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Antibacterial activities of *Plectranthus cyaneus* leaf extracts, against five bacterial wound pathogens in Uganda

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Abstract

Background: Although there is no doubt micro-organism plays a great role in the induction and healing progress of a wound, the debate about the extent of involvement and a simple attractive alternative to microbial resistance to imported antibiotics, may linger for a while until a universally accepted answer is outlined.
Objective: To evaluate the antibacterial activities of the leaf extracts of *Plectranthus cyaneus* against five bacterial isolates from wounds in Bushenyi Districts.
Material and methods: Standard Microbiological methods were adopted in analyzing the *in-vitro* activity of the leaf extracts of *Plectranthus cyaneus* against five bacterial isolates from wounds in Bushenyi Districts. This was evaluated based on the zone of inhibition produced using the agar well diffusion method at an extract concentration of 50mg/ml. The tested bacterial species were representative isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia,* and *Salmonella typhi* were involved in this investigation.

Results: Only *Staphylococcus aureus* was inhibited by various fractions of the leaf extracts. The greatest activity shown by a zone of inhibition of 22mm against *Staphylococcus aureus* was observed with the cold water extract, followed by a 16mm zone by the hot water extracts and the least zone of 12mm by the ethanolic extract. The phytochemical compounds were analyzed using the chemical method on the different extracts. The following phytochemicals were found; saponins, tannins, flavonoids, alkaloids, terpenoids, and steroids in all the extracts while only phenol was found in the cold water extract.

Conclusion: This study has thus justified in part the use of *Plectranthus cyaneus* in wounds treatment. Therefore the plant holds a promise as a potential ethnopharmacological source for treating infections caused by *S. aureus* on wounds.

Keywords: *Plectranthus cyaneus*, antibacterial activity, extracts.

Introduction

The use of medicinal plants for the treatment of various infections in traditional communities has been an age-long global practice. It has been estimated that 80% of African population use herbal regimen for treatment and control of diseases (1). *Plectranthus* is a large genus, with more than 350 species from the family of Lamiaceae. It has a rich diversity of ethnobotanical and medicinal uses by natives in Asia, East Hindis, and Africa. Several species are used as a folk medicine for skin irritations, antiseptics, vermicide, and nausea. Many species of the genus possess interesting medicinal properties such as the extract of *P. barbatus* used for the treatment of stomach ache and as a punitive, nausea, gastritis, and intestinal spasms agent in Brazil (2). Though most herbal medicines have confirmed antibacterial activity, they are not yet commercially available until their mode action and dosage are confirmed (3). Compared with most of the currently used commercial antibiotics, natural antibacterial compounds appear to have the benefits of lesser side effects and toxicity as well as higher stability (4). Recently, the emergence of multi, extensive, or total drug resistance due to the extensive drug abuse and/or over-use of antibiotics has become an increasingly serious problem, making the development of alternative antibiotics a very urgent issue.

Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi. We are in a post-antibiotic era, in which common infections causing minor injuries can now kill patients as if there were no remedy. It used to be an apocalyptic fantasy to talk about resistance or treatment failure when the antibiotic was newly discovered but now it is very real in this 21st century (5). In recent years, with increasing technology and medical knowledge, people have become more aware of the quality of medical care so much so that sick people expect more specialized attention from healthcare providers to reduce microbial infection to the barest minimum. . The livestock and aquaculture industry has included some antibiotics in animal feed with the ultimate goal of preventing infectious diseases commonly associated with livestock industries. This practice may lead to bacteria development of resistance to conventional antibiotics. Drug-resistant bacteria spread among different hosts, which in turn may lead to the emergence of multiple drug-resistant bacteria strains (6).

According to the clinical epidemiology analysis reports, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* have become the most common nosocomial infection and drug-

resistant strains, with infection rates as high as 50% (7). These clinical drug-resistant strains have increased the treatment difficulty or even led to the outbreak of severe nosocomial infections, and this lack of effective therapy has led to an urgent search for more diverse and novel antibacterial substances to improve the management of tropical diseases.

However, herbal medicine composition is a complex mixture of different phytochemical compounds acting by different mechanisms, which makes it difficult for pathogens to develop resistance (8). The primary benefit of using herbal drugs is that they are relatively safer and cheaper than the synthetic alternatives (9) or conventional antibiotics of which a good number of them have been found to have different side effects including being: neurotoxic, nephrotoxic, and hypertensive while few others cause severe damage to the liver and bone marrow depression (10). The natural products of medicinal plants may give new antibacterial, antifungal, and antimicrobial agents when organized research prospecting for bioactive compounds is conducted (11-12). This provides a rationalization for studying medicinal plant extracts as a possible source of alternative therapy against infectious diseases especially those due to resistant micro-organisms in a resource-limited setting. *Plectranthus caninus, P. laxiflorus, and P. barbatus* are used in the treatment of teeth and gum disorders. It is also reported that *P. amboinicus and P. barbatus* are used to treat a wide range of diseases such as treatment of digestive system disorders, skin conditions and allergies, infections and fever, genito-urinary conditions, pain, respiratory conditions, and muscular-skeletal conditions (13). *Plectranthus cyaneus* locally known as Kibwankulata in central Uganda, is an indigenous vegetable that has many medicinal uses, especially for the treatment of common illnesses such as cough, convulsions, malaria, stomach upset, headache, skin infection, asthma, and urinary conditions, and wound (13).

The plant extracts especially from the leaves have been reported to possess antioxidant, antibacterial, anti-inflammatory, and fungi-toxic activities (13). The geographical location of the study area from where the leaf was collected may have implications on the outcome of the investigation to be conducted with the leaf extracts, the activities of the leaf extracts against common tropical agents of infection, and the composition of the phytochemical compounds present in the leaf extract. With such abundant natural resources in our environment which contain active compounds against agents of infection, researchers that will search for and validate active compounds from these medicinal plants are welcome. It is based on these premises that local *P. cyaneus* were identified and analyzed for their potent antibacterial properties. This study was therefore aimed at providing preliminary scientific data on the antibacterial activities of *Plectranthus cyaneus* leaf extract as used by the local dwellers in wound treatment. Screening the plant extract for possible phytochemicals likely to be associated with any observed antibacterial activity will be a step forward in the ultimate goal of designing effective disease outbreak interventions with local medicinal plants.

Materials and methods.

Experimental preambles:

Fresh plants of *Plectranthus cyaneus* were collected from farmlands around Ishaka-Bushenyi Municipal Council of Uganda. These were then taken for identification by a botanist at Mbarara University of Science and Technology (MUST). The leaves were washed thoroughly and air-dried to crispiness under the shade for two weeks. The dried materials were reduced to coarse form using a pestle and

mortar and further pulverized using an electrical blender to very fine particles. The powdered extract obtained was stored separately in polyethylene bags awaiting analysis. All glassware used was washed with detergent and bleach, rinsed with distilled water, air-dried, and sterilized on a hot air oven at 160^0C for 1hour. Distilled water and all prepared media (Muller Hinton Agar) were sterilized in the autoclave at 121^0C at 15 psi for 15 minutes. The pure clinical isolates of the following bacterial pathogens were obtained from the microbial bank of pure clinical isolates, Microbiology laboratory unit, Kampala International University Teaching Hospital, Ishaka, Uganda. The microorganisms obtained from KIUTH included; *Staphylococcus aureus Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Salmonella typhi*. The cultures were maintained at 4°C on nutrient agar slants in a refrigerator.

Extraction of active agents from plant Material

Extraction was done in three different phases; Ethanolic extract, cold water extract, and hot water extracts. For **cold water extraction,** two hundred grams (200g) of the powdered *Plectranthus cyaneus* leaf was weighed and soaked in 1000ml of sterile distilled cold water, agitated manually until all the powder were fully dissolved in the water, and allowed to extract for 48hours or stand undisturbed for two days. Then each extract was centrifuged using 800-1 Centrifuge Machine at 5000 rpm for 10 minutes, filtered using a Whatmann No 1 filter paper. The filtrate was evaporated in a water bath at 50^0C to dryness. The extract was stored at 4ºC in a refrigerator until needed. For **hot water extraction**, two hundred grams (200g) of the powdered plant material was soaked in 1000ml of hot water (boiled for 30minutes) and allowed to extract for 48 hours or stand undisturbed for two days. The extract was centrifuged using an 800-1 Centrifuge Machine at 5000 rpm for 10 minutes, filtered, and evaporated to dryness using a water bath at 50ºC. The extract was then stored at 4ºC until needed. For **ethanolic extraction,** two hundred grams (200g) of the powdered plant material was soaked in 1000ml of absolute ethanol for 48h at 25ºC with occasional stirring. The extract was centrifuged using an 800-1 Centrifuge Machine at 5000 rpm for 10 minutes, filtered, and then evaporated to dryness in a water bath at 50ºC. The extracts were tested for sterility by inoculating 1ml of cold water, hot water, and ethanolic extracts on sterile Mueller Hinton Agar (MHA) and incubated at 37ºC for 24 hours. If no growth was seen in the media containing the inoculated extracts after incubation, this indicated that the extract was sterile. The sterile extract and culture media were then used to assess for the antibacterial activity of the extract.

Antimicrobial susceptibility test

The agar well diffusion method of (14-16) was adopted for this experiment. Briefly, the culture media,

(Mueller Hinton broth) was prepared as specified by the manufacturer (Mast diagnostics India). It was autoclaved, poured ascetically into sterile Petri dishes, and allowed for about two hours to gel. The standardized bacterial cell suspension (10^6cfu/ml) was poured evenly onto each gelled agar plate and dried for 5 minutes in the incubator at 37^0C. The plant extracts were then reconstituted using distilled sterile water to obtain the working concentrations of 50mg/ml. Two hundred microliters (200µl) of the extracts were then inoculated into the agar wells (6 mm diameters). The negative control was 200µl of sterile distilled water, while the positive control was 5µg/ml of ciprofloxacin. The plates were allowed to stand for 30minutes on the workbench for pre-diffusion of the extracts before the plates were incubated at 37°C for 24 h. The experiment was carried out in triplicate to ensure that the mean results obtained are

more reliable and reproducible. The antibacterial activity of the extracts was determined by measuring and recording the inhibition zones and later comparing the results with the known standard.

Determination of the minimum inhibitory concentration (MIC).

Plectranthus cyaneus leaf extracts that demonstrated significant antibacterial activity by the agar well diffusion method were subjected to MIC assay using micro-broth dilutions techniques (17) as modified by the published guidelines of the Clinical Laboratory Standard Institute (18). Briefly: one ml of 24 h culture of test organisms adjusted to 1.5 McFarland turbidity standard (10^7CFU/ml) were added to the prepared test tubes containing one ml Mueller-Hinton broth and the different extract concentration of 200, 400, 600, 800, 1000, 1200, 1400, µg/ml obtained by serial dilution and incubated at 37°C for 24h.The dilution with the lowest concentration of plant extractable to cause visible/doubtful turbidity (where turbidity indicates growth) after 18-24hours incubation, was considered the minimum inhibitory concentration (MIC) of the extract.

Phytochemical screening of the extracts

The different phytochemical compounds in the leaf extracts of *Plectranthus cyaneus* were analyzed according to standard procedures as described by (19-21). They were evaluated for the presence of the following; steroids, saponins, alkaloids, flavonoids, terpenoids, phenols, and tannins as follow;

To test for alkaloids: 0.5g of the sample was accurately weighed and defatted with 5% ethyl ether for 15mins. The defatted sample was then extracted for 20mins with 5.0ml of aqueous HCl on a steam bath. The resulting mixture was centrifuged for 10mins at 3000rpm to remove the filtrate (Supernatant). 1.0ml of the filtrate was treated with a few drops of Mayer‘s reagent. Turbidity or precipitation with this reagent was evidence of the presence of **alkaloids.** The ability of saponins to produce frothing in an aqueous solution was used as a screening test for the sample. 0.5g of dried extract was shaken with water in a test tube. Frothing which persists in warming shows evidence for the presence of **saponins.** Five (5.0g) grams of dried leaf extract were stirred with 10.0ml of distilled water, filtered and ferric chloride reagent added. A blue-black precipitate showed evidence for the presence of **tannins.** To 1.0ml of the extract contained in a test tube, 1.0ml of 10% lead acetate was added. The formation of a yellow precipitate indicated the presence of **flavonoids.** To 0.5g of the dried extract extracted using 2.5ml of chloroform in a test tube, 1ml of concentrated sulphuric acid was added to form a lower layer. A reddish-brown interface indicated the presence of steroids. Exactly 0.5ml 0f the chloroform extract of the dried extracts was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10minutes on a water bath. A grey color indicated the presence of **terpenoids**. To 1ml of extract, 2ml of distilled water was added and few drops of ferric chloride. The red color slightly changed to pale color indicates the presence of **phenols.**

Results

The result of the antibacterial activity of *Plectranthus cyaneus* against the tested bacterial pathogens showed that only *Staphylococcus aureus* was inhibited by the various fractions of the extract while the other organisms were not inhibited (Table 1). Of the different extracts that inhibited *Staphylococcus aureus,* cold water extract gave the highest zone of inhibition of 22mm followed by hot water extract with 16mm and the ethanolic extract with 12mm. All the isolates were not inhibited by water (negative control) while ciprofloxacin (positive control) inhibited

Salmonella typhi 26mm, *Klebsiella pneumonia* 24mm and *Staphylococcus aureus* 8mm. *Pseudomonas aeruginosa* and *Escherichia coli* were not inhibited by ciprofloxacin (Table 1)

Table 1: Mean diameter of zones of inhibition of the test bacterial species to the leaf extracts and controls.

Test isolate	Cold water 100 mg/ml	Hot water 100 mg/ml	Ethanolic 100 mg/ml	Cipro 5µg/ml (+ve control)	Water (-ve control)
P. aeruginо	Nil	Nil	Nil	Nil	Nil
E coli	Nil	Nil	Nil	Nil	Nil
S typhi	Nil	Nil	Nil	26mm	Nil
K pneumo	Nil	Nil	Nil	24mm	Nil
S aureus	22mm $\pm$0.02	16mm $\pm$0.01	12mm $\pm$0.2	8mm	Nil

Key: Results are means of three replica diameter zones of inhibition values (mm) $\pm$ *standardeviations (SD)*, Nil = no inhibition. *P. aerugino= Pseudomonas aeruginosa*

Results of the minimum inhibitory concentration showed that the MIC of cold water extract was 600 while that of hot water and ethanol was 800 respectively (Table 2). The result of MIC of cold water, hot water, and ethanol extracts against Staphylococcus strains were 600, 800, and 800 respectively. Results of the phytochemical analysis showed that all the tested phytochemicals were detected in the cold extract while both the hot water extract and the ethanolic extracts had all the other phytochemicals except phenol (Table 2).

Table 2: Qualitative analysis of the phytochemicals in the leaf extracts of *Plectranthus cyaneus*

PYTOCHEMICAL	COLD EXTRACT	HOT EXTRACT	ETHANOLIC EXTRACT
Saponins	+++	++	+++
Tannins	+++	++	++
Flavonoids	+	+	+
Alkaloids	+	+	+
Steroids	+	+	++
Terpenoids	+	+	+++
Phenols	+	-	-

Key: Not detected (-); detected little (+), detected moderate (++), detected intense (+++),

Discussion

Plectranthus cyaneus leaf extracts were found to produce significant diameters of inhibition against *Staphylococcus aureus* but showed no effect on the other tested microbes which included; *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, and Klebsiella pneumoniae*. Ciprofloxacin, which was the standard antibiotic used as positive control did not inhibit *E coli, and P aeruginosa*, while others such as *Salmonella typhi, Klebsiella pneumonia, and Staphylococcus aureus* were inhibited by the antibiotic (Table 1). This shows that Ciprofloxacin may not be the best control antibacterial agent for Pseudomonas species and E coli. We could have opted for another control antibiotic had it been the leaf extracts showed activity to

Pseudomonas and Escherichia coli while standard did not as shown in (Table 10. However, negative results obtained with both leaf extracts and control confirms the universal acceptability and specificity of ciprofloxacin for use as a control agent in this type of experiment.

Plectrathus cyaneus extracts inhibited *Staphylococcus aureus* test isolate. This finding corresponds to other works that show that Plectranthus cyaneus possess some antimicrobial activity (13). However, the different plant extracts showed no effect on the other test microbes (Table 1). The failure of all the extracts to exert antibacterial effect invitro on: *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Salmonella typhi* may not be enough evidence to conclude that they have no antibacterial activity because the potency of plant extracts depends on the extraction solvent and method used to obtain the extract, the age of the plant when harvested and the amount of the active constituent to be extracted from the plant, which can vary in quality and quantity from season to season (22). Thus, other future studies should use other extraction solvents such as methanol, in addition to water and ethanol tested in this investigation just to expand the research prospecting horizon in-search for active compounds from natural products before concluding that it has or has no activity against the tested organisms. Again, a wide spectrum of herbal parts such as a leaf, bark, root, and stems of young and older plants should be tested to enrich the herbal treatment database available for healthcare providers in resource-limited developing countries.

It may be logical to say that the extract of *Plectranthus cyaneus* is a candidate narrow-spectrum antibacterial agent which can be purified and explored for the full potential of its activities against common disease pathogens. This is because the extracts exhibited activity against gram-positive organisms and not gram-negative organisms. The mechanism of microbial resistance to gram-positive organisms has already been extensively studied and published in global and African literature. However, the inactivity of the extracts to the gram-negative organisms may be due to their cell wall which is a multilayered structure and complex (23) thus protecting the gram-negative organism from the action of the extracts and hence making them resistant to the leaf extracts.

The cold extract exhibited the largest diameter (22mm) followed by hot (16mm) and ethanolic (12mm). This result further supports the claim by natives about the effectiveness of the herb since the extraction method mostly used by them was cold water which produced the largest zone of inhibition (Table 1). The relatively high minimum inhibitory concentration value of *Plectranthus cyaneuos* against *Staphylococcus aureus* was indicative of low activity. The observed MIC of leaf extracts of Plectranthus cyaneuos although showed low activity was still within the acceptable range of 100–1000 μg/ml, to enable us to decide that the leaf extract had activity against *Staph aureus* and possibly other wound pathogens. Based on this observation, it can be inferred that the use of such leaf by the Ishaka-Bushenyi municipality dwellers is hereby validated its use for wound treatment confirmed for mono-microbial infections but may be less potent in polymicrobial infections involving gram-positive and negative organism. The reported minimum inhibitory concentration (MIC) of any plant extract higher than the range of 100–1000 μg/ml may be considered not active as previously suggested in the literature (24-25).

In Table 2**,** the phytochemical components identified in the leaf extracts, included; Flavonoids, tannins, saponins, phenols, steroids, alkaloids, and terpenoids, hence the wound healing effect of the leaf of Plectranthus cyaneuos and other of its observed biological effect may be due to the presence of the identified phytochemicals. Flavanoids are

phenolics structures containing one carbonyl group complexes with extracellular and soluble protein and with bacterial cell walls, thus exhibits antibacterial activity through these complexes (26). Tannins on the other hand have been found to form irreversible complexes with proline-rich proteins (27) resulting in the inhibition of the cell protein synthesis. Plants that have tannins as their main component are astringent and are used for treating intestinal disorders such as diarrhea and dysentery.

Terpenoids have been demonstrated to be active against bacteria, fungi, viruses, and protozoa (28), which has enabled food scientists to use terpenoids present in essential oils of plants to control *Listeria monocytogenes* (29). The mechanism of action of terpenes is by lipophilic membrane disruption. Indeed [30], found that increasing the hydrophilicity of kaurene diterpenoids by the addition of a methyl group drastically reduces their antimicrobial activity.

Conclusion

This study had demonstrated that the leaf extracts of *Plectranthus cyaneus* possess antibacterial activity against *Staphylococcus aureus* thus justifying its use by the natives as a traditional herbal medicine in wound treatment. Also, the other biological effects of the leaf of the plant observed by the natives might be due to the phytochemical components present.

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