

Evaluation of the Antibacterial Activity of *Jatropha multifida* sap and *Artemisia annua* Extract on some Clinical Strains Responsible of Urinary Tract Infections

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Abstract

Objectives: The present study aims to evaluate the antibacterial activity of *Jatropha multifida* sap and aqueous extract of *Artemisia annua* leaves on some clinical strains responsible of urinary tract infection. **Methods:** The bacterial strains were isolated after the cyto-bacteriological examination of the collected urine. The agar and liquid diffusion methods were used for the sensitivity test and the determination of the Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts on isolated strains. **Findings:** Ten bacterial strains isolated from the urine samples were collected. Isolated bacteria exhibited various resistant profiles. As for the antibacterial activity evaluated on the leaves and sap collected, the aqueous extract of the leaves of *Artemisia annua* had no effect on the strains studied. The MICs and CMBs of *Jatropha multifida* sap vary according to the bacterial strains studied. Thus, the sap of *Jatropha multifida* has a bactericidal activity on all strains studied with the exception of the strain of *Escherichia coli* on which its action is more moderate. At the end of the work, the most isolated bacterial species are respectively *Citrobacter freundii* (40%), *Staphylococcus aureus* (30%) and *Klebsiella pneumoniae* (20%). All isolated clinical strains are multiresistant. The aqueous extract of *Artemisia annua* at 600 mg/ml showed no antimicrobial activity on the strains studied. **Improvements:** The sap of *Jatropha multifida* shows a bactericidal activity against the various strains tested and could therefore be an alternative to antibiotics following transformations into improved traditional medicines.

Keywords: Antibacterial Activity, *Artemisia Annua*, Clinical Strains, *Jatropha Multifida*, Urinary Tract Infections

1. Introduction

Urinary tract infections can affect one or more parts

of the urinary system: Kidneys, ureters, bladder and urethra. They are most often manifested by pain or a

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burning sensation when urinating (the emission of urine), sometimes by abdominal pain and fever¹. The most commonly involved bacteria are *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa* and *Staphylococci* in descending order². In more than 80% of urinary tract infections, the causative organism is an intestinal *Escherichia coli*³. As for *Staphylococcus aureus*, it is often diagnosed at an advanced stage of genitourinary infections (43%)⁴.

Antibiotic therapy is the method used to treat this infection. But nowadays, bacterial resistance to antibiotics becomes recurrent and poses a global public health problem. The overall resistance rate of hospital and community enterobacterial strains to amoxicillin associated with the beta-lactamase, quinolone, fluoroquinolone, sulfamethoxazole + trimethoprim and nitrofurantoin inhibitors is high⁵.

In Africa and Asia, 80% of the population continues to use traditional medicines rather than so-called modern medicines for primary health care. The widespread use of traditional medicine in developing countries is due to the availability of plant resources used and often affordable. Healthcare professionals and the general public worldwide are struggling with the issues of safety, efficacy, quality, availability, preservation and future development of this type of health care⁶. Thus, many medicinal plants are used in traditional medicine among which include *Jatropha multifida* and *Artemisia annua*. *Jatropha multifida* with highly recognized medicinal properties is used to treat various diseases in Africa, Asia or Latin America⁷. The antibacterial effect of leaves, stems and roots has been reported in various studies⁷⁻⁹. The prescription of its juice in the management of oral candidiasis is a common practice among rural residents of western Nigeria. In Chinese medicine, the bark and leaves are used against itchy skin and eczema¹⁰. *Jatropha multifida* Linn. is one of the medicinal plants whose sap is widely used for healing wounds in Benin¹¹. As for *Artemisia annua* L. (Asteraceae), has been used for centuries in traditional Asian medicine for the treatment and prevention of fever and chills. Antioxidant, anti-inflammatory and antimicrobial

properties of leaf extracts of *Artemisia annua* have been reported¹².

It is in order to find a natural alternative, an affordable solution, accessible to all and effective to fight the resistance of the bacteria responsible for urinary tract infections that the present work is initiated. Thus, the general objective of this study was to evaluate the antibacterial activity of extracts of *Jatropha multifida* and *Artemisia annua* on some clinical strains incriminated in urinary tract infections.

2. Material

2.1 Plant Material

The plant material consists of the sap of *Jatropha multifida* Linn which was collected in the commune of Abomey-Calavi. Sampling was done directly in the sterile tubes after cutting branches of *Jatropha multifida* Linn and then stored at 4°C in the refrigerator.

With regard to *Artemisia annua*, the dried leaves and stems of the plant packaged and sold commercially by the house of Artemisia were used.

2.2 Biological Material

It consists of 45 urine samples of patients collected for two weeks at the Allada Zone Hospital Laboratory, Menontin Area Hospital and Calavi Area Hospital. All of these patients had come to the laboratory for reasons of infection etiology. Two reference strains (*Escherichia coli* ATCC 2552 and *Staphylococcus aureus* ATCC 2552) provided by the laboratory of U.R.M.A.Pha were also exploited.

2.3 Other Material and Equipment

The usual culture media in bacteriology have been used for culture and isolation of bacteria. Reagents and dyes such as: Kovacs reagent, gentian violet, lugol, 90° alcohol, fuschine solution diluted 1/10, methylene blue, hydrogen peroxide and fresh plasma rabbit were used to identify bacteria.

Several equipment were also used namely: The optical microscope, the refrigerator, the oven, the autoclave, the poupinel, the centrifuge, the balance of precision, the bunsen burner and the rack. Sterile Petri dishes, hemolysis tubes and sterile screw tubes, TPHA plates, swabs, antibiotic discs, anaerobiosis jar and gloves.

3. Methods

3.1 Determination of the Resistance Profile of Clinical Strains Isolated from Urinary Tract Infections

The prospective study for analytical purposes was conducted over a period of one month from March 18 to April 18, 2019. It took into account the cytobacteriological examination of 45 urine samples collected from patients received during the period collection. The bacteriological manipulations were carried out in four days. The first day was devoted to the reception of biological samples, the identification of samples, the macroscopic examination, the microscopic examination and the cultivation. The reading of the seeded media, the realization of Gram control and isolation for obtaining pure strain followed on the second day. On the third day, the identification at the classic Leminor gallery and the antibiogram were realized then read on the fourth day.

3.2 Antibacterial Activity of *Jatropha multifida* sap and *Artemisia annua* Leaf Extract on some Clinical Strains Responsible for Urinary Tract Infection

The sap of *Jatropha multifida* Linn has been directly tested on isolated strains. As for *Artemisia annua*, the aqueous extract of the leaves and stems of the plant was prepared and tested on isolated strains. The total aqueous extracts were obtained by an adaptation of the method developed by¹³. Fifty (50) grams of powder was macerated in 500 ml of distilled water. The mixture is stirred continuously (Stuart Bioblock Scientific Fisher shaker) for 72 hours at room temperature. The homogenate obtained was filtered

three times on hydrophilic cotton and once on Wattman No. 1 paper. This filtrate was then dried at 45°C. in the oven. The powder thus obtained is the total aqueous extract used.

3.3 Preparation of Extracts

The aqueous extract of *Artemisia annua* was taken up in distilled water at a rate of 100 mg per 1 ml. The stock solutions thus concentrated to 100 mg/ml were then autoclaved at 121°C. for 15 minutes. Sterility of stock extract solutions was verified by inoculating aliquots of each solution onto Mueller Hinton medium and incubated at 37°C for 24 hours. The absence of colonies on the Mueller Hinton medium after 48 hours confirmed the sterility of these mother extracts solutions. Regarding *Jatropha multifida*, sap was used. The concentration of the whole sap of this plant has been estimated at 320 mg/ml according to the work done by¹¹ on the exploration of the antibacterial properties and the healing power of *Jatropha multifida* Linn. sap in the rat Wistar.

The yield of the crude extract is defined as the ratio between the mass of the dry extract obtained and the mass of the treated plant material. This yield was calculated via the equation: $R (\%) = Me/Mv \times 100$ with R (%): Yield, Me: Mass of the extract after the evaporation of the solvent and Mv: Mass of the plant material used for extraction.

3.4 Test of Antimicrobial Activity carried out on Agar by the Diffusion Method

A bacterial preculture (1 colony in 5 ml of sterile distilled water) of 18 h was diluted to obtain a turbidity of 0.5 on the McFarland scale (ie 108 CFU/ml). Each inoculum was seeded by swabbing onto petri dishes containing Mueller Hinton agar (CA-SFM, 2012). Using the sterile pasteur pipette tip 6 mm diameter wells were dug. Then using a sterile cone and a micropipette 50 µl of each extract was deposited in previously dug wells. A well containing sterile distilled water served as a negative control. Standard antibiotic discs have also been used to serve as positive controls. The dishes were left for 15 minutes at room temperature for pre-diffusion before being incubated

Table 1. Standard used for reading the results of antibiogram tests of plant extracts

Inhibition zone diameter (Δ)	Degree of sensitivity of the germ	Symbol
$\Delta < 7\text{mm}$	Insensitive	-
$7\text{mm} \leq \Delta < 8\text{mm}$	Sensitive	+
$8\text{mm} \leq \Delta < 9\text{mm}$	Pretty sensitive	++
$\Delta \geq 9\text{mm}$	Very sensitive	+++

Δ : Inhibition zone diameter

- : insensitive

+ : Sensitive

++ : Pretty sensitive

+++ : Very sensitive

Source: WHO, 2002; Tsirinirindravo and Andrianarisoa, 2009

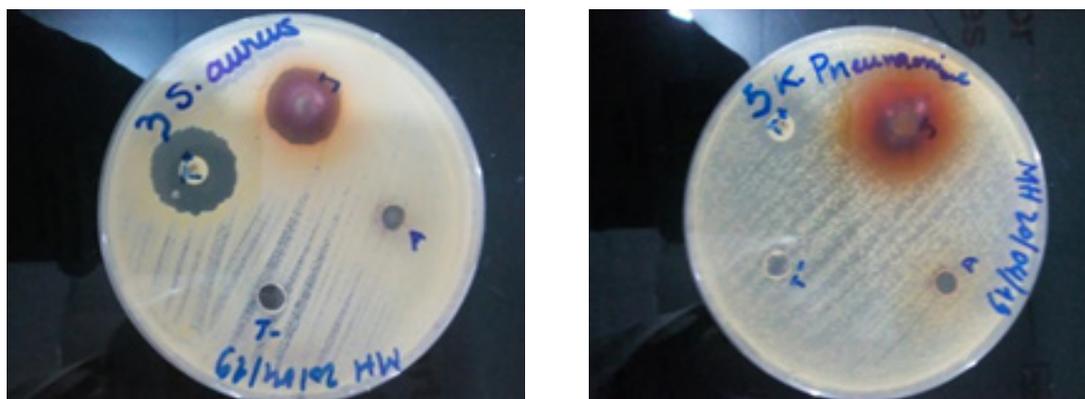


Image 1. Determination of inhibition diameter .



Image 2. Determination of inhibition diameters of *Artemisia annua*.

at 37°C for 24 hours. After the incubation period, the dishes were examined to record any zones of inhibition

(diameter measured in mm) (Table 1). All tests were done in duplicate. (Images 1, 2).

3.5 Determination of the Minimal Inhibitory Concentration (MIC) by Micro Dilution and the Minimal Bactericidal Concentration (CMB)

An extract stock solution was prepared at the concentration of 100 mg/ml in distilled water. 100 µl of Mueller-Hinton Broth medium (MHB) were placed in each well of the microplate (wells 1 to 8). 100 µl of the extract stock solution were deposited in the first well. After homogenization by suction-discharge using a micropipette, 200 µl of a solution of extract at 100 mg/ml are obtained. 100 µl of this new solution were taken and mixed with the MHB medium contained in the 2nd well and this series of dilution of reason 2 from wells to wells is continued until the 6th well, from which the 100 µl are discarded. Finally 100 µl of the bacterial suspension were added to each well. The 7th and 8th wells were respectively the positive control and the negative control and contain 100 µl of MHB + 100 µl of bacterial suspension for the positive control and 100 µl of MHB for the negative control. The microplates were covered for 24 hours in an oven at 37°C.

MICs were estimated visually compared to controls. As for the CMB, it was determined after seeding the contents of each well on the MH agar and incubated at 37°C for 24 hours. CMB was the lowest concentration of extract for which there were no bacterial colonies.

3.6 Data Processing and Analysis

The collection of information and the analysis of the tables were carried out using the Excel 2013 software. The graphs were made with the Graph Pad software. Descriptive statistics were performed using SPSS 20.ng.

4. Results

4.1 Bacterial Profile of Strains Isolated from Urine Samples

Of 45 samples collected, 10 were positive (22.22%) and 35 negative (77.78%). The majority of the samples came from women (78%), of which 20% were positive and

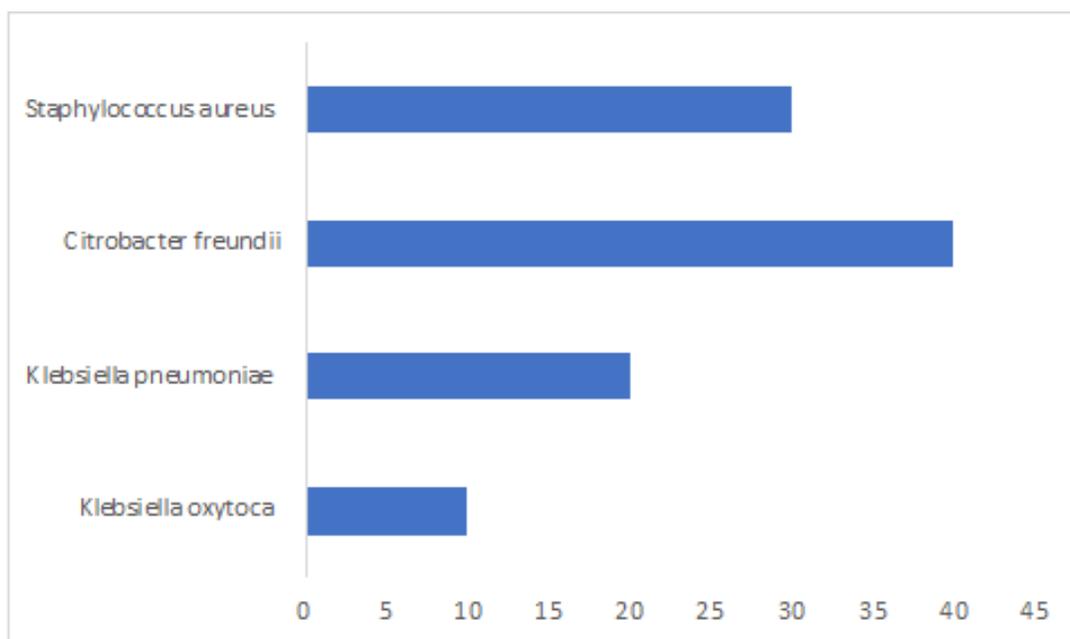


Figure 1. Frequencies of isolated microorganisms.

Table 2. Resistance profile of isolated bacterial strains

Strains		Resistance profile
Klebsiella oxytoca	1	AMC ^R NFE ^S CRO ^R COX ^R GMN ^R CIP ^R FAD ^R
Citrobacter freundii	1	AMC ^S COX ^S CRO ^S GMN ^S NFE ^R CIP ^R SXT ^R
	2	AMC ^R COX ^R CRO ^S GMN ^R NFE ^S SXT ^R CIP ^S FAD ^R
	3	AMC ^R GMN ^S CHL ^R CIP ^S COX ^R CRO ^S FAD ^R
	4	AMC ^R NFE ^S GMN ^R COX ^R CRO ^S FAD ^R CIP ^R
K. pneumoniae	1	AMC ^R NFE ^R GMN ^R CRO ^R COX ^R CIP ^R SXT ^R
	2	AMC ^S NFE ^R GMN ^S CHL ^R CIP ^S CRO ^R COX ^R
Staphylococcus aureus	1	AMP ^S OXA ^S NFE ^S GMN ^R ERY ^S LCN ^S FOX ^R CHL ^R
	2	AMP ^R AMC ^S NFE ^S GMN ^R ERY ^R LCN ^R FOX ^R CHL ^R
	3	AMP ^S AMC ^S NFE ^S GMN ^R ERY ^R LCN ^R FOX ^R CHL ^R

S = Sensitive; R= Résistant ;

AMP = Ampicillin ; AMC = Amoxicillin + clavulanicacid; NFE = Nitrofurantoin; GMN = Gentamycin; ERY = Erythromycin; LCN = Lincomycin; FOX = Cefoxitin; CHL = Chloranphenicol; CRO = Ceftriaxone; COX = Cefotaxime; GMN = Gentamicin; CIP = Ciprofloxacin; ADF = Fusidicacid;

58% negative. Only 22% of the samples came from men with 2% positive samples versus 20% negative samples. *Citrobacter freundii* was the most isolated bacterium (40%) followed by *Staphylococcus aureus* (30%) (Figure 1).

Table 2 shows the resistance profile of the isolated bacterial strains. It shows that all isolated bacterial strains have resistance to several families of antibiotics, most of which exceed three different families of antibiotics. These strains are therefore all multi-resistant (Table 2).

4.2 Antibacterial Activity of Extracts

4.2.1 Extraction Yields

The aqueous extract of the leaves and stems of *Artemisia annua* has a yield of 23.72%. The sterility tests revealed

the absence of contamination in the extract and sap of *Jatropha multifida*.

4.2.2 Sensitivity Test

The strains tested, at the concentration of 100 mg/ml showed a variable sensitivity to the extracts tested. The inhibition diameters of the extracts varied between 7 and 21 mm. The sap of *Jatropha multifida* was active on all bacterial strains tested. In contrast to the aqueous extract of *Artemisia annua* that showed no antimicrobial activity.

Increasing the concentration of the aqueous extract of *Artemisia annua* had no effect on the isolated strains including the reference strains. Thus, the aqueous extract tested at various concentrations (100, 200, 400 and 800 mg/ml) on the isolated strains and on the reference strains

Table 3. Inhibition diameter of extracts of the plants studied on isolated bacterial clinical strains

Species Plants / Bacteria	<i>C. freundii</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>C. freundii</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>C. freundii</i>	<i>C. freundii</i>	<i>K. oxytoca</i>	<i>E. coli</i> ATCC 2552	<i>S. aureus</i> ATCC 2552
SE <i>Jatropha multifida</i> (320 mg/ml/agar plate)	16	10	17	11	18	16	13	9	10	12	07	21
E. <i>Artemisia annua</i> (100 mg/ml/agar plate)	0	0	0	0	0	0	0	0	0	0	0	0

SE = Whole sap of *Jatropha multifida*; E = Aqueous extract of *Artemisia annua*

(*E. coli* ATCC2552 and *S. aureus* ATCC2552) showed no antibacterial activity (Table 3).

4.3 Minimal Inhibitory Concentration, Minimal Bactericidal Concentration and Antibiotic Potency of *Jatropha multifida* Sap

The Minimum Inhibitory Concentrations (MIC) obtained is variable depending on the types of strains. The

lowest MICs were obtained at a concentration of 40 mg. Minimum Bactericidal Concentrations (MBC) also varied with the type of susceptible strains. Antibiotic potency (a.p) was determined using the CMB/MIC ratio. The CMB/MIC ratio of the *Jatropha multifida* sap on *Klebsiella pneumoniae*, *Citrobacter freundii*, *Staphylococcus aureus* and *Klebsiella oxytoca* is less than 4. The *Jatropha multifida* sap therefore has a bactericidal effect on these strains (Table 4).

Table 4. MIC, CMB and antibiotic potency (a. p.) of the sap of *Jatropha multifida*

Extracts	Parameters	Bacterial strains											
		<i>C. freundii</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>C. freundii</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>C. freundii</i>	<i>C. freundii</i>	<i>K. oxytoca</i>	<i>E. coli</i> ATCC2552	<i>S. aureus</i> ATCC 2552
<i>Jatropha multifida</i> sap	MIC	40	-	20	-	80	40	-	40	40	80	-	40
	CMB	40	-	40	-	160	40	-	80	80	160	80	40
	p. a.	1	-	2	-	2	1	-	2	2	2	-	1

Pa = Antibiotic power; MIC = Minimum inhibitory concentration; CMB = Minimal Bactericidal Concentration

5. Discussion

The present study evaluated the antibacterial properties of plant extracts of *Artemisia annua* (leaf and stem), *Jatropha multifida* (sap) on bacterial species responsible for urinary tract infections. The study found that the most isolated bacteria are respectively *Citrobacter freundii* (40%) *Staphylococcus aureus* (30%), *Klebsiella pneumoniae* (20%), and *Klebsiella oxytoca* (10%). The resistance profile of the isolated strains shows that they are all multiresistant. The aqueous extract of leaf and stem of *Artemisia annua* showed no antibacterial activity on the strains tested regardless of the concentration. On the other hand, mainly with a minimum bactericidal concentration of 40 mg/ml, the sap of *Jatropha multifida* had a bactericidal activity on the majority of the strains tested despite their multi-resistance to the usual antibiotic disks.

The results obtained from the antibacterial activity showed that the 12 strains studied were sensitive to the sap of *J. multifida* including the two reference strains but to varying degrees. In fact, the growth of most of the strains studied was totally inhibited by the *Jatropha multifida* sap with a minimum MIC of 20 mg/ml whereas the *Escherichia coli* strain was only moderately sensitive. Indeed, the flavonoids, tannins and terpenes identified during phytochemical screening have strong antimicrobial activities^{14,15}. These compounds were responsible for the antimicrobial activity of the leaf extract of *Melastoma malabathricum* because of their antibacterial properties¹⁶.

Other authors have achieved results similar to ours by studying the antimicrobial activity of different parts of *Jatropha multifida*. Indeed, *Jatropha multifida* sap from the University of Ibadan in Nigeria has completely inhibited *S. aureus*, *E. coli* and other bacteria¹⁷. In¹⁸ 2007, aqueous and ethanolic extracts of *J. multifida* leaves were active on *E. coli* and *S. aureus*.

Similar results were noted in a study on the leaves, stems, roots of *Jatropha multifida*¹⁹. Although these results are similar, MICs differ in terms of effect on strains. In Nigeria, a MIC of 0.78 µg/ml for *S. aureus* was obtained

in 2008 with the leaves and roots of the plant (19) 16 mg/ml, 66 mg/ml and 263 mg/ml were noted respectively for *S. aureus*, *E. coli* and *K. pneumoniae* with the sap of *Jatropha multifida* in 2014²⁰. The difference in sensitivity obtained between the saps of Nigeria and Benin could be related to environmental factors (pedographic, climatic), the strains tested and the method used.

With regard to the aqueous extract of *Artemisia annua*, these results are contrary to the results obtained during the work carried out on the antibacterial, antioxidant and anti-inflammatory activity of Artemisinin extracted from *Artemisia annualinn*²¹. These results are explained by the fact that our study took into account only the aqueous extract of this plant and that the strains tested are different. The quality of the extract varies according to the solvent used during extraction²². It will be necessary to make other extracts of the plant with different solvent in addition to distilled water to better evaluate the antibacterial properties of this plant.

Medicinal plants are therefore a good alternative to antibiotics facing the multi-resistance bacterial observed today. It is very important to carry out various investigations on the properties necessary for a better qualification of these plants in order to minimize any risk to health.

6. Conclusion

At the end of this work, we can remember that the most isolated bacteria are enterobacteria including *Citrobacter freundii* and *Staphylococci*. All isolated strains show strong resistance to antibiotics. In the light of the results obtained from the antibacterial activity of the extracts, it should be remembered that the *Jatropha multifida* sap has good and well antibacterial properties on the multi-resistant strains of *K. pneumoniae*, *K. oxytaoca*, *C. freundii*, *S. aureus* isolated urine samples which is not the case for the aqueous extract of *Artemisia annua* (leaves and stems) which had no activity on isolated strains and reference strains.

It is therefore necessary to initiate additional in vitro and in vivo studies necessary to expand the database on

the antibacterial properties and toxicity of these plants. The craze for medicinal plants should shift from a mere fad to a research, investment and sustainable development perspective of aromatic and medicinal plants for health and well-being purposes.

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