Phytochemicals and Antibacterial Activity of Leaf and Stem Extracts of *Ageratum conyzoides* (Linn) on Some Clinical Isolates

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**Received Date:** May 31, 2019 / **Accepted Date:** June 25, 2019 / **Published Date:** June 27, 2019

**Abstract**

Phytochemicals and antibacterial activity of leaf and stem extracts of *Ageratum conyzoides* on four clinical isolates, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* were investigated. Filtrates from ethanol extract of each of the powdered samples of the leaves and stem of *A. conyzoides* were evaporated to thick residues. 1 g each of these residues was dissolved in 5 ml of distilled water followed by double dilution to obtain concentrations of 200, 100, 50, 25, 12.5 and 6.25 mg/ml. Ciprofloxacin and distilled water were used as positive and negative controls, respectively. Antibacterial activity was assayed using Agar-well diffusion technique while the MIC was determined from the curve of square of radius diameter of inhibition against log concentration of the extract. Results showed that the leaf and stem of *A. conyzoides* contain comparable amounts of the phytochemicals with higher contents of flavonoid (0.97-0.98 %), saponin (0.97-0.98 %), and alkaloids (0.90 %) than tannin (0.187-0.188 %) and phenol (0.022 %). The extracts of *A. conyzoides* showed concentration-dependent activity against all the test bacterial isolates. The test organisms were found to be susceptible to the extracts at concentration of ≥ 50 mg/ml except *S. typhi* and *P. aeruginosa* that were susceptible to the stem extract at ≥25 and ≥100 mg/ml, respectively, whereas, for ciprofloxacin, some were susceptible even at very low concentration of 6.25 mg/ml. The MIC values were in the range of 69.18-75.86 mg/ml, 36.3-144.54 mg/ml and 7.59-38.0 mg/ml for the leaves and stem extracts and ciprofloxacin, respectively. The extracts could therefore be used as a broad spectrum antibacterial agent.

**Keywords:** *Ageratum conyzoides*; Phytochemicals; Antibacterial; Minimum inhibitory concentration; Ciprofloxacin

Cite this article as: Iwuagwu Mary Oluchi, Ogbonna Nneka Constance, Okechukwu Chikodi Lilian. 2019. Phytochemicals and Antibacterial Activity of Leaf and Stem Extracts of *Ageratum conyzoides* (Linn) on Some Clinical Isolates. Int J Plant Sci Hor. 1: 95-105.

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Introduction

Infectious diseases are caused by some microorganisms including many types of bacteria species. These diseases have been reported to account for approximately 66.67% of all deaths in tropical countries [1]. The use of orthodox drugs/antibiotics has been the treatment method for these infections. However, bacterial strains that are resistant to many used antibiotics have emerged leading to increase treatment failures of antibiotics [2]. Currently, antibiotic resistance has become a global concern as it is being threatened by emergence of multidrug resistance-pathogens [3,4]. Hence, the shift back to the use of traditional medicine which has been since antiquity and the continuous screening of many plants for their medicinal properties and antimicrobial effect.

Extracts of medicinal plant parts are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites [5-7]. Many people in Nigeria particularly in the rural areas depend on traditional medicine for the treatment of their ailments; this practice has been in existence since prehistoric times [8] before the introduction of antibiotics and other modern medicine. The medicinal value of some plants lies in some chemical substances (phytochemicals) they contain, which are secondary metabolites that produce definite physiological actions in the body. These most important bioactive constituents include but not limited to alkaloids, tannins, flavonoids, terpens and phenolic compounds. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effect on all types of microorganisms in vitro [9]. Recently, secondary metabolites previously with unknown pharmacological activities have been examined as sources of medicinal and therapeutic agents.

In developing countries such as Nigeria, there has been a gradual revival of interest in the use of medicinal plants due to their availability, accessibility, cheap, safe and more or less no adverse effect when compared to synthetic drugs [10]. According to Ukaoma et al. [6], the use of medicinal plants in the treatment of ailments has been on the increase in Nigeria as many orthodox drugs are adulterated and therefore ineffective in the treatment of diseases.

_Ageratum_ is one of the genera which belongs to the family _Asteraceae_ and consists of 30 species [11]. _Ageratum conyzoides_ (Goat weed) is among the commonly known species of this genus. It is a tropical plant found commonly in western and eastern region of the African continent, in some regions of Asia and South America [12]. The leaves are consumed as vegetable [13] and has been traditionally used as a purgative, febrifuge, emetic, antispasmodic and antiasthmatic [11]. The most common use of this plant is to cure wounds and burns [14,15]. In Nigeria, it is reportedly used in the treatment of typhoid fever and diarrhoea [16]. It has also been reportedly used as styptic and anti-dysenteric, antimicrobial, anti-inflammatory, analgesic, antipyretic, antiparmeric, gastroprotective, anti-ulcer, insecticidal and herbicidal [2,7,17-19].

Historically, plant extracts have been used as a safe, effective and natural remedy for ailment and diseases in traditional medicine. Notwithstanding, there is need to validate claims made by Herbalists on the efficacy of plants in the treatment of ailments. Therefore, the aim of this study was to evaluate the phytochemical and antibacterial activity of leaves and stem extracts of _Ageratum conyzoides_.

Materials and Methods

Study Location

This study was conducted at the laboratory of Department of Plant Science and Biotechnology, Abia State University, Uturu, which is under the South East zone of Nigeria.
Collection of plant material

Leaves and stems of *Ageratum conyzoides* were collected from Uturu, in Isiukwuato Local Government Area of Abia State, Nigeria. The plant was certified by a taxonomist in Plant Science and Biotechnology Department, Abia State University, Uturu. The plant materials were washed separately under running water, cut into pieces; air-dried for one week and then oven-dried at a temperature of 40 °C for 2 days. The dried samples were pulverized in a mortar pestle and sieved through 1 mm mesh to obtain fine powdered samples. The powdered samples were kept in properly labelled air tight sterile containers until required.

Collection of test organisms

Four strains of pathogenic bacteria, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aureginosa* obtained from the Microbiology Laboratory of Federal Medical Centre (FMC) Umuahia, Abia State, Nigeria were used. These bacteria strains were maintained on nutrient medium at 37 °C for further study.

Preparation of plant extract

50 g each of the powdered plant samples were soaked in 200 ml 80% ethanol and was allowed to stand for 48 h to enable the constituents to diffuse into the solvent. The ethanol extract was filtered using Whatman No. 1 filter paper. The filtrate was evaporated to thick residue at 40 °C and preserved in a sterile test tube until when needed.

Phytochemical analysis

Quantitative analyses of the phytochemicals such as flavonoids, alkaloids, saponins, tannins and phenols present in the extracts of both leaves and stems of *A. conyzoides* were carried out using standard procedures described by Harborne [20], Trease and Evans [21] and Sofowora [22].

Estimation of flavonoid: 5 g each of the sample was boiled in 100 ml 2M HCl for 30 min. The extract was allowed to cool and then filtered through Whatman No. 42 filter paper. The filtrate was re-extracted with 20 ml ethyl acetate. This was again filtered through Whatman No. 42 filter paper. Each sample filtrate was later transferred into a crucible of known weight and evaporated to dryness over a water bath. The crucible together with the residue was further dried in an oven until constant weight was obtained. It was then cooled in a desiccator and weighed. The percentage of flavonoid was calculated as:

\[
\% \text{ Flavonoid} = \frac{W_2 - W_1 \times 100}{W}
\]

Where: \(W\) = weight of sample
\(W_1\) = weight of crucible
\(W_2\) = weight of crucible + residue

Estimation of alkaloids: Alkaloids were determined using alkaline gravimetric method. 5 g of each of the samples was weighed separately into a beaker and 200 ml of 10% acetic acid in ethanol was added. The mixture was shaken, covered and allowed to stand for 4 h at room temperature. After which, the mixture was filtered and the filtrate (extract) was concentrated in a water bath to one quarter of its original volume. Concentrated ammonium hydroxide solution was added drop wise to the extract until precipitation was complete. The mixture was allowed to settle and the alkaloid precipitate was collected, washed with dilute ammonium hydroxide solution and then filtered using Whatman No. 42 (of known weight). The filter paper containing the precipitate was dried in an oven, cooled in a desiccator and weighed. The percentage of alkaloid in the sample was calculated as:

\[
\% \text{ Alkaloid} = \frac{W_2 - W_1 \times 100}{W}
\]

Where: \(W\) = weight of sample
\(W_1\) = weight of filter paper
\(W_2\) = weight of filter paper + precipitate
**Estimation of saponins:** 5 g of the sample was weighed into a conical flask. 100 ml of 20% ethanol was added to the sample. The mixture was heated over a hot water bath for 4 h at a temperature of 55 °C and stirred continuously. The mixture was then filtered and the residue re-extracted with another 100 ml of 20% ethyl alcohol. The combined extracts were reduced to 40 ml over a water bath at a temperature of 90 °C. The concentrate was then transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added to the extract and vigorously agitated. The aqueous layer was recovered while the diethyl ether layer was discarded and the purification process was repeated. 60 ml of n-butanol was added and the combined n-butanol extracts was extracted twice with 10 ml of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was evaporated to dryness over a water bath in a crucible of known weight. This was further oven-dried to a constant weight, cooled in a desiccator and then weighed. The percentage saponin was calculated using the formula below:

\[
\% \text{ Flavonoid} = \frac{W_2 - W_1}{W} \times 100
\]

Where: 
- \(W\) = weight of sample
- \(W_1\) = weight of crucible
- \(W_2\) = weight of crucible + residue

**Estimation of tannins:** The quantity of tannins was determined by using Folin-Denis reagent and reading the absorbance with a spectrophotometer. Folin-Denis reagent was made by dissolving 50 g of sodium tungstate (Na₂WO₄) in 37 ml of distilled water. 5 g each of the plant samples was separately mixed with 50 ml of distilled in a beaker, stirred for 1 h, filtered into a 50 ml volumetric flask and made up to mark. 2 ml of the extract was mixed with 35 ml of distilled water in a 50 ml volumetric flask. To this mixture, 1 ml of Folin-Denis reagent and 2.5 ml of saturated Na₂CO₃ solution were added for colour development. This was agitated and then allowed to stand for 90 min at room temperature. The absorbance was read at 700 nm with a spectrophotometer. Tannin acid was used as standard and same quantity of the acid was treated in the same way as the sample extract and its absorbance was also read at 700 nm. Percentage tannin was calculated as thus:

\[
\% \text{ Tannin} = 100 \times \frac{A_u \times V_t \times C}{W \times A_s \times V_a \times 1}
\]

Where: 
- \(W\) = weight of sample
- \(A_u\) = absorbance of sample
- \(A_s\) = absorbance of standard tannin
- \(V_t\) = total extract volume
- \(V_a\) = volume of extract analysed
- \(C\) = concentration of standard tannin

**Estimation of Total phenols:** Total phenolic content of the extracts was determined by Folin Ciocalteu reagent method. 0.1 g each of the sample was treated separately with 10 ml concentrated methanol and vigorously shaken for 50 min at room temperature. The mixture was centrifuged at 500 rpm for 15 min. 1 ml of the supernatant (extract) was mixed with 0.1 ml 1 N Ciocalteu reagent (0.1 ml, 1 N) and allowed to stand for 15 min. Then 5 ml of saturated Na₂CO₃ was added. The mixtures were allowed to stand for 30 min at room temperature and the total phenols were determined spectrophotometrically at 760 nm. Gallic acid was used as a standard and treated the same way as the extract. The total phenol expressed as percentage was calculated as:

\[
\% \text{ Phenol} = 100 \times \frac{A_u \times V_t \times C}{W \times A_s \times V_a \times 1}
\]

Where: 
- \(W\) = weight of sample
- \(A_u\) = absorbance of sample
- \(A_s\) = absorbance of standard phenol
- \(V_t\) = total extract volume
- \(V_a\) = volume of extract analysed
- \(C\) = concentration of standard phenol
Preparation of antibiotic stock solution

The antibiotic used for this investigation was ciprofloxacin (500 mg). A tablet of the antibiotic was dissolved in 2.5 ml of distilled water in a sterile test tube to get a concentration of 200 mg/ml. Double dilutions of this concentration were made [23] to give the following concentrations - 100, 50, 25, 12.5 and 6.25 mg/ml. These concentrations were used for antimicrobial susceptibility test on each of the bacterial isolates.

Antibacterial activity assay

Antibacterial activity determination was done using Agar Well Diffusion Technique [24]. 1 g of the residue obtained from evaporation of the ethanolic leaf and stem extracts of A. conyzoides each was dissolved in 5 ml of distilled water to make a stock of 200 mg/ml. This was followed with double dilutions to obtain concentrations of 100, 50, 25, 12.5 and 6.25 mg/ml. Cultures of the selected organisms were prepared by inoculating each of the bacterial strain into already prepared culture media dispensed in Petri dishes and labelled appropriately. The inoculation was such that there was an even distribution of the organism. Three uniform wells were made on the agar surface with 6 mm cork borer. The extracts were poured into the wells in their respective plates using sterile Pasteur pipette. Thereafter, the plates were incubated at 37 °C for 24 h. The evaluation of antimicrobial activity of the plant extracts was determined by measuring the average sizes of inhibitory zones (including the diameter of wells) on the agar surface around the wells measured to the nearest millimeter with a transparent meter rule. To account for the inhibitory effect of the extract, the different concentrations of Ciprofloxacin solution (used as the positive control) and distilled water (used as the negative control) were also poured into the wells made in their respective culture plates for the different isolates. The plates were incubated and diameter of zone of inhibition measured.

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is the lowest concentration of which antibiotic completely inhibits visible growth of bacteria after 18-24 h of incubation at 37 °C. The method described by Kareem et al. [25] and Otto et al. [26] was used in the determination of MIC. In this method, the radius of the diameter of zones of inhibition was squared and then plotted against the log concentrations of the extract. A suitable curve was drawn from the plots of each extract. The MIC was determined by extrapolation of the curve to the X-axis (log concentration) and the actual value determined as the antilog.

Results

The quantitative analysis of some of the phytochemicals present in the leaves and stem of A. conyzoides showed that both plant parts contain comparable amounts of flavonoids, alkaloids, saponin, tannin and phenols (Table 1). Flavonoids, alkaloids and saponins were found in higher concentration in the leaves and stem of this plant in relation to the other phytochemicals. The Phenol content was very low.

In all the tested bacterial isolates, the activity of the extracts including that of the positive control (ciprofloxacin) decreased with decrease in concentration of the extracts / ciprofloxacin. Both the leaves and stem extracts were effective in inhibiting the growth of S. aureus at concentrations of 50 mg/ml and above (Table 2). Below this concentration, no inhibition occurred. On the contrary, the positive control was effective at concentration of 25 mg/ml and above while the negative control (distilled water) showed no inhibition at all in all the tested bacterial strains. The highest zone of
inhibition was recorded with the positive control while the least was the stem extract.

Table 1: Quantitative result of the phytochemical constituents of the leaves and stem of *A. conyzoides*.

<table>
<thead>
<tr>
<th>Phytochemical (%)</th>
<th><em>A. conyzoides</em> Plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>0.98±0.02</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>0.90±0.01</td>
</tr>
<tr>
<td>Saponin</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.188±0.03</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.022±0.01</td>
</tr>
</tbody>
</table>

Values are mean of triplicates

Table 2: Antibacterial activity of the leaves and stem extracts of *A. conyzoides* on *S. aureus* measured as the diameter (mm) of inhibition zone.

<table>
<thead>
<tr>
<th>Extract/control</th>
<th>Concentration of extract (mg/ml)</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td></td>
<td>13.0±1.4</td>
<td>11.0±0.2</td>
<td>9.0±0.4</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td>12.0±0.7</td>
<td>7.0±0.5</td>
<td>7.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>26.0±0.1</td>
<td>23.0±0.0</td>
<td>18.0±0.5</td>
<td>11.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are mean of three replicates

The leaves extract was effective in inhibiting the growth of *S. typhi* at concentration of 50 mg/ml and above where as the stem extract recorded inhibition even at low concentration of 25 mg/ml (Table 3). The positive control inhibited the growth of *S. typhi* up to the low concentration of 12.5 mg/ml and recorded higher zone of inhibition in relation to the extracts. However, the stem extract showed relatively higher diameter of zone of inhibition compared to the leaves extract as the concentration decreased from 200 mg/ml to 25 mg/ml. The growth of *E. coli* was inhibited by both extracts at concentration of 50 mg/ml and above, below which no inhibition occurred (Table 4). On the other hand, the positive control inhibited the growth of *E. coli* at concentration of 25 mg/ml and above. It also recorded higher inhibition zone in comparison with the extracts. The diameters of inhibition zone for the two extracts were at par except at 50 mg/ml where the leaves extract showed higher inhibition.

Table 3: Antibacterial activity of the leaves and stem extracts of *A. conyzoides* on *S. typhi* measured as the diameter (mm) of inhibition zone.

<table>
<thead>
<tr>
<th>Extract/control</th>
<th>Concentration of extract (mg/ml)</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td></td>
<td>15.0±0.2</td>
<td>12.0±0.7</td>
<td>8.0±0.4</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td>14.0±0.5</td>
<td>13.0±0.2</td>
<td>12.0±0.7</td>
<td>10.0±1.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>26.0±0.7</td>
<td>23.0±0.5</td>
<td>16.0±0.1</td>
<td>13.0±0.0</td>
<td>9.0±0.8</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are mean of three replicates
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DOI: https://doi.org/10.36811/ijpsh.2019.11001

Table 4: Antibacterial activity of the leaves and stem extracts of *A. conyzoides* on *E. coli* measured as the diameter (mm) of inhibition zone.

<table>
<thead>
<tr>
<th>Extract/control</th>
<th>Concentration of extract (mg/ml)</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td></td>
<td>14.0±0.3</td>
<td>12.0±0.7</td>
<td>10.0±0.2</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td>13.0±0.5</td>
<td>12.0±0.4</td>
<td>7.0±0.7</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>26.0±1.2</td>
<td>20.0±0.5</td>
<td>16.0±0.1</td>
<td>13.0±0.8</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are mean of three replicates.

Concentration of 50 mg/ml and above of the leaves extract was effective in inhibiting the growth of *P. aeruginosa* (Table 5). The stem extract showed inhibition of this organism at high concentration of 100 mg/ml and above, below which there was no inhibition. Contrary to this, the positive control inhibited the growth of *P. aeruginosa* at all the concentration assayed. Again, the positive control recorded the highest zone of inhibition and the stem extract the least. The result on MIC is depicted in Table 6. The leaves extract had comparable MIC values for all the tested bacterial strain which ranged from 69.18 mg/ml against *E. coli* to 75.56 mg/ml against *P. aeruginosa*. The MIC of the stem extract against the bacterial strain varied with the least value obtained against *S. typhi* and the highest value against *S. aureus* and *P. aeruginosa*. However, the MIC of the positive control was in the range of 7.59 mg/ml against *P. aeruginosa* to 38.0 mg/ml against *S. aureus* and *E. coli*.

Table 5: Antibacterial activity of the leaves and stem extracts of *A. conyzoides* on *P. aeruginosa* measured as the diameter (mm) of inhibition zone.

<table>
<thead>
<tr>
<th>Extract/control</th>
<th>Concentration of extract (mg/ml)</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td></td>
<td>12.0±0.1</td>
<td>10.0±0.2</td>
<td>8.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td>11.0±1.0</td>
<td>7.0±0.5</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>30.0±0.1</td>
<td>27.0±0.2</td>
<td>23.0±0.7</td>
<td>20.0±1.0</td>
<td>16.0±1.0</td>
<td>11.0±1.5</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are mean of three replicates.

Table 6: Minimum inhibitory concentration (mg/ml) of the different extracts and the control against the different isolates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bacterial isolates</th>
<th><em>S. aureus</em></th>
<th><em>S. typhi</em></th>
<th><em>E. coli</em></th>
<th><em>P. aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves extract</td>
<td></td>
<td>72.44</td>
<td>72.44</td>
<td>69.18</td>
<td>75.86</td>
</tr>
<tr>
<td>Stem extract</td>
<td></td>
<td>144.54</td>
<td>36.3</td>
<td>69.18</td>
<td>144.54</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>38.00</td>
<td>17.37</td>
<td>38.00</td>
<td>7.59</td>
</tr>
</tbody>
</table>

Discussion

The phytochemicals present in extracts of *A. conyzoides* had been documented in previous works [2,7,27,28]. This study thus quantified the amount of some of the important phytochemicals earlier reported to be present in this plant extract. The results obtained revealed that the leaves and stem extracts of *A. conyzoides* contain higher quantities of flavonoids, alkaloids and saponin compared to tannin and phenol. These phytochemicals...
Phytochemicals and Antibacterial Activity of Leaf and Stem Extracts of *Ageratum conyzoides* (Linn) on Some Clinical Isolates

DOI: [https://doi.org/10.36811/ijpsh.2019.11001](https://doi.org/10.36811/ijpsh.2019.11001)

The leaves and stem extract of *A. conyzoides* showed inhibitory effect on all the tested bacteria strains. An indication that the extract of *A. conyzoides* is a broad-spectrum antibacterial agent as the test bacterial strains included both Gram positive and Gram-negative bacterial strains. This was also reported by Odoleye et al. [31]. This result confirms the earlier report of Nascimento et al. [32] that some plant extracts have shown activity on both Gram negative and Gram-positive organism. Antibacterial activity of extracts of *A. conyzoides* has been reported by previous workers [2,28,31]. The phytochemicals present in the extract of this plant have been implicated in its antibacterial activity. Flavonoids have been reported to possess biological activity such as antibacterial, anti-inflammatory, antiallergic, antimutagenic, and antiviral. Therefore, the antibacterial activity of *A. conyzoides* could be due to the presence of alkaloids and flavonoid [33]. According to Adetutu et al. [34], the presence of tannins, saponins, and phenolic compounds aside alkaloids and flavonoids, may be responsible for the antibacterial activity of the extracts. Tannins cause antimicrobial action by precipitating microbial proteins [35].

However, extracts of the leaves and stem of *A. conyzoides* showed concentration-dependent activity against all the tested bacterial isolates. The activity of the extracts decreased as the concentration decreased and vice versa. The same trend was also observed for the positive control. This is an indication that the bioactive compound(s) responsible for the inhibitory activity of the extracts/drug decreases as the concentration of the extract/drug decreases since the potency of the extract depends on the concentration of bioactive substances in the plants [35]. It also shows that the susceptibility of the test bacterial strains decreases with decrease in the concentration of the extracts/drug and vice versa. The decrease in activity of extracts of *A. conyzoides* against some bacteria has also been reported by Okwori et al. [2].

There was variation in the susceptibility of the test bacterial strains to the plant extracts. The leaves extract showed inhibition of growth of all the test organisms at concentration of 50 mg/ml and above. On the contrary, the stem extract recorded growth inhibition at concentrations of ≥ 100, 50 and 25 mg/ml against *P. aeruginosa*, *S. aureus/E. coli*, and *S. typhi*, respectively. This result is in agreement with that of Odeleye et al. [31], who also reported susceptibility of some bacterial isolates to extracts of *A. conyzoides* at concentration of ≥ 50 mg/ml. This result revealed that *S. typhi* was more susceptible to the stem extract in comparison with the other isolates as well as the leaves extract while *P. aeruginosa* was more susceptible to the leaves extract in relation to the stem extract. This variation in the susceptibility of the bacterial isolates may be attributable to differences in their bioactive constituents. Nevertheless, the susceptibility of *S. typhi* to the stem extract of *A. conyzoides* confirms its traditional use in Nigeria, in the treatment of typhoid fever as reported by Adodo [16]. The positive control showed inhibition even at very low concentrations of 25, 12.5 and 6.25 mg/ml against *S. aureus/E. coli*, *S. typhi* and *P. aeruginosa*, respectively. This shows that the test bacterial strains were more susceptible to the antibiotics, ciprofloxacin compared to the plant extracts. This is an indication of the superiority of the positive control over the plant extracts. The higher activity of the positive control, ciprofloxacin was expected since the extracts have various impurities in relation to the drug which is already a synthetically processed substance and has undergone refining processes and thus have been established as a standard antibacterial agent.
The antibacterial activity of the ethanolic extracts of the leaves and stem of *A. conyzoides* gave varying diameters of inhibition zones against the test bacterial strains. The diameters of inhibition zone of the leaf and stem extracts range from 9.0 - 13.0 mm and 7.0 - 12.0 mm, respectively against *S. aureus*, 8.0 - 15.0 mm and 10.0 - 14.0 mm, respectively against *S. typhi*, 10.0 - 14.0 mm and 7.0 - 13.0 mm, respectively against *E. coli*, 8.0 - 12.0 mm and 7.0 - 11.0 mm, respectively against *P. aeruginosa*. Ciprofloxacin, the positive control showed the widest zone of inhibition in the range of 11.0 - 26.0 mm, 9.0 - 26.0 mm, 13.0 - 26.0 mm and 11.0 - 30.0 mm against *S. aureus*, *S. typhi*, *E. coli* and *P. aeruginosa*, respectively. These results further revealed the existence of variations in the susceptibility of the bacterial isolates to the plant extracts and the superiority of the positive control in comparison with the plant extracts. This confirms the effectiveness of the drug in the treatment of ailments/diseases associated with the test bacterial strains.

The MIC values were in the range of 69.18 mg/ml against *E. coli* - 75.86 mg/ml against *P. aeruginosa* for the leaves extract, 36.3 mg/ml against *S. typhi* - 144.54 mg/ml against *S. aureus* / *P. aeruginosa* for stem extract, 7.59 mg/ml - 38 mg/ml against *S. aureus/E. coli* for the positive control. This is suggestive that the leaves extract was bactericidal at lower concentration in comparison with the stem extract except for *S. typhi* in which the leaves extract was bactericidal at higher concentration in relation to the stem extract. Okwori et al. [2], reported minimum inhibitory concentrations 25-100 mg/ml of aqueous and methanolic leaf, stem and root extracts of *A. conyzoides* against some bacterial isolates. The positive control was found to be bactericidal at lower concentrations for all the test bacterial isolates when compared to the plant extracts. This result corroborates with the already established use of ciprofloxacin as a broad-spectrum antibiotic.

**Conclusion**

It is evidenced from the results that the plant extracts (leaves and stem extracts) have inhibitory effects on the growth of the bacterial isolates and therefore can be used as an alternative to the confirmed antibiotic, ciprofloxacin if properly harnessed.

**References**

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**IJPSH: June-2019: Page No: 95-105**


