https://rijournals.com/scientific-and-experimental-sciences/

RESEARCH INVENTION JOURNAL OF SCIENTIFIC AND EXPERIMENTAL SCIENCES 3(3):49-56, 2024

ISSN: 1597-2917

Evaluation of Antibacterial and Phytochemical Properties of Leaf Extract of *Plectranthus cyaneus* against some five Human Pathogens

Bbira Mike

Faculty of Pharmacy, Kampala International University, Western Campus Uganda.

ABSTRACT

The in-vitro antibacterial activity of leaf extracts from *Plectranthus cyaneus* was examined to determine their antimicrobial effects on five human clinical isolates. The evaluation was conducted by measuring the diameter of the zone of inhibition created using the agar well diffusion technique, with an extract concentration of 100 mg/mL. The antibiotic ciprofloxacin was utilized as a positive reference standard at a concentration. The bacterial strains that underwent testing were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*. The results indicated that the leaf extracts inhibited exclusively *Staphylococcus aureus*. The antibacterial activities of the various leaf extracts did not show significant differences. However, the cold extract exhibited the highest activity (22 mm), followed by the crude extract (18 mm) and hot extracts (16 mm), while the ethanolic extract had the lowest activity (12 mm). The phytochemical substances detected by chemical examination of several extracts included saponins, tannins, flavonoids, alkaloids, phenols, terpenoids, and steroids. The plant shows potential as an ethanol-pharmacological resource for developing drugs to treat infectious disorders caused by *Staphylococcus aureus*. Overall, this research has provided evidence supporting the use of *Plectranthus cyaneus* by the indigenous population.

Keywords: Antibacterial activity, Leaf extracts, *Plectranthus cyaneus*, Phytochemical, Clinical isolates.

INTRODUCTION

The use of medicinal plants for the treatment of many illnesses in traditional cultures has been a longstanding worldwide practice. According to a study by Hugo and Russell [1], almost 80% of the African population relies on herbal remedies for the treatment and management of ailments. Chinese herbal remedies have played significant roles in the treatment of several ailments throughout history. Nevertheless, the majority of herbal medicines have not been formulated into commercial medications since their doses and mechanisms of action have not been elucidated [2]. According to Marasini et al., [3], natural antibacterial compounds offer many advantages over most commercially used antibiotics. These advantages include reduced side effects and toxicity, as well as increased stability. Drug resistance has become a pressing issue owing to the widespread misuse and overuse of antibiotics, necessitating the urgent development of alternative antibiotics [4]. Antimicrobial resistance poses a significant challenge to the successful prevention and treatment of a growing number of illnesses caused by bacteria, parasites, viruses, and fungi. The prospect of a post-antibacterial world where ordinary illnesses and mild injuries might be fatal is not a far-fetched doomsday scenario but rather a genuine concern for the 21st century [5]. The livestock and aquaculture industries have used antibiotics in animal feed excessively as a preventive measure against infectious illnesses, resulting in the development of antibiotic resistance in microorganisms. The dissemination of drug-resistant bacteria occurs across various hosts, facilitating the transmission of drug-resistant genes among different drug-resistant bacteria. Consequently, this process gives rise to the formation of many drug-resistant bacteria [6]. Based on clinical epidemiology study reports, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have emerged as the predominant drug-resistant strains causing nosocomial infections, with infection rates reaching up to 50% [7]. The presence of these drug-resistant strains in clinical settings has significantly complicated treatment and even caused serious nosocomial infections. The absence of effective therapy has created an urgent need to discover new antibacterial agents. Herbal medication composition is an intricate blend of several phytochemicals that operate via distinct pathways, making it challenging for infections to acquire resistance [8]. Plants are a valuable source of antibacterial agents that may effectively combat infectious diseases, such as bacteria [9,10,11,12]. One major advantage of using herbal drugs is that they are more cost-effective than synthetic

alternatives or conventional antibiotics. Many conventional antibiotics have been found to have neurotoxic, nephrotoxic, and hypertensive side-effects, while a few others can cause severe damage to the liver and bone marrow depression. Medicinal plants provide a potential reservoir of antibacterial, antifungal, and antimicrobial compounds. Jahan et al. [14], and Runyoro et al., [15], offered a justification for investigating medicinal plant extracts as a potential alternative treatment for infections. Plectranthus is a vast genus, with over 350 species belonging to the Lamiaceae family. It exhibits a broad range of ethnobotanical and therapeutic applications that are indigenous to Asia and East India and extensively grown in Africa. Various species of them are used as traditional remedies for skin irritations, antibacterial properties, deworming, and alleviating nausea [16]. Several species within the genus have notable therapeutic characteristics. *Plectranthus scaninus*, *Plectranthus laxiflorus*, and *Plectranthus barbatus* are used for the management of dental and periodontal ailments. P. *amboinicus* and P. *barbatus* have been documented as being utilized for the treatment of various ailments, including digestive system disorders, skin conditions, allergies, infections, fever, genito-urinary conditions, pain, respiratory conditions, and muscular-skeletal conditions [17]. *Plectranthus cyaneus*, also known as Kibwankulata in central Uganda, is a native vegetable with numerous medicinal properties. The plant extracts, particularly the volatile essential oils derived from the leaves, have been found to exhibit antioxidant, antibacterial, antimicrobial, anti-inflammatory, and fungi toxic properties [17]. However, the activity and composition of essential oils may vary depending on the geographical region and variety. Hence, it is crucial to examine the indigenous P. *cyaneus* to evaluate its possible antibacterial properties in the local area. Antibacterial resistance is a growing concern as it undermines the ability to prevent and treat a wide range of bacterial infections. This poses a significant threat to the treatment of common medical conditions, highlighting the urgent need for new drug discoveries to combat resistant microorganisms. There has been less research undertaken on the efficacy of herbal remedies, despite claims made by consumers. This has impeded the advancement in the exploration of novel, efficacious, and safe medications, therefore leading to the emergence of resistance towards the current ones. Leaf extracts of *Plectranthus cyaneus* have historically been used against some bacterial infections. The discovery of the antibacterial characteristics of this plant will help to counter the growing danger and enhance general health. However, it also raises concerns over the appropriate dosage, effectiveness, and safety of its usage. The study was designed to gather scientific evidence about the antibacterial properties of the leaf extract from *Plectranthus cyaneus*, as reported by the local population. Additionally, the study attempts to analyze the plant extract for potential phytochemicals that may be responsible for any observed antibacterial activity.

METHODOLOGY

Study Design

The research design included a brief experimental control. A subset of pathogens obtained from the KIU-TH microbiology lab was used as a model to forecast the antibacterial characteristics of *Plectranthus cyaneus* leaf extract. The research also included a positive control consisting of a conventional antibiotic, ciprofloxacin, and a negative control consisting of sterilized distilled water.

Area of Study

The study was conducted in the microbiology lab, KIU-TH lab, and the School of Pharmacy pharmaceutics labs at KIU-WC.

The study organism

The investigation was done on five clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeurignosa*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumonia*. The pure culture of each pathogen was standardized to a known estimated number of microorganisms using the McFarland turbidity standard technique [18].

Determining the Size of the Sample

The sample size consisted of roughly 108 pathogens, as determined by the McFarland (0.5) method [18].

Procedures for collecting data

Plectranthus cyaneus plants were freshly gathered from agricultural areas around Ishaka Municipal and Bushenyi communities in Uganda. Leaves specimens were subsequently authenticated by a Botanist from the Mbarara University of Science and Technology (MUST) The leaves were meticulously cleansed and left to desiccate in the shade for two weeks. The desiccated materials were further crushed into a coarse consistency using a pestle and mortar, and further pulverized or ground into very tiny particles using an electric blender. The powdered extract was thereafter kept individually in plastic bags, pending examination.

Material Sterilization

All glassware used in this study underwent a thorough cleaning process including the use of detergent (jik), followed by rinsing with distilled water. Subsequently, the glassware was left to air dry and then subjected to sterilization in a hot air oven at a temperature of 121°C for 3 hours. Distilled water and prepared medium (Muller Hinton Agar) were sterilized in the autoclave at a temperature of 121°C for 15 minutes. Before being subjected to a Bunsen burner flame, cork borers and glass rods were sanitized by immersing them in 70% alcohol and then

scorching them. In addition, the workbench was sterilized with a 75% alcohol solution both before and after every experiment.

Origin of Test Microorganisms

The clinical isolates of the following pathogens were collected from the KIU-TH Lab. The bacteria that were included in the study were *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. The cultures were stored at a temperature of 4°C on a nutrient agar medium in a refrigerator. Standardization of test organisms is the process of establishing uniformity and consistency in the organisms used for testing purposes. Before the antimicrobial sensitivity test, the pure culture of each pathogen was standardized. A 0.2 ml sample of each organism's overnight culture was added to sterile distilled water (20 ml) and then compared to the Macfarland turbidity standard.

Plant material extraction.

The extraction process was conducted in three distinct phases: ethanolic extraction, and aqueous extraction using both cold and hot water.

The process of extracting substances using cold water.

A quantity of 200 grams of the powdered sample was measured and immersed in 500 millilitres of sterile distilled water. The mixture was stirred by hand and left to extract for 48 hours. Subsequently, each extract underwent centrifugation using an 800-1 centrifuge machine at a speed of 1400 revolutions per minute for 15 minutes. The resulting mixture was then filtered using a Whatmann No. 1 filter paper. The liquid that passed through the filter was removed by heating it in a water bath at a temperature of 50 °C until it completely dried out. The % yield was determined, and the extract was kept under sterile conditions at a temperature of 4 °C in a refrigerator until it was required. For the hot water extraction, 200 grams of the powdered plant materials was measured and immersed in 500 millilitres of hot water that had been cooked for 30 minutes. The mixture was placed in a conical flask and left for 48 hours. The solution was subjected to centrifugation using an 800-1 centrifuge machine at a speed of 1400 rpm for 15 minutes. The resulting mixture was then filtered using Whatman No. 1 filter paper and then evaporated to dryness using a water bath set at a temperature of 50 °C. The yields were computed and documented. The extract was thereafter kept under sterile conditions at a temperature of 4 °C until it was required.

Ethanol Extraction

To perform ethanol extraction, a plant sample weighing 200 g was immersed in 500 ml of 100% ethanol for 48 hours at room temperature. The mixture was stirred intermittently throughout this period. The crude extract was subjected to centrifugation using an 800-1 centrifuge machine at a speed of 1400 revolutions per minute for 15 minutes. The resulting mixture was then filtered and then evaporated to dryness in a water bath maintained at a temperature of 50 °C. The yield was determined using the following formula: % yield=actual yield / theoretical yield. The samples were gathered and kept in a sterile manner in the refrigerator at a temperature of 4 °C until they were needed for future usage.

Sterility refers to the state of being free from living microorganisms or germs. Plant extract testing. The aforementioned extracts, including ethanolic and aqueous (cold and hot) extracts, were examined for their potential to promote or inhibit the development of pollutants. This was accomplished by introducing 1 ml of each sample onto a sterile Mueller-Hinton agar plate and allowing it to incubate at a temperature of 37 °C for 24 hours. The extracts exhibited growth upon incubation, indicating their lack of sterility. The extracts were incubated again, and upon doing sterility tests, it was determined that they were free from any living organisms. Subsequently, they were used to evaluate their efficacy against microorganisms.

Assessment of the susceptibility of microorganisms to antimicrobial agents.

The agar-well diffusion technique developed by Perex et al. [19], and further studied by Alade and Irobi [20] is being referred to here. Another study by Nweze and Onyishi, [21], was adopted. The prescribed protocol was that Mueller Hinton broth was produced according to the manufacturer's instructions, then sterilized using an autoclave. It was then carefully placed onto sterile Petri plates and left to solidify. The bacterial cell suspension, with a concentration of 10^6 colony-forming units per millilitre (cfu/ml), was equally distributed over each agar plate and dried for 5 minutes in a 37 °C oven. The plant extract was diluted with distilled, sterilized water to achieve a working concentration of 1 mg/mL. Next, 200µ1 of the extract was introduced into pre-made wells (6 mm in diameter) that were previously labelled and created using a sterile cork borer on each plate. The negative control consisted of 200 µ1 of sterile distilled water, while the positive control included 5µg/ml of ciprofloxacin. The plates were left undisturbed for 30 minutes on the workstation to enable the extracts to diffuse before the organism started growing. The plates were thereafter placed in an incubator set at a temperature of 37 °C for 24 hours. The antibacterial efficacy of the extract was assessed by measuring the average diameter of the zones of inhibition caused by the extracts against the test organisms, after the incubation time. Measurements were documented in millimetres (1m11) using a clear ruler.

Determination of the MIC

Cultures that showed notable antibacterial activity using the agar well diffusion method were further tested using the macro broth dilution method todetermine the minimum inhibitory concentration (MIC). This methodology follows the guidelines set by the Clinical and Laboratory Standard Institute (CLSI, 2006) after reconstitution. A volume of 1 ml from a 24-hour culture of test organisms, adjusted to the McFarland turbidity standard of 107 CFU/ml, was introduced into test tubes containing 1 ml of Muellar-Hinton broth. The test tubes also contained varying concentrations of plant extract obtained through serial dilution (400, 600, 800, and 1000 µg/ml). The mixture was then incubated in physiological saline at a temperature of 37 °C for 24 hours. The minimum inhibitory concentration (MIC) was determined as the concentration with the lowest dilution that showed no detectable bacterial growth during incubation.

Determination of MBC

This was likewise conducted according to the findings of the aforementioned experiment (MIC). The Minimum Bactericidal Concentration (MBC) is the lowest concentration of a drug necessary to eliminate 99.9% of the cells in a given inoculum. The minimum bactericidal concentration (MBC) was calculated by quantifying subcultures obtained from clear tubes or wells of a dilution series of minimum inhibitory concentration (MIC). Tubes that exhibit no growth are then inoculated into the agar medium and placed in an incubator. The plate exhibiting no growth and the lowest concentration of MIC represents the minimal bactericidal concentration [22].

Phytochemical analysis was conducted on the extract

The leaf extract of *Plectranthus cyaneus* was subjected to analysis of its various secondary metabolites using established protocols outlined by Sofowora [23], Trease and Evans [24], and Harbone [25]. The plant extract underwent analysis to determine the presence of the following phytochemical secondary metabolites: steroids, saponins, alkaloids, flavonoids, terpenoids, steroids, phenols, and tannins, as outlined below:

Evaluation criteria

The sterility of the equipment was verified by the lack of any growth seen when swabs taken from the equipment were cultured. The MIC (minimum inhibitory concentration) was determined by measuring the diameter of the zones of inhibition using a clear ruler and recording the measurements in millimetres. MBC refers to the minimum concentration of the minimum inhibitory concentration (MIC) tube that will exhibit no growth on plates after overnight incubation at 37°C. Screening was warranted based on the presence of positive test results obtained from doing the specific test for each secondary metabolite. The lack of growth seen after overnight incubation suggested the absence of any plant extracts.

Methods for Analyzing Data

The experiment was replicated three times and the means were calculated from the raw data. The antibacterial activity of the medicinal extract was assessed by comparing it to that of a reference antibiotic using the Activity Index [26]. The activity index is calculated by dividing the zone of inhibition of extracts by the zone of inhibition of antibiotics.

RESULTS

Moisture content

Table 1: Percentage yield of all the leaf extracts

Type of extract	Percentage yield *(%)*
Cold	7.45
Hot	6.95
Ethanol	4.25

The concentration of ethanol

The sterility test findings for leaf extracts are 4.3. The sterile broth exhibited no microbial growth after inoculation and incubation for 24 hours at 37 °C with various plant extracts, showing its sterility and suitability for use.

Antimicrobial action test for the extracts.

The antimicrobial activity tests of *Plectranthus cyaneus* revealed that some of the test isolates were not responsive to the ethanolic and aqueous extracts at a concentration of 100 mg/mL, as utilized in the experiment. Several test isolates exhibited resistance to the conventional antibiotic, Ciprofloxacin (5 micrograms/ml), except *Salmonella typhi*, which displayed a significant zone of inhibition of roughly 26 mm in diameter. *Staphylococcus aureus*, on the other hand, showed very little inhibition.

Table 2: Results of antimicrobial sensitivity against test organisms

Testisolate P aeuroginosa	Cold l00mg/ml NI	Hot l00mg/ml NI	Ethanoliclo 0mg/ml NI	Crude extract NI	Ciprofloxacin 5µg/ml NI	Water NI
E coli	NI	NI	NI	NI	NI	**NI**
Styphi	NI	NI	NI	NI	26	**NI**
K pneumomea	NI	NI	NI	NI	24	**NI**
S aureus	**22mm**	**16mm**	**.121m11**	**181nm**	**81IB11·**	**NI**

NI means No Inhibition.

The concentration of ethanol is 4.25 units.

The sterility test findings for leaf extracts

The sterile broth exhibited no microbial growth after inoculation and incubation for 24 hours at 37 °C with various plant extracts, showing its sterility and suitability for use.

The test findings of the extracts' antimicrobial efficacy

The antimicrobial activity tests of *Plectranthus cyaneus* revealed that some of the test isolates were not responsive to the ethanolic and aqueous extracts at a concentration of 100 mg/mL, as utilized in the experiment. Several test isolates showed resistance to the conventional antibiotic, Ciprofloxacin 5 microgram/mL, except for *Salmonella typhi*, which exhibited a significant zone of inhibition of roughly 26 mm in diameter. *Staphylococcus aureus* also showed slight inhibition.

Table 3: Results of Minimum Inhibitory Concentration of the hot and cold leaf extracts of *Plectranthus cyaueouos* against *Staphylococcus aureus*.

Test Isolate	MIC of the leaf extracts	
	Hot Extract	IICold extract
S.aureus	4000 µg/mL	4000 ug/ml

The plant is considered weak when the minimum inhibitory concentration (MIC) of the leaf extract falls outside the range of 100–1000 µg/mL, as stated by Cowan (1999).

The activity index is calculated by dividing the zone of inhibition of extracts by the zone of inhibition of antibiotics.

Table 4: Displays the activity index (AI) of extracts from medicinal plants and antibiotic discs against *Staphylococcus aureus.*

Extracts of *PlectrautImscaucus*	·Active Index
Cold	2.75
Hot	2
Ethanolic	1.5
Crude	2.25

The plant is considered weak when the minimum inhibitory concentration (MIC) of the leaf extract falls outside the range of 100-1000 µg mL-1, as stated by Cowan (1999).

Table 4 presents the activity index (AI) of extracts from medicinal plants and antibiotic discs against Staphylococcus aureus. The activity index is calculated by dividing the zone of inhibition of extracts by the zone of inhibition of antibiotics.

Table 5: Results of the phytochemical analysis

	Cold extract	Hot extract	Ethanolic extract
Saponins	+++	++	+++
Tannins	+++	++	++
Phenols	+	-	-
Flavonoids	+	+	+
Alkaloids	+	+	+
Steroids	+	+	
Terpenoids	+	+	+++
Phlobatanins	+	+	+

Presence of phytochemicals; Intense (+++), Moderate (++), little (+), Absent (-)

Table 6: Results of the phytochemical analysis

	Cold extract	Hot extract	Ethanolic extract
Saponins	+++	++	+++
Tannins Phenols	+++	++	++
	+	-	-
Flavonoids	+	+	+
Alkaloids	+	+	+
Steroids	+	+	
Terpenoids	+	+	+++
Phlobatanins	+	+	+

Presence of phytochemicals; Intense (+++), Moderate (++), little (+), Absent (-)

DISCUSSION

According to data from Table 1, the cold and hot extracts had the greatest percentage yield (7.45% and 6.95%, respectively) of the plant extract, compared to the ethanolic extract, which had a yield of 4.25%. This might be attributed to the greater solubility of the plant's phytochemicals and other constituents in water compared to ethanol. The observed large-diameter zone of inhibition against *Staphylococcus aureus* in the cold and hot extracts (22 mm and 16 mm) may have been influenced by this factor as compared to the ethanolic extract (12 mm). The majority of the compounds are water-soluble, which enhances the quantity of substances recovered from the plants. The extracts of *Plectrathus cyaneus* have shown inhibitory effects on *Staphylococcus aureus*, surpassing all other test organisms. The leaf extract had no impact on other test microorganisms, such as *S. typhi*, *P. euroginosa*, *K. pneumonia*, and E. *coli*. These specific microorganisms were chosen because they are often associated with respiratory and wound diseases, for which the herb is traditionally used by the local population. The *Plectrathus cyaneus* extracts in Table 2 exhibited considerable inhibition against the *Staphylococcus aureus* test isolate. The significant increase in the width of the zones of inhibition seen in the pure extract (18 mm) against *S. aureus* provides strong evidence of the plant's efficiency, particularly because S. *aureus* is the primary causal organism associated with the diseases for which the plant is utilized. The results align with those presented in the assessment of ethanol-botanical applications of plectranthus species conducted by Lukhoba in 2006 [17]. The effectiveness of extracts is influenced by factors such as the solvent and extraction method employed, the maturity of the plant at the time of harvest, and the concentration of the active ingredient, which can vary in both quality and quantity across different seasons [27]. The cold extract had the greatest diameter (22 mm), followed by the pure extract (18 mm), the hot extract (16 mm), and the ethanolic extract (12 mm). These findings provide more

evidence to support the assertion made by indigenous people about the efficacy of the plant. This is because the extraction procedures predominantly used by them yielded the largest widths of zones of inhibition. The minimum inhibitory concentration (MIC) of *Plectranthus cyaneuos* against *Staphylococcus aureus*, as shown in Table 3, was very high. This suggests that the activity of *Plectranthus cyaneuos* is low at low doses. The leaf extract's stated minimum inhibitory concentration (MIC) being outside the range of 100–1000 µg mL1 indicates that the plant is less potent compared to the standards mentioned in the studies by Iqbal et al. [28]. In Table 4, the utilization of aqueous and alcohol extracts of medicinal plants with the conventional antibiotic Ciprofloxacin was determined using the Active index. The presence of an activity index (AI) value greater than 1 indicates a significant contribution of herbal extracts, whereas a value below zero indicates a substantial action of antibiotics against the tested pathogens. The significance of the findings obtained from the plant extracts surpassed that of the conventional antibiotic when more AI values were assessed. The observed considerable increase in the active index value in this instance may be due to the use of extremely high quantities of plant extracts during the determination of MIC, in contrast to the low concentration of Ciprofloxacin utilized as a positive control. The locals' use of the plant for wound healing may be attributed to the presence of phytochemicals, as shown in Table 5. The combination of these phytochemicals effectively inhibits the development of *Staphylococcus aureus*, a primary culprit behind skin and soft tissue infections. Flavonoids are phenolic compounds that have a structure including one carbonyl group. They form complexes with extracellular and soluble proteins, as well as with bacterial cell walls. As a result, they demonstrate antibacterial action via these complexes. [29]. Tannins, on the other hand, have been discovered to form irreversible complexes with proline-rich proteins [30], leading to the suppression of cellular protein synthesis. Plants containing tannins as their primary constituent have astringent properties and are used in the treatment of intestinal ailments such as diarrhoea and dysentery. This study suggests that these plants might be used as a therapy for diarrhoea and dysentery due to their antibacterial properties [31]. Terpenoids have shown efficacy against bacteria, fungi, viruses, and protozoa This has allowed food scientists to use the terpenoids found in the essential oils of plants to manage *Listeria monocytogenes* [32,33] Terpenes exert their effects by disrupting lipophilic membranes. It has been shown that enhancing the hydrophilicity of kaurene diterpenoids by adding a methyl group significantly decreases their ability to fight against microorganisms [34].

CONCLUSION

The research has shown that the leaf extract of *Plectranthus cyaneus* possesses antibacterial properties against *Staphylococcus aureus*, which supports its traditional usage as an herbal medication for treating wounds. The observed biological impact of the plant by the native might perhaps be attributed to the phytochemical constituents found in the plant.

REFERENCES

1. Hugo, W.B., and A.D. Russell, 2003. Pharmaceutical Microbiology, 6th Edn., Blackwell Science Publishers
2. Xu, X.M.; Fan, Y.F.; Feng, W.Y.; Mi, Z.H.; Weng, X.B. Antibiotic resistance determinants of a group of multidrug-resistant *Acinetobacter baumannii* in China. *The Journal of Antibiotics*, *2014;67*, 39–44.
3. Marasini, B.P.; Baral, P.; Aryal, P.; Ghimire, K.R.; Neupane, S.; Dahal, N.; Singh, A.; Ghimire, L.; Shrestha, K., 2015. Evaluation of the antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. *Biomedical. Research. International.*, 2015;2015:265425; doi:10.l 155/2015/265425.
4. Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. *Pharmaceuticals* (Basel), 2023;16(11):1615. doi: 10.3390/ph16111615.
5. WHO, Antimicrobial Resistance, Global Report on Surveillance, 2014
6. Rossolini, G.M.; Arena, F.; Pecile, P.; Pollini, S. Update on the antibiotic resistance crisis. *Currentt Opinion in Pharmacology*, *2014;18*, *56–60.*
7. Edelsberg, J.; Weycker, D.; Ban-on, R.; Li, X.; Wu, B.; Oster, G.; Badre, S.; Langeberg, W.J.; Weber, DJ. Prevalence of antibiotic resistance in US hospitals. *Diagnostic Microbiology and Infectious Disease*, *2014;78*, 255-262.
8. Daferera, DJ., B.N. Ziogas, and M. Polission. The effectiveness of plant essential oils on the growth of botrytis cinerea, fusarium sp., and clavibactermichiganensis subsp. Michiganensis. *Crop Prot*ection, 2003;22: 39–44.
9. Ume-Kalsoom, Siddique S., Shahzad N., Ghous T., and Andleeb S. *In vitro* screening of herbal extracts and antibiotics against bacteria isolated from fish products at retail outlets. *British Microbial Research Journal*, *2013*;3(1): 19-31
10. Ahmad M, Muhanm1ad N, Mehjabeen, Jahan N, Alunad M, Obaidullah, Malunood QM, and Jan SU. Spasmolytic effects of *Scrophularia nodosa* extract on an isolated rabbit intestine. *Pakistan. Journal. Pharmaceutical Sciences*, 2012; 25(1): 267–275.

11. Nasim F, Andleeb S, Iqbal M, Ghous T, Khan AN, and Akhtar K. Evaluation of the antimicrobial activity of extracts of fresh and sour-spoiled *Spinacia oleracea* against some mammalian pathogens. *African Journal of Microbiology Research*., *2012*;6(29): 5847–5851.
12. Bhalodia NR, Nariya PB, and Shukla VJ. Antibacterial and antifungal activity from lower extracts of *Cassia fistula*L: An Etlmomedicinal Plant. *International Journal of Pharmaceutical Research and Technology*, *2011*;3(1): 160–168.
13. Jahan N, Ahmad M, Mehjabeen, Zia-ul-Haq M, Alam SM, and Qureshi M. Antimicrobial screening of some medicinal plants in Pakistan. *Pakistan Journal of Botany*, *2010*;42(6): 4281–4284.
14. Runyoro DKB, Matee M., Olipa N., Joseph C., and Mbwambo H. Screening of Tanzanian medicinal plants for anti-Candida activity. *BM* C. *Complementary and Alternative Medicine*, *2006;* 6: 11.
15. Schmidt, G.J., and E.E. Schilling. Phylogeny and biogeography of eupatorium (asteraceasereupatorieae) based on its nuclear sequence. *American Journal of Botany*, 200;87(5): 716-726.
16. Arumugam G, Swamy MK, Sinniah UR. Plectranthus amboinicus (Lour.) Spreng: Botanical, Phytochemical, *Pharmacological and Nutritional Significance. Molecules*, 2016;21(4):369. doi: 10.3390/molecules21040369.
17. Lukhoba, Mathenge, *P. edulis (Vatke) Agnew. Herbarium specimens* held at NAI were collected from Kenya *(Lukhoba&Mathenge 220) 1993*.
18. Collins CH, Lynes PM, and Grange JM. Microbiological Methods (7th edn), Butterwort Heinemann Ltd., Britian, p. 175–180. 1995
19. Perex, C.C., M.L. Paul, and P. Bazerque. Antibiotic assay by the agar-well diffusion method. *Experimental Medicine and Biology*, 1990;15: 113–115.
20. Alade, P.I., and N.O. Irobi. Antibacterial activities of crude extracts of Acalypha wilsiana *Journal of Ethnopharmacology*, 1993;39 (171–174).
21. Nweze, E.I., and M.C. Onyishi. In vitro antimicrobial activity of ethanolic and methanolic extracts of *Xylopia aethiopica* and its combination with disc antibiotics against clinical isolates of bacteria and fungi. *Journal of Rural and Tropical Public Health*, 2010;9: 16.
22. Kayser, Fritz H. Kurt A. Bienz, Johairnes Eckert, and Rolf M. Zinkernagel, *Medical Microbiology*, 2005;10, (210),
23. Sofowora A. Screening plants for bioactive agents. medicinal plants and traditional medicines in Africa. 2nd Ed. Spectrum Books Ltd., Sunshine House, Ibadan, Nigeria, pp. 134–156. 1993
24. Trease, E., and W.C. Evans. Phannacognosy. Williams, Charles Evans, 15th Edn., London: Saunders. Publishers. 2004
25. Harbone, N.V. Phytochemical methods. A guide to modern techniques of plant analysis, 2nd edn., London, Chapman and Hall. 1994
26. Shekhawat N. and Vijayvergia R. Evaluation of the Antimicrobial Potential of Some Medicinal Plants Against Plant and Human Pathogens. *Journal of Pharmaceutical Research*., *2010*;3(4): 700–702.
27. 27 Rios JL, Recio MC. Medicinal plants and antimicrobial activity. *Journal of Ethnophamacology*, 2005; 100:80–81.
28. 28 Iqbal Ahmad, FarrukhAqil, and Mohammad Owctis. Turning Medicinal Plants into Drugs. *Modern Phytomedicine*, 2006;12, *564-582*.
29. Tsuchiya H, Sato M, Miyazaki T, Tanigaki S, Ohyama M, Tanaka T, and Iinuma M. A comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *Journal of Ethnophamacology*, 1996; 50: 27–34.
30. Akinpelu DA, Onakoya TM. Antimicrobial acts of medicinal plants used in folklore remedies in southwestern Africa. *African. Journal of Traditional and Complementary Medicine*, 2006; 3:112–115.
31. Odugbemi T., Odunayo A. Nature of medicinal plants. outline and pictures of medicinal plants from Nigeria. Tolu Odugbemi (Editor). University of Lagos Press, Lagos, Nigeria, p. 73–161. 2006
32. Aurelli P., Costantini A., and Zolea S. Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. *Journal of Food Protection*, 1992; 55: 344-348.
33. Tassou CC, Drosinos EH, and Nychas GJE. Effects of essential oils from mint *(Menthapiperita)* on *Salmonella enteritidis and Listeria monocytogenes in* model food systems at 4 and 10°C. *Journal of Applied Bacteriology*, 1995; 78:593-600.
34. Mendoza L, Wilkens M, Urza A. Antimicrobial study'of the resinous exudates and of diterpenoids and flavanoids isolated· from Chilean *PseudognL.phalium (AM., anderaceaA.). Journal of Ethnophamacology*, 1997;58:85–88.

CITE AS: Bbira Mike (2024). Evaluation of Antibacterial and Phytochemical Properties of Leaf Extract of Plectranthus cyaneus against some five Human Pathogens. RESEARCH INVENTION JOURNAL OF SCIENTIFIC AND EXPERIMENTAL SCIENCES *3*(3):49-56.