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# Evaluation of the antioxidant and antibacterial activity of aqueous and ethanoic extracts of two medicinal plants used to treat diarrhea in Côte d'Ivoire

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## Abstract

This study was initiated to evaluate the antioxidant and antibacterial activities of aqueous and ethanoic extracts of *Anthocleista djalonensis* and *Uvaria chamae*, two plants of the African pharmacopoeia used by the peoples of the northern Côte d'Ivoire to treat severe cases of diarrhea. A series of extraction by maceration was carried out. The extracts obtained were assayed by the colorimetric method and their antioxidant activity determined by spectrophotometer using the free radical scavenging (DPPH) and iron reduction (FRAP) methods. Dilution and agar diffusion methods on Mueller Hinton medium were used to determine MICs and inhibition diameters. The assay of the extracts determined the polyphenol content ranging from 6.8 to 21.1 mg. The total phenol content of the ethanol extract of *Uvaria chamae* was the highest. Evaluation of the free radical scavenging activity and the reducing power of ferric ions to ferrous ions of the extracts of both plants indicated variable IC50 values ranging from 4 to 14 (µg/mL). The lowest value (4 µg/mL) close to that of vitamin C (reference molecule) (3.5 µg/mL) was obtained with the aqueous and ethanoic extracts of *Anthocleista djalomensis* and *Uvaria chamae* respectively. Furthermore, all germs were found to be sensitive to the action of the extracts with inhibition diameters greater than 10 mm and MICs greater than 3.12 mg/mL. This sensitivity could be due to the presence of phytochemicals such as total phenols, flavonoids, tannins, alkaloids, saponins whose antimicrobial activities have already been shown. These investigations justify the traditional use of these plants in the treatment of diarrhea and dysentery.

**Keywords:** *Anthocleista djalonensis*; *Uvaria chamae*; Abdominal pain; Antibacterial activity; Antioxidant; Phytochemical screening

## 1. Introduction

Diarrheal diseases continue to be one of the main causes of morbidity and mortality in the world in general, and in Africa and sub-Saharan Africa in particular. It affects mostly children under 5 years of age [1]. Nearly 4 billion cases of diarrhea occur each year with 2.2 million deaths, 37% of which occur in sub-Saharan Africa. In this part of the world, diarrhea is *responsible* for approximately 7.7% of all deaths recorded annually [2]. In Côte d'Ivoire, a multiple indicator cluster survey conducted in 2006 [3] showed that 17% of children under five years of age had experienced diarrhea in the two weeks prior to the survey and that this pre valence was 19% in rural areas compared to 15% in urban areas. Many

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bacteria are involved. In tropical countries, these are generally entero-invasive bacilli with a predominance of *Shigella flexneri* (52.8%) [4]. The increase in the number of infected people as well as the recrudescence of multi-resistant strains to the antibiotics available on the market constitutes a major concern in the management of this pathology [5]. Therefore, effective control of diarrhea requires a comprehensive approach integrating therapeutic case management, biomedical, environmental and socio-anthropological research on pathogens, risk factors and their perception by communities in each local context [6]. The literature teaches us that plants are powerful remedies in the treatment of abdominal infections, particularly dysentery, diarrhea and gastroenteritis, due to the presence of several bioactive molecules [7]. An ethno pharmacological survey conducted by [8] in Bagoué region (Northern Côte d'Ivoire) revealed that several plants from this region are used for their anti-diarrheal virtue. These plants include *Anthocleista djalonensis* and *Uvaria chamae*. The puspose of the present work is to explore the antibacterial potential of some extracts of *Anthocleista djalonensis* and *Uvaria chamae* for the development of an Improved Traditional Medicine (ITM).

## 2. Material and methods

### 2.1. Material

The plant material consisted of root bark of *A. djalonensis* and *U. chamae* collected in the region of Bagoué in northern Côte d'Ivoire. Two reference strains and five multi-resistant strains implicated in occurrence of diarrhea were obtained through researchers from Bacteriology and Virology Laboratory of Pasteur Institute of Côte d'Ivoire.

### 2.2. Methods

#### *2.2.1. Preparation of plant extracts by maceration*

The extracts were prepared according to the method described by [9].

#### *2.2.2. Phytochemical screening of the extracts*

The qualitative analysis of the extracts was carried out on the basis of the colouring and precipitation tests characteristic of the major chemical groups. The methods described by [10]; [11] were used. The results are either positive (+) or negative (-).

#### *2.2.3. Assay of phytochemicals*

Total phenol content

The content of total phenols was determined by method described by [12]. To 0.5 mL of each plant extract with a concentration of 100 µg/mL, 5 mL of Folin-ciacolteu diluted 1:10 in distilled water and 4 mL of sodium carbonate (1M) were added respectively. The mixture is incubated at room temperature for 15 minutes. The optical density (O.D.) was then read at 765 nm spectrophotometer against a blank. The total phenol content of the extracts is expressed as Gallic acid equivalents (mg GAE/g extract).

Flavonoid content

The determination of total flavonoids was carried out according to method described by [13]. A volume of 0.5 mL of methanolic extract was introduced into a test tube. To the contents of the tube, are successively added 0.5 mL of distilled water, 0.5 mL of aluminium chloride, 0.5 mL of potassium acetate and 2 mL of distilled water. The tube is left to stand for 20 min in the dark and the optical density (OD) is read at 415 nm against a blank. A standard range established from a stock solution of quercetin (0.1 mg/mL) under the same conditions as the test, is used to determine the amount of flavonoids in the sample.

#### *2.2.4. Antioxidant activity*

Measurement of the anti-free radical capacity

Concentration range is prepared by successive double dilution from a stock solution of plant extract at 0.1 mg/mL. Then, to each concentration of extract, the same volume of methanolic solution of DPPH is added. After 30 minutes of incubation at room temperature (37°C) and protected from light, the absorbance was read with a spectrophotometer at 517 nm against a sample blank (0 mg/mL of extract). Vitamin C (0.1 mg/mL) was prepared under the same conditions [14]. The percentages of DPPH radical inhibition are calculated by the following equation:

Inhibition (%) = [(Absorbance blank - Absorbance sample/ Absorbance blank)] × 100

Measuring the reducing power

Method used by [15] measured the capacity of extracts to reduce ferric ion ($Fe^{3+}$) to ferrous ion ($Fe^{2+}$). One (1) mL of plant extract or vitamin C at different concentrations (1000; 500; 250; 125; 62.5; 31.5) was mixed separately with 2.5 mL of phosphate buffer (0.2 mM; pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture is incubated in water bath at 50°C for 30 minutes. After addition of 2.5 mL of 10% trichloroacetic acid, the reaction medium is centrifuged at 3000 rpm for 10 minutes. To 2.5 mL of the supernatant, is added 0.5 mL of 0.1% iron III chloride. After 10 minutes of incubation at room temperature, the absorbance is measured with a spectrophotometer at 700 nm. The absorbance-inducing concentrations of 0.5 ($IC_{50}$) of the extracts are determined and compared to that of vitamin C.

*2.2.5. Antibacterial activity of the extracts*

Preparation of the inoculum

The bacterial inoculum was prepared from youth colony of 18 hours, isolated in 10mL of Mueller Hinton Broth (MHB) and incubated for 3-5 h at 37°C to pre-culture. A volume of 0.1mL was taken and added to 10 mL of twice concentrated BMH. This bacterial suspension produced is estimated to be approximately $10^6$ cells/mL and constitutes the $10^0$ dilution or pure inoculum.

Sensitivity testing

Sensitivity of strains to plants root bark extracts was carried out by agar diffusion technique. Mueller Hinton medium was inoculated by flooding. Approximately, 2-6 mL of the inoculum was poured into the agar surface. Using a sterile cookie cutter, wells approximately 6mm in diameter were drilled on the agar. Each well received 80 µL of the test substance at a concentration of 100 mg/mL. After 30 min as time of diffusion at laboratory temperature, the plates were incubated at 37 °C for 18-24 h. The presence or absence of a zone of inhibition was observed.

### 2.3. Statistical Analysis

The data was processed using the Graph Pad Prism 5.0 software (Microsoft, USA). The statistical analysis of results was carried out using Anova One-Way variance analysis followed by Dunett tests for comparison between extracts activity to reference molecules. The values expressed were the means of three experiments with the standard error of the mean (mean ± SEM). Probability values $P \leq 0.05$ were considered significant.

## 3. Results and discussion

### 3.1. Phytochemical screening

**Table 1** Phytochemical screening of extracts

| | ***Uvaria chamae*** | | ***Anthocleista djalonensis*** | |
|---|---|---|---|---|
| | **Aqueous Ext.** | **Ethanol Ext.** | **Aqueous Ext.** | **Ethanol Ext.** |
| Alkaloid | - | + | + | - |
| Saponoside | + | + | + | + |
| Flavonoid | + | + | + | + |
| Phenol | + | + | + | + |
| Tannins | + | + | + | + |
| Quinones | - | - | + | - |
| Sterols et Terp. | - | - | + | + |
| Cardio glucoside | + | + | + | + |

Ext: Extract Terp : Terpene; - : absence; + : presence

Phytochemical screening carried out from the aqueous and ethanoic extracts of the plants revealed the presence of total phenols, tannins, flavonoids, sterols and terpenes, saponosides, cardiotonic glycosides and alkaloids. The results are shown in Table 1. These secondary metabolites are more concentrated in ethanoic extracts than aqueous extracts of *U. chamae*. This implies a high solubility of these compounds in ethanol. Identical results were obtained by [16]; [17] during their studies on *A. djalonensis* and *U. chamae* respectively. These results are similar to those obtained by [18]. These authors, during their work on the different parts of the artichoke (*Cynara scolymus*) flower showed that ethanol and acetone are the preferable solvents for high extraction of total phenols and flavonoids. Therefore, ethanoic extracts would be more concentrated in polyphenolic compounds than aqueous extracts. The difference in biomolecule composition of the extracts could be the basis for the difference in biological activities observed between the plants extracts studied. Ethanolic extracts should have more interesting microbicidal properties compared to aqueous extracts in view of the proven antimicrobial activity of phenolic compounds.

### 3.2. Assay of phytochemicals

The quantification of polyphenols in this study revealed variable contents (Table 2).

Content of total phenols in the extracts of *A. djalonensis* and *U. chamae* was determined from the calibration line (y= 1.66x + 0.0203, $R^2$= 0.980). It is expressed in milligrams of gallic acid equivalent per gram of extract (mg EAG / g of extract) while that of the flavonoids of the same extracts was determined from quercetin calibration line (y= 6.2x + 0.0067, $R^2$= 0.994) and therefore expressed in gram of quercetin equivalent per milligram of extract (mg EQ/g of extract). The ethanoic extracts recorded contents ranging from 12.1 to 21.1±0.5 mg while the aqueous extracts obtained contents varying from 6.8 to 15.7±2.0 mg. Statistical analyzes indicate that there is a significant difference at $p<0.05$ between the contents of phenols on the hand and flavonoids on the other depending on the plant and the extraction solvent. These results indicate that polyphenolic compounds extracts were more concentrated in ethanoic extracts than aqueous extracts. This difference in content observed between the both extracts on one hand and between the two plants on other hand, could be explained by biogenetic and environmental factors [19]. Furthermore, the work carried out by [20] on the polyphenol composition of the leaves and bark of the trunk of *A. djalonensis* showed relatively lower contents than in our study. The high concentration of these phytoconstituents in the root barks of this plant would justify its use in cases of severe diarrhea. According to [21], all parts of this plant are active but the roots are more potent and therefore recommended in severe cases. The richness of root barks of this plant in phenolic compounds could give it an excellent antioxidant activity.

**Table 2** Content of polyphenolic compounds in extracts

| **Content** | ***Uvaria chamae*** | | ***Anthocleista djalonensis*** | |
|---|---|---|---|---|
| | **Aqueous Ext.** | **Ethanol Ext.** | **Aqueous Ext.** | **Ethanol Ext.** |
| Total Phenol (mg GAE/g extracts) | 8.9±0.9 | 21.12±0.5 | 10.1±0.0 | 16.9±0v1 |
| Flavonoids (mg EQ/g d'extracts) | 6.8±0.0 | 12.1±0.0 | 15.7±2.0 | 20.8±0.7 |

**Ext:** Extract

### 3.3. Antioxidant activity

*3.3.1. DPPH radical Scavenging*

Antioxidant activity of all extracts is dose-dependent. Indeed, the $IC_{50}$ value is inversely proportional to the antioxidant capacity of a compound. Thus, if the $IC_{50}$ is small, antioxidant activity of the compound will be greater [22]. The $IC_{50}$ of ethanoic extract of *U. chamae* and aqueous extract of *A. djalonensis* are similar (4.1±0.3 µg/mL) and close to that of the reference molecule, vitamin C (3.5±0.4µg/mL) (**Table 3)**. This difference between $IC_{50}$ of the ethanolic extract of U. chamae and aqueous of A. djalonensis is not statistically significant at $p<0.05$. This indicates that these extracts present an interesting anti-free radical activity. The $IC_{50}$ of ethanoic and aqueous extracts of *U. chamae* and *A. djalonensis* respectively are equal to that of the methanolic extract of *Chrysophyllum perpulc*hrum ($IC_{50}$ = 4.0 ± 0.3 µg/mL) obtained by [11] and lower than that of *Combretum sp*. (6.0 ± 0.5 µg/mL) obtained by [23]. These authors showed that *Chrysophyllum perpulchrum* and *Combretum sp*. have good antioxidant activity. In the light of these observations, *A. djalonensis* and *U. chamae* are good antioxidants. This interesting antioxidant activity of plant extracts may be due to the richness of these extracts in polyphenols. According [23], there is a relationship between polyphenol content and anti-radical activity. Indeed, polyphenols are compounds rich in hydroxyl groups that can act as electron donors and react with free radicals [24], [25]. Antioxidants are considered to be reducers and inactivators of oxidants [26].

*3.3.2. Iron reducing power*

The reducing power of an extract is associated with its antioxidant power. $EC_{50}$ corresponds to the concentration which induces an absorbance of 50%. The $EC_{50}$ values indicate that the reduction capacity is proportional to the increase in the concentration of the extracts. Vitamin C has the lowest $EC_{50}$ value (6.1±0.4 µg/mL) followed by ethanolic extract of *A. djalonensis* (6.5±0.9 µg/mL). The highest value being obtained with aqueous extract of *U. chamae* (14.4±4.9 µg/mL). These values are recorded in **Table 3** The difference between the $EC_{50}$ values of the ethanolic extracts of *A. djalonensis* and vitamin C is not statistically significant at $p<0.05$. This means that, our plant extracts cause a strong reduction of ferric ions ($Fe^{3+}$) into ferrous ions ($Fe^{2+}$) through the formation of a blue ferricyanic complex [27]. More or less identical results were obtained by [28] through the methanolic extract of *Satureja calamintha*. The reducing power of the two plant species is probably due to the presence of hydroxyl groups in the phenolic compounds which can serve as electron donors [25]. Antioxidants are considered as reducers and inactivators of oxidants [26]. The reducing power of the ethanolic extracts of *U. chamae* and *A. djalonensis* therefore constitue a significant indicator of their antioxidant activity.

**Table 3** Antioxidant activity of extracts

| **Extracts** | **Anti-free radical activity CI50 (µg/ml)± standard deviation** | | **Reducing power EC50 (µg/ml)± standard deviation** | |
|---|---|---|---|---|
| | ***Anthocleista djalonensis*** | ***Uvaria chamae*** | ***Anthocleista djalonensis*** | ***Uvaria chamae*** |
| Aqueous | 4.1±0.3ns | 12.6±2.8 *** | 11.5± 0.2 *** | 14.4± 4.9 *** |
| Ethanoic | 6.5 ± 0.2 *** | 4.0±1ns | 6.5±0.9 ns | 9.0±0.7 ns |
| Vitamin C | 3.5±0.4 | 3.5±0.4 | 6.1±0.4 | 6.1±0.4 |

ns : difference no significant at $p<0.05$; *** : difference very significant at $p<0.05$

### 3.4. Antibacterial activity

Antibacterial activity of aqueous and ethanoic extracts of the target plants on *in vitro* growth of *Escherichia coli* (ATCC 25922), *Yersinia enterocolitica* IP 480 1A/O: 14, *E. coli* BLSE, *Shigella flexneri* BLSE, *Shigella sp* and *Yersinia sp* showed inhibition diameters greater than 10 mm and MICs greater than 3.12 mg/mL (Table 4). The highest inhibition diameter (15 mm) was obtained with the hydroalcoholic extract of *A. djalonensis* on *in vitro* growth of *Shigella flexneri* BLSE and *Yersinia*.

**Table 4** Antibacterial activity of extracts

| **Germs** | ***Uvaria chamae*** | | | | ***Anthocleista djalonensis*** | | | | **CIPRO (5 ug)** |
|---|---|---|---|---|---|---|---|---|---|
| | **Aque Ext.** | | **Etha Ext.** | | **Aque Ext.** | | **Etha Ext.** | | |
| | **D (mm)** | **MIC (mg/mL)** | **D (mm)** | **MIC (mg/mL)** | **Dia (mm)** | **MIC (mg/mL)** | **Dia (mm)** | **MIC (mg/mL)** | |
| *E. coli* ATCC | 12 | Nd | 12 | 6.25 | 12 | 50 | 12 | 12.50 | S |
| *E. coli* BLSE | 12 | 6.25 | 12 | 50 | 12 | 25 | 12 | 12.50 | R |
| *S. flexneri* BLSE | 13 | 6.25 | 13 | 3.125 | 10 | 6.25 | 15 | 6.25 | S |
| *Shigella* sp | 10 | 100 | 11 | 12.50 | 10 | 12.50 | 11 | 100 | S |
| *Yersinia* IP 480 | 13 | 25 | 13 | 25 | 12 | 12.50 | 15 | 12.50 | S |
| *Yersinia* sp | 11 | Nd | 10 | 25 | 10 | 100 | 11 | 25 | R |

D (mm) : inhibition diameter; MIC (mg/mL): minimum inhibitory concentration; Aque Ext.: aqueous extract; Etha Ext. : ethanoic extract; CIPRO: ciprofloxacine; S: susceptible; R: resistant; Nd: no determined

The lowest MIC value was obtained with the ethanoic extract of *U. chamae* on growth of *S. flexneri* BLSE. These results indicate that *S. flexneri* is the most sensitive strain to plant extracts tested. Present results are similar to those obtained by [29]. These authors, using hydroalcoholic extracts, of *A. djalonensis* leaves, obtained largest inhibition diameters. The

bacteriostatic action of the recorded plant extracts is believed to be due to the polyphenolic compounds present in high doses in the ethanoic extracts.

## 4. Conclusion

The aim of this study was to evaluate *in vitro* the antioxidant and antibacterial activity of two medicinal plants, *Anthocleista djalonensis* and *Uvaria chamae* on the growth of some strains of enterobacteria involved in diarrhea. The study showed that the plants studied have a good antioxidant activity close to that of vitamin C. This activity is justified by the high content of polyphenolic compounds present in the plant extracts. Moreover, these plants present an interesting antibacterial activity on the growth of the studied germs. *Shigella flexneri* BLSE was found to be the most sensitive germ and the ethanoic extract the most active. The phytochemistry of the extracts showed a high concentration of alkaloids, tannins and polyphenols. The activity of these extracts would be partly due to these secondary metabolites. The antibacterial activity of the target plants against the germs involved in diarrhea would explain their use in treatment of this diseases in traditional environments. It would therefore be interesting, to consider the formulation of an improved traditional anti-diarrheal medicine from the aqueous and ethanoic extracts of these two plants.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

There is no conflict of interest between the authors of this manuscript

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