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Research Article
Assessment of Phytochemical and Antibacterial Activities of the Leaf and Stem Extracts of *Gomphrena celosioides* Mart. (Amaranthaceae)

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Abstract

Background and Objective: The use of medicinal plants for therapeutic potentials and treatment of microbial diseases is popular and has been archived since time immemorial. Plants contain phytochemicals, which when utilized by humans give therapeutic and antibacterial effects. *Gomphrena celosioides* has not been reported to be edible rather it is mostly used for medicinal and ornamental purposes. The present study was carried out to evaluate the medicinal potentials by analyzing phytochemical and antibacterial activities of ethanolic leaf and stem extracts of *Gomphrena celosioides.* **Materials and Methods:** phytochemical analysis was done using standard gravimetric and spectrophotometric techniques whereas the antibacterial assay of leaf and stem extracts was carried out at different concentrations against some selected human microbes (*Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus)* using standard agar diffusion method. The results were analyzed using Analysis of Variance (ANOVA). **Results:** Qualitative and quantitative phytochemical results indicated varied compositions of the secondary metabolites in leaf and stem extracts of *G. celosioides*. Antibacterial results indicated that the ethanolic leaf and stem extracts of *G. celosioides* inhibited the growth of the microbes assayed but at varying levels and the inhibition was extracted concentration-dependent. The leaf showed significantly higher inhibitory effects against the tested pathogens when compared to stem extract. **Conclusion:** The data obtained from the research indicated that the plant possessed antibacterial potentials and its uses in ethnomedicine are thus justified.

Key words: Antibacterial, phytochemical, *Gomphrena celosioides,* ethnomedicine, microbes, clinical isolates, inhibition

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

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

INTRODUCTION

Plants and other substances of natural origin have been in use throughout the world for human and animal health care since ancient times[1]. Africa, with a range of regions, has a long and impressive list of medicinal plants spread through the forest and woodland regions of the continent[2]. Many African plants are used in traditional medicine as antimicrobial agents though only a few have been documented. A wide range of substances has been found in plants which are used in traditional medicine to treat chronic as well as infectious diseases[3]. The medicinal values of plants are attributed to the presence of some chemical substances which produce a definite physiological action on the human body[4]. These substances are called secondary metabolites or phytochemicals. Plants manufactured these chemical substances, to help them grow or halt competitors, pathogens or predators[4].

Antimicrobial compounds derived from plants might inhibit bacteria through different mechanisms and provide clinical values for the treatment of infection caused by resistant microbes[5]. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used to fight bacteria and antifungal is used to fight fungi. They can also be grouped based on their roles. Biocidal compounds or substances whose function or role is to reduce the infectivity of microbes are called microbicidal, while those that inhibit or multiply their growth are called biostatic. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds[4,6,7]. Different crop species have been examined for phytochemical and anti-microbial potentials but very great numbers have not yet been fully assessed[1]. Among the medicinal plants, locally used in our community for treatment and management of various ailments is *Gomphrena celosioides* Mart.

Gomphrena celosioides Mart., a plant in the genus *Gomphrena* of the family Amaranthaceae is an herbaceous perennial and cosmopolitan pioneer plant. It is a short-lived perennial or an annual woody plant, whose branches lie upon or just above the ground, with a deep taproot and is often mat-forming. *G. celosioides* is widely used by the rural people of West Africa to cure a variety of sicknesses[8]. The extract of this plant is generally employed for medicinal and therapeutic uses[9]. The leaf paste is used to treat Malaria[10]. The whole plant is used in the treatment of skin diseases in Nigeria[11]. Different compounds, including aurantiamide and aurantiamide acetate, a selective cathepsin suppressor, also produced by *Aspergillus penicilloides* has been found in this species. These compounds suppress the growth of microorganisms, even in very small quantities. Studies have shown that *G. celeosioides* contain high contents of moisture, ash, fiber and carbohydrate[12].

G. celosioides is a species with immense medicinal and ornamental purposes. However, no scientific study has been done to ascertain its inhibitory potentials on the growth of bacteria especially in the eastern region thus, the need for the present study. Accordingly, the objectives of this research were to evaluate the phytochemical and antibacterial effects of ethanolic leaf and stem extracts of *G. celosioides* against pathogenic bacteria to ascertain its potentials as an antibacterial agent.

MATERIALS AND METHODS

Study area: The experiments were carried out at the Department of Botany, Laboratory of Nnamdi Azikiwe University, Awka, Anambra State.

Collection and identification of plant materials: The plant species *G. celosioides* used in this work were collected between April-June 2018 from Nnamdi Azikiwe University, Awka, Anambra State (6°12N', 7°04E'). The plant was identified by a taxonomist of the Botany Department, Nnamdi Azikiwe University, Awka. The voucher specimens were deposited in the herbarium of Nnamdi Azikiwe University, Awka with the accession No. NAUH 285 (Fig. 1, Ilodibia *et al.*[12]).

Materials used for phytochemical and antibacterial studies: The materials and instruments used included plant specimen, blender (grinder), masking tape, mortar and pestle, moisture cans, crucibles, Whatman filter paper No. 42 (SIGMA-ALDRICH Laboratories, USA), burettes, volumetric flasks, beakers, conical flasks, sample tubes, desiccators, spectrophotometer (Analytik

Fig. 1: *Gomphrena celosioides* in its natural habitat
Source: Ilodibia *et al.*[12]

Jena, Germany), muslin cloth, oven, measuring cylinder, spatula, electric scale, Bunsen burner (stove), funnels, aluminum foils, test tubes, syringes, pipettes, cotton wools, etc.

Chemical and reagents used: Ethanol (alcohols), concentrated acetic acid, sulphuric acid, diluted ammonia, water, ferric chloride, potassium ferrocyanide, ethyl acetate, hydrochloric acid, petroleum ether, sodium hydroxide, potassium hydroxide (potassium permanganate). Hydrogen peroxide, sodium chloride, copper sulphate, sodium picrate, methyl red, cresol green, folin-ciocalteu reagent, folin-denis reagent, Eriochrome black and sole chrome dark blue. Listed reagents (SIGMA-ALDRICH Laboratories, USA),

Preparation of plant samples: Fresh Leaves and stem of *G. celosioides* were cut into bits with a knife and oven-dried at 70°C for 12 h to remove all moisture. The samples were then ground into *a* fine powder.

Extraction of plant material: The ethanolic extract of the plant was prepared by soaking the ground sample of the leaf and stem in 100 mL of ethanol. The concentration of each extract was determined by adding 100, 150, 200 and 250 g in 100 mL of ethanol. The experimental set-up was left for 24 h at room temperature and thereafter filtered using Whatman filter paper No. 42 (SIGMA-ALDRICH Laboratories, USA). The extract was then concentrated to 50 mL of the original volume of the extract and stored in an airtight container in a refrigerator at 4°C until when needed.

Phytochemical analysis: Qualitative phytochemical screening of the extracts was conducted to determine the presence of these Phytochemicals: saponins, tannins, flavonoids, alkaloids, sterols and phenols. This was done using the standard procedure as described by Harborne[13].

The quantitative phytochemical test of the extracts was conducted to determine the percentage quantitative contents of above phytochemicals using standard procedure described by Harborne[13], Kirk and Sawyer[14].

Antibacterial studies

Test microorganisms: The following microorganisms: Bacterial strains (*Salmonella typhii* (NR 201), *Escherichia coli* (NR 202), *Pseudomonas aeruginosa* (NR 203) and *Staphylococcus aureus* (NR 204) were collected based on their clinical and pharmacological importance.

Sources of test microorganisms: The pure cultures of the microorganisms were obtained from the Department of Pathology, National Root Crop Research Institute, Umudike, Abia State. The isolates were checked for purity and are maintained on nutrient broth at 4°C in the refrigerator until when required.

Antibacterial activity: The agar diffusion method as described by Ilodibia *et al.*[15] and Osadebe and Ukwueze[16] was adopted for the study. Standardized Nutrient broth culture of the test isolate containing approximately 107 cells mL^{-1} organisms was used. 0.1 mL of the broth culture was introduced into sterile Petri dishes and 15 mL of molten nutrient agar poured into the Petri dishes. The contents were thoroughly mixed and allowed to solidify. Three holes each measuring 5.0 mm in diameter were made in each of the solid agar plates using a sterile cork borer. 0.04 mL of the different concentrations of plant extracts were transferred into the holes using a Pasteur pipette. Two Petri dishes containing a particular bacterium were used for each concentration of the extracts. The plants were thereafter allowed to stand for 1 hour for pre-diffusion of the extracts[15] and were subsequently incubated at 37°C for 24 hours. After incubation, the plates were collected and the zones of growth inhibition were measured. The extent of inhibition was expressed in terms of the diameter of the inhibition zone as measured with a transparent meter rule. The effects of the extracts on bacteria pathogens were compared with those of the standard antibiotic ampicillin as a standard control.

Statistical analysis: The results were analyzed using ANOVA. Duncan's multiple range test significance was used to test the difference among treatments. All analyses were carried out at 5% level of significance.

RESULTS

Qualitative phytochemical screening of stem and leaf extracts of *G. celosioides*: It was revealed from the result that all phytochemicals (saponin, flavonoid, alkaloid, sterol and phenol) assayed except tannin were present in the leaf and stem extracts of *G. celosioides* (Table 1).

Table 1: Qualitative phytochemical screening of stem and leaf extracts of *G. celosioides*

Plant parts	Saponins	Tannins	Flavonoids	Alkaloids	Sterol	Phenols
Leaf	+	-	+	+	+	+
Stem	+	-	+	+	+	+

+: Presence and -: Absence

Table 2: Percent Quantitative phytochemical screening of stem and leaf extracts of *G. celosioides*

Plant parts	Saponins	Tannins	Flavonoids	Alkaloids	Sterol	Phenols
Leaf	1.10±0.2	-	0.77±0.2	2.18±0.0	0.60±0.0	0.95±0.0
Stem	0.47±0.1	-	0.44±0.1	1.77±0.1	0.55±0.0	0.92±0.0
p-value	**	-	**	**	**	**

Results are in mean±standard deviation

Table 3: Inhibitory activity of leaf and stem extracts of *G. celosioides at* 100 mg/100 mL of ethanol

Microorganisms	Leaf	Stem	Control	p-value
S. typhi	7.24±0.0^{a}	6.03±0.1^{a}	14.26±0.1^{c}	**
E. coli	8.24±0.1^{c}	7.26±0.1^{c}	14.18±0.0^{b}	**
P. aeruginosa	9.54±0.4^{d}	8.77±0.2^{d}	15.00±0.1^{d}	**
S. aureus	8.74±0.4^{b}	7.17±0.1^{b}	14.04±0.2^{a}	**

Result values are in Mean±Standard Deviation, **p<0.05, columns followed by the same letter are not significantly different

Table 4: Inhibitory activity of leaf and stem extracts of *G. celosioides* at 150 mg/100 mL of ethanol

Microorganisms	Leaf	Stem	Control	p-value
S. typhi	8.40±0.0^{a}	7.40±0.1^{a}	16.92±0.1^{c}	**
E. coli	8.70±0.2^{b}	8.01±0.1^{b}	16.86±0.1^{b}	**
P. aeruginosa	10.44±0.4^{d}	9.25±0.2^{d}	17.54±0.1^{d}	**
S. aureus	9.42±0.1^{c}	8.16±0.1^{c}	16.74±0.1^{a}	**

Result values are in Mean±Standard Deviation, **p<0.05, columns followed by the same letter are not significantly different

Table 5: Inhibitory activity of leaf and stem extracts of *G. celosioides* at 200 mg/100 mL of ethanol

Microorganisms	Leaf	Stem	Control	p-value
S. typhi	9.24±0.0^{a}	8.13±0.1^{a}	18.26±0.1^{a}	**
E. coli	9.84±0.1^{b}	9.26±0.1^{c}	19.18±0.0^{d}	**
P. aeruginosa	11.54±0.4^{d}	10.27±0.2^{d}	18.34±0.1^{b}	**
S. aureus	10.74±0.4^{c}	9.07±0.1^{b}	19.04±0.2^{c}	**

Result values are in Mean±Standard Deviation, **p<0.05, columns followed by the same letter are not significantly different

Quantitative Phytochemical Screening of stem and leaf extracts of *G. celosioides*: Results indicated that the stem and leaf extracts of *G. celosioides* contained all Phytochemicals assayed in varied proportions except tannin (Table 2). The leaf contained higher quantities of the Phytochemicals than the stem (Table 2).

Antibacterial result: Antibacterial activity of ethanolic extracts (leaf and stem) of *G. celosioides* was studied at different concentrations (100, 150, 200, 250 mg/100 mL) against four pathogenic bacterial strains (*Salmonella typhi, Escherichia coli*, *Pseudomonas aeruginosa and Staphylococcus aureus*). Antibacterial potential of extracts was assessed in terms of the zone of inhibition of microorganisms' growth. The results in Table 3-6 showed that *G. celosioides* extracts at 100, 150, 200 and 250 mg/100 mL all exhibited inhibitory effects on tested pathogens but at varying levels. The exhibited antibacterial effect was concentration-dependent and proportional. From the study, the leaf showed

Table 6: Inhibitory activity of leaf and stem extracts of *G. celosioides* at 250 mg/100 mL of ethanol

Microorganisms	Leaf	Stem	Control	p-value
S. typhi	10.24±0.0^{a}	9.03±0.1^{a}	19.26±0.1^{a}	**
E. coli	11.24±0.1^{b}	10.26±0.1^{c}	20.18±0.0^{d}	**
P. aeruginosa	13.54±0.4^{d}	12.77±0.2^{d}	19.34±0.1^{b}	**
S. aureus	12.74±0.4^{c}	10.17±0.1^{b}	20.04±0.2^{c}	**

Result values are in Mean±Standard Deviation, **p<0.05, columns followed by the same letter are not significantly different

significantly higher inhibitory effects against the tested pathogens when compared to stem extract. Similarly, in comparison with the control, the inhibition is significantly higher in the control than in plant extract (Table 3-6).

DISCUSSION

This study indicated that the leaf and stem extracts of *G. celosioides* possessed the investigated secondary metabolites but in varied compositions. The curing properties of medicinal plants are usually associated with the presence of bioactive compounds and these differ (in type and concentration) from one plant to another, accounting in part for the difference in Pharmaceutical and therapeutic effects of medicinal plants[4]. Therefore, the presence of various secondary metabolites in *G. celosioides* (Table 1 and 2) justified their use locally for the treatment of infectious diseases and also suggests that *G. celosioides* possess medicinal and therapeutic values. The leaf showed the significantly higher composition of all the Phytochemicals assayed except for tannin that was absent in both extracts (Table 1 and 2). The leaf therefore serves as a better source of this Phytochemicals for medicinal purposes than the stem. Saponin has been reported to have a wide range of pharmacological and medicinal activities. Excitedly, it has been indicated to usually have low oral toxicity in humans[17]. Plants containing saponins are used to heal wounds[18,19] because saponins can precipitate and thicken Red Blood Cells (RBCs)[20]. Alkaloids are highly bitter in taste and many are excessively poisonous[21]. The wonderful effect of these alkaloids on man has led to the manufacture of pain-relief drugs, spiritual medications and important inclusions by those who are ignorant of the potentials of the powerful chemical[22]. They are physiologically active in animals, usually even at very low concentrations and many are widely used in medicine

(e.g., cocaine, morphine, atropine, colchicines, quinine and strychnine). Flavonoids possessed antioxidant properties and act as a converter which alters the body's reactions to carcinogens and viruses[20]. They can act as powerful anti-cancerous, anti-inflammatory, anti-microbial and anti-allergic agents[23] and may, therefore be of pharmacological and medicinal values[24]. Phenols are known to inhibit the mutagenicity of cell DNA and neutralize free radicals[25]. They also act as antimicrobial compounds manufactured by some plants to protect them from pathogens[23]. Antibacterial activity indicated that the leaf and stem extracts of *G. celosioides* at various concentrations (100, 150, 200, 250 mg/100 mL) all showed antibacterial activity and the inhibition was extract concentration-dependent. This could be attributed to the presence of bioactive compounds (saponin, alkaloid, flavonoid, tannin, phenol and sterol) in the extracts (Table 3-6). These phytochemicals are known to have pharmacological and medicinal properties. However, the leaf extract showed higher antibacterial activity against the microbes than the stem extract. This according to Hassan *et al.*[26] could be attributed to the presence of higher bioactive compounds in leaf extracts. The inhibition of bacterial strains (*Salmonella typhi, Escherichia coli, P. aeruginosa* and *Staphylococcus aureus*) suggests that the plant possesses a broad spectrum of antibacterial properties which could be used in the treatment of bacterial diseases and food poisoning of which the pathogens are usually implicated.

CONCLUSION

The results of the study offer a scientific basis for the ethnomedicinal uses of extracts of *G. celosioides.* The study revealed that the plant extracts possessed secondary metabolites that have antibacterial activities against some human microbes, which justified their use in ethnomedicine for the treatment of infectious diseases. The leaf and stem extracts of *G. celosioides* both showed antibacterial activities however, the leaf extract showed better inhibition than the stem extract indicating that it is a better antibacterial agent than the stem. The data obtained from the research indicated that the plant possessed antibacterial potentials.

SIGNIFICANCE STATEMENT

This study discovered a new antibacterial agent (*G. celosioides*) from the eastern region that could be used as a natural antibiotic against multidrug-resistant bacteria. The study discovered also that the plant extracts possessed secondary metabolites that have antibacterial activities against some human bacterial pathogens. The study will be beneficial to the drug manufacturers who isolate the secondary metabolites for the development of new drug supplements. It also offers a scientific basis for the optimum utilization and usefulness of *G. celosioides* in ethnomedicine as drugs. This study helps the researchers to discover critical areas of new sources of antibacterial drugs and alternative treatments for multidrug-resistant bacteria which many researchers are not able to explore. Thus, a new theory on plant extracts and their Phytochemicals as antibacterial agents may be arrived at.

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