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Research Article

Evaluation of the antiviral activity of different extracts of *Gliricidia sepium* and *Xylopia aethiopica* on enteroviruses isolated in Côte d'Ivoire

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Abstract

Objective : The emergence of viral infections has highlighted the need for new antiviral molecules. This study is part of an exploration into finding new antiviral compounds from medicinal plants used in traditional pharmacopoeia in Côte d'Ivoire. The aim is to determine which of the extracts from Gliricidia sepium leaves and Xylopia aethiopica fruits (hydroalcoholic, hexane/water, ethyl acetate/water, aqueous) exhibits the best antiviral activity.

Methodology: The cytotoxicity of each partition P1 (hydroalcoholic), P2 (hexane/water), P3 (ethyl acetate/water), P4 (aqueous) of Gliricidia sepium leaves and P1' (hydroalcoholic), P2' (hexane/water), P3' (ethyl acetate/water), P4' (aqueous) of Xylopia aethiopica fruits is determined on RD cells. The antiviral activity of these partitions was then evaluated against a type 1 enterovirus.

Results: The hexane-water partition showed high antiviral activity against type 1 enterovirus, with an inhibition of 65.78±9.21% at a concentration of 1 µg/mL for Gliricidia sepium leaves, compared to a non-significant inhibition of 86.84±5.26% at 31.25 µg/mL for Xylopia aethiopica fruits. Additionally, the aqueous partition of Gliricidia sepium induced an inhibition of 50±0.01% at a concentration of 1.95 µg/mL.

Conclusion: Hexane-water and aqueous partitions of Gliricidia sepium showed potent antiviral activity against enterovirus 1 than those of Xylopia. aethiopica.

Keywords: plant partition, RD cells, enterovirus type 1.

INTRODUCTION

Combating viral infections represents a constant challenge in the medical field, due to the ability of viruses to evolve and develop resistance to available treatments [1]. Faced with this complexity, the World Health Organization (WHO) recommends exploring traditional medicine as a potential source of new antiviral agents. This approach has led to a growing interest in medicinal plants, recognized for their wealth of bioactive compounds with antiviral properties [2, 3, 4].

In the Third World in general, and in Africa in particular, populations rely on traditherapy, whose recipes have been used to alleviate certain illnesses. Numerous plant species, including 50,000 in Africa [5] and 1,421 in Côte d'Ivoire [6], are prescribed in medicinal recipes for the treatment of many ailments.

Since the 1990s, the use of medicinal plant extracts and their pharmaceutical derivatives has generated significant interest in the search for solutions to viral infections. Gliricidia sepium and Xylopia aethiopica are among the 61 species used to treat viral diseases sold on markets in the Abidjan district [7]. Their respective leaves and fruits are used in decoctions to treat fever. Scientific studies have shown a viral inhibition rate of 74.57% with Gliricidia sepium and 44.52% with Xylopia aethiopica on poliovirus 1 [8].

Following on from previous studies, the antiviral properties of the hydroalcoholic, hexane/water, ethyl acetate/water and aqueous fractions of Gliricidia sepium leaves and Xylopia aethiopica fruits will be tested. Specifically, the cytotoxicity of the different fractions on RD cells and their antiviral activity against enterovirus type 1 will be evaluated.

MATERIALS AND METHODS

Material

The plant material used for this study consists of Gliricidia sepium leaves and Xylopia aethiopica fruits.

The leaves of Gliricidia sepium were harvested during October 2021 at the Centre National de Floristique (CNF) of the Université Félix Houphouët-Boigny (see Figure 1), while the fruits of Xylopia aethiopica were collected in the Azaguié region of Côte d'Ivoire (see Figure 2). After harvesting, the leaves and fruits were carefully washed, cut into small pieces and dried in the sun for two weeks before being reduced to a fine powder. Plant identification was carried out at the national floristics center (CNF).

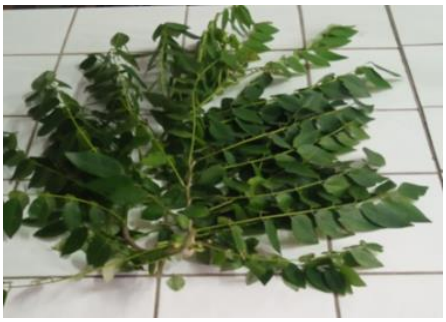

Figure 1 : Feuilles de *Gliricidia. Sepium* (Jacq.) Kunth ex Walp

Figure 2 : Fruits de *Xylopia aethiopica* (Dunal) A. Rich

Methods

1) Preparation of extracts

1-1) Hydroalcoholic extracts

The crude extract was prepared from the powders of Gliricidia. sepium leaves and Xylopia. aethiopica fruits according to the method of Zirihi et al [9]. To this end, 100 g of powder from each plant organ was extracted separately in 1000 mL of a solvent containing 70% ethanol and 30% distilled water. Each mixture was homogenized in a blender. The total homogenate from each extraction was filtered once on a sieve. The resulting mixture containing plant debris was filtered a second time using a square of clean white cloth, then filtered three (03) times on absorbent cotton and once (01) on filter paper. The final filtrate is oven-dried at 45°C. After drying, the hydroalcoholic extracts from Gliricidia. sepium leaf and Xylopia. aethiopica fruit powders are stored in a refrigerator.

1-2) Hexane-water extracts

Hydroalcoholic extracts were partitioned by maceration in hexane-water (immiscible solvent). Extraction was carried out at room temperature. Partitioning involved progressively dissolving 20 grams of hydro-ethanol extract in 200 mL of distilled water. During stirring, 200 mL of hexane were added to the previous mixture. This heterogeneous mixture was hermetically sealed and left on the magnetic stirrer for 24 hours. The mixture was then transferred to a separating funnel to separate the two phases of the solution [10,11]At the end of phase partitioning, we obtained the hexane-water extract.

1-3) Ethyl acetate-water extracts

Hexane-water extracts were partitioned by maceration. After concentration of the different phases of the hexane-water mixture, the aqueous fraction was partitioned again using an ethyl acetate-water mixture (50/50; v/v). To the aqueous phase obtained, 200mL of ethyl acetate was added. This heterogeneous mixture was hermetically sealed and left on the magnetic stirrer for 24 hours. The mixture was then transferred to a separating funnel for separation of the two phases of the solution [10,11]. Ethyl acetate extract and aqueous extract were obtained at the end of the phase partition, ethyl acetate-water extract.

1-4) Aqueous extracts

The aqueous extract was obtained after immiscible solvent partitions (ethyl acetate-water) [10,11].

2) Study of the cytotoxicity of plant substance fractions

2-1) Determination of the cytotoxicity of hydroalcoholic extracts on RD cells

Stock solutions of hydroalcoholic extracts were prepared in 1% dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. Next, 1/2 ratio serial dilutions of concentrations ranging from 1000µg/mL to 1 µg/mL were obtained as before. These different extract concentrations were respectively contacted with cells grown in 96-well plates (2.5*106 cells/well). These tests were carried out in triplicate for each concentration. This resulted in a total number of 33(thirty-three) samples for each extract. In addition, cells with maintenance medium alone were used as negative controls, while cells in contact with Triton served as positive controls. Concentrations with no cytotoxic effect on the cells were used for the antiviral activity test.

2-2) Determination of cytotoxicity of hexane-water extracts on RD cells

Stock solutions of hexane-water extracts were prepared in 1% dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. Next, 1/2 ratio serial dilutions of concentrations ranging from 1000µg/mL to 1 µg/mL were obtained as before. These different extract concentrations were respectively contacted with cells grown in 96-well plates (2.5*106 cells/well). These assays were carried out in triplicate for each concentration, resulting in a total number of 33 samples for each extract. In addition, cells with maintenance medium alone were used as negative controls, while cells contacted with Triton served as

positive controls. Concentrations with no cytotoxic effect on cells will be tested for antiviral activity.

2-3) Determination of the cytotoxicity of ethyl acetate-water extracts on RD cells

Stock solutions of ethyl acetate-water extracts were prepared in 1% dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. Next, 1/2 ratio serial dilutions of concentrations ranging from 1000µg/mL to 1 µg/mL were obtained as before. These different extract concentrations were respectively contacted with cells grown in 96-well plates (at 2.5*106 cells/well). These assays were performed in triplicate for each concentration, resulting in a total number of 33 samples for each extract. In addition, cells with maintenance medium alone were used as negative controls, while cells contacted with Triton served as positive controls. Concentrations with no cytotoxic effect on the cells will be tested for antiviral activity.

2-4) Determination of the cytotoxicity of aqueous extracts on RD cells

Stock solutions of aqueous extracts were prepared in 1% dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. Next, 1/2 ratio serial dilutions of concentrations ranging from 1000µg/mL to 1 µg/mL were obtained as before. These different extract concentrations were respectively contacted with cells grown in 96-well plates (2.5*106 cells/well). These assays were carried out in triplicate for each concentration, resulting in a total number of 33 samples for each extract. In addition, cells with maintenance medium alone were used as negative controls, while cells contacted with Triton served as positive controls. Concentrations with no cytotoxic effect on the cells will be tested for antiviral activity.

3) Antiviral test

3-1) Determination of antiviral activity of hydroalcoholic extracts

The antiviral properties of hydroalcoholic extracts of Gliricidia sepium leaves and Xylopia aethiopica fruits were determined by a cytopathic effect inhibition (CPE) assay. RD cells were infected with an enterovirus suspension (S1) of 50 µL/well, and incubated for 1. 5 h at 37°C. An aliquot of each of the thirty-three sample concentrations of hydroalcoholic extracts (C0=1000 µg/mL; C1= 500 µg/mL; C2= 250 µg/mL; C3= 125 µg/mL ; C4= 62.5 µg/mL; C5=31.25 µg/mL; C6=15.625 µg/mL; C7=7.8125 µg/mL; C8=3.9625 µg/mL; C9=1.95 µg/mL; C10 =1 µg/mL) was then added to the cells. Cells were re-incubated for 3 days. These tests were performed in triplicate for repeatability. The occurrence of total CPE was then observed relative to the positive control. Absorbance was also measured with a microplate reader at 450 nm to determine correlation. Finally, inhibition of viral infection at different concentrations of hydroalcoholic extracts was calculated using the formula below:

$$\text{ECP inhibition \%} = (A\,t - Ap) / (An - Ap) \times 100$$

An: Average uptake of negative control (without virus and natural compound)

Ap : Average uptake of positive control (without natural compound)

At : Average absorbance of test sample

3-2) Determination of the antiviral activity of hexane-water extracts

The antiviral properties of hexane-water extracts of Gliricidia sepium leaves and Xylopia aethiopica fruits were determined by a cytopathic effect inhibition (CPE) test using the same procedure as for hydroalcoholic extracts.

3-3) Determination of the antiviral activity of ethyl acetate-water extracts

The antiviral properties of ethyl acetate-water extracts of Gliricidia sepium leaves and Xylopia aethiopica fruits were determined by a cytopathic effect inhibition (CPE) test using the same procedure as for hydroalcoholic extracts.

3-4) Determination of the antiviral activity of aqueous extracts

The antiviral properties of aqueous extracts of Gliricidia sepium leaves and Xylopia aethiopica fruits were determined by a cytopathic effect inhibition (CPE) test, using the same procedure as for hydroalcoholic extracts.

RESULTS AND DISCUSSION

Results

1) Extraction yields

Extraction yields from different partitions are summarized in percentage terms in the table below.

Table I: Extraction yields for the various partitions

	Derived extracts	Mass of partitions (g)	Percentage
Powder of leaves of Gliricidia sepium:	P1 : Hydroalcoholic 70%	200	18.46
	P2 : Hexane-water phase (100%)	2.78	13.9
	P3 : Ethyl acetate-water phase (100%)	0.50	2.5
	P4 : Aqueous phase (100%)	15.25	76.25
Fruit powder of Xylopia aethiopica	P1 : hydroalcoholic 70%	200	14.44
	P2 : Hexane-water phase (100%)	13.80	69
	P3 : Ethyl acetate-water phase (100%)	0.40	2
	P4 : Aqueous phase (100%)	4.61	23.05

The highest yields were observed with the aqueous phase (76.25%) for Gliricidia sepium leaf powder and with the Hexane-water phase (69%) for Xylopia aethiopica fruit powder.

2) Cytotoxic activity of fractions on RD cells

After 72 hours of interaction with RD cells, the cell carpet remained intact for the hydroalcoholic extract of X. aethiopica at concentrations ranging from 1 µg/mL to 500 µg/mL. However, at the concentration of 1000 µg/mL, damage to the cell mat was observed (Figure 3).

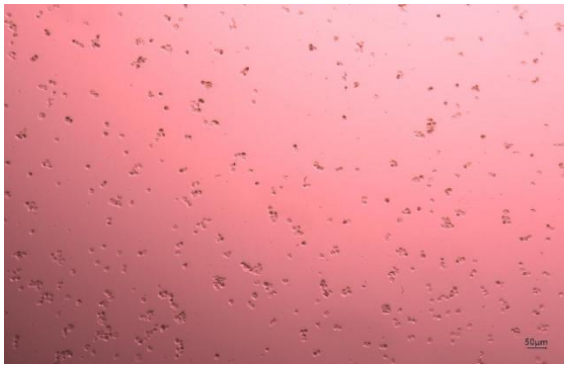

Figure 3: Intact mat observed after 72h incubation of X. aethiopica hydroalcoholic extract (500 µg/mL).

At concentrations ranging from 1 µg/mL to 500 µg/mL of Xylopia aethiopica hydroalcoholic extract, the cell mat remained intact after 72 hours of contact with RD cells. At 1000 µg/mL, however, the cell mat was destroyed.

In the case of Gliricidia. sepium hydroalcoholic extract, the cell mat remained intact over a concentration range from 1 µg/mL to 250 µg/mL after 72 hours of contact with RD cells. Above 250 µg/mL up to 1000 µg/mL, cell mat degradation was observed.

For the hexane-water extract of Xylopia. aethiopica, the cell mat remained intact over a concentration range from 1 µg/mL to 15.62 µg/mL after 72 hours of contact with RD cells. However, at higher concentrations ranging from 31.25 µg/mL to 1000 µg/mL, cell mat degradation was observed.

In the hexane-water extract of Gliricidia. sepium, the cell mat remained intact for concentrations ranging from 1 µg/mL to 31.25 µg/mL after 72 h of contact with RD cells. However, damage was observed for concentrations ranging from 62.5 µg/mL to 1000 µg/mL.

Concerning ethyl acetate-water extracts of Xylopia. aethiopica and Gliricidia. sepium, the cell mat remained intact over concentration range from 1 µg/mL to 15.62 µg/mL after 72 hours of contact with RD cells. However, damage was observed for concentrations ranging from 31.25 µg/mL to 1000 µg/mL.

Finally, for the aqueous extract of Gliricidia. sepium, the cell mat remained intact for concentrations ranging from 1 µg/mL to 500 µg/mL after 72 hours of contact with RD cells. However, a concentration of 1000 µg/mL resulted in damage to the cell mat. In contrast, the cell mat of the aqueous extract of Xylopia. aethiopica remained intact from concentrations ranging from 1 µg/mL to 1000 µg/mL (Figure 4).

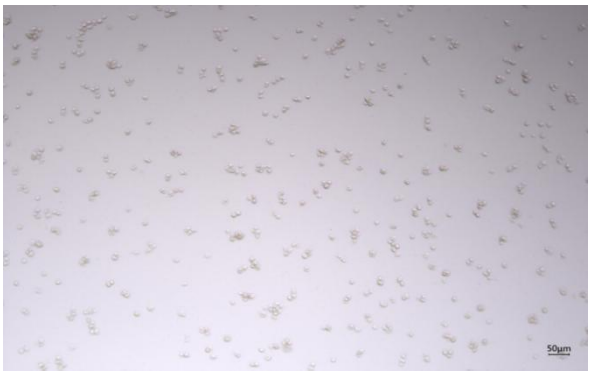

Figure 4: Damage observed after 72h incubation of Gliricidia. sepium hydroalcoholic extract (1000 µg/mL)

Microscopic observation of the different mats 72 hours after administration of concentrations of Xylopia aethiopica and Gliricidia sepium extracts to the RD cell is summarized in the table below.

Table II: Cytotoxicity of Xylopia aethiopica and Gliricidia sepium extracts on the RD cell after 72h

Extracts	Solvents	Concentration (µg /mL)											Negative Control	Positive Control
		1000	500	250	125	62.5	31.25	15.62	7.81	3.9	1.95	1		
Xylopia aethiopica	Hydroalcoholic	+	-	-	-	-	-	-	-	-	-	-	-	+
	Hexane-water	+	+	+	+	+	+/-	-	-	-	-	-	-	+
	Ethyl acetate-water	+	+	+	+	+	+/-	-	-	-	-	-	-	+
	aqueous	+	-	-	-	-	-	-	-	-	-	-	-	+
Gliricidia sepium	*Hydroalcoholic*	+	+	+/-	-	-	-	-	-	-	-	-	-	+
	Hexane-water	+	+	+	+	+	-	-	-	-	-	-	-	+
	Ethyl acetate-water	+	+	+	+	+	+	-	-	-	-	-	-	+
	aqueous	-	-	-	-	-	-	-	-	-	-	-	-	+

+ : cytotoxic concentration - : non-cytotoxic concentration

After 72 h of incubation, the highest non-cytotoxic concentration for each extract on RD cells resulting from the different observations is summarized in the table below.

Table III: Highest non-cytotoxic concentration of extracts on RD cells

Plant extracts	Solvents	Highest non-cytotoxic concentration in ug/mL
Xylopia aethiopica	Hydroalcoholic	500
	Hexane-water	15.62
	Ethyl-acetate -water	15.62
	Aqueous	500
Gliricidia sepium	Hydroalcoholic	125
	Hexane-eau	31.25
	Ethyl-acétate water	15.62
	Aqueous	1000

3) Antiviral activity of different extracts on enterovirus

After an incubation period of 72 h at 37°C, the hydroalcoholic partition of Xylopia. aethiopica caused a maximum inhibition of 15±1.25% of enterovirus 1 at the concentration of 500 µg/ml, while the hydroalcoholic partition of Gliricidia. sepium caused an inhibition of 27.5±3.75% at the concentration of 125 µg/ml.

The hexane-water fraction of Xylopia. aethiopica caused 86.84±5.26% inhibition of enterovirus 1 at a concentration of 31.25µg/ml, after 72 h incubation at 37°C while that of Gliricidia. sepium caused 65.78±9.21% inhibition at a concentration of 1 µg/ml.

After 72 h incubation at 37°C, the ethyl-water partition of Xylopia. aethiopica caused 36.36±0.1% inhibition at the concentration 15.62 µg/ml while that of Gliricidia. sepium caused 27.27±0.01% inhibition at the concentration 1.95µg/ml.

Finally, the aqueous fraction of Xylopia. aethiopica caused 40±0.02% inhibition at concentration 125µg/ml after 72 hours incubation at 37°C, while the aqueous partition of Gliricidia. sepium caused 50±0.01% inhibition at concentration 1.95µg/ml.

Discussion

The extraction yield by partition reveals that the aqueous extract has a high yield (76.25%) for *Gliricidia sepium*, while the hexane-water extract (69%) has a high yield for *Xylopia aethiopica*. These results indicate that the aqueous extract of *Gliricidia sepium* and the hexane-water extract of *Xylopia aethiopica* are richest in secondary metabolites, as there is a correlation between extraction yield and biological activities according to Sunday et al. [12].

Cytotoxicity testing of fractions of both plant species on RD cells indicated that they do not have the same highest non-toxic concentrations on said cells. Apart from the aqueous partition of Gliricidia sepium, the fractionations carried out on the species led to an increase in cytotoxic activity[8]. Referring to the work carried out by Riss et al.[13], the quantity of cells killed (cytotoxicity) could be measured by detecting these two processes, namely the quantity of living cells (viability) and cell death (apoptosis).

According to Bouagnon et al.[8], the antiviral efficacy of the whole *G. sepium* extract (at a concentration of 2 µg/mL) resulted in a 74% inhibition of the cytopathic effect, compared to its hexane and aqueous partitions, which caused 65.78±9.21% and 50±0.01% inhibition at concentrations of 1 µg/mL and 2 µg/mL, respectively. This effectiveness could be attributed to the numerous antioxidants present in the aqueous whole extract compared to its fractions[14].

The 86.84±5.26% inhibition caused by the hexane-water partition of *X. aethiopica* on enterovirus type 1 (at a concentration of 31.25 µg/mL) is not significant. However, previous work has shown a moderate inhibition of the whole *X. aethiopica* extract of 44.52% (at a concentration of 2 µg/mL) on poliovirus type 1. Further exploration on enveloped viruses would be warranted based on previous studies that revealed flavonoids derived from plants effectively act against respiratory syncytial virus (RSV) and herpes simplex virus (HSV)[15]. Additionally, according to Kahn et al.[16], polyphenols could have antiviral activities.

CONCLUSION

Following a previous study showing superior antiviral activity of aqueous extracts of Gliricidia sepium leaves to that of Xylopia aethiopica fruits on poliovirus 1, hexane-water and aqueous partitions of Gliricidia sepium showed superior inhibition on enterovirus 1 to those of Xylopia aethiopica partitions. Further work will be needed to assess the antiviral activity of totums and their partitions on enveloped viruses, in order to advise on the best use in traditional environments.

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Conflict of Interest

There are no conflicts of interest declared by the authors

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