Fig 1). The polyphenol contents were reported in mg of gallic acid equivalent/g of dry extract (mg EAG/g ES). The *G. sepium* extract (0.13± 0.02 mg EAG/g ES) was richer in polyphenols while the extract of *O. gratissimum* and *X. aethiopica* showed low values for the same solvent used. These contents were respectively 0.08 ±0.003 mg EAG/g ES and 0.05±0.01 mg EAG/g ES (Fig 2).

Table 1: Phytochemical compounds in three Ivorian medicinal plants

Excerpts	Sterols and Polyterpenes	Polyphenols	Flavonoids	Tannins		Quinones	Alkaloids		Saponins
				Cat.	Gal.		B	D	
G. sepium	+	+	+	+	-	+	+	+	+
O. gratissimum	+	+	+	+	-	+	-	-	+
X. aethiopica	+	+	+	+	-	+	+	+	+

Cat = Catechic, Gal = Gallic, B - Bouchardat, D = Dragendorff, + = Presence of the metabolite, **-** = Absence of the metabolite.

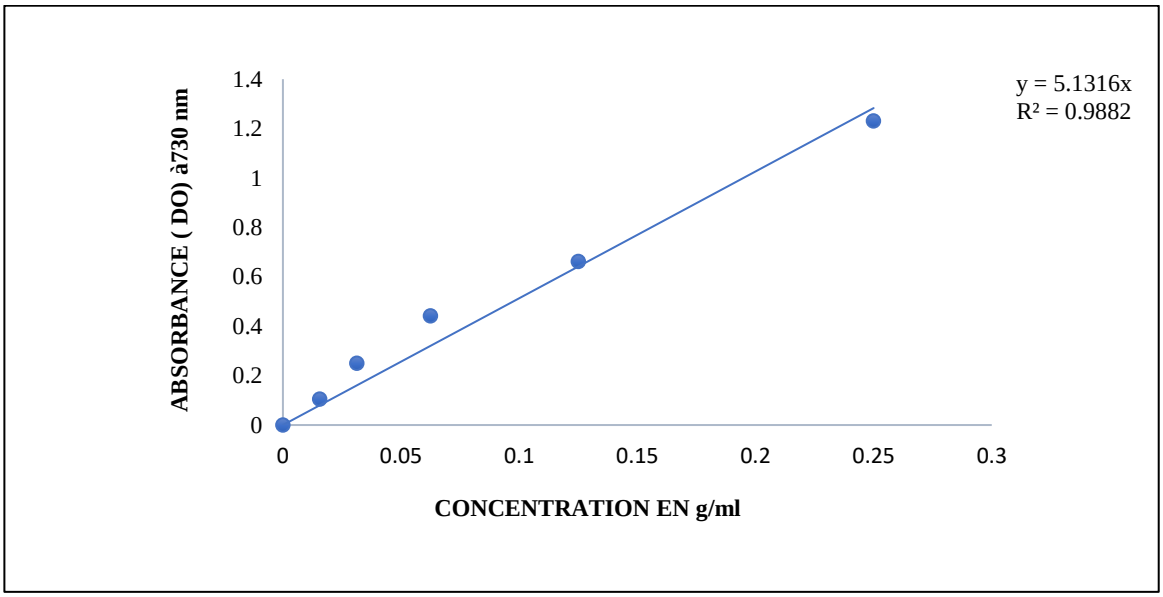

Fig 1: Gallic acid calibration curve

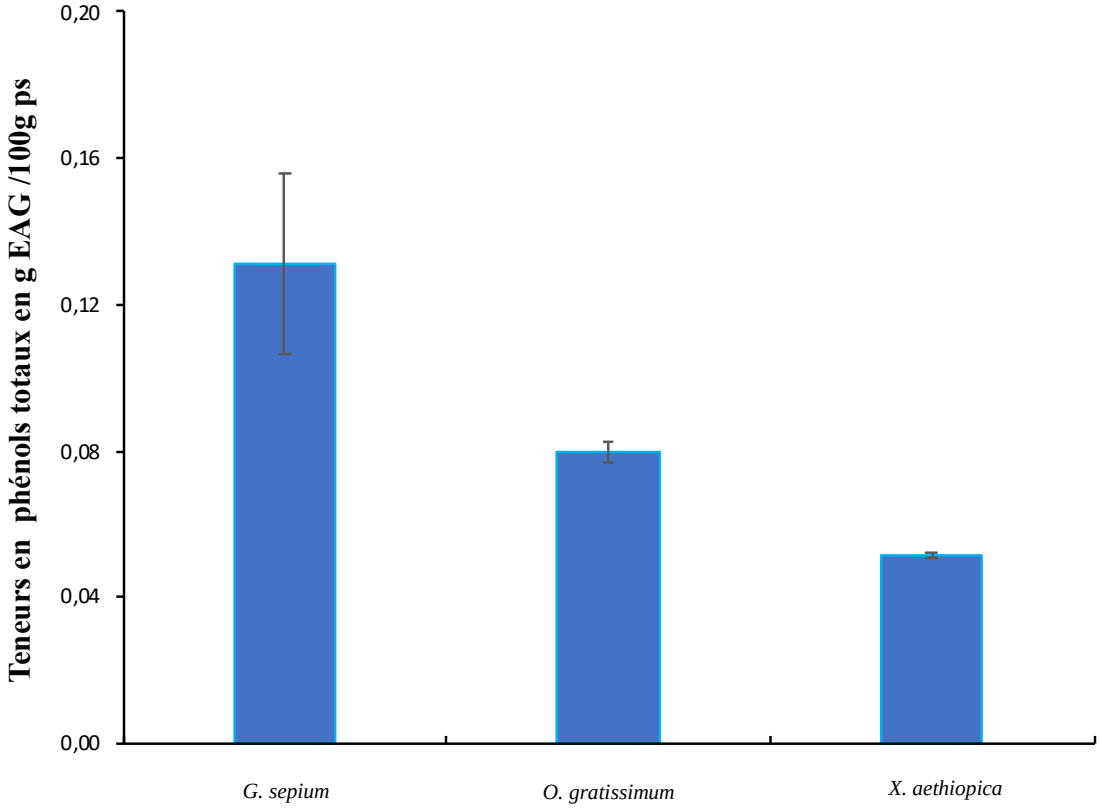

Fig 2: Polyphenol content of aqueous leaf extracts of *Gliricidia sepium*, *Ocimum gratissimum* and fruits of *Xylopia aethiopica*.

Cytotoxicity to L20B cell lines

An intact cell layer was observed after 72 hours of contact of the L20B cells with each extract at concentrations ranging from 2 (1.953125) to 1000 µg/ml (Table 2). The lowest concentration which had no cytotoxic effect on the L20B cells was 1.953125 µg/ml (~ 2 µg/ml), which was used to evaluate the antiviral activity of the three plant extracts.

Antiviral activity

The antiviral activity of the extracts of the three plants on poliovirus 1, evaluated by the percentage inhibition of the effect of the cytopathic effect, is shown in Table 3. The aqueous extract of *G. sepium* showed significantly higher antiviral activity (74.569%) than the extracts of *O. gratissimum* and *X. aethiopica* ($p<0.01$). The latter two extracts had statistically identical antiviral activity (45.6112 and 44.5247% respectively) on poliovirus 1 (Fig 3).

Discussion:

The experiments carried out at the phytochemical level made it possible to identify

Table 2: Effect of crude extracts on L20B cells

Extraits	Cellules	CONCENTRATION (µg/ml)										Contrôle négatif	Contrôle positif
		1,953125	3,90625	7,8125	15,625	31,25	62,5	125	250	500	1000		
G. sepium	L20B	-	-	-	-	-	-	-	-	-	-	-	+
O. gratissimum													
X. aethiopica													

(-): absence of toxicity, (+): presence of toxicity.

Table 3: Inhibition of the PCE of poliovirus 1 by the three plant extracts (at a concentration of 2 µg/ml)

Extraits	% moyen inhibition ± Ecart-type			Paramètre statistique d'ANOVA		
				dl	*F*	*P*
G. Sepium	74,569	±	0,6096[a]	2	122,5	<0,01
O. gratissimum	45,6112	±	3,1324[b]			
X. aethiopica	44,5247	±	2,0099[b]			

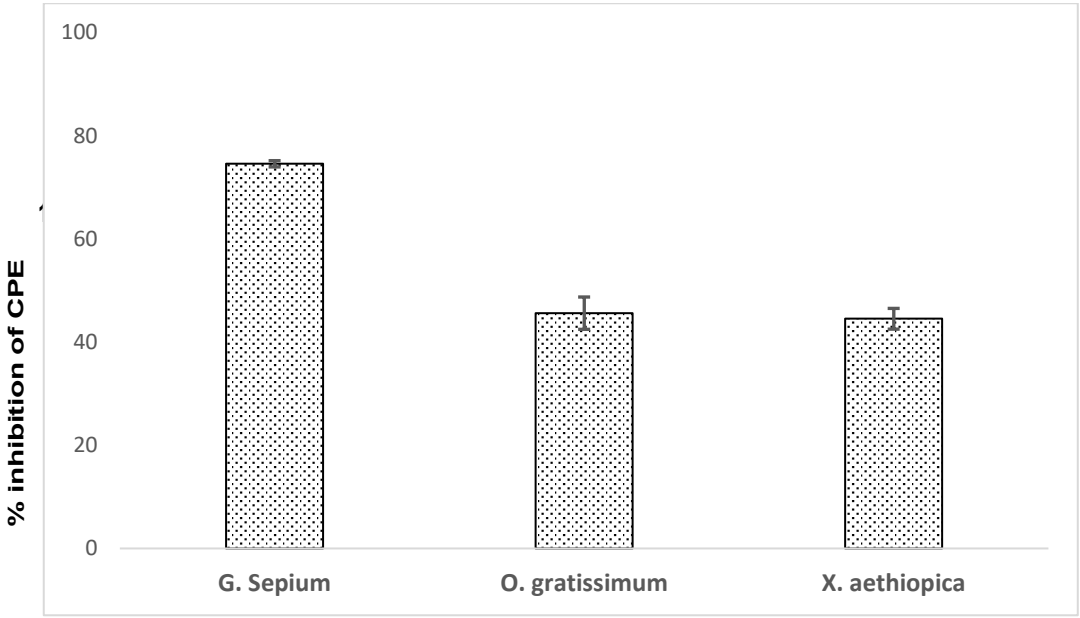

Fig 3: Inhibition of the PCE of poliovirus 1 in the presence of aqueous extracts of *G. sepium*, *O. gratissimum* and *X. aethiopica*

several secondary metabolites present in the extracts of the leaves of *G. sepium, O. gratissimum* and the fruit of *X. aethiopica*. The aqueous extraction yields revealed that *G. sepium* has a higher yield (13.01%) followed by *O. Gratissimum* (10.61%) and *X. aethiopica* (9.81%). This means that *G. sepium* could be richer in water-extractable metabolites because a link exist between extraction yields and biological activities (21).

In addition, the three extracts qualitatively contain polyphenols, flavonoids, catechetical tannins, saponins, quinones, sterols and polyterpenes. However, the lack of gallic tannins in the three extracts and alkaloids in the extract of *O. gratissimum* could indicate a lack of affinity with the extraction solvent. Recent work has indicated the presence of polyphenols, tannins and flavonoids in the aqueous extract of leaves of *O. gratissimum* (22), and the works of Okwu and Omodamiro (23) revealed the presence of tannins, alkaloids, flavonoids, saponins and polyphenols in the fruits of *X. aethiopica.* The polyphenol content of the *G. sepium* extract (0.13±0.02 mg EAG/g ES) was significantly higher than that of *O. gratissimum* and *X. aethiopica* (0.08 ±0.003 mg EAG/g ES and 0.05±0.01 mg EAG/g ES, respectively). Some researchers have reported that polyphenols are endowed with antioxidant capacity (24), but in addition, polyphenols, in particular flavonoids, are endowed with antiviral properties (25).

No cytotoxicity of the three plant extracts (at concentrations ranging from 2 µg/ml to 1 mg/ml) was seen on L20B cell (a genetically modified mouse cell line). Indeed, researchers have considered as active a plant extract which antiviral activity is detectable in at least two subsequent dilutions of the maximum non-toxic concentration in order to ensure that the activity is not directly correlated with the toxicity of the extract (26). The antiviral efficacy of the extract of *G. sepium* (at a concentration of 2 µg/ml resulted in a rate of inhibition of 74% of the cytopathic effect. As non-enveloped viruses have indeed been reported in the literature to exhibit high intrinsic resistance because of their structure (27), this result indicates a promising activity

of the extract of *G. sepium* in the development of plant antivirals.

Our study constitutes an avenue of investigation into the use of these types of antiviral drugs in the face of the phenomenon of microbial resistance to antimicrobial agents. In addition, previous study has revealed that polyphenols derived from plants, particularly flavonoids, act effectively on various viruses such as poliovirus, respiratory syncytial virus (RSV) and herpes simplex virus (HSV) (28). However, further study is needed to elucidate the mechanism of action of *G. sepium* extract. The extracts of *O. gratissimum* and *X. aethiopica* for their part inhibited the cytopathic effect of poliovirus 1 by 45.6112 and 44. 5247% respectively. These levels, although being moderate, constitute an avenue for further exploration study of these two extracts plants on viruses.

Conclusion:

The antiviral activity of three medicinal plants selected from the Ivorian flora against poliovirus 1 was reported in the present study. The extract of *G. sepium* showed potent antiviral activity against poliovirus 1 than that of *O. gratissimum* and *X. aethiopica*. This was justified by its higher polyphenol content than the two extracts. Further research is needed to elucidate their mechanism of action of the extract by *G. sepium*. To our knowledge, this is the first report on the antiviral activity of *G. sepium*, *O. gratissimum* and *X. aethiopica* following the ethnobotanical survey carried out on the plants.

Conflicts of interests:

No conflict of interest is declared.

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