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Research Article
Aspects of Medicinal Activities of the Stem Bark Extracts of *Curtisia dentata* (Burm. F.)

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Abstract

Background: Dysentery is a chronic disease causing intestinal inflammation as a result of severe diarrhoea with mucus or blood in the faeces. This is caused either by infectious or non-infectious agents and the severity ranges from asymptomatic to severe dehydration resulting into death. **Methodology:** Phytochemical, antioxidant and antimicrobial analyses were carried out using standard methods and the crude extracts were screened against 12 bacteria strains. Agar well diffusion and broth micro-dilution techniques were used to determine the diameters of zone of inhibition and the Minimum Inhibition Concentrations (MICs), respectively. **Results:** Phytochemical assay revealed the presence of high content of flavonoids, total phenol and tannins, low equivalent quantity of saponins and proanthocyanidin and very low alkaloids. Antioxidant activity showed high nitric oxide, low ferric reducing activities, moderate/low DPPH and ABTS scavenging activities of the plant. The degree of inhibitions varied significantly with different solvents used. *Curtisia dentata* activity against all the tested bacterial demonstrated an inhibition mean zone diameter of 10-25 mm. The MIC of the acetone extract ranged from >5 to 0.01 mg mL^{-1} and was active against 10 out of the 12 bacteria isolates with *Escherichia coli* (20 ± 1.1) being the most susceptible organism. **Conclusion:** This study provides scientific evidence for ethnomedicinal uses of *Curtisia dentata* stem bark as a good source of free radical scavenging and antimicrobial agents. This appreciably justifies the ethnomedicinal importance of the plant.

Key words: Antibacterial, *Curtisia dentata*, dysentery, phytochemicals, radical scavenging, agar well diffusion, McFarland, minimum inhibitory concentration

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dysentery is a chronic disease causing intestinal inflammation as a result of severe diarrhoea with characteristic mucus or blood in the faeces. The severity of diarrhoea ranges from asymptomatic to severe dehydration resulting in death. Globally, diarrhoeal disease is the second leading cause of death in children under 5 years old with an estimation of about 60,000 cases of childhood diarrhoea per month and approximately 9,000 child diarrhoeal deaths in the same[1,2].

Human pathogens are able to cause diseases such as dysentery which have been reported as one of the most serious infectious bacterial diseases, causing a threat to healthcare globally despite the availability of drugs and care centres[3]. There are other risk factors causing the prevalence of this infectious diseases, these are lack of proper sanitary facility such as and unhygienic habits of not washing hands before eating. The unsanitary environments allow diarrhoea-causing pathogens to spread more easily[4,5]. It is pertinent to know that in South Africa, approximately six million households in South Africa (46%) do not have access to portable water supply in their homes. In addition, 1.4 million households (11%) still lack access to sanitation services[6]. These issues could be major risk factors for diarrhoeal diseases since the sanitation services in over 3.8 million households (26%) does not meet the required standards due to infrastructure deterioration[6].

Hence, the need to look for a more accessible, affordable and reliable means of reducing the morbidity/mortality due to the cost of infectious diarrhoea diagnostic treatment as a result of lack of portable water supply and/or improper sanitation services. Interests in examining the effects of antidysenteric traditional medicine as potential sources of new antimicrobial agents are on the increase. This is because they are easily accessible, cheaper and have fewer side effects as compared to most synthetic drugs[7]. Plant extracts are known to have antispasmodic effects, delay gastrointestinal transit, suppress gut motility, stimulate water adsorption or reduce electrolyte secretion[8]. All these activities may explain the benefits of using certain plants in the treatment of diarrhoeal disease[9].

Curtisia dentata (Burm. F.) C.A. Sm, locally called Assegaai (Afrikaans) and umLahleni (Xhosa, Zulu) belongs to the Cornaceae family. It is an evergreen tree with a dark fissured square patch bark. The leaves are smooth, glossy and ovate, broadly elliptic and up to 10 cm in length. The flowers are about 10 mm in diameter with round oval shaped fleshy bitter berries[10]. In Southern Africa, the stem bark of *C. dentata* (CD) is used traditionally for the management of stomach ailments, diarrhoea, as a blood purifier and aphrodisiac[11,12]. In the Eastern Cape Province, it is used in the treatment of heartwater disease in cattle[10]. Previous studies on different organs of CD have isolated phytonutrients (betulinic acid, ursolic acid and 2-α-hydroxyursolic acid) and demonstrated their pharmacological significance as antimicrobial, anti-obesity, anti-parasitic and anthelminthic[10,12]. In light of these, the present study was also designed to enrich the ethnopharmacological information of the plant through investigations on the antioxidants and antibacterial properties of its different extracts (aqueous, acetone and ethanol) against selected pathogens of dysentery importance which is lacking in scientific literature till date.

MATERIALS AND METHODS

Plant material: Fresh mature whole plant of CD was collected in December, 2014 at Ntselamanzi area of the Eastern Cape Province of South Africa. The sample was authenticated by Prof. DS Grierson, a botanist at the University of Fort Hare, Alice South Africa. Specimen sample was prepared and deposited at the Giffen's Herbarium (Win 2014/2).

Sample processing and preparation of extracts: The collected sample was oven-dried to constant weight at 40°C and subsequently milled into a homogeneous powder. A portion (50 g each) of the powdered sample was extracted separately in 500 mL each of distilled water, acetone and ethanol with consistent agitation on an orbital shaker (140 rpm) for 48 h. Each extract was filtered (Buchner funnel and Whatman No. 1 filter paper) and the filtrates obtained were concentrated to dryness under reduced pressure (37°C). The extracts were reconstituted in their respective solvents to give the desired concentrations used in the study.

Phytochemical quantification

Determination of total phenols: The Folin-Ciocalteu assay described by Wintola and Afolayan[13] was used for the quantification of total phenolic content. Absorbance at 765 nm was read using an AJI-C03 UV-VIS spectrophotometer after incubating the reaction mixtures at room temperature for 30 min using a blank containing other working reagents without the test extract. The assay was done in triplicate. Results were expressed as mg g^{-1} TAE using the calibration curve:

$$Y = 0.1216x, R^2 = 0.936512$$

where, Y is the absorbance and x is the tannic acid equivalent.

Estimation of flavonoids: Flavonoid content was measured using the aluminium chloride colorimetric method as described by Oyedemi *et al.*[10]. Absorbance at 420 nm was read using an AJI-C03 UV-VIS spectrophotometer against a blank. The samples were analysed in triplicate and total flavonoids content were calculated as mg g^{-1} QE using the following equation based on the calibration curve:

$$Y = 0.0255x, R^2 = 0.9812$$

where, Y is the absorbance and x is quercetin equivalent.

Determination of total proanthocyanidin: Proanthocyanidin content was measured using the vanillin-ethanol procedure described by Cheng *et al.*[14]. Absorbance was taken at 500 nm against blank and total proanthocyanidin content was evaluated at a concentration of 0.1 mg mL^{-1} and expressed as mg g^{-1} CE using the calibration curve equation:

$$Y = 0.5825x, R^2 = 0.9277$$

where, Y is the absorbance and x is the catechins equivalent.

Tannin determination: The procedure of Mbaebie *et al.*[15] using Folin-Denis reagent tannin determination was adopted. The absorbance of the tannic acid standard solutions as well as the sample was spectrophotometrically measured at 760 nm following colour development. Results were expressed as mg g^{-1} TAE using the calibration curve:

$$Y = 0.0593x-0.0485, R^2 = 0.9826$$

where, Y is the absorbance and x is tannic acid equivalent.

Determination of saponins: Quantitative determination of saponins was done using the method of Eze and Airouyuwa[16]. About 5 g of each extract was dispersed in 50 mL of 20% (v/v) aqueous ethanol. The suspensions were heated in a water bath (55°C) with continuous stirring for 4 h. The resulting mixture in each case was filtered and the residue re-extracted with another 50 mL of 20% aqueous ethanol. The combined infusions were reduced to 20 mL over water bath maintained at 90°C. The concentrated solution obtained was shaken vigorously with 10 mL of diethyl ether in a 250 mL separating funnel and the aqueous layer was collected while the ether layer was discarded. The purification process was repeated. About 20 mL of butanol was added to the filtrate and then washed twice with 10 mL of 5% w/v aqueous sodium chloride. The whole mixture was heated to evaporation in a water bath and subsequently oven-dried at 40°C to constant weight. The saponins content was calculated using the formula:

$$\text{Saponins (\%)} = \frac{\text{Final weight of sample}}{\text{Initial weight of extracts}} \times 100$$

Determination of alkaloids: Alkaloids were quantitatively determined according to the method of Wintola and Afolayan[13]. Each extract sample (2 g) was extracted with 200 mL of 10% acetic acid in ethanol. This was allowed to stand for 4 h at room temperature, filtered and the filtrate was then concentrated on a water bath to 1/4th of its original volume. Concentrated ammonium solution was added drop-wise to basify the extract until the precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with dilute ammonium solution and then filtered. The residue was dried and weighed. The alkaloid content was determined using this formula:

$$\text{Alkaloid (\%)} = \frac{\text{Final weight of sample}}{\text{Initial weight of extracts}} \times 100$$

Antioxidant activity

Nitric oxide scavenging activity assay: The modified method described by Oyedemi *et al.*[10] was used to determine the nitric oxide radical scavenging activity. A volume of 2 mL of 10 mM of sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of plant extracts, gallic acid and BHT individually to make different concentrations from 0.025-0.5 mg mL^{-1}. The mixture was incubated at 25°C for 150 min. About 0.5 mL of incubated solution was then mixed with 0.5 mL of Griess reagent (1.0 mL sulfanilic acid reagent 0.33% prepared in 20% glacial acetic acid) at room temperature for 5 min with 1 mL of naphthylenediamine dichloride (0.1% w/v). The mixture was incubated at room temperature for 30 min, followed by the absorbance reading at 540 nm. The percentage of nitric oxide inhibitory potential of the extracts was subsequently evaluated.

Ferric reducing power assay: The reducing power of each extract of CD was determined by the method of Otang *et al.*[17] with slight modifications. Briefly, different concentrations (0.025-0.5 mg mL^{-1}) of extract (0.5 mL) were mixed with 0.5 mL 0.2 M phosphate buffer (pH 6.6) and 0.5 mL 0.1% potassium hexacyanoferrate followed by incubation at 50°C

in a water bath for 20 min. After incubation, 0.5 mL 10% TCA was added to terminate the reaction. The upper portion of the solution (1 mL) was mixed with 1 mL of distilled water and 0.1 mL 0.01% $FeCl_3$ solution added. The reaction mixture was left for 10 min at room temperature and the absorbance measured at 700 nm against the appropriate blank solution. All tests were in triplicates. A higher absorbance of the reaction mixture indicated greater reducing power.

DPPH radical scavenging activity assay: The method of Mathangi and Prabhakaran[18] was used for the determination of DPPH radical scavenging activity. Briefly, a solution of 0.135 mM 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) in methanol was prepared. Exactly 1.0 mL of this solution was mixed with 1.0 mL of the extracts (0.025-0.5 mg mL^{-1}) and standard drugs (BHT and ascorbic acid). The reaction mixture was then vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The actual decrease in absorbance was measured against that of the control. All tests and analysis were run in triplicates. The scavenging ability of each extract was then calculated using this equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

where, Abs control is the absorbance of DPPH+methanol and Abs sample is the absorbance of DPPH radical+sample (sample or standard).

2, 2'-aazino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging assay: The method described by Olajuyigbe and Afolayan[19] was adopted for the determination of ABTS scavenging activity. Briefly, the stock solutions including; 7 mM ABTS solution and 2.4 mM potassium persulfate solution were prepared. The working solution was then prepared by mixing the two stock solutions in equal proportions and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS+solution with 60 mL of methanol to obtain an absorbance of 0.708±0.001 U at 734 nm using spectrophotometer. Each extract/control (1 mL) was allowed to react with 1 mL of the ABTS+solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS+scavenging capacity of the extract was then compared with that of the standards. The percentage inhibition was then calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

where, A_{blank} is the absorbance of ABTS radical+methanol used as control, A_{sample} is the absorbance of ABTS radical+sample extract/standard. All the tests were carried out in triplicates. The activity was expressed as 50% inhibitory concentration (IC_{50}). The lower the IC_{50} value, the higher the antioxidant activity.

Antibacterial assay

Test organisms: Reference bacterial strains were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. These included *Salmonella typhimurium* (ATCC 13311), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *Bacillus cereus* (ATCC10702), *Shigella sonnei* (ATCC 29930), *Streptococcus pyogens*, *Bacillus subtilis* KZN, *Shigella flexneri* KZN, *Vibrio cholerae* (Laboratory isolate), *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538). The strains were kept at 4°C on agar slant and sub-cultured at 37°C for 24 h on nutrient agar before any susceptibility test. The antibacterial assays were carried out using nutrient agar (Biolab) and broth.

Antibacterial susceptibility test: The agar well diffusion technique was employed as previously described by Nethathe and Ndip[20] with modifications. Using the micropipette, 100 µL of 0.5 McFarland standard of bacterial cultures in 0.9% NaCl was inoculated on the surface of the nutrient agar plate and spread using a sterile swab stick. Wells were bored into each agar plate with a sterile cork borer (6 mm diameter). The wells were filled with the prepared concentration of the extract and care was taken not to allow spillage. Ciprofloxacin (0.0125 mg mL^{-1}) was used as positive control while the diluents used for extract was used as negative control. The tests were carried out in triplicates and the plates were incubated at 37°C for 24 h after which the zones of inhibitions were measured.

Microdilution assay: The microdilution method was employed to determine the Minimum Inhibitory Concentrations (MICs) of the plant extracts using 96 well microtitre plates[17]. Initially, 120 µL of SDW was added into each well of the first (A) and last (H) rows and also into all the wells of the last column. Then, 120 µL of Nutrient Broth (NB) was added into each well of the second row (B) and 150 µL of NB was added into the remaining wells of the first column and

100 µL into the rest of the wells from the second column rightward. About 50 µL of the plant extract were then added into the third well of the first column while 50 µL of the positive and negative control were separately added into the remaining wells of the first column.

A two-fold serial dilution was done by mixing the contents in each well of the first column (starting from the third row) and transferring 100 µL into the second well of the same row and the procedure was repeated up to the 11th well of the same row and the last 100 µL from the 11th well was discarded. Hence, various concentrations of the plant extracts ranging from 5-0.005 mg mL^{-1} were prepared in the wells, following the two-fold dilution method. Thereafter, 20 µL of 0.5 McFarland bacteria suspensions was inoculated into the wells except those which contained SDW. The plates were then incubated at 37°C for 24 h after which the growth of the bacteria was measured by determining the absorbance at 620 nm with a microtitre plate reader before and after incubation. The lowest concentration of the test extract resulting in inhibition of 50% of bacterial growth was recorded as the MIC.

Statistical analysis: All experiments were done in triplicates and where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between mean values were determined by Duncan's multiple range test using the Minitab program (version 12 for windows). The $p<0.05$ were regarded as significant.

RESULTS

Phytochemicals: The phytochemical contents of *C. dentata* extracts reveal the presence of alkaloids, saponins, tannins, anthocyanins, flavonoids and phenols. The solubility of these phytochemicals in different solvents as functions of their concentrations showed the value range for flavonoids (4.18-12.47 mg g^{-1}), saponin (0.23-3.09 mg g^{-1}) and proanthocyanidin (0.22-1.58 mg g^{-1}). The concentrations of the total phenol and tannin extracted by the different solvents were higher than other phytochemicals (Fig. 1).

The amount of total phenol in the water and acetone extract were higher that the amount in the ethanol extract. On the other hand, the amount of tannins in the ethanol extract was higher than the amount of tannins in the water extract and acetone extract, respectively. In a similar way, the amount of flavonoids in the ethanol extract was higher than the amount in the aqueous extract and far lower than that in the acetone extract. The amount of proanthocyanidin in the water and ethanol extracts were higher than the amount in the acetone extract. Similarly, the amount of saponin in the acetone extract was higher than that of ethanol extract and least in the water extract. The concentration of alkaloids in the stem bark extracts of CD was very low compared to other phytochemicals evaluated. However, alkaloids extracted were not significantly different from each other (Fig. 1).

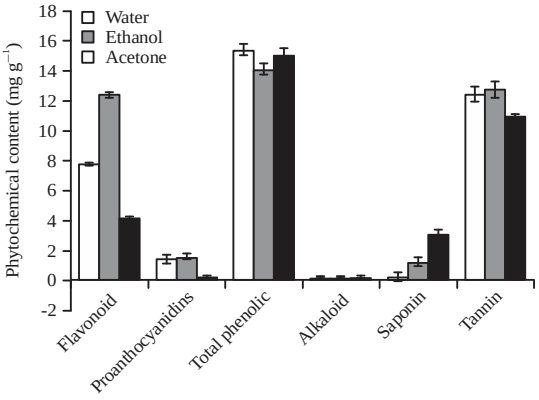

Fig. 1: Phytochemical contents of *Curtisia dentata* stem bark extracts

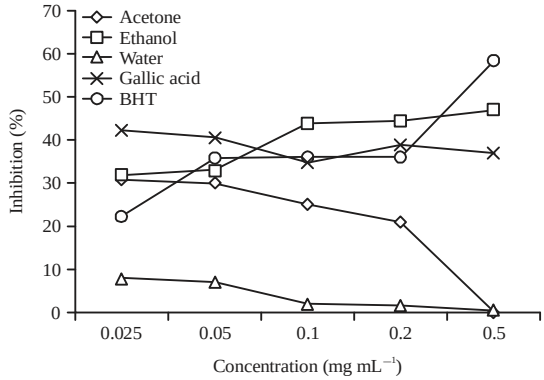

Fig. 2: Nitric oxide scavenging effect of extracts of *Custisia dentate*

Antioxidant

Nitric oxide: The nitric oxide scavenging activities of the different extracts of CD are shown in Fig. 2. The nitric oxide scavenging activity of the ethanol extract (IC_{50} = 0.15) and standard BHT (IC_{50} = 0.23) were concentration dependent. On the other hand, for the water (IC_{50} = 0. 30) and acetone (IC_{50} = 0.20) extracts, their nitric oxide scavenging activity decreases with increasing concentration. However, the scavenging activity of the water extract was the lowest compared with the controls. The nitric oxide scavenging activity of standard gallic acid (IC_{50} = 0.27) was almost stationary despite the increasing concentration.

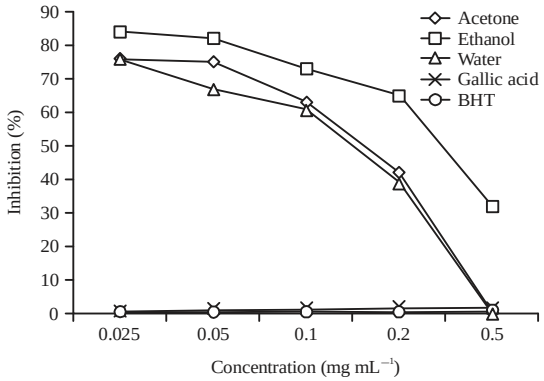

Fig. 3: Reducing power of the various solvent extracts of *Curtisia dentata* and the standards

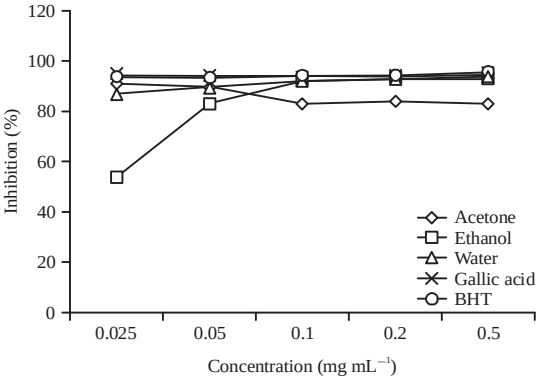

Fig. 4: DPPH radical inhibitory effects of *Curtisia dentata* stem bark extracts

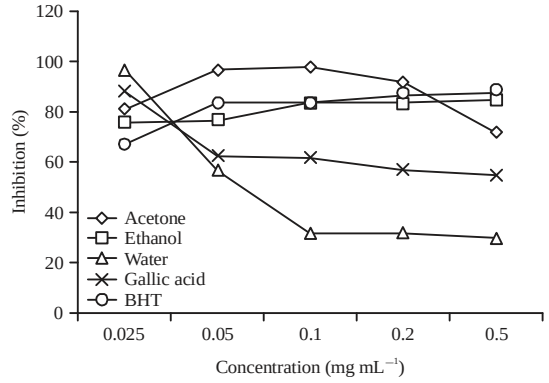

Fig. 5: ABTS radical scavenging abilities of stem bark extracts of *Curtisia dentata* antimicrobial activity

Ferric reducing power: The ferric ion reducing effects of the different extracts of CD are shown in Fig. 3. The IC_{50} values for ethanol, acetone and water extracts were 0.68, 0.26 and 0.26, while the IC_{50} for gallic acid and BHT were 0.11 and 0.12 mg mL^{-1}, respectively. All the extracts exhibited good ferric ion reducing activities at lower concentrations (0.025-0.05 mg mL^{-1}). The FRAP scavenging activity was highest in the ethanol extract of CD.

DPPH: Figure 4 showed the DPPH scavenging activities of *C. dentate* extracts. Judging by the respective IC_{50} values of 0.11, 0.12 and 0.14 mg mL^{-1} for the water, acetone and ethanol extracts which were not significantly different from those of gallic acid ($IC_{50} = 0.11$) and BHT ($IC_{50} = 0.12$), it may be inferred that the extracts exhibited remarkable DPPH scavenging activity. Although, the ethanol extract of CD at lower concentration elicited least inhibition of DPPH radical, its overall effect on this radical was also commendable particularly at higher concentrations.

ABTS: The ABTS scavenging activities of the different stem bark extracts of CD are shown in Fig. 5. The ABTS scavenging activities of the acetone and ethanol extracts are almost stationary despite the increasing concentrations. The scavenging activities of the water extract decrease with increasing concentration. At all concentrations, the scavenging activity of BHT was comparable with the acetone and ethanol extracts at 0.2 mg mL^{-1}. The ABTS scavenging activity of the gallic acid was however lower than that of acetone and ethanol but higher than that of water extract. This clearly shows that this plant is not showing ABTS scavenging activities.

Antibacterial: Of the three extracts tested against 12 pathogenic bacteria, the acetone extract was active against 10 of the bacteria with zones of inhibition ranging from 20-25 mm, followed by ethanol and water extracts which were active against 7 of the bacterial cells with zones of inhibition ranging from 15-20 mm. The aqueous extract was found to be very active against *Vibrio cholera* (20±1.1 mm), while the ethanol and acetone extracts were less active against *Vibrio cholera* (10±1.0 mm). *Bacillus cereus* was not sensitive to the acetone extract (Table 1).

The highest activity was observed with acetone extract against *Shigella sonnei* and *Streptococcus pyogens* (25±1.6 and 25±3.1 mm) as well as *Bacillus subtilis* 21±1.6 mm. The zone of inhibition was 20±1.0 mm for *Salmonella typhimurium*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. While, its result for *Vibro cholerae* was 10±1.0 mm, it had no activity against *Bacillus cereus.* For the ethanol extract, the inhibition zones was 20 mm against *Shigella sonnei*, *Escherichia coli* and *Pseudomonas aeruginosa* and 10±1.0 mm against *Staphylococcus aureus* (Table 1).

The MICs of the various extracts against the bacterial strains is presented in Table 2.

Table 1: Average zones of inhibition produced by different stem bark extracts of *Curtisia dentata* and ciprofloxacin against selected bacterial strains

		Zone of inhibition			
Bacteria	Gram	Water	Ethanol	Acetone	Positive control (Ciprofloxacin)
Salmonella typhimurium	-	18±1.1*	20±1.0	20±1.0	25±1.1
Enterococcus faecalis	+	19±1.1*	20±1.1	20±3.1	24±1.2
Escherichia coli	-	20±1.1	20±1.1	20±1.2	25±0.2
Pseudomonas aeruginosa	-	15±1.1*	15±2.1*	20±1.5	24±1.1
Bacillus cereus	+	20±1.1	20±1.6	NA	24±1.2
Shigella sonnei	-	15±1.1*	15±1.4*	25±1.6	24±2.2
Streptococcus pyogens	+	20±1.1	20±0.1	25±3.1	23±2.1
Bacillus subtilis	+	20±1.1	20±1.5	21±1.6	22±0.2
Shigella flexneri	-	20±1.1	20±1.5	20±3.1	24±1.1
Vibrio cholerae	-	20±1.1	10±1.0*	10±0.3*	20±1.3
Kleb pneumoniae	-	18±1.1*	15±1.1*	20±1.4	24±2.2
Staphylococcus aureus	+	20±1.1	10±0.2*	20±1.2	24±0.2

*Statistically different from the positive control, NA: Not active

Table 2: Minimum inhibitory concentration (mg mL^{-1}) of different extracts of *Curtisia dentata* against selected bacteria strains

		Extract			
Bacteria	Gram	Aqueous	Ethanol	Acetone	Ciprofloxacin
Salmonella typhimurium	-	0.04*	0.02	0.02	0.01
Enterococcus faecalis	+	2.5*	0.02	0.02	<0.1
Escherichia coli	-	0.01	0.02	0.01	<0.01
Pseudomonas aeruginosa	-	2.5*	2.5*	0.02	0.01
Bacillus cereus	+	0.02	0.01	Na	<0.01
Shigella sonnei	-	2.5*	2.5*	0.01	<0.01
Streptococcus pyogens	+	0.02	0.01	0.01	<0.01
Bacillus subtilis	+	0.02	0.02	0.02	0.02
Shigella flexneri	-	0.02	0.02	0.02	<0.02
Vibrio cholerae	-	>5*	>5*	>5*	<0.01
Klebsiella pneumonia	-	2.5*	2.5*	0.02	<0.01
Streptococcus aureus	+	0.02	>5*	0.02	<0.1

*Statistically different from the positive control, NA: Not active

The lowest MICs were obtained in the aqueous extracts against *E. coli* (0.01 mg mL^{-1}) and on *B. cereus*, *S. pyogens*, *B. subtilis*, *S. flexneri* and *S. aureus* at 0.02 mg mL^{-1}. The ethanol extract had the lowest MICs of 0.01 mg mL^{-1} against *B. cereus* and *S. pyogens* and 0.02 mg mL^{-1} against *S. typhimurium*, *E. faecalis*, *E. coli*, *B. subtilis* and *S. flexneri.* While the acetone extract had the lowest MIC of 0.01 mg mL^{-1} against *E. coli*, *Shigella sonnei* and *S. pyogens*, it was 0.02 mg mL^{-1} against *S. typhimurium*, *E. faecalis*, *P. aeruginosa*, *B. subtilis*, *S. flexneri*, *K. pneumonia* and *S. aureus*. The positive drug had the lowest MICs of 0.01 mg mL^{-1} against *S. typhimurium* and *P. aeruginosa*, 0.02 mg mL^{-1} against *B. subtilis* and others are >0.01 mg mL^{-1} (Table 2).

DISCUSSION

Medicinal significance of plants in healing human diseases has been adduced to their phytochemical constituents[21]. These phytoconstituents are known to be biologically active and thus aid pharmacological effects of botanicals including antioxidative and antimicrobial activities[22,23]. In addition to aiding medicinal attributes of plants, metabolites such as alkaloids have been reported to protect human cells against foreign organisms. Hence, the presence of alkaloids in the extracts of CD may suggest protective effect of the plant as a probable antibacterial and antidiarrhoeal agent[24]. The result of this study is in line with the report of Karuppusamy and Rajasekaran[25] and Shrisha *et al.*[26] who studied the antimicrobial activities of seeds extract of *Holarrhena antidysenterica* used in the treatment of dysentery.

Saponins are useful in biological activities such as cell growth and division[10]. The presence of saponins in the extracts of CD may thus be helpful in the treatment of cell related diseases. The moderate result obtained in this study is slightly different from the report of Oyedemi *et al.*[10], who reported a higher quantity of saponin in their study of the phytochemicals contents of the hydroalcoholic extract from *Curtisia dentata* stem bark. However, the quantity obtained in this study is consistent with the reports of Otshudi *et al.*[27] who reported the *in vitro* antimicrobial activity of six medicinal plants traditionally used for the treatment of dysentery and diarrhoea in Democratic Republic of Congo.

Polyphenolic compounds such as phenols and flavonoids are important plant components with significant antioxidant activity and a wide range of biological activities including antibacterial, anti-inflammatory, analgesic and anti-allergic properties[28]. The presence of these phenolics in high quantity in this plant supported its antimicrobial activities and whose mechanism of action could have been to inhibit the growth of dysentery-causing bacteria. This study agrees with previous reports[29,30], who linked antimicrobial potential of plant extracts to their phytoconstituents concentrations. The quantity of phytochemicals extracted from the plant sample revealed the vital role played by the solvent of extraction in the yield. All the three solvents contributed to the quantity of phytochemicals extracted from CD. However, the best solvent of extraction for the plant could be said to be ethanol followed by water and acetone.

Nitric oxide is a reactive gas molecule with an unpaired electron and this property is important for its signalling and ability to undergo diverse reactions[31]. The radical compounds such as nitric oxide and nitrogen dioxide play a dual role in human as both toxic and beneficial compounds. This delicate balance between their two opposite effects is undoubtedly a key aspect of life[32,33]. Judging by the IC_{50} obtained for the different extracts that competed favourably with the standards used, it could be inferred that all the stem bark extracts of CD demonstrated good activity to scavenge nitric oxide.

The ferric ion reducing effects of botanical extracts have been reported as one of their antioxidative potential[28]. These were estimated from their ability to reduce Fe^{3+} (yellow colour) to Fe^{2+} (green-blue colour). The reducing ability of all the plant extracts was significantly lower to that of gallic acid and BHT. A similar trend was observed by Oyedemi *et al.*[10] with regards to the ferric ion reducing potential of the hydroalcoholic stem bark extract of *C. dentata*.

The plant extracts were able to reduce the stable free radical of DPPH to the yellow coloured diphenyl picrylhydrazine. This proves that the stem bark extracts of DC contains some active constituents that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The DPPH radical scavenging method has been proven to be good because its results are not affected by substrate polarity[34]. Scavenging ability of the stem bark extracts of CD shows that the extracts possess high DPPH scavenging activity.

The ABTS, a protonated radical has characteristic absorbance which decreases with the scavenging of the proton radicals[35]. The scavenging activities of the stem bark extracts of CD and BHT correlated well with increasing concentrations. All the extracts possessed strong ABTS scavenging activity an observation that is supported by other researchers[36,37]. This study demonstrated the capability of the plant extract to scavenge ABTS radicals and thus suggest its usage in the management of degenerative diseases associated with free radical species including those of the microbial cell wall and genome synthesis.

Plant-derived compounds or crude extracts have proven to exhibit a protective and therapeutic effect in a variety of ailments including microbial infections[38]. Antibacterial activities of plant extracts have been linked to the presence of some bioactive compounds or secondary metabolites, which protect the plants themselves against bacterial, fungal and viral infections[39]. Compounds such as flavonoids, polyphenols, tannins and alkaloids have been reported to possess antimicrobial properties and thus could be responsible for the observed results in this study[40].

The results of this experiment showed considerable antibacterial activity of the acetone extract more than other extracts, especially against Gram-negative strains. This was clearly shown in this study and the reason could be that the extracts were able to penetrate the lipopolysaccharide layers of Gram-negative bacteria more readily thereby making them susceptible.

The acetone, ethanol and water extract of *C. dentata* tested against *E. coli* were active at 0.01-0.02 mg mL^{-1}. This was contrary to the report of Oyedemi *et al.*[10], which showed potent activity of hydroalcoholic extract of CD at 500 mg mL^{-1} against the organism. This disparity may be adduced to differences in the solvents of extraction because different solvents extract different antibacterial compounds. The differences in the experimental conditions of work could also affect the results. The aqueous extract (2.5 mg mL^{-1}) in this study was not very active against *E. faecalis* as does the other solvent extracts with 0.02 mg mL^{-1}. This is in consonant with the reports of Oyedemi *et al.*[10], who reported the activity of 0.019 mg mL^{-1} for the hydroalcoholic extract. In the same vein, the ethanol extract of CD in this report (>5 mg mL^{-1}) was also not very active against *S. aurues* as reported by Oyedemi *et al.*[10], who reported a significant antimicrobial activity against the two bacteria isolates. Moderate activity of the extracts recorded in this experiment against *B. cereus*, *P. aeruginosa* and *K. pnuemoniae* confirmed to the previous reports against these organisms. Earlier observation by McGaw *et al.*[41], reported antibacterial activity of 70% ethanol extract and aqueous stem bark extracts of the plant against *Bacillus subtilis.* The findings of this study is also comparable to data reported by Shai *et al.*[42] who demonstrated a moderate inhibitory effect of acetone extract of plant spp., on *Pseudomonas aeruginosa* using microdilution method.

The higher antibacterial potentials exhibited by the acetone extract against the test bacteria might be due to the high concentration of phytoconstituents in the acetone extract than in ethanol and water extracts. Our observation strongly suggests that *Curtisia dentata* can be an effective herbal remedy for the treatment of infections caused by these pathogens.

Though this study did not establish the antidysenteric effect of this plant to human cells, the plant has demonstrated its potential as a source of novel antibacterial agents for the treatment of bacteria causing dysentery infections. Furthermore, the study presents a report on the antidysenteric activity of *Curtisia dentata* extracts against various bacteria causing dysentery infections. Future study to determine the possible effects of the extract on dysenteric animal and its phytotoxicity is our next target.

CONCLUSION

The extracts showed good antioxidative and antibacterial activities. The phytochemicals of the plant extracts with their pharmacological properties allied to these bioactivities. This may suggest that this plant could be a good source of antioxidant and antimicrobial agents of natural origin. The activities could also account for the multi-pharmacological uses of *C. dentata* in the treatment of infectious diseases like dysentery and some chronic diseases. The study showed that it has the potential to be used for the development of therapeutic agents for the treatment of infection caused by the tested strains. Further studies to characterise the active constituents, their mechanisms of action, toxicological profile and *in vivo* antidysenteric potentials of *C. dentata* extracts are ongoing to appreciably lend scientific credence to its ethnomedicinal usage.

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