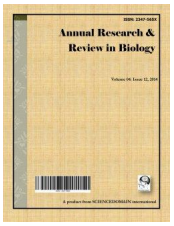

Annual Research & Review in Biology

24(5): 1-7, 2018; Article no.ARRB.39314
ISSN: 2347-565X, NLM ID: 101632869

Phytochemical Screening and Antimicrobial Studies of Stem and Root Extracts of *Crateva adansonii*

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Authors' contributions

This work was carried out in collaboration with all authors. The design, performance and manuscript development of the research study was carried out by authors COA, JOE and RCM. Authors TFO, OST and OE carried out the phytochemical and antimicrobial analysis while author JUA collated the review of literature searches. All authors approved the final manuscript for publication.

Article Information

DOI: 10.9734/ARRB/2018/39314
Editor(s):
(1) Manikant Tripathi, Department of Microbiology, Dr. Ram Manohar Lohia Avadh University, Faizabad, India.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Rajeev K. Singla, Netaji Subhas Institute of Technology, India.
(2) Gulab Khan Rohela, Kakatiya University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/23419

Original Research Article

Received 20th November 2017
Accepted 3rd February 2018
Published 4th March 2018

ABSTRACT

Aim: This study was designed to explore the phytochemical and antimicrobial screening of the stem and root extracts of *Crateva adansonii*.
Place and Duration of Study: Sample: Iyesi village, Ota, Ogun State, and analysis carried out at Department of Chemistry and Department of Biological Sciences, Covenant University, Ota, Ogun State and for duration of three months (November 2016 to February 2017).
Methodology: Standard universal procedures were employed for both phytochemical and antimicrobial analysis.
Results: The result obtained from the stem and root extracts of *Crateva adansonii* indicated the presence of flavonoids, terpenoids, alkaloids, and cardiac glycosides. Root extract was found to be richer in source of phytochemicals when compared to the stem extract. However, the highest

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antibacterial activity was observed against selected bacteria by both stem and root extracts. The potency of the root extract was observed to be higher than the stem extract against *Bacillus cereus, Staphylococcus aureus, Aspergillus niger* and *Serratia spp*.
Conclusion: The preliminary studies on the stem and the root of *Crateva adansonii* extracts revealed their antimicrobial potential which could be further investigated for global utilization in pharmaceutical treatment, natural therapies, food preservation and cosmetic applications.

Keywords: Phytochemicals; antimicrobial; antibacterial assay; inhibitory concentration.

1. INTRODUCTION

The discovery of numerous benefits inherent in plants has been time immemorial. Right from the early days, man has found plants as his source of feeding, shelter, clothing, health, etc. Various ailments, ranging from mild to more complex situations, have been treated with the knowledge of the use of herbs. Impairment of the normal functioning of the body could be attributed to the presence of pathogenic microbial agents such as: bacteria, fungi, viruses, protozoa or multicellular organisms [1]. The invasion and multiplication of these microbes on the human cells and release of toxins, results in infectious diseases, commonly referred to as illness. Diverse ailments are accompanied with their variable symptoms. Advancement in technology by introducing modern medicine, has not eradicated the role of plants; rather, it has recognized its importance, as well as enhanced its role in contemporary medicine.

Crateva adansonii, is generally found in Northern Nigeria referred to as 'Garlic pear tree' with a native name of 'ungududu'. In the Eastern Nigeria, the Igbo refer to it as 'amakarode'. In the Western part of Nigeria, the Yoruba expresses the plant as 'ẹgun-ọ́run' or 'Taniya'. *Crateva adansonii* belongs to the family of Capparaceae. It is a deciduous shrub or a small tree which is about ten metres tall, having the main stem short and irregular. The leaves are trifoliate and alternate; with the flowers having a creamy colour, with many terminal corymbs. The bark is grey, turning light-brown when old [2].

It has been reported that various parts of *Crateva adansonii* are used for traditional medicine in West Africa. In Senegal, the roots are used in the treatment of sterility, syphilis, ear infections, yellow fever and jaundice [3]. The plant is considered as diuretic, anti-inflammatory, hepatoprotective, laxative, anti-oxidant, contraceptive and anthelmintic [4]. The bark has a disagreeable smell and taste slightly bitter and pungent while the leaves are considered to be stomachic and tonic. The leaf powder is administered externally to treat headache, poor vision, fever, cyst, rheumatism and asthma [2]. The bark has found good use in the treatment of skin diseases and snake bite [5] while the powdered form is useful in the treatment of kidney, bladder and urinary tract infection [6]. The bark is taken in form of an enema for constipation and piles. This was also ascertained by earlier inhibitory activity studies of the fractions of the plant [7].

The aim of this work is to ascertain the ethnobotanical benefits of *Crateva adansonii* by carrying out phytochemical screening to identify the secondary metabolites present; at the same time investigate its antibacterial and antifungal potentials through antimicrobial analysis, thereby gaining insight into the microorganisms which the components are active against.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Fresh stem and root of *C. adansonii* plant were 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 4ºC.

2.2 Preparation of Plant Extracts

Fresh stem and roots were hand-plucked and cleaned for debris using tap water while distilled water was used in rinsing the plant samples. The air drying of the stem and roots was carried out at room temperature for two weeks. Dried

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. The filtration process was carried out using Whatman number 1 filter paper and the filtrates obtained was concentrated using rotary evaporator under vacuum. 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 procedures [8-10]. Screening involved tests for alkaloids, saponins, quinones, tannins, phenols, terpenoids, flavonoids, oxalates and cardiac glycosides.

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, and *Aspergillus niger*, using sabouraud dextrose agar as the medium [11,12].

2.5 Determination of Antibacterial Well Diffusion Assay

Determination of minimum inhibitory concentration (MIC) was carried out on the stem and root of *Crateva adansonii* [13]. The procedure was carried out on the different fractions of *Crateva adansonii* stem and the roots that showed sensitivity against the growth of some selected organisms. The medium used was nutrient agar which was prepared according to the manufacturers' standard. The solvent used during antibacterial screening for the dissolution of various fractions of stem and roots was Dimethyl sulphoxide (DMSO).The concentrations of the extracts obtained from the chloroform, methanol and hexane fractions after fractionation were adjusted to 50, 25, 12.5, 6.25, 3.125 and 1.562 mg/mL by serial dilution. The sterile nutrient agar plates were seeded using swab sticks with the test organisms or isolates of 0.5% Mcfarland standard. sterile cork borer was used to bore wells of about 9 mm in diameter into the sterile nutrient agar plates. Sterile pipette of 1 mL were used to measure 0.2 mL of each extract of different concentrations into the bored wells on the inoculated nutrient agar plates. The plates were observed for growth or death of test organisms after incubation at 37ºC for 24 hours. The minimum inhibitory concentration, MIC, was taken as the lowest concentration inhibiting the growth of organisms. Gentamycin, with a concentration of 10 µg was used as the standard antibiotic.

3. RESULTS

The qualitative result of the phytochemical screening discovered the presence of alkaloids, saponins, quinones, phenols, oxalates, terpenoids, flavonoids and cardiac glycosides (Table 1). After partitioning of the stem extract into the various solvents, we found that all the solvents showed the presence of saponins and oxalates; terpenoids and cardiac glycosides are present in all the solvents except for the aqueous fraction; alkaloids in the chloroform fraction. Methanol and aqueous fractions showed the presence of quinones whereas the hexane fraction showed the occurrence of flavonoids and the chloroform fraction exhibited the presence of phenols.

Table 1. Phytochemicals present in stem extract of *Crateva adansonii*

Constituent	Intensity				
	Methanol fraction	**Hexane fraction**	**Chloroform fraction**	**Aqueous fraction**	**Crude fraction**
Alkaloids	-	+	++	+	+
Saponins	++	++	+	++	+
Quinones	++	-	-	++	-
Tannins	-	-	-	-	-
Phenols	-	-	++	-	-
Terpenoids	++	++	++	-	++
Flavonoids	-	++	-	-	-
Oxalates	++	++	++	+	++
Cardiac glycoside	++	+	++	-	++

Key: + = trace, ++ = moderate +++ = intense - = not present

The results of the phytochemical screening of the root extract of *Crateva adansonii* revealed the presence of alkaloids, saponins, quinones, phenols, terpenoids, flavonoids, oxalates and cardiac glycosides, as shown in Table 2. All the solvents showed the presence of alkaloids, saponins and cardiac glycosides. Quinone is present in all the solvents except hexane; terpenoids are present in all except the aqueous fraction. Hexane solvent shows the presence of flavonoids and oxalates.

The results from Table 3 showed that methanol, chloroform, crude and aqueous fractions showed zone of inhibition (25 mm) against *E. coli, Pseudomonas, Serratia, Bacillus sp and Staphylococcus*. We see also that the methanol and chloroform fractions and the crude showed zones of inhibition (20 mm) against *Pseudomonas, Bacillus sp.* and *Staphylococuss aureus*.

Table 4 showed the results of inhibition for the root extract of *Crateva adansonii*. The crude extract, the hexane and methanol fractions showed high zone of inhibition (30 mm) against *E. coli, Bacillus, Aspergillus niger* and *Staphylococcus*. The Crude, methanol and hexane fractions showed inhibition (25 mm) against *Pseudomomnas, Aspergillus* and *Staphylococus*.

Table 2. Phytochemicals present in root extract of *Crateva adansonii*

Constituent	Intensity				
	Methanol fraction	**Hexane fraction**	**Chloroform fraction**	**Aqueous fraction**	**Crude fraction**
Alkaloids	+	+	++	+	++
Saponins	+	++	+	++	++
Quinones	++	-	++	++	++
Tannins	-	-	-	-	-
Phenols	-	-	++	-	+
Terpenoids	++	++	++	-	++
Flavonoids	-	++	-	-	-
Oxalates	-	+	-	-	-
Cardiac glycoside	++	++	++	++	++

Key: + = trace, ++ = moderate, +++ = intense, - = not present

Table 3. Zones of inhibition of stem extract of *Crateva adansonii* against selected microorganisms

Organism	Zone of inhibition (in mM)					
	Methanol fraction	**Hexane fraction**	**Chloroform fraction**	**Aqueous fraction**	**Crude fraction**	**Control (Gentamycin)**
Eschericia coli	28	-	25	18	20	-
Pseudomonas aerogenosa	20	-	25	25	-	15
Bacillus subtilis	20	-	-	-	25	20
Aspergillus niger	-	-	-	-	-	-
Serratia spp.	25	-	30	-	15	-
Staphylococcus aureus	20	-	24	-	10	15
Saccharomycisspp	-	-	-	-	-	-
Micrococcus variance	-	-	-	-	-	-

Table 4. Zones of inhibition of root extract of *Crateva adansonii* against selected microorganisms

Organism	Zone of inhibition (in mM)				
	Methanol fraction	Hexane fraction	Chloroform fraction	Crude fraction	Control (Gentamycin)
Eschericia coli	15	30	18	30	10
Pseudomonas aerogenosa	15	-	18	25	10
Bacillus subtilis	30	20	-	10	15
Aspergillusniger	30	-	20	25	15
Serratia spp.	25	18	-	22	-
Staphylococcus aureus	30	25	-	25	-
Saccharomycisspp	-	-	-	-	15
Micrococcus variance	-	-	-	-	-

4. DISCUSSION

The results obtained for the phytochemical screening of the stem and root extracts of *Crateva* adansonii revealed the presence of saponins, flavonoids, terpenoids, alkaloids and cardiac glycosides.This result agrees with earlier studies carried out on the phytochemical screening of the leaf extract of *Crateva adansonii* [14,15]. Particularly, methanol, aqueous and chloroform fractions show high potentials in transporting the secondary metabolites mentioned earlier. This implies that these fractions are suitable solvents in extracting the active components in the plant. Flavonoids were detected in the hexane fraction of both stem and root extracts. Flavonoids are known to possess antioxidant, anti-inflammatory, anti-microbial properties [16]. It has been shown that there is a positive correlation between high ingestion of flavonoids with reduced risk of cancer-related diseases [17]. The extracts from the stem and root of *Crateva adansonii* showed the presence of saponins and cardiac glycosides. These families of compounds are known to cause haemolysis of blood [18] and have anti-parasitic, anti-inflammatory and anti-viral properties [19] as well as their usefulness in the treatment of heart-related diseases [20]. Terpenoids, such as diterpenes or triterpenes which are known to possess insecticidal, antibiotic, as well as antiseptic properties [21,22] were also detected in all the solvents except in aqueous solvent for both stem and roots extracts. The presence of alkaloids is shown in all the solvents of the root extract, while it is only present in the chloroform solvent of the stem extract. This indicates the potential benefits inherent in the root portion of the plant, particularly as a medicinal agent against analgesic, bacterial and antimalarial activities [23,24]. Quinones were present in all the polar solvents in the root extract except in hexane solvent. However, it was present in the methanol and aqueous solvent of the stem extract. This suggests the potency of the root part in the treatment of malaria. For all the solvent fractions of the stem and the root extracts, hexane fraction was found to have the minimum number of phytochemicals present. The chloroform fraction of the stem was found to have rich source of phytochemicals as compared to the other fractions, whereas in the root, all the solvents were found to be rich in phytochemicals except the hexane fraction. This observation that the root portion of *Crateva adansonii* is a rich source of phytochemicals implies that the root portion will contain the most active ingredients for the treatment of ailments.

The highest zone of inhibition for the stem of *Crateva adansonii* was found in the chloroform and methanolic fraction of the stem part of the plant. It was found to be active against *Stappylococci, E. coli, Pseudomonas, Serratia*, while the hexane fraction showed no activity against any of the test micro-organisms.

The highest form of zone of inhibition for the root part of the plant was found in the methanol fraction. It was found to be active against *E. coli, Bacillus, Aspergillus, Serratia* and *Staphylococcus*. However, the chloroform fraction showed the least activity against the test organisms. The results also showed that no solvent fraction, both in the stem and root part of the plant, was found to be active against *Saccharomyces spp* and *Micrococcus* variance. This result confirms previous studies carried out on *Crateva adansonii* [25,26].

5. CONCLUSION

The results of preliminary phytochemical screening suggest that both stem and root extracts of *Crateva adansonii* are good sources of beneficial phytochemicals. Among the various prepared extracts, there was an observed correlation between the solvents having rich source of phytochemicals as well as solvents showing high activity against selected micro-organisms. Generally, the chloroform solvent of the stem confirms rich source of phytochemicals along with showing high degree of activity against the micro-organisms. The antibacterial analysis reveals that the crude, chloroform and methanol fractions of the root contain bioactive constituents which can effectively inhibit the growth of micro-organisms. Further research will be required to determine the efficacy of these extracts against other pathogenic bacterial and fungal species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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