Qualitative Phytochemical ScreeningandIn VitroAntibacterialActivitiesof Crude Akaziraruguma Leaf Extracts

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Abstract

The resistance of pathogenic bacteria is a global health dynamic and the time is nowtofind alternative solutions from plant secondary metabolites.*Ageratum conyzoides*L. is a plant known as akaziraruguma in Rwandaand used to treat wounds and ulcers. This study aimedto evaluate the phytochemical profile and germicidal efficacy of ethanolic and aqueous leaf extracts of *Ageratum conyzoides* L. grown in Rwasave wetland.Theleaves were collected and dried under the shed for 10 days, blended into powder by electric blender and macerated with water and 96% ethanol. Phytochemical screening wasperformed by followingthe standard procedures and antibacterial activity of the extracts wasexamined by agar well diffusion method and the inhibition zones were recorded.Phytochemical screening revealed the presence of different secondary metabolites including alkaloids, tannins, flavonoids, steroids, terpenoids and saponins . In this assessment,aqueous and ethanolic extracts exhibited significant inhibitory activity against tested pathogens with inhibition zones that ranging from6±0.9 mm to 20±0.5 mm of diameter. The minimum inhibition concentrations range between 0.47 mg/mL and 15.00 mg/mL.The resultsconfirm that the leaves of akaziraruguma could be the credible source ofantibacterial agents that should be used for therapeutic purposes and in production of pharmaceuticals.

Keywords*: Ageratum conyzoides L., Antibacterial, Phytochemical screening, Zone of Inhibition*

Introduction

Akaziraruguma *(Ageratum conyzoides* L.)is an ethnomedicinal plant by which one or more parts can be used for therapeutic intensions. Itwas named akaziraruguma by Rwandan citizens due to the fact that it is mostly employed to treat wounds. It is believed tohaveoriginated from America yet, nowadays it is able to be cultivatedall over demographic areas including tropical and subtropical regions [1].

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The leaves of *Ageratum conyzoides* L.contain flavonoids(eupalestin, kaempferol, sinensetin,quercetin), alkaloids (echinalineand lycopsamine), steroids (β-sitosterol, stigmasterol, brassicasterol) and sesquiterpenes (linalool, limonene, eugenol)[1,2] which possess various medicinal importance including anti-oxidant[2] , analgesic, anti-inflammatory,[3] anti-malarial,[4] anti-cancer due toits potent hepatotoxic and carcinogenic nature associated with pyrrolizidine[5,20,] anti-hyperglycemic,[6] anti-ulcerogenic,[7] antimicrobialand insecticidal [8] properties.

This plant hasand ability to heal woundsand thehaemostaticeffects by decreasing the bleeding time through the production of vasoconstriction, precipitate proteins at the bleeding sites and promote the natural

process of blood coagulation. The hemostatic activity of *Ageratum conyzoides* L. could be observed through the lowering of prothrombin and clotting times and also by increasingplasma fibrinogen concentration in rats of theexperimental group.[9]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 curetetanus,itchiness and leprosyin India and it is as well considered as an anti-gynecological disease in Vietnam.[9]The leaves of this plant are used to treat headache and skin diseases [9], ringworm infections [12] and haveanti- nematocidal activities against *Taenia solium*.[11,8]

The leaves of this plant have active pharmacological substances such as chromenes and chromans.Chromans consist of 6-amino and 6-acetamido derivatives,which can help to relieve the symptoms of depression and also demonstrated the ability to reduce fever by causing the hypothalamus to override a prostaglandin-induced increase in temperature.They also used to treat the infections caused by worms that are classified in the order of trematodes. The other secondary metabolites that are available in *Ageratum conyzoides* L. are the derivatives from 2,2-dimethyl chromene like 6-(1-hydroxyethyl)-7,8-dimethoxy-2,2-dimethylchromene and 6-hydroxy-7,8-dimethoxy-2,2-dimethyl chromene which demonstrate the capability to impedethe life processes of various microorganisms.[13]

The polyhydroxyflavones include scutellarein-5,6,7,4′-tetrahydroxyflavone, quercetin, quercetin-3-rhamanopiranoside,kaempferol-3-rhamnopiranosideand kaempferol3,7-diglucopiranoside[14] which have virustatic ability to inhibit HIV syncytium and viral p24 antigen formations.[15] 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.[16]The leaf extracts of this plant have demethoxyageratochromene with antifungal activity against*Penicillium chrysogenum*and *Paphiopedilum javanicum*[17]and antibacterial potential against *Vibrio cholerae*, *Streptococcus pyogenes* ,*Corynebacterium diphtheriae* and *Salmonella typhi*. [8]By considering all scientific researches done in different parts of the world, there are no published findings onphytochemical evaluation and germicidal efficacy of ethanolic and aqueous leaf extracts of *Ageratum conyzoides* L. grown in Rwasave wetland, Rwanda. The novelty of this study is to provide the primary data on medicinal profile of this plant by taking into account that the abundance of bioactive substances in plants depend on seasonality, climate, geographical location and other environmental conditions. From that standpoint, the current study is specifically aimed to evaluatephytochemical constituentsand antibacterialactivity 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 plant was independently authenticated by Chief Herbarium Officer at National Herbarium ofRwanda which is located in Huye district, 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

During extraction, 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 N°1. Ethanolic crude extract was obtained by evaporating the solvent using rotary evaporator.[19]Aqueous crude extract was obtained by lyophilization process[8,21,22] and the yielded thick

extracts were dissolved in 10% DMSOand 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 such observations, sterilization of the extracts under UV light was not carried out.

Phytochemical screening

Qualitative phytochemical screening was carried out to evaluate the presence of bioactive components in crude leaf extracts according to standard method.[23] 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 crude extracts with 3mL of chloroform and eventually added 2mL of concentrated sulphuric acid. Theformation of brown ring confirmed the availability of terpenoids in the examined extracts.

Source of test bacteria

Staphylococcus aureus, *Escherichia coli* and *Pseudomonas aeruginosa* are the bacteria that were used in this work and they were allobtained from University Teaching Hospital of Butare. Each bacteriumwas tested for viability by reviving it in peptone broth and finally sub-cultured into nutrient agar followed by incubation 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 antibacterial assay of extracts was examined by agar well diffusion method according to National Committee for Clinical Laboratory Standards. About 20 µL of diluted bacterial cultures 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. During this experiment, 30 µL of 30mg/mL vancomycin was used as a standard antibiotic. The plates were incubated in the upright position at 37ºC. *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa* plates were incubated for 24 hours. Following the incubation, the antifungal and antibacterial assays of the extracts were evaluated by observing and measuring the inhibition zones. These assays were repeated three times each and the data were noted as mean±standard deviation. The ration between inhibition zone of the sample and the inhibition zone showed by the standard antibiotic was reported as the activity index ofthe extracts.

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

The yields of the ethanol and water extraction were 12.5 and 7.5mg/g, respectively. Ethanol disclosed the highest capability to extract bioactive substances from plant leaves. In reference to the results presented in table 1, 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.

Table1: The results of the chemical tests of the crude epicarp extracts of *Ageratum conyzoides* L.

Extracts	Flavonoids	steroids	Terpenoids	Saponins	Tannins	Proteins	Alkaloids
Ethanolic	+	+	+	+	+	+	+
Aqueous	+	+	_	+	_	+	+

Key: (+): Presence, **(-):** Absence

The findings of antimicrobial assay revealed that vancomycin had more potential compared to ethanolic and chloroform extracts. Both extracts were able to inhibit Gram positive bacteria (*Staphylococcus aureus* ATCC 25923) stronger than Gram negative bacteria (*Pseudomonas aeruginosa ATCC* 27853and *Escherichia coli* ATCC25922) in Table 2. This potential was also confirmed by their activity indexes presented in Table 3and their respective MICs as shown in Table 4.

Table 2: Antimicrobial activity of leaves extracts of *Ageratum conyzoides* L.

	Inhibition zones (mm)		
Microorganisms	**Ethanolic extract (mg/mL)**	**Aqueous extract (mg/mL)**	**Vancomycin (30mg/mL)**
Pseudomonas aeruginosa*ATCC* 27853	16±3.3	6±0.9	26.92
Staphylococcus aureusATCC 25923	20±0.5	15±0.2	24
Escherichia coliATCC25922	14±0.2	7±2.5	21±3.1

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

	Activity index Inhibition zone of extracts/ inhibition zone of standard antibiotic	
Microorganisms	**Ethanolic extracts**	**Aqueous extracts**
Pseudomonas aeruginosa *ATCC* 27853	0.59	0.22
Staphylococcus aureus ATCC 25923	0.83	0.63
Escherichia coli ATCC25922	0.66	0.33

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

	MIC (mg/mL)	
Microorganisms	**Ethanolic extract**	**Aqueous extract**
Pseudomonas aeruginosa *ATCC* 27853	3.75	7.50
StaphylococcusaureusATCC 25923	0.47	15.00
Escherichia coli ATCC25922	1.88	3.75

Discussion

The current investigation was carried out to evaluate the phytoconstituents and antibacterial activity ofethanolic 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 findings are in conformity with the results published by other researchers which highlighted that the secondary metabolites from plants have ability to hinder the cell wall synthesis of different microorganisms by creating substances that are rich in prolene growth factor. [24]

Once the plant extracts associatewith the compounds that do not have slightly negative and positive charges in the water-fearing interior of the membrane, they impede the microbial growth. This growth should also be hindered through the formation of hydrogen bonds between the polar lipids and the hydrophilic flavonoids at the interface of the membrane. The antimicrobial activity of flavonoids is explained by the fact that they decrease elasticity in hydrophilic and hydrophobic regions of cell membrane and induce biofilm disturbance. [25]

The observed outstanding antibacterial capacity of flavonoids is attributed to the presence of 3-O-octanoyl-epicatechin which enhance membrane affinity of their long acyl chains. The absence of hydroxyl groups on the B rings of the flavonoids play major role to inhibitmicrobial membranes than the flavonoids which have hydroxyl(OH) groups on their B rings.[26]

The plant derived antimicrobials control bacterial growth by altering their membrane permeability or decreasing their pH.[8] These membrane disruptionsalong with the activity of β-lactams on the transpeptidation of the cellular membrane increase the inhibitory activity of the extracts. [28] The extracts evidently demonstratedextensive capability to cause leakage of different growth factors and enzymes from the cell. These plant secondary metabolites perform antibacterial activity by agitatingcellular binary fission, interacting with extracellular proteins and by damaging the integrity of bacterial cell walls.[29,9]

The differences in antimicrobial potential of the extractsis also attributed to the amount of secondary metabolites available in tested plant parts. This finding could be explained by the fact that the distribution of bioactive substances in roots, leaves, fruits, stems and seeds are different.[31] The abundance of the bioactive compounds in plants depends on the stage of maturity, rainfall, seasonality, soil salinity and other agroecological

conditions [32] which repress or induce water absorption, physiological and chemical processes during plant metabolism. [33]

Both ethanolic and aqueous extracts demonstrated great antimicrobial activity against tested microorganismsbut organic solvents demonstrated high ability to dissolve plant secondary metabolites due to their polarity [34].This finding is as well in agreement with the findings of Idris [35] who published that the ability of extractants to extract the compounds from the leaves plays a pivotal role during extraction of plant metabolites. This finding is in contrast with the research published by Cowan[9]which highlighted that water may not be able to extract aromatic and saturated antibacterial compounds that can inhibit the growth of microorganisms.

The results obtained from this study showed that all leaf extracts exhibited high antibacterial activities against Gram-positive than Gram-negative bacteria.[30] 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.[9]The diversity in antimicrobial activity of extracts to Gram-positive and Gram-negative bacteria can be as well explained by the fact that catechinsinduce an oxidative stress due to reactive oxygen species (ROS) that cause changes in the membrane porosity and its destruction.[31]

Gram-negative were not highly sensitive due to the liposomes which contain high amounts of negatively charged lipidswhich make catechins weak to inhibit Gram-negative bacteria due to negatively charged lipopolysaccharides of the outer bacterial membrane.[27]*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 [34] 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 Mn^{2+}, Hg^{2+}, Co^{2+}, and Cd^{2+} complexes of quercetin exhibit bactericidal upshot against *Staphylococcus. aureus*, *Bacillus cereus* and *Klebsiella pneumoniae*. [9]

In this research, antibiotic disc of vancomycinmanifested 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.[34]

Conclusion

The conclusion that can be drawn from the findings of the current study is that *Ageratum conyzoides* L. has high medicinal importance and alsogive a great promise that the leaves of this plant should be considered in production of antibacterial agentstofight off the pathogens that are resistant to typical antibiotics in current use.

Conflict of Interest: Authors did not declare any conflict of interest associated with this article

Source of Funding: Self-funding

Ethical Clearance : Not applicable

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