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## Antibacterial pharmacochemical activity “*in vitro*” of total alkaloid extracts of *Crateva religiosa* G. forst. (Capparidaceae) versus amoxicillin + Clavulanic acid on germs responsible of human common affections

Ferdinand M Adoukpe, Thierry CM Medehouenou, John R Klotoe and Victorien T Dougnon

### Abstract

The phytochemical screening of *Crateva religiosa* G. Forst, a plant used in Benin in traditional veterinary medicine, has shown its richness in alkaloids. The objective of this study was to evaluate the antibacterial pharmacochemical activity “*in vitro*” of total alkaloids extracts of *C. religiosa* leaves and roots on pathogenic germs in comparison to Amoxicillin + clavulanic acid (AMC), a conventional broad-spectrum antibiotic. The extraction of these alkaloids was made by the Stas-Otto method followed by thin layer chromatography. Total alkaloid extracts from leaves and roots were found to be more active than AMC against species of *Staphylococcus aureus* (38), *Escherichia coli* (28), *Klebsiella pneumoniae* (26), *Streptococcus agalactiae* (23), and *Citrobacter freundii* (12) following agar-well diffusion method using two concentrations (50 mg/ml and 200 mg/ml). Total alkaloids extracts of *C. religiosa* leaves and roots (200 mg/ml) gave the largest inhibition diameters against all microorganisms tested compared to AMC. The minimum inhibitory concentration was estimated at 50 mg/ml. The results obtained confirmed the long-established antimicrobial activity of this plant and brought novelty that its alkaloids would intervene in its therapeutic effect.

**Keywords:** *Crateva religiosa*, total alkaloids, leaves and roots extracts

### 1. Introduction

*Crateva religiosa* G. Forst. is a medicinal plant of Capparidaceae family widely used in Africa and known in "Fon", one of Benin language, as "*Wontonzonzwen*" to treat painful affections [8]. It is well known for its diuretic, analgesic, anti-inflammatory, laxative, anti-oxidant, hepatoprotective, antimycotic, contraceptive, antipyretic, antilithiatic, antiemetic, antihelminthic, anti-arthritis and vesicant remarkable properties [1-7]. *C. religiosa* is used in Benin traditional veterinary medicine against bacterial infection of *Thryonomys swinderianus* (class of mammals, family of Thryonomyidae) commonly called “*agouti*” or “*kholan*” [8]. Studies also showed that raw extracts of *C. religiosa* contain sugars, alkaloids, steroids, terpenes, flavonoids, polyphenols, saponins, tannins, fibers and proteins [9-11]. This plant has been the subject of some studies in Benin. In 1986, the total extracts of *C. religiosa* leaves and roots were obtained with Soxhlet by heat via petroleum ether and methanol. These extracts have been tested on Staphylococci and Streptococci [12]. Two difficulties were encountered: 1) the non-diffusion of the ether extracts in the agar, and 2) the uncontrolled diffusion from a certain concentration of the methanolic extracts. Two years later and to solve these difficulties, Yevessin resumed extraction of the active substances from leaves and roots by aqueous decoction followed by cold desiccation for one part and nebulization for the other part. He also tested the total aqueous extracts on the same germs [13]. This study has, on the one hand revealed the presence of alkaloids, flavonoids, terpenes, sugars and steroids in the roots and leaves total extracts of *C. religiosa* and on the other hand, concluded that the tested germs were sensitive to the different total extracts.

The present study was initiated after a spectacular result obtained with *C. religiosa* in the treatment process of a young lady who came to give birth by caesarean in Senegal local clinic: this plant has allowed the rapid healing of the wound remained incurable in this woman despite the use of conventional antibiotics.

In order to promote this medicinal plant in the perspective of standardized drugs preparation and especially to know if its alkaloids would be responsible of the antibacterial pharmacochemical properties that are recognized to it, this study aims to assess the antibacterial pharmacochemical activity *in vitro* of total alkaloid extracts of *C. religiosa* leaves and roots on pathogenic germs in comparison to Amoxicillin + clavulanic acid (AMC).

## 2. Material and Methods

### 2.1. Description of Plant Material

*Crateva religiosa* is a shrub about 10 m tall and has a smooth bark of the Capparaceae family also called Capparidaceae (Photo1). Its leaves are alternate and trifoliate, the lateral

leaflets being asymmetrical. The leaflets are obovate, 6 to 10 cm long, 3 to 4 cm wide, with a base at the corner and a pointed acuminate tip; the leaf has 5 to 7 lateral veins and a very detailed network of nerves, visible below, but not salient. The limbus surface is glabrous. The petiole is about 4 to 10 cm long, with a thin gutter on it. Young twigs are lenticellate with white dots. Their flowers develop during the leafless phase. The flowers are white and grouped into corymbiform panicles at the tops of the branches [14]. The flower has 4 white petals and about 20 stamens with purple-long net of 4 cm. The pistil surpasses them a little, bearing on top the piriform ovary. The ovary is inserted in the center of a crown with 4 sides in beads. Its fruits are spherical of 3 to 8 cm wide [15-17].



Photo 1: Some botanical aspect of *Crateva religiosa* [16].

### 2.2. Collection of plant material

The leaves and roots of *C. religiosa* were collected in Cotonou city, Department of Atlantic (southern Benin), in March 2017. The botanical identification of the collected material was performed by botanists from the botanic Garden at the University of Abomey-Calavi in Benin.

### 2.3. Preparation of extracts

The collected leaves and roots of *C. religiosa* were neatly washed and shade dried at room temperature (20 ° C) in the laboratory for two weeks. Samples were chopped into smaller pieces and then ground into fine powder using a dry grinder RETSCH Haan SM1-33241 (Comptoir Scientific, Benin). Each powder of leaves and roots was kept in a desiccator before extraction. Roots and leaves total alkaloids extractions were done by the Stas-Otto method. The diagram in Figure 1 shows the different steps of the extraction of total alkaloids.

#### 2.3.1. Extraction / release of total alkaloids

An amount of 500 g of leaf and root powder, each contained in separated basins was respectively treated in a hood with 320 ml of ammonium hydroxide and left for 24 hours for better alkaloid release. The total alkaloids are then extracted until each powder is depleted with 6 L of ethyl acetate for 24 hours using a Soxhlet.

#### 2.3.2. Purification / removal of impurities

Each organic extract of leaf and root powders containing the total alkaloids in the base state was concentrated by

evaporation using a Rotavapor Vacuum type 349/2. Too much chlorophyll and other impurities were removed by precipitation using the solvent system methanol / acetic acid (60:40). The purification phase was continued by a succession of acidification (treatment with 2% sulfuric acid) and alkalization (treatment with ammonia) and then a salification step followed by washing with sodium sulfate according diagram in Figure 1.

#### 2.3.3. Characterization

This is a step of identifying or confirming the actual presence of alkaloids in the total extracts obtained. Dragendorff test [20, 21] carried out throughout the purification process has kept the useful phases containing the extracted alkaloids. The appearance of a red precipitate turning orange indicates the presence of alkaloids. The various extracts of leaves and roots containing the total alkaloids are concentrated by evaporation and kept in an oven.

### 2.4. Thin layer chromatography (TLC)

A volume of 50 ml of each extract of roots and leaves total alkaloid was evaporated to dryness and then taken up with 1 ml of ethyl acetate. The chloroform-methanol (95: 5) solvent system was used for the migration of each leaf and root extract and the separation of their various constituents.

## 2.5. Antibacterial pharmacological tests

### 2.5.1. Bacterial strains

The tested bacterial strains were supplied by the laboratory of

the National University Hospital Center of Microbiology (CNHU-HKM) in Cotonou where they were isolated on Mueller-Hinton Agar (MHA), then identified and confirmed by standard bacteriological methods. The strains are made up of *Staphylococcus aureus* (38), *Escherichia coli* (28), *Klebsiella pneumoniae pneumoniae* (26), *Streptococcus agalactiae* (23) and *Citrobacter freundii* (12).

### 2.5.2. Dilution Preparation of total alkaloid extracts and impregnation of sterile discs

An amount of 200 mg of each total alkaloid extracts from leaves and roots in acidic aqueous phase was weighted and

diluted in 1 mL of water. This stock solution (200 mg/ml) was diluted to a quarter (50 mg/ml). In Petri dishes, non-impregnated sterile antibiotic test discs GE Healthcare - Whatman 710-0635 (Comptoir Scientific, Benin) were placed and were each soaked with two drops of total alkaloid extracts from leaves of *C. religiosa* with the two (2) concentrations each prepared per dish. Non-impregnated sterile discs were even impregnated with total alkaloid extracts from *C. religiosa* leaves and roots. Non-impregnated sterile discs soaked with concentrations of 50 mg/ml and 200 mg/ml of leaf and root extracts were dried in the oven at 37 °C for one hour.

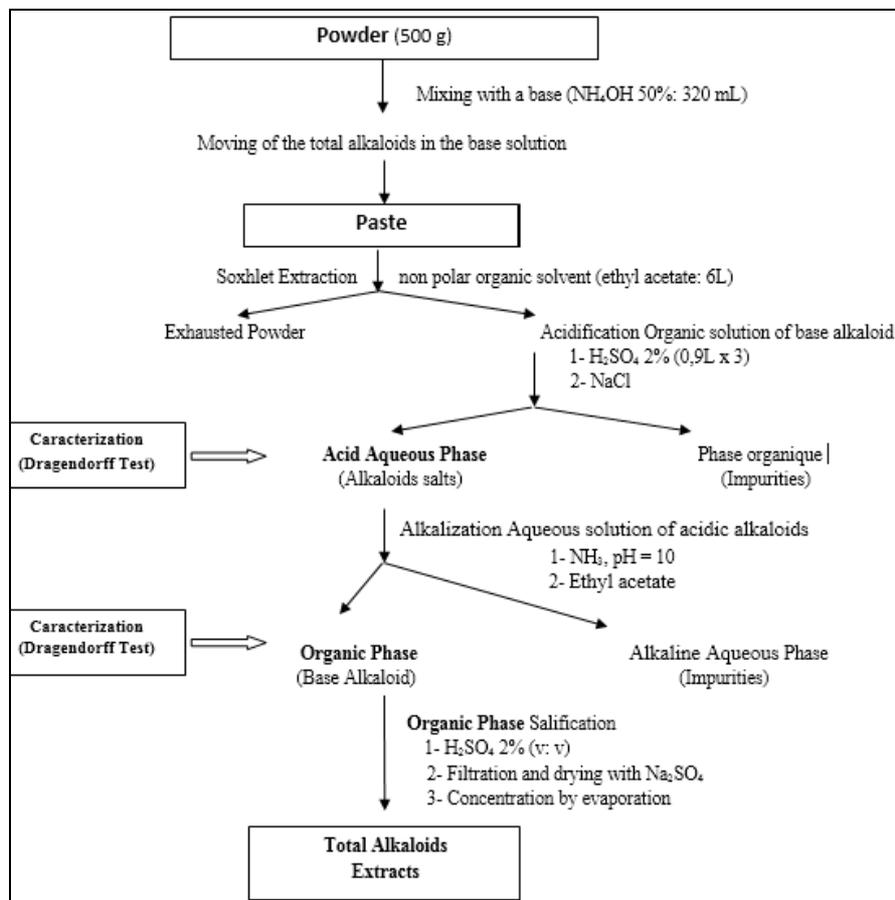


Fig 1: Extraction scheme of the total alkaloids by Stas-Otto the method.

### 2.5.3. Dilution Preparation of total alkaloid extracts and impregnation of sterile discs

An amount of 200 mg of each total alkaloid extracts from leaves and roots in acidic aqueous phase was weighted and diluted in 1 mL water. This stock solution (200 mg / ml) was diluted to a quarter (50 mg / ml). In Petri dishes, non-impregnated sterile antibiotic test discs GE Healthcare - Whatman 710-0635 (Comptoir Scientific, Benin) were placed and were each soaked with two drops of total alkaloid extracts from leaves of *C. religiosa* with three (3) concentrations each prepared per dish. Non-impregnated sterile discs were even impregnated with total alkaloid extracts from leaves of *C. religiosa*. Non-impregnated Sterile discs soaked with concentrations of 50 mg / and 200 mg / ml of leaf and root extracts were dried in the oven at 37 °C for one hour.

### 2.5.4. Bacterial culture and impregnated disc deposition and amoxicillin + clavulanic acid (AMC) disc

The Petri dishes containing MH agar were inoculated by flooding with a suspension each of the five (5) bacterial strains to be tested. Then, an impregnated disc with total

alkaloid extracts from leaves and another from the roots of *C. religiosa* were placed per Petri dish simultaneously with a disc of leaves and roots of AMC. Series of Petri dishes so inoculated containing an AMC disc and impregnated discs with the total alkaloid extracts at 50 mg/ml and 200 mg/ml from leaves and roots were incubated at 37°C for 24 hours. The effect of the total alkaloid extracts was compared with that of the antibiotic AMC reference (impregnated AMC disc dosed at 20/10µg). The agar-well diffusion method was used to test antibacterial activity [18].

### 2.6. Inhibition diameters measure

The inhibitions due to the effects of total alkaloids extracts of the leaves and roots of *C. religiosa* and the AMC were observed with naked eye and inhibition diameters were measured manually with the help of a flat millimeter ruler on the back of Petri dishes. The inhibitory effects of leaf and root extracts were compared with each other, and then with those of AMC. The minimum inhibitory concentrations were subsequently estimated.

### 3. Results and Discussion

#### 3.1. Yield of total alkaloids extraction from leaves and roots

Total alkaloids extraction from leaves and roots was successful. The Dragendorff test performed on the obtained leaves and roots extracts gave a characteristic red precipitate of the presence of alkaloids. This result confirms the one of Yevessin, who showed in 1988 that *Crateva religiosa* contained alkaloids, sugars, terpenes, flavonoids and steroids [13].

The yield of total alkaloids extraction from leaves and roots of *C. religiosa* was shown in Table 1.

**Table 1:** Yield of total alkaloids extraction from *C. religiosa* leaves and roots.

	Leaves	Roots
Powder quantity (g)	500	500
Amount of extracted alkaloids (g)	5,62	1,86
Yield (%)	1,12	0,37

For the same quantity of powder, the leaves allowed to have about three (3) times more total alkaloids extracts than the roots.

#### 3.2 Thin layer chromatography (TLC)

Only the chromatography on one-dimensioned layer was

performed to separate the constituents of total alkaloid extracts from leaves and roots. The frontline report (Rf) of the five (5) different spots appeared at level of roots and leaves extracts are recorded in Table 2.

**Table 2:** Rf of each of the five spots appeared at the level of total alkaloid extracts from leaves and roots of *C. religiosa*.

	Leaves (Rf of spots)	Roots (Rf of spots)
Spot 1	5%	5%
Spot 2	6%	7%
Spot 3	18%	18%
Spot 4	75%	77%
Spot 5	97%	97%

Each of total alkaloid extracts from leaves and roots presented five (5) spots with very close Rf suggesting that they are made up of at least five (5) same groups of alkaloids.

#### 3.3 Antibacterial pharmacological tests

- Antibacterial tests performed by the diffusion technic through the agar gave results that are recorded in Table 3, where the inhibition average diameters of total alkaloid extracts of leaves and roots were compared with those of AMC.

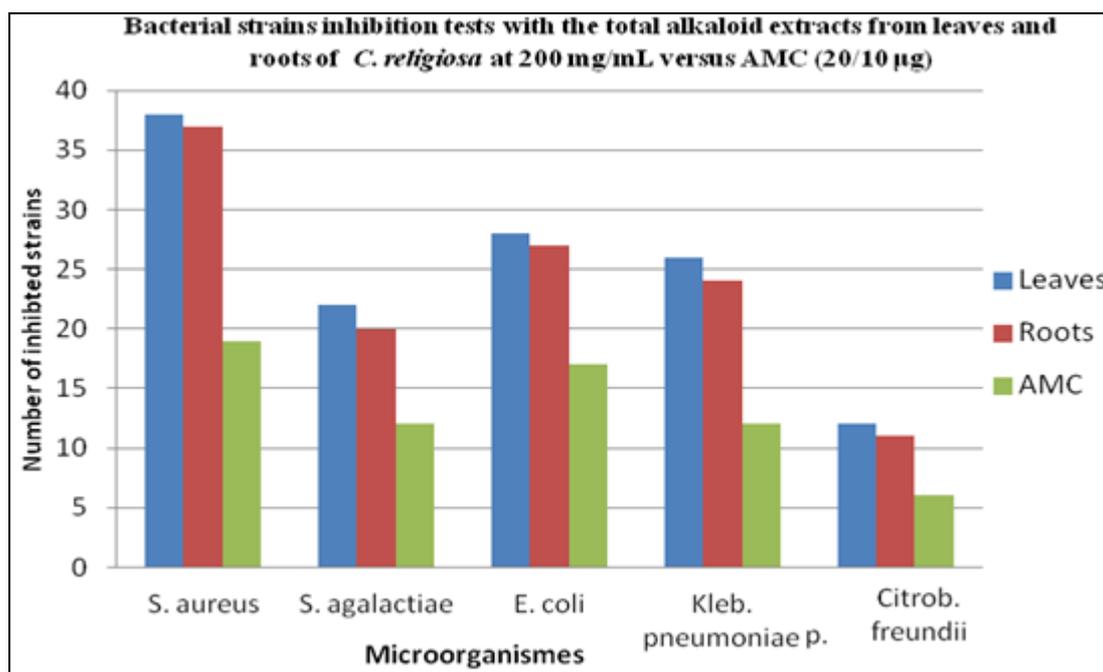
**Table 3:** Inhibition tests of bacterial strains with total alkaloid extracts from leaves and roots of *C. religiosa* at 50 mg/mL and AMC (20/10 µg).

Inhibition diameter (mm) Micro-organisms	AMC (20/10µg)	Aqueous extracts of total alkaloids from roots (50 mg/mL)	Aqueous extracts of total alkaloids from leaves (50 mg/mL)
<i>Staphylococcus aureus</i>	21,17±0,05	23,10±0,02	25,35±0,02
<i>Staphylococcus agalactiae</i>	22,12±0,01	22,85±0,05	23,15±0,03
<i>Escherichia coli</i>	20,75±0,03	21,80±0,01	23,08±0,01
<i>Klebsiella pneumoniae p.</i>	19,33±0,02	21,16±0,01	22,74±0,01
<i>Citrobacter freundii</i>	20,15±0,03	21,50±0,03	22,27±0,02

At the same concentration, the inhibitions diameters of bacterial strains tested that is obtained with the total alkaloid extracts from leaves are higher than those obtained with the extracts from roots. The total alkaloid extracts from leaves and roots at 50 mg/mL produced larger inhibition diameters

than those of the AMC antibiotic (20/10 µg).

- The bacterial strains inhibition tests results by the total alkaloid extracts from leaves and roots at 200 mg/ml compared with those of AMC are presented in the form of histograms (Figure 2)



**Fig 2:** Bacterial strains inhibition tests with the total alkaloid extracts from leaves and roots of *C. religiosa* at 200 mg/mL versus AMC (20/10 µg).

Aqueous extracts of the total alkaloids from leaves and roots of *C. religiosa* inhibited the development of all the strains of tested *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae pneumoniae*, *Streptococcus agalactiae* and *Citrobacter freundii*. The use of *C. religiosa* leaves and roots in traditional medicine to heal human infections related to micro-organisms is hence fully justified [15]. Similar results had also been obtained by Latifou and *al.*, who reported that the extract with ethyl acetate had a strong antimicrobial activity on bacteria strains such as *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus*, *Pasteurella pestis* and *Yersinia enterocolitica* [8].

The total alkaloids extracts from leaves and roots of *C. religiosa* revealed themselves more efficient than AMC on the tested bacterial strains. This well justifies the use of this plant in Senegal in the healing process of the wound remained incurable on a caesarianized woman despite the use of the conventional antibiotics. Our results are confirmed by those of Ajali and *al.*, who reported that the extract of methanol leaves of *C. religiosa* had shown a healing effect more important than the one of penicillin [2]. In fact, the wound surfaces treated with the extract dried more quickly, indicating that the extract exhibited an extensible healing mechanism compared to that of penicillin.

Regarding the obtained results in this study and conscious that alkaloids are complex nitrogenous organic compounds of plant of natural origin endowed with important therapeutic properties even at low doses [19], it seems clear that the total alkaloids of leaves and roots play a sure role in the therapeutic capacity recognized to *C. religiosa* against infections related to micro-organisms.

The inhibitory minimum concentration of the total alkaloids extract from leaves and roots of *C. religiosa* is estimated to 50 mg/ml.

#### 4. Conclusion

Aqueous extracts of total alkaloids of leaves and roots of *C. religiosa* contain at least five (5) groups of alkaloids which are efficient against strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae pneumoniae*, *Streptococcus agalactiae* and *Citrobacter freundii*.

The leaf extracts produced higher inhibition diameters of microbial strains than root extracts. The total alkaloids extract either of leaves or roots at 50 mg/mL are neatly more efficient than the Amoxicillin + Clavulanic acid (20/10µg).

The results we obtained are worthy of interest and bring the novelty that *C. religiosa* alkaloids intervene in its therapeutic effects widely requested in traditional medicine.

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