

## Phytochemical Screening and Antimicrobial Studies of *Crateva adansonii* Leaf Extract

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**Abstract:** Diverse challenges of microbial infections and upsurge of multi-drug resistant microbes informed the investigation into the phytochemical and antibacterial properties of *Crateva adansonii*. Cold extraction was carried out using methanol solvent. The crude extract of *Crateva adansonii* was fractionated into the *n*-hexane, methanol and chloroform layers successively. The phytochemical screening indicated the presence of alkaloids, saponins, terpenoids, flavonoids and cardiac glycosides. The antimicrobial assay showed that, for *Bacillus* spp, the organism was sensitive to the chloroform fraction of leaf extract at 1.562 mg/ml. For *Micrococcus varians*, result showed organism was sensitive to the crude extract at 3.125 mg/ml. According to the result of antifungal screening, the *n*-hexane fraction and crude extract showed activity against *Aspergillus niger* at 12.500 mg/ml and 3.125 mg/ml respectively. From these results, the crude extract of the leaf of *Crateva adansonii* shows activity against both bacteria and Fungi; hence, it may might be a good source of new drug for treating infections caused by these pathogens.

**Keywords:** Phytochemicals, antimicrobial, antibacterial assay, inhibitory concentration.

### 1.0 Introduction

Plants have been a major source of food and energy for man. The various ways to which plants have been of immense benefit ranges from feeding, tonic, treatment of illnesses, laxative, energy booster, preventive as well as curative (Moharam and Ali, 2012). The specific plants to be used and methods of application for particular ailments were

passed down through oral history (Robert *et al.*, 2003). *Crateva adansonii* is a deciduous tree with grey, very smooth bark. It is called the sacred garlic pear and temple plant. The plants' common name in Hausa is 'Ungundu' in Yoruba, it is 'eegun-orun'; in Igbo, it is called 'Amakarode' (Peter, 1997). The plant belongs to the family *Capparidaceae* (Ryan and Ray, 2004).

The wood is soft and yellow, having a strong smell when exposed via cut. It is a small tree that grows up to 3-10 m tall with 3 foliated leaves. Studies have shown that *C. adansonii* has tonic properties; has a counter-irritant effect for headache; applied for rheumatic conditions after powdering and boiling in oil (Ainslie, 1937). The powdered leaves are applied to cysts and swellings, while the bark is used for treating sterility (Kerhero & Adam, 1974). According to Burkill (1985) and Tsado *et al.* (2015) its leaves are used to treat ear infections. In Senegal, the roots are used in the treatment of syphilis, jaundice and yellow fever Ayodeji *et al.* (2011). The aim of the work is to identify medicinal plants that are active against infectious diseases. The phytochemical screening will reveal the secondary metabolites present while the antibacterial studies will give insight into the range of microorganisms which the plant are active against.

## 2.0 Materials and Methods

### 2.1 Collection of Plant Samples

Fresh leaves of *C. adansonii* plant was collected in Iyesi village, within Ota environment, Ogun State, Nigeria, in the early hours of the day, during the rainy season between June and October 2015. Taxonomic identification and confirmation were carried out by a botanist at the Forestry Herbarium, Ibadan with No. FHI 110016. The test bacteria, which were environmental organisms, *Bacillus* spp, *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcus varians*, *Serratia* spp, *Aspergillus niger* were sourced from the Department of Biological Sciences College of Science and Technology, Covenant University,

Ota, Ogun State, Nigeria. The bacteria strains were maintained at 0.5% on Nutrient agar at 4 °C.

### 2.2 Preparation of Plant Extracts

Fresh leaves were hand-plucked and cleaned for debris using tap water while distilled water was used in rinsing the leaves. The leaves were air-dried in the shade at room temperature for two weeks. Dried leaf samples were pulverized. 100 g of pulverized plant materials was weighed and soaked in 500 ml of methanol and left for 72 hours in order to prepare the extracts. Whatman number 1 filter paper was used to filter the extracts and filtrates were concentrated under vacuum below 40 °C using rotary evaporator. The crude extract was partitioned into aqueous, chloroform, hexane and methanol fractions by liquid-liquid extraction.

### 2.3 Determination of Phytochemical Constituents

The phytochemical constituents of the plant leaves were screened qualitatively using the Standard steroids procedures described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973). Screening involved tests for alkaloids, saponins, tannins, flavonoids and terpenoids.

### 2.4 Antibacterial Activity Bioassay

The extracts were evaluated for antibacterial activity against *Bacillus* spp, *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcus varians*, *Serratia* spp, *Aspergillus niger*, using the methods of Ogbulie *et al.* (2007); Doughari and Manzara (2008).

### 2.5 Determination of Minimum Inhibitory Concentration (MIC)

Determination of minimum inhibitory concentration (MIC) was carried out on

the leaf of *Crateva adansonii*, using the method described by Mahesh and Satish (2008). The procedure was carried out on the different fractions of *C. adansonii* leaves that showed sensitivity against the growth of some selected organisms from the microbiology laboratory and Applied Biology and Biotechnology unit of Covenant University, Ota, Ogun State, Nigeria. The medium used was nutrient agar which was prepared according to the manufacturers' standard. Extracts concentrations, the chloroform, methanol and hexane fractions obtained from fractionation were adjusted to 50, 25, 12.5, 6.25, 3.125 and 1.562 mg/ml by serial dilution method. The sterile nutrient agar plates were seeded using swab sticks with the test organisms or isolates of 0.5% Mcfarland standard and were properly labeled. Wells were bored into the sterile nutrient agar plates, with the aid of a sterile cork borer of 9 mm diameter. With sterile 1ml pipettes, 0.2

ml of each extract of different concentrations was dispensed into the already bored wells on the inoculated nutrient agar plates and plates were incubated at 37°C for 24 hours, after which they were observed for growth or death of the test isolates or organisms. The lowest concentration inhibiting growth was taken as the MIC. The antibiotic used was Gentamycin.

### 3.0 Results

The result of the qualitative phytochemical screening revealed the presence of Alkaloids, Saponins, Terpenoids, Flavonoids and Cardiac glycosides (Table 1). This report revealed that the aqueous, methanol and crude extracts contain alkaloids, while all solvents showed the presence of saponins. However, the *n*-hexane extract revealed the trace presence of terpenoids, flavonoids and cardiac glycosides.

**Table 1:** Phytochemical Analysis for the Leaves of *Crateva adansonii*

Phytochemical	Aqueous	Chloroform	Methanol	<i>n</i> -Hexane	Crude
Alkaloids	++	-	++	-	++
Saponins	+	++	++	++	++
Terpenoids	-	-	-	+	-
Flavonoids	-	-	-	+	-
Cardiac glycosides	-	-	-	+	++

**Key:** - is Absent, + is Present, ++ is Significant and +++ is Very significant

**Table 2: Antimicrobial Activity of Leaf Extracts of *C. adansonii* (mm)  
(Zones of inhibition measured in mm)**

Fraction	<i>Bacillus spp</i>	<i>E. coli</i>	<i>S.aureus</i>	<i>P. aeruginosa</i>	<i>Serratia spp</i>	<i>M. varians</i>	<i>A. niger</i>	Yeast
<i>n</i> -Hexane	16	-	-	-	-	10	20	-
Methanol	-	-	-	25	-	-	-	-
Chloroform	25	-	-	23	-	10	-	-
Aqueous	-	-	-	16	-	-	-	-
Crude	-	15	-	17	-	25	20	-

*B. spp* = *Bacillus sp.*; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *M. varians* = *Micrococcus varians*; *A. niger* = *Aspergillus niger*.

The result from Table 2 showed that the methanol, chloroform and crude extracts exhibited the highest zone of inhibition (25 mm) for *P. aeruginosa*, *Bacillus spp.* and *Micrococcus varians* respectively. The chloroform fraction showed zone of inhibition (23 mm) for

*P. aeruginosa*, while the *n*-hexane, and crude fraction exhibited zone of inhibition at 20 mm for *A. niger*. The antimicrobial activity demonstrated by the leaf extract against some bacterial pathogens could be due to some bioactive constituents of the extracts.

**Table 3: Result of Minimum Inhibitory Concentration in mg/ml of Leaf Extracts of *C. adansonii* in (mg/ml )**

Fractions	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>M. varians</i>	<i>E. coli</i>	<i>Bacillus spp</i>	<i>Serratia spp</i>	<i>A. niger</i>
Chloroform	-	-	-	-	1.562	-	-
Methanol	-	-	-	-	-	-	-
<i>n</i> -Hexane	-	-	-	-	-	-	12.5
Crude	-	-	3.125	-	-	-	3.125

*B. spp* = *Bacillus sp.*; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *M. varians* = *Micrococcus varians*; *A. niger* = *Aspergillus niger*

The result from Table 3 showed that the MIC value of *C. adansonii* was 1.562 mg/ml for the chloroform extract against *Bacillus spp.* and 12.5 mg/ml for the *n*-hexane extract against *Aspergillus niger*, while the crude extract was 3.125 mg/ml

against *M. varians* and *Aspergillus niger*. The crude, hexane and chloroform fractions have shown some lethal effect on the test organisms as indicated by the values of the median lethal dose, LD<sub>50</sub>.

**Table 4:** Antimicrobial Activity of the Organic Chemicals Used (mm)  
(Zones of inhibition measured in mm)

Fraction	<i>Bacillus spp</i>	<i>E.coli</i>	<i>S.aureus</i>	<i>P. aeruginosa</i>	<i>Serratia spp</i>	<i>M. varians</i>	<i>A. niger</i>	Yeast
<i>n</i> -Hexane	-	10	-	10	-	-	-	-
Methanol	-	11	-	10	-	-	-	-
Chloroform	-	12	-	11	-	-	-	-
Aqueous	-	-	-	-	-	-	-	-

*B. spp* = *Bacillus sp.*; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *M. varians* = *Micrococcus varians*; *A. niger* = *Aspergillus niger*

The results from table 4 shows that the test organisms used were not sensitive to the organic chemicals when used alone without the fractions from the leaf of *C. adansonii*. The observed values shown for *E. coli* and *P. aeruginosa* indicated resistance to the organic chemicals. This implies that it is only the dissolved fractions that showed activity on the test organisms.

#### 4.0 Discussion

Phytochemicals are known to be responsible for the pharmacological and toxic activities of plants (Lawal *et al.*, 2005). Plants are known to produce phytochemicals so as to defend them against predators; however, studies have also shown that they can be used to protect human against diseases. The results from the phytochemical analysis of *Crateva adansonii* is in agreement with previous studies: Agbankpe *et al.*, (2015); Borokinni and Omotayo, (2012). Report has shown flavonoids to exhibit anticarcinogenic potentials due to their antioxidant and anti-inflammatory properties. Likewise, saponins are used as adjuvant in the production of vaccines (Asl and Hosseinzadeh, 2008).

Alkaloid has been used as CNS stimulant and powerful painkillers, among other uses. The cardiac glycoside has been used for over two centuries as stimulant in cases of cardiac failure and diseases. Igboko, (1983) reported that plants containing tannin have been used for healing of wounds, hemorrhoids and burns in herbal medicine.

The antibacterial analysis shows that the crude, chloroform and methanolic fractions contains bioactive constituents which can effectively inhibit the growth of some microorganisms. This result confirms previous studies by Agbankpe *et al.*, (2016) and Tsado *et al.*, (2016).

#### 5.0 Conclusion

This study reveals that *C. adansonii* is a highly potent plant which lends credence to its traditional use as a medicinal plant. However, further studies are currently being carried out on the anti-pyretic, anti-hypertensive, anti-malaria, anti-tubercular and analgesic activities of *C. adansonii* for a better bioactivity profiling and documentation for possible future drug design.

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