Original Research

Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of *Cyathula prostrata* (L) Blume against some human pathogens

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Abstract
The antimicrobial activities of aqueous (cold and hot) and ethanolic extracts of leaf stem bark and root of *Cyathula prostrata* were investigated against some human clinical isolates of *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhi*, and *Candida albicans* using the Agar well diffusion method at extract concentration of 25mg/ml. Ciprofloxacin (5μg/ml) and Fluconazole (20μg/ml) drugs was used as positive reference standards to determine the sensitivity of the strains. Results obtained showed that all the test isolates were inhibited by various fractions of the leaf, root and stem bark extracts. The antimicrobial activities of the different plant parts were not significantly different (P<0.05), though the greatest activities were observed with the ethanolic fractions (14.0-25.5 mm), followed by the hot water (12.0-24.2 mm) and cold extracts (13.0-18.5 mm). An inhibition range of 24.0-25.5mm and 28.5mm were observed from ciprofloxacin and fluconazole drugs respectively. The percentage susceptibility of the most sensitive bacterial isolate (*E. coli*) was 95.9% while the least (*K. pneumoniae*) had 40.0% sensitivity. *Candida albicans* had a percentage susceptibility of 57.5%. The minimum inhibitory concentration (MIC) ranged between 400 and 800μg/ml. The observed phytochemical compounds were saponins, tannins, flavonoids, alkaloids, cardiac glycosides and steroids. This study has justified the applications of *Cyathula prostrata* in the traditional herbal medicines and therefore holds a promise as a potential source of novel broad spectrum drug for treating infectious diseases.

INTRODUCTION
Over the years, plants and their extracts have been applied as herbal remedies for diverse human ailments. Presently, plant is still being utilized by numerous developing countries as sources of therapeutic agents because they believe medicinal plants are readily available, accessible, affordable, potent, and with relatively lower incidences of adverse reactions compared to modern conventional drugs [1]. Base on the growing knowledge of potency of traditional medicinal plants and coupled with fact that numerous infections agents are becoming resistant to synthetic drugs, researchers all over the world have intensified the screening of these acclaimed medicinal plants in order to provide a documented scientific backing and ultimately recommend them as novel sources of future antimicrobial agents. Therefore, the continuous screening of these acclaimed medicinal plants by scientists cannot be overemphasis.

Currently, several researchers have reported that numerous tropical plant possess antimicrobial properties against pathogenic micro-organisms. In their report, [2] demonstrated the activity of *Terminalia avicennioides* against *Vibrio cholerae* and *Salmonella typhi*. In the same vein, [3] reported the antimicrobial
activity of the leaf extract of 

Anacardium occidentale and Gossypium hirsutum against Escherichia coli, Shigella dysenteriae, Salmonella typhimurium, Staphylococcus aureus and Pseudomonas aeruginosa. The leaf extract of Kalanchoe pinnata displayed antimicrobial activity against Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa and Candida albicans [4]. In their studies, [5] reported that the root, stem bark and root extracts of Parkia Clappertoniana demonstrated significant antimicrobial activities against commonly implicated human clinical pathogens. Also, the stem bark extracts of Vitellaria paradoxa and Caesalpinia pulcherrima were reported possess significant antibacterial activities against some enteric pathogens like Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Proteus mirabilis, and Shigella dysenteriae [6, 7]. Furthermore, the seed extracts of African nutmeg (Monodora myristica) were currently documented to possess broad spectrum antibacterial properties against some human pathogens [8]. With these promising results, there is still the need to search for more plants of medicinal value so as to complement the available arsenal of drugs and ultimately, increase the array of choices required for effective chemotherapy.

Cyathula prostrata (L) Blume (Amaranthaceae) is an annual, branched herb/shrub reaching up to 1 m with stem trails on the ground and bears leaves which are rhomboid-oblong and adhesive fruits [9]. Traditionally, various preparations of the leaves, stems and roots of this plant are used to treat a range of illnesses including articular rheumatism, cough, skin diseases, scabies, scrapes, snake bites, bruises, liver problem, dysentery, diarrhoea, nausea, cholera vomiting blood, and many others in Nigeria and other African countries [9-11]. Among the Kurichayas tribe of Kannur District, a tea spoon of the dried powdered root is boiled in water and taken thrice daily as cure for fever [12]. When mixed with other plants (Syndrella nodiflora and Aframomum melegueta), and clay, it is used to treat heart trouble and bronchial infections while the fruit has been claimed to prevent miscarriages [9]. Scientically, the methanolic extract of Cyathula prostrata has been documented to be relatively non toxic in albino mice [11]. Also, it was recently documented that the methanolic extract of this plant possesses anti-inflammatory and analgesic properties, justifying its application in the traditional management of ailments associated with pains among others [13].

Despite the arrays of traditional applications to which the leaf, stem and root of Cyathula prostrata are subjected to, available literature revealed that there is paucity of information on the scientific elucidation of these plants as remedy for the acclaimed related ailments.

This study was therefore undertaken to evaluate the phytochemical properties and antimicrobial activities of aqueous (cold and hot water extract) and ethanolic extracts of this plant against Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi and Candida albicans which are routinely implicated in gastro-intestinal infections, oral diseases, wounds and other skin diseases in order to establish a scientific evidence for their traditional usage in Africa.

MATERIALS AND METHODS

Collection and Preparation of Plant material

Fresh plants of Cyathula prostrata were collected from farmland around the Amai Campus of Novena University, Delta State, Nigeria and were identified at the Department of Biological Sciences of the university courtesy of Prof. J. M. O. Eze (Botany unit). The leaves, stem, and roots were then washed thoroughly, separated and air-dried to crispiness on the laboratory workbench (prevailing room temperature of 30 ± 2°C) for two weeks. The dried materials were reduced to coarse form using a pestle and mortar and further pulverized to very fine particles with an electric blender (Super Search Model 2815). The powdered leaves stem and root samples obtained were stored separately in polyethylene bags until needed for analysis.

Sterilization of materials

All glassware used in this research were washed with detergent, rinsed with distilled water, air dried and sterilized on a hot air oven at 121°C for 2 hours. Each of the materials was wrapped with aluminum foil before sterilization. Distilled water and all prepared media were sterilized in the autoclave at 121°C for 15 minutes. Cork borers and glass rods were sterilized by dipping into 70% alcohol prior to flaming in a Bunsen burner. The working bench was swabbed with 75% alcohol before and after each experiment.

Source of test micro-organisms

The pure clinical isolates of Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, and Candida albicans were collected from Lahore Research Diagnostic and Environmental Consortium (LRDEC) in Benin City, Nigeria. The cultures were maintain at 35°C on nutrient agar (bacteria) and sabouraud dextrose agar (fungi) and used for the study.

Standardization of test organisms

Prior to antimicrobial sensitivity test, 0.2 ml of
overnight culture of each organism was dispensed into 20 ml of sterile Mueller Hinton Broth (Hi-Media, India) and then incubated about 16-24h to standardize the cultures to approximately 10^6 cfu/ml [14].

**Extraction of Plant Material**

**Cold water Extraction:** 200g of each powdered sample was soaked in 500ml of sterile distilled water, agitated manually, and allowed to extract for 48hours, before each extract was filtered using Whatmann No 1 Filter paper. The filtrates were evaporated in a water bath at 50°C to dryness. The yields were 13.5% w/w, 13.9% w/w, and 14.0% w/w the leaf, stem and root extracts respectively. The extracts were stored at 4°C until needed.

**Hot water Extraction:** 200g of each weighed plant materials was soaked in 500ml of hot water boiled for 30minutes into a conical flask for 48 hours. Each extract was filtered using filter paper and evaporated to dryness using water bath at 50°C. The yields were 14.5% w/w, 13.8% w/w, and 14.0% w/w the leaf, stem and root extracts respectively. The extracts were stored at 4°C until needed.

**Ethanol extraction:** 200g of the plant samples were soaked in 500ml of absolute ethanol for 48h at room temperature with occasional stirring. The content was filtered and evaporated to dryness in a water bath at 50°C. The yields were 14.5% w/w, 15.4% w/w, and 14.0% w/w the leaf, stem and root extracts respectively. The extracts were collected and stored in the refrigerator at 4°C until required for assay.

**Sterility Test of the Plant Extracts**

Each of the above extracts ethanolic and aqueous (cold and hot) extract were tested for growth or contaminants. This was carried out by inoculating 1ml of each of them on sterile Mueller Hinton Agar and incubated at 37°C for 24 hours. The plates were observed for growth. No growth in the extracts after incubation indicated that they were sterile. The different extracts were then assessed for antimicrobial activity.

**Antimicrobial susceptibility testing**

The agar well diffusion method of [14] with slight modification, was adopted for this assay. Mueller Hinton Broth (Hi-Media, India) was prepared as specified by the manufacturer, autoclaved and poured ascetically into sterile Petri dishes and allowed to gel. Then a loopful of the standardized bacterial cell suspension (10^6 cfu/ml) was streaked evenly on each gelled agar plate. The leaf, stem bark and root extracts were reconstituted in 20% DimethylSulfoxide (DMSO) to obtain the working concentrations of 25mg/ml. 200μl of each extract was inoculated into three wells (6 mm Diameters) earlier bored with a sterile cork borer in each plate. The negative control was 200μl of 20% DMSO, while the positive control were 5μg/ml of ciprofloxacin (Ranbaxy Pharmaceuticals India) and 20μg/ml of fluconazole (Greenlife, pharmaceuticals India). The plates were allowed to stand for 30minutes on the work bench for pre-diffusion of the extracts to proceed before the growth of the organism commenced. The plates were incubated at 37°C for 24 h. The whole experiment was carried out in triplicate and the antibacterial activity of the extracts were determined after incubation period by measurement of mean diameter zones of inhibition produced by the extracts against the test organisms and results were recorded in millimeters(mm) using a transparent ruler.

**Determination of the minimum inhibitory concentration (MIC)**

The plant extracts that demonstrated significant antibacterial activity by the agar well diffusion method were subjected to MIC assay using the broth dilution method of [15]. One ml of 24 h culture of test organism (10^7 CFU/ml) adjusted to McFarland turbidity standard were incubated in serial dilution of 100, 200, 400, 600, 800 and 1000 μg/ml of plant extracts in physiological saline at 37°C for 24 h. The concentration at which the lowest dilution with no detectable bacterial growth was considered as minimum inhibitory concentration (MIC).

**Phytochemical screening of Extracts**

The different extracts of Cyathula prostsrata were tested for the presence of phytochemicals such as steroids, saponins, alkaloids, flavonoids, terpenoids, cardiac glycosides, and tannins using the standard procedures described by [16].

**Test for alkaloids.** 0.5g of the sample was accurately weighed and defatted with 5% ethyl ether for 15mins. The defatted sample was extracted for 20mins with 5.0ml of aqueous HCl on a steam bath. The resulting mixture was centrifuged for 10mins at 3000rpm to remove filtrate (Supernatant). 1.0ml of the filtrate was treated with a few drops of Mayer’s reagent and a second 1.0ml portion was treated similarly with Dragendorff’s reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids.

**Test for saponins:** The ability of saponins to produce frothing in aqueous solution was used as screening test for the sample. 0.5g of dried extract was shaken with water in a test tube, frothing which persist on warming was taken as evidence for the presence of saponins.

**Test for tannins:** 5.0g of dried extract was stirred with 10.0ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate. A blue-black precipitate was taken as evidence for the presence of...
tannins

**Test for cardiac glycosides:** 0.5g of dried extract was dissolved in 2.0ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under laid with 1.0ml of concentrated H₂SO₄. A brown ring obtained at the interface indicated the presence of a cardenolides.

**Test for flavonoids:** 1.0ml of 10% lead acetate was added to 1.0ml of the extract contained in a test-tube. A formation of a yellow precipitate was taken as positive for flavonoids.

**Test for steroids:** 0.5g of the dried extract was extracted with 2.5ml of chloroform in a test tube and 1ml of concentrated sulphuric acid added to form a lower layer. A reddish-brown interface indicated the presence of steroids.

**Test for Terpenoids:** 0.5ml of the chloroform extract of the dried extracts was evaporated to dryness on a water bath. A grey colour indicated the presence of terpenoids.

**Statistical treatment of the results**

The results were expressed as means ± standard error (SE). Significance of differences compared to the control groups was determined using students t-test.

**RESULT**

The antimicrobial activity test of *Cyathula prostrata* showed that all the test isolates were susceptible to the organic and aqueous extracts at 25mg/ml concentration used (Table 1, 2, and 3). Generally, the root extract demonstrated the most inhibitory activity followed by the leaf extract and then the stem bark being the least, though their activities were not significantly different (P<0.05). The zones diameter of inhibition observed ranged between 14.0 – 25.5 mm for ethanolic extracts, 12.0 – 24.2 mm for cold water extracts and 13.0 – 18.5 mm for hot water extracts as against an inhibition range of 24.0-26.5mm and 28.5mm observed from ciprofloxacin and fluconazole drugs respectively. With the ethanolic extracts, at concentration of 25mg/ml, the most susceptible organisms (inhibition diameter>19.5mm) were *E. coli, S. aureus, P. aureuginosa, and S. typhi*, while the least susceptible isolates (inhibition diameter<14.5mm) were *K. pneumoniae* and *B. cereus* (Table 1). With the hot aqueous extracts, at concentration of 25mg/ml, only *E. coli* and *P. aureuginosa* were the most sensitive (inhibition diameter>19.5mm) isolates, while the least susceptible isolates (inhibition diameter<14.5mm) were *P. mirabilis, E. coli*, and *P. aureuginosa* (Table 2). The result in Table 3 however shows that none of the cold aqueous extracts at 25mg/ml exhibited antimicrobial inhibition greater than 19.5mm, but had several inhibition zones that were less than 14.5mm against *S. mutans, C. albicans, P. mirabilis, E. coli* and *P. aeruginosa*. When compared with the reference standard drugs (Ciprofloxacin -5μg/ml and Fluconazole- 20μg/ml) used, it was observed that the percentage susceptibility of the most sensitive bacterial isolate (*E. coli*) was 95.9% while the least (*K. pneumoniae*) had 40.0% sensitivity. While *C. albicans* had a percentage susceptibility of 57.5% (Table 1, 2, and 3).

**Table 1. Diameter of Zones of inhibition of the test bacterial species to the ethanolic extracts and controls**

<table>
<thead>
<tr>
<th>Test Isolate</th>
<th>Leaf (25mg/ml)</th>
<th>Stem bark (25mg/ml)</th>
<th>Root (25mg/ml)</th>
<th>CIP (5μg/ml)</th>
<th>FLU (20μg/ml)</th>
<th>DMSO 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>22.4±0.01</td>
<td>22.5±0.11</td>
<td>24.0±0.01</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans</td>
<td>15.0±0.00</td>
<td>15.1±0.01</td>
<td>15.5±0.05</td>
<td>25.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>E.coli</td>
<td>25.0±0.00</td>
<td>24.5±0.04</td>
<td>25.5±0.03</td>
<td>26.5</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>B.cereus</td>
<td>14.4±0.11</td>
<td>14.2±0.33</td>
<td>14.0±0.25</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>24.2±0.01</td>
<td>22.5±0.11</td>
<td>22.0±0.00</td>
<td>24.5</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>K.pneumoniae</td>
<td>14.3±0.01</td>
<td>14.0±0.15</td>
<td>14.4±0.02</td>
<td>25.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>P.mirabilis</td>
<td>15.5±0.11</td>
<td>15.0±0.12</td>
<td>16.0±0.00</td>
<td>24.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>S.typhi</td>
<td>20.5±0.04</td>
<td>21.1±0.21</td>
<td>22.0±0.00</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>C.albicans</td>
<td>16.4±0.11</td>
<td>15.2±0.14</td>
<td>16.0±0.24</td>
<td>ND</td>
<td>28.5</td>
<td>-</td>
</tr>
</tbody>
</table>

* Results are means of three replicate diameter zones of inhibition values (mm) ± standard deviations (SD); CIP = Ciprofloxacin; FLU = Fluconazole; 20% DMSO=Dimethylsulfoxide; ND=No Inhibition.
Table 2. Diameter of zones of inhibition of the test bacterial species to the hot water extracts and controls

<table>
<thead>
<tr>
<th>Test Isolate</th>
<th>Leaf (25mg/ml)</th>
<th>Stem bark (25mg/ml)</th>
<th>Root (25mg/ml)</th>
<th>CIP (5μg/ml)</th>
<th>FLU (20μg/ml)</th>
<th>DMSO 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>18.2±0.01</td>
<td>19.0±0.21</td>
<td>19.0±0.51</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans</td>
<td>14.5±0.00</td>
<td>15.0±0.02</td>
<td>14.5±0.12</td>
<td>25.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>E.coli</td>
<td>24.0±0.03</td>
<td>23.5±0.04</td>
<td>24.2±0.05</td>
<td>26.5</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>B.cereus</td>
<td>13.1±0.11</td>
<td>13.5±0.03</td>
<td>13.5±0.15</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>20.2±0.31</td>
<td>19.6±0.01</td>
<td>20.0±0.10</td>
<td>24.5</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>K.pneumonia</td>
<td>13.4±0.00</td>
<td>13.0±0.10</td>
<td>13.2±0.32</td>
<td>25.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>P.mirabilis</td>
<td>14.0±0.21</td>
<td>14.2±0.22</td>
<td>13.0±0.10</td>
<td>24.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>S.typhi</td>
<td>19.2±0.03</td>
<td>18.1±0.22</td>
<td>19.9±0.10</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>C.albicans</td>
<td>15.1±0.21</td>
<td>14.5±0.24</td>
<td>14.5±0.04</td>
<td>ND</td>
<td>28.5</td>
<td>-</td>
</tr>
</tbody>
</table>

*Results are means of three replicate diameter zones of inhibition values (mm) ± standard deviations (SD), CIP= Ciprofloxacin; FLU= Fluconazole; 20% DMSO=Dimethylsulfoxide; =No Inhibition.

Table 3. Diameter of zones of inhibition of the test bacterial species to the cold water extracts and controls

<table>
<thead>
<tr>
<th>Test Isolate</th>
<th>Leaf (25mg/ml)</th>
<th>Stem bark (25mg/ml)</th>
<th>Root (25mg/ml)</th>
<th>CIP (5μg/ml)</th>
<th>FLU (20μg/ml)</th>
<th>DMSO 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>14.5±0.02</td>
<td>14.8±0.01</td>
<td>15.0±0.11</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans</td>
<td>12.8±0.50</td>
<td>13.0±0.01</td>
<td>13.5±0.02</td>
<td>25.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>E.coli</td>
<td>18.4±0.13</td>
<td>18.0±0.14</td>
<td>18.2±0.02</td>
<td>26.5</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>B.cereus</td>
<td>12.5±0.01</td>
<td>12.2±0.05</td>
<td>12.7±0.35</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>18.0±0.01</td>
<td>16.6±0.41</td>
<td>18.5±0.20</td>
<td>24.5</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>K.pneumonia</td>
<td>12.3±0.10</td>
<td>12.0±0.00</td>
<td>12.5±0.22</td>
<td>25.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>P.mirabilis</td>
<td>13.1±0.11</td>
<td>13.5±0.22</td>
<td>13.5±0.50</td>
<td>24.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>S.typhi</td>
<td>16.4±0.12</td>
<td>15.1±0.24</td>
<td>16.0±0.30</td>
<td>26.0</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>C.albicans</td>
<td>13.1±0.01</td>
<td>13.0±0.04</td>
<td>13.0±0.02</td>
<td>ND</td>
<td>28.5</td>
<td>-</td>
</tr>
</tbody>
</table>

*Results are means of three replicate diameter zones of inhibition values (mm) ± standard deviations (SD), CIP= Ciprofloxacin; FLU= Fluconazole; 20% DMSO=Dimethylsulfoxide; =No Inhibition.

The result of the minimum inhibitory concentrations presented in Table 4, shows that the values of the different extracts ranged between 400 and 800μg/ml. With the ethanolic extracts, the MIC ranged from 400μg/ml on S. aureus, E. coli, C. albicans and P. aeruginosa, to 600μg/ml on S. mutans, and S. typhi, to 600-800μg/ml on K. pneumonia, B. cereus and P. mirabilis. The hot aqueous extracts however, had MIC ranged between 600μg/ml for S. aureus, E. coli, S. typhi, P. aeruginosa and C. albicans and 800μg/ml for S. mutans, K. pneumonia, B. cereus and P. mirabilis, while the cold water extract had MIC value of 600μg/ml on S. aureus and E. coli, and 800μg/ml on S. typhi, P. aeruginosa, C. albicans, S. mutans, K. pneumonia, B. cereus and P. mirabilis(Table 4).

The result of the phytochemical analysis of Cyathula prostsrata presented in Table 5 shows that only alkaloids was detected in the ethanol extracts of leaf and stem bark, while terpenoids, tannins, flavonoids, saponins, glycosides and steroids were found in the leaf, stem bark and root extracts.
Table 4. Minimal Inhibitory Concentration (MIC μg/ml) of Ethanol, and aqueous leaf, stem and root extracts against test isolate

<table>
<thead>
<tr>
<th>Test Isolate</th>
<th>Ethanol Extract</th>
<th>Hot Aqueous Extract</th>
<th>Cold Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Root</td>
</tr>
<tr>
<td>S. aureus</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>S. mutans</td>
<td>600</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>B. cereus</td>
<td>600</td>
<td>800</td>
<td>600</td>
</tr>
<tr>
<td>E. coli</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>600</td>
<td>800</td>
<td>600</td>
</tr>
<tr>
<td>S. typhi</td>
<td>600</td>
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<td>K. pneumonaiae</td>
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<td>P. aeruginosa</td>
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<tr>
<td>C. albicans</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
</tbody>
</table>

Table 5. Qualitative analysis of the phytochemicals in Leaf, Stem bark, Root extracts of Cyathula prostsrata

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Leaf</th>
<th>Stem Bark</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiacglycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: AqE = Aqueous extract ETE = Ethanol extract, + = Detected, - = Not detected

DISCUSSION

Medicinal plants play a central role not only as traditional medicines but also as commercial commodities meeting the demand of distant markets. To compete with the growing market, there is need to expeditiously utilize and scientifically validate more medicinally useful plants. Because of the appearance of drug resistance to antimicrobial agents, more effort is being made to find alternative antimicrobial components. It had been suggested that natural products are a preferable option to synthetic ones. Literature indicates that medicinal plants are the backbone of traditional medicine [17], and the antimicrobial activity of plant extract is due to different chemical agent in the extract with antimicrobial compounds [18]. Many studies have been undertaken with the aim of determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant [7,19,20].

In this study the leaf, stem bark, and root extracts of C. prostsrata were found to inhibit all the test bacteria and fungus, indicating that this plant possesses significant in vitro antimicrobial properties. The slightly greater activities, though not significant (P<0.05), exhibited by the ethanol fractions of the various plant parts shows that this solvent dissolved a greater percentage of the actual bioactive ingredient of this plant than the hot and cold aqueous counterparts. This justifies the preference of local gin “ogogoro” as extraction solvent by herbal physician in the preparation of crude drugs from medicinal plant materials. Local gin obtained from fermented palm wine distillation is known to contain a high concentration of alcohol. When these solvents are used as herbal extractants, it may be possible that bioactive substances that are less soluble in water would then be dissolved by the solvent [21]. This observation is in consonance with the works of [22] and [23] who reported that the ethanol extract of Piliostigma reticulatum and Aspila africana generally
displayed the highest activities, followed by the hot aqueous and cold aqueous extract against S. aureus, S. faecalis, P. aeruginosa, K. pneumonia, E. coli, S. dysentriae, and S. typhimurium. But the result of this study is however in disagreement with the work of [5], where the hot water extracts of root, stem bark and leaf of Parkia clappertonia were more active than their ethanol and cold water extracts against E. coli ATCC 11775, P. aeruginosa ATCC10145, S. aureus ATCC 12600. The relatively high antimicrobial activities of hot aqueous extract also indicate that significant amount of the bio-active components were extracted at elevated temperature. This finding is in line with the report of [24], that hot aqueous extraction expressed greater amount of the inherent bioactive chemicals thereby making them more available for antimicrobial activities over the cold water counterpart. This observation also suggests that the secondary biocompounds of these plant extracts are to some extent stable at relatively high temperature, thereby justifying the efficacy of the whole plant extract even when boiled in water and utilized as herbal remedy.

The slightly greater antimicrobial activities recorded by root and leaf extract over the stem bark extracts in this study, suggest that more of the bioactive ingredients are lodged in these parts. Many practitioners may have observed these in the past that they almost always recommend the use of leaf and root extracts over that of stem bark of a medicinal plant for native medicine. This finding is in agreement with the observation of [5].

The significant antibacterial activities recorded by the ethanol and hot water extract of leaf, stem and root extract of this plant against E. coli, S. aureus, P. aeruginosa, and S. typhi, are worth-noting especially now that they are multiple-drug resistant species of these bacteria commonly implicated in several cases of human diseases such as gastro-intestinal, urinary tract and wound infections in Nigeria and other African countries [25-27]. The significant antibacterial activity of this plant extract against E. coli was however in disagreement with the observations of [28], who documented the antibacterial activity of C. prostrata and some other traditional Medicinal Plants (Leucas aspera, Murraya koengigii, Oxalis corniculata, Alternanthera sessilis, Pagostemon benghalensis, Hydrocotyl rotendifolia, Cyathula prostrata, Piper peepaloides, Potentilla mooniana) of North East India on Escherichia coli. They noted that the aqueous leaf extracts of C. prostrata, P. benghalensis, H. rotendifolia and P. mooniana could not inhibit the growth of E. coli. The failure of some extracts to exert antibacterial effect on test organisms in not enough to to conclude lack of antimicrobial property because the potency of extracts depends on the solvent and method used to obtain the extract, the age of plant when harvested and the amount of the active constituent, which can vary in quality and quantity from season to season [29, 30] (Rios and Recio, 2005). In addition, the greater. Thus, the variations of this finding with the previous report could be attributed to some of the aforementioned reasons.

The inhibition of S. mutans, P. mirabilis, B. cereus, and K. pneumonia equally suggests that this plant possesses broad spectrum antimicrobial properties and could be used in the treatment of dental caries, food poisoning, wound infections, and urinary tract infections(UTIs) of which these pathogens are commonly implicated [25,31]. Inhibition of C. albicans by this plant extracts also suggest that it possesses antifungal properties, and can thus be tried as antifungal agent for the treatment of refractory candidiasis (oral) that has been a major global challenge with HIV/AIDS patients [32]. This observed antifungal property justifies their applications as cure for craw-craw, scabies, ringworm and other skin diseases in Nigeria and several African countries [10-12]. This result is however not in agreement with the findings of [33], who reported that the aqueous extracts of some medicinal plants including C. prostrata could not reduced the mycelia growth of Fusarium moniliforme, crop spoilage mould. In this study significant inhibitory activity was observed from the ethanolic extracts than the aqueous extracts. Thus, the slight variation could be attributed to extracting solvent used as well as resistant nature of spores of moulds. This result further suggests more investigations on antifungal properties of this plant on a wider range of fungi.

The relatively low MIC values recorded by the extracts against the test isolates confirm the high activity of the extract at low concentrations. In their studies on the toxicity of the methanolic leaf extract of this plant (C. prostrata), [11] documented that the extract could be administered at a dose range of 100 mg/kg/BW without any side effects in mice. High activity of antimicrobial agent at low concentration, in relation to the standard reference drug is very essential for chemotherapeutic purposes because of their toxicity to the patient’s system. This research was conducted on crude extract; it is believed that if the extract is further purified, stronger inhibitory results will be achieved.

The significant antimicrobial properties of the leaf, stem bark and root extracts of Cyathula prostrate could be attributed to the presence of the bioactive compounds detected in this study. Earlier researchers had demonstrated their antimicrobial activities [34-40]. Flavanoids are phenolics structure containing one carbonyl group complexes with extra cellular and soluble protein and with bacterial cell wall [35], thus exhibits antibacterial activity through these complexes [41]. Tannins on the other hand have been found to
form irreversible complexes with proline-rich proteins [19] resulting in the inhibition of the cell protein synthesis. Plants that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery [42]. This could be the basis for the antimicrobial use of such plants in the treatment of diarrhoea and dysentery [10-12]. Saponins, however, are a special class of glycosides which possesses antifungal activities [43]. The significant activities of the extracts against C. albicans in this study might be attributed to the action of this bioactive ingredient. Terpenoids have been demonstrated to be active against bacteria, fungi, viruses and protozoa [37, 44], which has enabled food scientists to use terpenoids present in essential oils of plants to control Listeria monocytogenes [36]. The mechanism of action of terpenes is by lipophilic membrane disruption. Indeed, [35] found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduces their antimicrobial activity. The presence of cardiac glycosides and steroids have been documented to inhibit the many bacteria and found to possess antioxidant potentials [40].

In conclusion, this study has demonstrated that the leaf, stem bark and root extract of Cyathula prostrata possess bioactive ingredient with in vitro antibacterial and antifungal activities against some human pathogens, thereby justifying the application of their extracts as traditional herbal medicine. It is worth-noting that in vitro finding is not always dependable because plants which are effective in vitro might not work when used in vivo and some plants which showed little or no effect in vitro study might also be effective when evaluated in animals due to various factors that affect or favor the release of active ingredients in animal bodies. Therefore, it is recommended that further identification of the active constituents is needed to exploit them in evaluating efficacy and safety in vivo against the test pathogens.

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