PHYTOCHEMICAL EVALUATION AND ANTI-GERMICIDAL EFFICACY OF ETHANOLIC AND AQUEOUS LEAF EXTRACTS OF AGERATUM CONYZOIDES L GROWN IN RWASAVE WETLAND, RWANDA

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**Abstract. Background and aim:** The resistance of pathogenic microorganisms to the current antibiotics is considered as a global health dynamic. This has grasped the attention of scholars to find the effective solutions from plant secondary metabolites. Ageratum conyzoides L is a medicinal plant which is mostly used to treat wounds and ulcers in Rwanda. This study is intended to light its beams on the phytochemical evaluation and anti-germicidal efficacy of ethanolic and aqueous leaf extracts of Ageratum conyzoides L grown in Rwaseave wetland, Rwanda.

**Material and methods:** The mature fresh leaves were rigorously collected and dried under the shed for 10 days. The dried leaves were blended into powder and macerated with water and 96% ethanol. Phytochemical screening was carried out by following the specific standard procedures. Anti-germicidal efficacy of the extracts was examined by agar well diffusion method and the inhibition zones were recorded.

**Results:** Phytochemical screening disclosed the presence of alkaloids, tannins, flavonoids, steroids, terpenoids, saponins and proteins. In this evaluation, all the solvents showed the ability to dissolve plant bioactive compounds. Both extracts exhibited significant inhibitory activity against all test pathogens with inhibition zones ranging from 6±0.9 mm to 20±0.5 mm of diameter. The Minimum Inhibition Concentration values range between 0.46875 mg/mL and 15mg/mL.

**Conclusion:** The results confirm that the leaves of Ageratum conyzoides L could be the credible source of anti-germicidal agents that can be used for therapeutic purposes and in production of pharmaceutical products.

**Keywords:** Ageratum conyzoides L, Anti-germicidal, Efficacy, Phytochemical screening, Zone of Inhibition

**Introduction**

Ageratum conyzoides L is an ethnomedicinal plant by which one or more parts can be used for therapeutic intensions. It has been originated from America yet, nowadays it is able to be cultivated all over the demographic areas including tropical and subtropical regions.

The leaves of Ageratum conyzoides L contain flavonoids such as eupalestin, kaempferol, sinensetin, quercetin, alkaloids (echinaline and lycopsamine), steroids (β-sitosterol, stigmasterol, brassicasterol) and sesquiterpenes (linalool, limonene, eugenol) which possess various medicinal importance including anti-oxidant, analgesic, anti-inflammatory, anti-malarial, anti-cancer due to its potent hepatotoxic and carcinogenic nature associated with pyrrolizidine anti-hyperglycemic, anti-ulcerogenic, anti-microbial and insecticidal properties.
This plant has haemostatic effects and ability to heal wounds. Indeed, this treatment is not only used in modern medical treatment but also people are familiar with it in traditional ways to cure different types of diseases. It has been used to treat pneumonia in Cameroon, to cure antitetanus, anti-itch, anti-leprosy in India and it is as well considered as an anti-gynecological disease in Vietnam.

The leaves of this plant are used to treat headache and skin diseases, ringworm infections and have anti-nematocidal activities against T. solium and P. posthuman.

The leaves of this plant have pharmacological active substances such as chromenes and chromans. Chromans have 6-amino and 6-acetamido derivatives with anti-depressant and antipyretic properties. They also known to fight against flat worms of the order of trematodes. These leaves also consist of 2,2-dimethyl chromene derivatives, such as 6-(1-hydroxyethyl)-7,8-dimethoxy-2,2-dimethylchromene and 6-hydroxy-7,8-dimethoxy-2,2-dimethyl chromene which have the ability to inhibit the life processes of different disease-causing pathogens.

The polyhydroxyflavones include scutellarein-5,6,7,4′-tetrahydroxyflavone, quercetin, quercetin-3-rhamnopiranoside, kaempferol-3-rhamnopiranoside and kaempferol 3,7-diglucopiranoside which have virustatic ability to inhibit HIV syncytium and viral p24 antigen formations. They also contain afzelin and quercetin 3-O-d-arabinopyranoside which have repulsive potential against herpes simplex virus type 1, Aujeszky's disease virus and adenovirus type-3 by inhibiting acyclovir-resistant HSV-1.

The leaf extracts of this plant have demethoxyageratochromene with antifungal activity against P. chrysogenum and P. javanicum and antibacterial potential against V. cholerae, S. shigae, S. pyogenes, C. diphtheriae and S. typhi.

The current study is specifically aimed to evaluate phytochemical constituents and anti-germicidal efficacy of Ageratum Conyzoides L. leaves grown in Rwasave wetland, Rwanda.

**Materials and methods**

**Collection of plant materials**

The mature healthy fresh leaves of Ageratum conyzoides L were collected from Rwasave wetland which is located in Huye district, Southern province, Rwanda. The leaves were washed thoroughly with running tap water and rinsed properly with distilled water. The leaves were air-dried at room temperature for 10 days and blended into powder using electric blender.

**Plant material extraction**

The extraction experiment was aseptically performed in Biotechnology laboratory at room temperature. Twenty grams of Ageratum conyzoides L leaf powder was macerated with 100 mL of 96% ethanol (1:5) and water for 3 days using rotary shaker for better extraction. After extraction, the extracts were decanted and then filtered through Whatman filter paper No.1. Ethanolic crude extract was obtained by evaporating the solvent using rotary evaporator. Aqueous crude extract was obtained by lyophilization process. The yielded thick extracts were dissolved in 10% DMSO and kept in labelled containers at 4°C for future use.

**Sterility proofing of the extracts**

To be sure about the sterility of the extracts, 2mL of the extracts were introduced into 10 mL of Mueller Hinton broth and incubated at 37°C for 24 hours. The absence of microorganism growth on the broth after the period of incubation signifies the presence of a sterile extract. After seeing that, there was no need of sterilizing the extracts under UV light.

**Phytochemical screening**

Qualitative phytochemical screening was carried out to evaluate the presence of bioactive components in crude leaf extracts according to standard method. The qualitative analysis tests were performed for various phytoconstituents such as flavonoids (Shinoda's test), steroids (Salkowski test), tannins (Ferric chloride test), alkaloids (Wagner’s test), saponins (Froth’ test), proteins (Xanthoproteic’s test) and terpenoids were tested by mixing 5 mL of the crude extracts with 3mL of chloroform and eventually added 2mL of.
concentrated sulphuric acid. The formation of brown ring confirmed the availability of terpenoids in the examined extracts.

**Source of test microorganisms**
The clinical isolates of *S. aureus*, *E. coli* and *P. aeruginosa* used in the work were obtained from the Microbiology laboratory of the University Teaching Hospital of Butare, Huye district, Rwanda. Viability test of each isolate was carried out by resuscitating the organisms in buffered peptone broth and eventually sub-cultured into nutrient agar medium and incubated at 37 °C for 24 hours. A single colony of each microorganism was diluted in 9 mL of peptone water and eventually acclimatized to give the equal concentration of bacterial cells to density of 10^4 CFU/mL.

**Antibacterial and antifungal assay of extracts**
The antimicrobial assay of extracts was examined by agar well diffusion method according to National Committee for Clinical Laboratory Standards. 24 20 µl of diluted microorganisms were swabbed on respective nutrient agar plates. After spreading, Pasteur pipette was used to create 3 wells in the inoculated agar and filled up with 20mg/mL, 40mg/mL and 60 mg/mL, respectively. The plates were incubated in the upright position at 37°C. *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* plates were incubated for 24 hours. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of inhibition zones in millimeter. Three replicates were carried out for each extract against each of the test microorganism and data were recorded as mean±standard deviation. The activity index for each extract was calculated by dividing the inhibition zone of the sample with the inhibition zone of the standard antibiotic.

**Determination of minimum inhibition concentration (MIC)**
The MIC is explained as the lowest concentration that inhibits the observable growth of microorganism after nightlong incubation. In this study, the MIC was examined by preparing the inoculum of microorganisms from nutrient broth cultures. With broth dilution technique, the extracts were serially diluted from 60mg/mL to 0.0585mg/mL with 2 mL of distilled water.1 milliliter suspension of the test microorganisms was inoculated with Mueller Hinton broth as a positive control and Vancomycin as a standard reference antibiotic. It was incubated for 18-20 hours at 37ºC and determined the MIC by observing the presence or absence of turbidity in the test tubes. The least concentration where no turbidity observed was noted as the MIC value.

**Results**

**Phytochemical analysis**
Phytochemical screening clearly confirmed the presence of alkaloids, proteins, terpenoids, tannins, flavonoids and steroids in ethanolic extracts and absence of terpenoids and tannins in aqueous extracts.

<p>| Table1: The results of the chemical tests of the crude epicarp extracts of <em>Ageratum conyzoides</em> L. |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th></th>
<th>Flavonoids</th>
<th>steroids</th>
<th>Terpenoids</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Proteins</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: (+): Presence, (-): Absence
Table 2: Antimicrobial activity of leaves extracts of *Ageratum conyzoides* L

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
<th>Vancomycin (30mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>16±3.3</td>
<td>6±0.9</td>
<td>26.92</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>20±0.5</td>
<td>15±0.2</td>
<td>24</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC25922</td>
<td>14±0.2</td>
<td>7±2.5</td>
<td>21±3.1</td>
</tr>
</tbody>
</table>

Table 3: The activity indexes of each extract in accordance to the standard antibiotic

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanolic extracts</th>
<th>Aqueous extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>0.59</td>
<td>0.22</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>0.83</td>
<td>0.63</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC25922</td>
<td>0.66</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Table 4. The Minimum Inhibitory Concentrations of the extracts against the tested pathogens

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>3.75</td>
<td>7.5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>0.46875</td>
<td>15</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC25922</td>
<td>1.875</td>
<td>3.75</td>
</tr>
</tbody>
</table>

Discussion
The current investigation was carried out to evaluate the phytoconstituents and anti-germicidal efficacy of ethanolic and aqueous leaf extracts of *Ageratum conyzoides* L. grown in Rwasave wetland, Rwanda. The core findings of this study revealed that leaf extracts of *Ageratum conyzoides* L contain secondary metabolites with antiseptic properties.

The results disclosed the availability of tannins, steroids, saponins, alkaloids and flavonoids in ethanolic extract which have ability to inhibit the visible growth of various pathogens. These results are in tandem with the findings reported by other researchers that the plant bioactive substances have ability to impede microbial cell wall synthesis by creating irremediable compounds with abundant prolene growth factor. 25

The plant extracts inhibit microbial growth by associating with non-polar compounds in the hydrophobic interior of the membrane and by the formation of hydrogen bonds between the polar head groups of lipids and the more hydrophilic flavonoids at the membrane interface. The antimicrobial activity of flavonoids is explained by the fact that they reduce fluidity in hydrophilic
and hydrophobic regions of both inner and outer plasma membrane and cause biofilm perturbation. The high antimicrobial activity of flavonoids is owed to 3-O-octanoyl-epicatechin which enhance membrane affinity of their long acyl chains. The flavonoids lacking hydroxyl groups on their B rings are the most effective to hinder microbial membranes than those with hydroxyl (OH) groups.

The plant derived antimicrobials control bacterial growth by altering their membrane permeability or decreasing their pH. These membrane disruptions along with the activity of β-lactams on the transpeptidation of the cellular membrane increase the inhibitory activity of the extracts. The extracts evidently demonstrated extensive capability to cause leakage of different growth factors and enzymes from the cell. These plant secondary metabolites perform anti-germicidal activity by agitating cellular binary fission, interacting with extracellular proteins and by damaging the integrity of bacterial cell walls.

The variability in antipathogenic capabilities of the extracts also depends on the quantity of active substances present in extracted plant parts. Roots, leaves, fruits, stems and seeds have different allotment of chemical compounds. The abundance of the bioactive compounds in plants depends on the stage of maturity, rainfall, seasonality, soil salinity and other agroecological conditions which repress or induce water absorption, physiological and chemical processes during plant metabolism.

Both ethanolic and aqueous extracts demonstrated great antimicrobial activity against tested microorganisms. This result absolutely unveiled the effectiveness of organic solvents to dissolve bioactive compounds from plants due to their polarity which influenced their biological activities. This finding is as well in fair correlation with the results of Idris who reported that solvent solubility has a critical importance to extract the plant natural products. This finding is in contrast with the research published by Cowan which highlighted that water may not be able to extract aromatic and saturated antibacterial compounds that can inhibit the growth of microorganisms.

The findings of the current evaluation emphasized that Gram-positive bacteria were more susceptible to all extracts than Gram-negative bacteria. This statement could be explained by the fact that cell wall make-ups of Gram-positive and Gram-negative bacteria are slightly different. Gram-negative outer membrane consists of phospholipids and lipopolysaccharides that act as a barrier which block the entrance and reaction of antimicrobial agents through cell envelope. The diversity in antimicrobial activity of extracts to Gram positive and Gram-negative bacteria can be as well explained by the fact that catechins cause an oxidative burst by the generation of reactive oxygen species that cause alteration in the membrane permeability and membrane damage. Gram-negative were not highly sensitive due to the liposomes which contain high amounts of negatively charged lipids which make catechins weak to inhibit Gram-negative bacteria due to negatively charged lipopolysaccharides of the outer bacterial membrane. Staphylococcus aureus ATCC 25923 as a Gram-positive bacterium was more sensitive to the ethanolic extracts with the activity index of 0.83. This finding is absolutely correlated with the results of Zaika who reported the significant sensitivity of Staphylococcus species due to their cell walls and outer membranes. This result is also in line with the research of Bravo and Anacona which demonstrated that Mn2+, Hg2+, Co2+, and Cd2+ complexes of quercetin exhibit bactericidal upshot against Staphylococcus aureus, Bacillus cereus and Klebsiella pneumoniae.

In this research, antibiotic disc of vancomycin manifested high inhibitory activity than the prepared plant extracts. This outstanding effectiveness of antibiotic than the plant extracts is obviously correlated with the fact that antibiotics are refined and naturally purified while plant extracts are crude states.

**Conclusion**

To sum up, the findings of this study confirmed the
high medicinal importance of *Ageratum conyzoides* L. and also give a great promise that the leaves of this plant should be considered in production of antimicrobial agents to fight pathogens resistant to typical antibiotics in current use.

References


