PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF CRATEVA ADANSONII DC LEAVES AND STEM BARK EXTRACTS AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT

Plants have widely been used as sources of both traditional and modern medicine especially in the era of antimicrobial resistance. this necessitated the need to embark on current research on phytochemical analysis and antibacterial activity of Crateva adonsonii DC leaves and stem bark crude extracts against Escherichia coli, Staphylococcus aureus and Salmonella typhi. The extraction was done using chloroform, hexane and methanol. Phytochemical analysis was determined while the antibacterial activity of the plant extracts was carried out using agar-well diffusion method with the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Phytochemical constituents of leaves and stem bark of the plant revealed the presence of anthraquinones, flavanoids, alkaloids, balsams, saponins, saponins glycosides, steroids, tannins and volatile oil. Stem extract of the plant indicated no antibacterial activity against Escherichia coli and Salmonella typhi but highly effective against Staphylococcus aureus with the highest zone of inhibition of 30 mm and 20 mm as the lowest. However, the leaf extracts of hexane and methanol indicated antibacterial activity against all the test organisms with 30 mm as the highest zone of inhibition and 10 mm as the lowest. Methanolic extracts of both leaves and stem bark had an MIC value of 12.5 mg/ml against Staphylococcus aureus while the same extracts have the MBC of 25 mg/ml. The study showed that there is a possibility of using Crateva adonsonii DC extracts serve as remedy for infections caused by the test isolates.

Keywords: Antibacterial, Phytochemical, *Crateva adonsonii* DC, Bacteria.

1.0 INTRODUCTION

Conventional treatment of bacterial infections rely on commercial antibiotics. However, there is an overwhelming issue of antibiotic resistance and a continuous quest for new solutions. Antibiotic resistance is an age long process and the "resistome" is a dynamic and mounting issue. Reasons for the worldwide resistome are overpopulation, enhanced global migration, expanded use of Phone: +2348036318987

antibiotics in health facilities and animal production units, selection pressure, poor sanitation, wildlife spread and poor sewerage disposal framework (Singer *et al.*, 2016).

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Natural Products and their derivatives are used by locals to treat various illnesses including bacterial, parasitic and viral infections (Ganjhu *et al.*, 2015). There has been a rising interest worldwide in the investigation of medicinal plants as a source of pharmacologically potent compounds. Plants are known to be a rich repository of bioactive secondary metabolites (or natural Products, NPs), for instance, the anti-malarial medications quinine and artemisinin (AT) are both plant derivatives (Pinheiro *et al.*, 2019). About 20% of the plants across the globe have been subjected to pharmacological or biological test, a vast number of antimicrobials found in the market today are from natural or semi-synthetic sources (Mothana and Lindequist, 2005).

Crateva adonsonii DC is commonly found in Northern Nigeria popularly called 'Garlic Pear Tree' with a local name as '*ungududu*'. In the Eastern Nigeria, the Igbo call it '*amakarode*'. In the Western region of Nigeria, the Yoruba referred to the plant as '*egun-ór un*' or '*Taniya*'. *Crateva adonsonii* DC belongs to the family of Capparaceae. It is a deciduous shrub or a tree of average size which is around ten meters tall having a short stem and irregular. The leaves are trifoliate and alternate; with a cream-coloured flowers having numerous terminal corymbs. The bark is grey, turning light-brown as it ages out (Igoli et al., 2012).

The results obtained from the previous study for the phytochemical screening of the stem and root extracts of *Crateva adonsonii* DC revealed the presence of saponins, flavonoids, terpenoids, alkaloids and cardiac glycosides which have the potential to show antibacterial activity against some bacteria especially Enterobacteriaceae family (Ajanaku *et al.*, 2016; Tsado *et al.*, 2015). *Escherichia coli*, a bacterium belonging to a larger group of Gram negative rods known as enterobacteria are normally found in the intestinal tract, in soil and water. It is the commonest pathogen found in patients with cystitis (urinary tract disease) persistent in

women. *E. coli* can be spread via contaminated water or food or through contact with individuals and different animals (CDC, 2017). *Staphylococcus aureus* is an important cause for a wide range of clinical diseases including bacteraemia and infective endocarditis, osteoarticular, skin and soft tissue, pleuropulmonary, and device related infections (Tong *et al.*, 2015). *Salmonella typhi* poses a significant danger to human wellbeing in different parts of the world, particularly in non-industrial nations where there is open defecation where fecal matter collection and disposal is inefficient or recycled in horticulture and for other agricultural purposes and where there is lack of access to safe drinking water. Typhoid fever specifically, is an endemic infection in several South Asian and sub-Saharan African nations. Outbreak can happen even in endemic settings if an environmental variable, population immunity or circulating strain characteristics shift occur (Obaro *et al.*, 2017).

The present research focus on the screening of phytochemical components of *Crateva adonsonii* DC and afterward test for antibacterial activity of the plant's leaves and stem bark extracts against three bacterial species namely *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*.

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Fresh leaves and stem bark of *Crateva adonsonii* DC were collected and washed at Kalgo town of Kebbi State, Nigeria in the early hours of the day and transported to Plant Science and Biotechnology Laboratory of Federal University, Gusau for identification and authentication with the number CV 23/FUG.

2.2 Preparation of Plant Material

The samples were dried at room temperature under shade for two weeks. 50 g of powdered leaves and stem were soaked into 250 ml of chloroform, hexane and methanol. They were allowed to stand for 24 hours with regular shaking after time interval. The preparations were filtered using muslin cloth. The solvents were evaporated using rotary evaporator and the extracts were recovered and stored in the refrigerator for further use.

2.3 Preparation of Extracts Concentrations

Extracts concentration were prepared as described by Oloninefa *et al.* (2018). Two hundred and fifty milligram (250 mg) of the chloroform, hexane and methanol crude extract were weighed in 5 ml each of 20% Dimethyl sulfoxide (DMSO) to give 50 mg/ml concentrations respectively. The concentrations of 100 mg/ml, 150 mg/ml and 200 mg/ml were obtained following the same procedure.

2.4 Isolation and Identification of Bacterial Isolates

The test organisms namely, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were obtained from Microbiology Laboratory, Federal University, Gusau. However, they were subcultured in order to test for their sterility and to obtain fresh and young cultures of the organisms. The isolates were then characterized by cultural and biochemical tests as described by Cheesbrough (2006).

2.5 Determination of Phytochemical Constituents

The phytochemical screening of the plant leaves and stem were done in accordance with the standard procedures described by Sofowora (1993), Trease and Evans (1989) and Harbone (1973). The screening involved tests for flavanoids, saponins, tannins, alkaloids, cardiac glycosides, steroids, saponin glycosides, balsams anthraquinones and volatile oil.

2.5.1 Test for alkaloids

About 2-4 drops of Dragendroff"s reagent were added to 5 ml of ethanolic or methanolic extract. A change of colour confirmed the presence of alkaloids.

2.5.2 Test for Anthraquinones

Ten (10) ml of benzene was added in 6 g of the Ephedra powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds and pink, violet, or red color indicated the presence of anthraquinones in the ammonia phase.

2.5.3 Test for Balsams

Three (3) ml of extract was mixed with equal volume of 90% ethanol. 2 drops of 5% alcoholic ferric chloride was added to the mixture. A dark green colour indicates the presence of balsams.

2.5.4 Test for Cardiac Glycosides

Two (2) ml of 3.5 % was added to the plant extract and allowed to stand for one minute. Concentrated H_2SO_4 was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring indicates the presence of cardiac glycosides.

2.5.5 Test for Flavonoids

A few drops of concentrated sodium hydroxide (NaOH) were added to 2 ml of the extracts, followed by the addition of a few drops of dilute hydrochloric acid and observed for the presence of flavonoids.

2.5.6 Test for Glycosides

Two (2) ml of the extract was boiled, followed by the addition of a few drops of concentrated hydrochloric acid (HCI). The mixture was boiled again for a few minutes to hydrolyze any glycoside present. The mixture was changed to alkaline by the addition of a few drops of aqueous ammonia solution. Five drops of the mixture were added to 2 ml of Benedict's reagent, boiled and observed for the presence of glycosides.

2.5.7 Test for Saponins

About 1 g of the extracts was dissolved in distilled water, vigorously shaken and observed for the presence of saponins.

2.5.8 Test for Saponin Glycosides

2.5 ml of the extract was mixed with equal volume of Fehling solution A and B. A bluish green precipitate shows the presence of saponin glycosides.

2.5.9 Test for Steroids

Approximately 2 drops of chloroform were added into 2 ml of the extracts followed by the addition of concentrated sulphuric acid (H_2SO_4) and observed for the presence of steroids.

2.5.10 Test for Tannins

Two drops of ferric chloride were added to 2 ml of ethanolic or methanolic extract and observed for the presence of tannins.

2.5.11 Test for Volatile Oils

1 ml of the extract was mixed with equal volume of diluted HCI. A white precipitate shows the presence of volatile oils.

2.6 Standardization of Inocula

The inocula were standardized by dissolving 1-2 colonies of the test organisms into a test tube containing 9 ml of sterile normal saline. The tubes were mixed thoroughly and matched with 0.5 McFarland turbidity standards (approximate cell count density: 1.5x10⁸).

2.7 Antibacterial Activity

The extracts from the leaves and stem bark were investigated for their antibacterial activities concentrations of 200, 150, 100 and 50 mg/ml against E. coli, S. aureus and S. typhi by agar well diffusion method as described by Azam et al. (2012). Sterile swab stick was immersed into the tube containing standardized inocula, excess fluids were drained by pressing the sticks against the inner walls of the tubes and swabbed on to plates containing Mueller Hinton Agar. Three wells were punched using sterile cork borer of 6 mm diameter. A 100 µl of each of the concentrations was placed into the wells and allowed to stand for one hour for the extracts to diffuse into the agar and incubated for 24 hours at 37°C. A 50 mg/ml Ciprofloxacin was used for positive control while dimethylsulfuroxide (DMSO) served as the negative control. The diameter of the zones of inhibition for different plant extracts. Ciprofloxacin and DMSO against different bacteria were measured in millimeter (mm). An agar well (6 mm) showing no zone of inhibition was considered as no antimicrobial activity (Farjana et al., 2014).

2.8 Determination of Minimum Inhibitory Concentration

Different concentrations of the effective plant extracts (200, 100, 50, 25, 12.5 and 6.25 mg/ml) were prepared by two-fold serial dilution of the stock solution in test tubes. Test organisms were inoculated into test tubes containing the extracts and incubated for 24 hours. The result was taken based on the tubes with clarity and those with turbidity. In each experiment, two control tubes were prepared alongside by mixing the extracts and growth medium without the standardized inocula in test tubes (positive control) and tube containing the growth medium and the inocula (organism control).

2.9 Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing all tubes that showed no visible bacterial growth from the MIC on fresh solid media and incubated for 24 hours at 37°C.

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Phytochemical Profile of Stem Bark Extracts

Table 1 shows the result of phytochemical profile of stem bark extracts. Tannins were present in hexane, chloroform and methanol extracts while anthraquinones, cardiac glycosides, flavonoids and glycosides were absent in all the three extracts. Alkaloids were present in hexane and methanol extracts but absent in chloroform extract. Balsams were present in chloroform and methanol extracts but absent in hexane extract while saponins were present in methanol extract but absent in hexane and chloroform extracts. Similarly, saponins glycosides were only present in chloroform extract, absent in hexane and methanol extracts. Steroids were only present in hexane extract but not found in chloroform and methanol extracts. On the other hand, volatile oils were absent in methanol extract but found in both hexane and chloroform extracts (Table 1).

Table 1: Phytochemical Profile of Stem Bark E

Parameters	Hexane	Chloroform	Methanol
	Extract	Extract	Extract
Alkaloids	++	-	+++
Anthraquinones	-	-	-
Balsams	-	+++	+++
Cardiac Glycosides	-	-	-
Flavanoids	-	-	-
Glycosides	-	-	-
Saponins	-	-	++
Saponins Glycosides	-	+++	-
Steroids	+++	-	-
Tannins	+++	+++	++
Volatile oils	+++	+++	-

Key: + = Trace, ++ = Moderate, +++ = Intense, - = Absent

3.1.2 Phytochemical Profile of Leaf Extracts

Table 2 shows the result of phytochemical profile of stem bark extracts. Cardiac glycosides and steroids were intensely present in hexane, chloroform and methanol extracts while anthraquinones, balsams, flavonoids, saponins glycosides and tannins were absent in all the three extracts. Alkaloids and glycosides were intensely present in hexane extract but absent in chloroform and methanol extracts. However, volatile oils were intensely found in hexane and chloroform extracts but not found in methanol extract.

Table 2: Phytochemical Profile of Leaf Extracts

Parameters	Hexane	Chloroform	Methanol
Alkaloids	+++	-	-
Anthraquinones	-	-	-
Balsams	-	-	-
Cardiac Glycosides	+++	+++	+++
Flavanoids	-	-	-
Glycosides	+++	-	-
Saponins	-	-	-
Saponins Glycosides	-	-	-
Steroids	+++	+++	+++
Tannins	-	-	-
Volatile oils	+++	+++	-

Key: + = Trace, ++ = Moderate, +++ = Intense, - = Absent

3.1.3 Antibacterial Activity of *Crateva adonsonii* Stem Bark Extract

Table 3 shows the antibacterial activity of *Crateva adonsonii* stem bark extract against the selected isolates (*E. coli, S. typhimurium* and *S. aureus*). Chloroform, hexane, methanol extracts and DMSO (Negative control) had no activity against *E. coli* and *S. typhimurium*. Hexane and methanol extracts had antibacterial activity against *S. aureus*. The highest value of 30.31 mm was obtained when 150 mg/ml of hexane extract was used while the lowest value recorded was 20.34 mm when 50 mg/ml of methanol extracts was used (Table 3). The positive control (200 mg/ml Ciprofloxacin) recorded the highest value of antibacterial activity of 57.29 mm against *E. coli* and the lowest value of 40.45 mm against *S. aureus* (Table 3).

3.1.4 Antibacterial Activity of Crateva adonsonii Leaf Extract

Table 4 shows the antibacterial activity of *Crateva adonsonii* leaf extract against the selected isolates (*E. coli,* S. *typhimurium* and *S. aureus*). Chloroform extract had no activity against *E. coli,* S. *typhimurium* and *S. aureus*. In the same vein, methanol extracts had no activity against *E. coli* and *S. typhimurium*. Hexane and methanol extracts had antibacterial activity against *E. coli,* S. *typhimurium* and *S. aureus*. The highest value of 30.30 mm was obtained while the lowest value of 10.24 mm was obtained when 200 mg/ml and 50 mg/ml of methanol extract were used respectively (Table 4). Ciprofloxacin) had the highest value of antibacterial activity of 58.04 mm against *E. coli* and the lowest value of 41.72 mm against *S. typhimurium* (Table 3). The DMSO had no activity on the tested organisms.

Table 3: Antibacterial Activity of Stem Bark Extract (50-200 mg/ml) of Crateva adonsonii (mm)

	Escherichia coli				Salmonella typhimurium			Staphylococcus aureus				
Extracts/Control	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml
Chloroform	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª
Hexane	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	22.22±0.17	24.45±0.04	30.31±0.06⁵	0.00±0.00ª
Methanol	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	20.34±0.09	27.40±0.04°	30.22±0.02⁵	0.00±0.00ª
Ciprofloxacin	49.39±0.49°	52.40±0.52	55.39±0.46	57.29±0.74	41.72±0.16	43.48±0.39	44.91±0.44	47.66±0.57⁵	40.45±0.11	42.31±0.17ª	44.68±0.05℃	48.42±0.31°
DMSO	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at p<0.05

Table 4: Antibacterial Activity of Leaf Extract (50-200 mg/ml) of Crateva adonsonii (mm)

	Escherichia coli			Salmonella typhimurium				Staphylococcus aureus				
Extracts/Control	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml
Chloroform	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª
Hexane	0.00±0.00ª	25.20±0.22	25.27±0.19	29.99±0.28°	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	20.37±0.10°	0.00±0.00ª	20.75±0.35	25.21±0.11	30.10±0.11⁵
Methanol	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	10.24±0.14	25.11±0.14	25.41±0.02⁵	30.30±0.10 ^b
Ciprofloxacin	50.43±0.13	53.30±0.16	54.48±0.26¢	58.04±0.26℃	41.72±0.16	43.48±0.39	44.91±0.44	57.29±0.74℃	48.77±0.21	50.86±0.23ª	52.86±0.23¢	54.44±0.06⁰
DMSO	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at p<0.05

3.1.5 Minimum Inhibitory Concentration (mg/ml)

Table 5 shows the minimum inhibitory concentration (MIC) obtained when hexane leaf extract (HLE), methanol leaf extract (MLE), hexane stem bark extract (HSBE) and methanol stem bark extract were used. the MIC of 12.5 mg/ml was obtained from MLE, HSBE and MSBE respectively against *S. aureus*.

Table 5: Minimum Inhibitory Concentration (mg/ml)

Organisms	HLE	MLE	HSBE	MSBE
E. coli	25	-	-	-
S. typhimurium	100	-	-	-
S. aureus	50	12.5	12.5	12.5

Key:

HLE: Hexane Leaf Extract MLE: Methanol Leaf Extract HSBE: Hexane Stem Bark Extract MSBE: Methanol Stem Bark Extract

3.1.6 Minimum Bactericidal Concentration (mg/ml)

Table 6 shows the minimum bactericidal concentration (MBC) obtained when hexane leaf extract (HLE), methanol leaf extract (MLE), hexane stem bark extract (HSBE) and methanol stem bark extract were used. the MIC of 25 mg/ml was obtained from MLE, HSBE and MSBE respectively against *S. aureus*.

Table 6: Minimum Bactericidal Concentration (mg/ml)

Organisms	HLE	MLE	HSBE	MSBE
E. coli	50	-	-	-
S. typhimurium	100	-	-	-
S. aureus	50	25	25	25

Key:

HLE: Hexane Leaf Extract MLE: Methanol Leaf Extract HSBE: Hexane Stem Bark Extract MSBE: Methanol Stem Bark Extract

3.2 DISCUSSION

The result of the qualitative phytochemical screening on the stem bark of Crateva adonsonii reveals the presence of alkaloids, balsams, saponins, saponins glycosides, steroids, tannins and volatile oil. However, there are some variations in the result of the qualitative screening on the leaves of the plant which revealed the presence of alkaloids, cardiac glycosides, glycosides, steroids and volatile oil. This report also shows that both stem bark and leaf extracts have the presence of alkaloids, glycosides, steroids and volatile oil. Consequently, they lack anthraguinones and flavanoids. The result obtained from phytochemical screening disagree with the one reported by Ajanaku et al. (2016) but similar to Mann et al. (2009) and Ajanaku et al. (2018) who reported the presence of alkaloids and cardiac glycosides in their studies. Phytochemicals play diverse roles in plants including the provision of vigour to plants, attraction of insect for pollination, feeding defence against predators and provision of colours. They are also known to be responsible for the pharmacological and toxic activities of plants (Lawal et al., 2005; Lawal et al., 2014).

The result shows that neither leaf nor stem chloroform extracts exhibits any antibacterial activity against the tested organisms which is similar to the result obtained by Ajanaku *et al.* (2016) but contrary to the recent study by Ajanaku *et al.* (2018) that recorded antibacterial activity against the tested organisms. This may be due to lack of or insufficient bioactive compounds in the extract to effect antibacterial activity against the tested organisms. The difference in results may also be due to the solvents used for extraction, geographical location and soil characteristics among other factors as reported in the previous study carried out by Ughachukwu *et al.* (2014). However, Mignanwandé *et al.* (2020) reported the possibility of using *Crateva adonsonii* extracts to tackle antibacterial resistance.

Furthermore, hexane and methanol extracts of the stem bark exhibit antibacterial activity against *S. aureus* only while chloroform shows no activity against all the test organisms but the methanol extracts of the leaves demonstrated antibacterial activity against *S. aureus* alone while hexane on all the organisms. The report by Bibitha *et al.* (2002) that there is a variation in the antibacterial activities of different plant extracts might have been responsible for this.

The results obtained for minimum inhibitory concentration (MIC) and minimum bacteriacidal concentration (MBC) in this study differ from the results obtained by Mignanwandé *et al.* (2020) possibly due to the differences in the laboratory procedures, reagents, solvents and plant extracts that were used (Bonini *et al.*, 2002; Wallack, 2007). The geographical locations where *Crateva adonsonii* was obtained might have also contributed to this (Ughachukwu *et al.*, 2014).

4.0 Conclusion

The study revealed that *Crateva adonsonii* had phytochemicals in both leaf and stem extracts. hexane and methanolic extracts had antibacterial activities on the tested organisms but chloroform extracts (obtained from leaf and stem) had no antibacterial activity on the tested organisms. The MIC and MBC of both the hexane and methanol extracts against tested organisms were also determined. The study showed that there is a possibility of using *Crateva adonsonii* extracts to remedy infections caused by these test isolates and may also serve to counter their antibiotic resistance..

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