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PHYTOCHEMICAL AND ANTIBACTERIAL ANALYSIS OF LIVINGSTONE POTATO (*Plectranthus esculentus* N.E.BR) TUBERS

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ABSTRACT: *Plectranthus esculentus* N.E.Br. is a dicotyledonous perennial shrub that grows up to 2m tall and belongs to Lamiaceae family. Presently, there is dearth of publications on its Phytochemical and antibacterial analysis. The study was conducted on phytochemical and antibacterial analysis of the plant tubers. The aim was to evaluate the phytochemical constituents and antibacterial activity. The phytochemicals were qualitatively accessed by adopting the standard procedures, while the antibacterial activity was tested via agar-well diffusion method. The result of the phytochemical analysis revealed the presence of Phenols, flavonoids, terpenoids, steroids, saponins, alkaloids and tannins all in higher reactions. 500, 250, 125 and 62.5 µg/ml were used as the antibiotic concentrations with Ofloxacin 5 µg/ml as the positive control, against; *S. aureus*, *E. coli* and *K. pneumoniae*. *S. aureus* showed the highest value of inhibition zone at 500 µg/ml, while the lowest value was recorded on *K. pneumoniae.* At 250 µg/ml, highest and lowest values were recorded on *E. coli* and *K. pneumoniae* respectively. *E. coli* showed the highest activity at 125 µg/ml, while *K. pneumoniae* showed the lowest activity. At 62.5 µg/ml, highest and lowest values were recorded on *E. coli* and *K. pneumoniae.* This study therefore concludes that the ethanol tuber extract harbors various Phytochemicals. The presence of phyto-constituents is useful for treating different ailments and it also has a potential of providing useful drugs for human use. It therefore recommends tuber extract of *P. esculentus* should be used as natural antibiotic in place of the chemically-combined.

Keywords: Antibiotic, Phytochemical screening; sensitivity test; agar-well diffusion

INTRODUCTION

Plants have been integral to mankind's healthcare system, either directly or indirectly. Directly, plant parts such as fruits, leaves, stem back, roots, and even whole plants themselves are used to treat ailments in animals. The medicinal value of drug plants is attributed to the presence of chemical substances in plant tissues that produce specific physiological effects on the human body (Uttpal *et al.,* 2019). These chemical substances, known as phytochemicals, are biologically active compounds found naturally in plants. They provide health benefits to humans as medical ingredients and nutrients (Uttpal *et al.,* 2019).

Plants synthesize secondary metabolites, which are small organic molecules that are not essential for their normal growth or development. However, these metabolites play crucial roles in reproduction and defense mechanisms against bacteria, fungi, viruses, vertebrates, and other organisms (Uttpal *et al.,* 2019). Phytochemicals possess various biological properties, including antioxidant activity, antimicrobial effects, immune system stimulation, and anticancer properties (Mamta *et al.,* 2013). Phytochemicals can be classified into two categories: primary metabolites (common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls, etc.) and secondary metabolites (remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, and glucosides). These compounds have formed the basis of medical treatments throughout much of human history and continue to be widely practiced today (Mamta *et al.,* 2013). Indirectly, modern medicine utilizes numerous plant-derived compounds as the foundation for evidence-based pharmaceutical drugs. While herbalism may apply modern standards of effectiveness testing to herbs and medicines derived from natural sources, high-quality clinical trials and standards for purity or dosage exist (Abera, 2014). Herbal medicines are available in various forms such as tablets, capsules, powders, teas, extracts, fresh or dried plants. People use herbal medicines to maintain or improve their health. These secondary metabolites have the potential to act as drugs (Abera, 2014).

Plants are widely recognized as significant sources of numerous natural medicinal compounds (Abera, 2014). Phytochemicals, which are secondary metabolites derived from plants, have been found to perform various functions such as pathogen defense, protein synthesis, protection against adverse weather conditions, nutrient

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uptake, and enzyme activity (BNT, 2022). Plant polyphenols exhibit antioxidant properties and are beneficial in neutralizing free radicals and preventing reactive oxygen-related diseases (Mamta *et al.,* 2013). They are known to help prevent and reduce the risk of diseases such as diabetes, cancer, cardiovascular diseases, cataracts, liver toxicity, stroke, and age-related disorders (Mamta *et al.,* 2013). Medicinal plants hold immense value for individuals and communities due to their health benefits. The exploitation of the biological properties of medicinal plants has been driven by their natural origin, cost-effectiveness, and minimal side effects (Tesfahuneygn and Gebreegziabher, 2019). Medicinal plants have often been described as a precious gift from nature to humankind. Additionally, these properties have been investigated for the development of novel therapies to treat various microbial infections and combat microbial resistance against conventional antibiotics (Abera, 2014).

P. esculentus, also known as the kaffir potato or Livingstone potato, is a species of plant in the dicot family Lamiaceae (Masimba *et al.,* 2019). It is indigenous to Africa, where it is grown for its edible tubers (Masimba *et al.,* 2019). The aim of this study is to determine the phytochemical and antibacterial properties of the *P. esculentus*

MATERIALS AND METHODS

Description of the Study Area

Fresh and healthy tubers of *P. esculentus* were collected from Baganje village in the Billiri Local Government Area of Gombe State, Nigeria. The collection area is located between Latitude: 9049' 0" N and Longitude: 11010'0" E, within the Northeastern zone of Nigeria. The region experiences a mean annual rainfall ranging from 600-1200 mm, with the highest concentration occurring between July and September (Ajuo *et al.,* 2014). During the collection process, strict measures were taken to avoid any form of adulteration. *P. esculentus* is widely cultivated in the area as a supplementary food crop. The selection of this area was based on its status as one of the major producers of *P. esculentus* tubers in Gombe State and Nigeria.

Materials

All chemical reagents used were of analytical grade and purchased from reputable suppliers. The clinical isolates used in the study were sourced from the Federal Teaching Hospital Gombe (FTHG). The *P. esculentus* tubers (2.50 kg) were obtained from farmers residing in Tal village of the Billiri Local Government Area in Gombe State.

Sample Identification

Botanical identification was performed by plant taxonomists at the Herbarium unit of the Department of Botany in Gombe State University. A herbarium specimen (voucher number) was deposited for future reference (Masimba *et al.,* 2020).

Drying and Grinding

To eliminate foreign contaminants, the tubers underwent a thorough cleaning process with running tap water, followed by washing with sterilized distilled water. Fresh *P. esculentus* tubers were then finely chopped using a sterile knife to expedite the drying process. The plant parts were subsequently subjected to shade drying to remove any remaining moisture. Afterward, the plant materials were pounded into a homogeneous mixture using a mortar and pestle (Masimba *et al.,* 2020). To prevent any potential fungal attack, the resulting powder was stored in an airtight container and kept in a dark, cool, and dry place until the extraction process commenced.

Extraction of Phytochemicals

For the extraction of phytochemicals, a common solvent extraction technique was employed, as demonstrated by Divya and Manimegalai (2015). A separate 5.38g crushed tuber sample was extracted using 500 mL ethanol (40.0%) as the extraction solvent. The mixture was placed on a laboratory shaker for 24 hours. The residue plant material was then separated from the extract by filtering it through a Whatman number 1 filter paper. Subsequently, the extract was concentrated to dryness using rotary vacuum evaporation at 400°C, accompanied by air drying as suggested by Dima et al. (2008) and Koksal and Gulcin (2008) . The resultant solid extract was weighed and stored for further experiments.

Phytochemical Screening

The phytochemical test involved subjecting the coarse powdered sample to Wagner's, Libermann-Burchard, Foaming, Shinoda and Ferric Chloride tests to test for alkaloids, steroids and sterols, saponins, flavonoids, tannins, and phenols. According to the method adopted by Krishveni and Dhanalakshmi (2014).

Sensitivity Test

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To evaluate the antimicrobial activity of the crude extract of *P. esculentus* tubers, agar-well diffusion method was employed to assess the antimicrobial assay against Gram-positive bacterium *S. aureus* and Gram-negative bacteria *E. coli* and *K. pneumoniae*. The bacterial cultures were inoculated onto prepared Nutrient agar and incubated at 37°C for 24 hours. The prepared agar was dispensed into three petri dishes and allowed to solidify for the inoculation of the bacterial strains, as described by Cheesbrough (2006).

Preparation of Antibacterial Agent Concentration

To prepare the antibacterial agent concentration, five different sample containers were used. One container was loaded with 1.0 gm of the crude ethanolic extract of *P. esculentus* tuber to prepare the stock solution. The remaining four sample containers were numbered 1, 2, 3, and 4. In all five sample containers, 2 ml of Dimethyl Sulfoxide (DMSO) was added. Serial dilution was performed to prepare concentrations of 500, 250, 125, and 62.5 µg/ml in all four test tubes (Ochei and Kolhatkar, 2008).

Standardization of the Inoculum

For standardization of the inoculum, bacterial strains were inoculated into a prepared Mueller-Hinton agar and incubated overnight (24 hours). The resulting turbidity was adjusted to a 0.5 McFarland turbidity standard using the same broth medium. The McFarland turbidity standard was prepared by mixing 99.5 ml of 1% v/v sulfuric acid and 0.5 ml of 1.175% w/v $BaCl_2.H_20$. The mixture was then dispensed into three sterilized tubes in amounts of 3-4 ml (Ochei and Kolhatkar, 2008).

Sensitivity Test

To evaluate the antibacterial activity of the extract from *P. esculentus*, an agar-well diffusion assay was performed. The entire surfaces of Mueller-Hinton agar plates were inoculated with adjusted bacterial strains. After the plates had dried aseptically, 6 mm wells were punched into the agar using a sterile cork borer. The prepared concentrations (500, 250, 125, and 62.5 µg/ml) were loaded into the four punched wells, and the plates were incubated at 37°C for 24 hours. As a positive control, the antibiotic Ofloxacin (5 µg/disk) was used. The antimicrobial activity was evaluated by measuring the diameter of the circular inhibition zones around the wells. A positive result was defined as an inhibition zone (halo size) of 9 mm or more appearing around the wells, indicating the presence of an antibacterial substance in the extract tested (Hassan *et al.,* 2012).

RESULTS AND DISCUSSION

Phytochemical Analysis

The qualitative assessment of phytochemicals is presented in Table 1. The findings of this study suggest the presence of phenolic compounds, flavonoids, terpenoids, steroids, sterols, alkaloids, and tannins, with stronger reactions.

Table 1. Phytochemical Analysis

S.No	Name of the test	Results
1.	Test for Alkaloids	+++
	a) Wagner's test	
2.	Test for steroids and sterols	+++
	a) Libermann-Burchard test	
3.	Test for Saponnins	+++
	a) Foaming test	
4.	Test for Flavonoids	+++
	a) Shinoda test	
5.	Test for tannin and Terpenoid	+++
	a) Ferric chloride test	
6.	Test for Phenols	+++
	a) Ferric Chloride test	

+++ Stronger reactions

Sensitivity Test

The antimicrobial activity of the crude extract from *P. esculentus* tubers was evaluated using the agar-well diffusion method. The results are summarized in Table 2. The inhibition of each microorganism by the crude extract was measured as the average of two cross diameters in irregular zones after 24 hours of inoculation. The crude extract exhibited moderate antibacterial activity against *S. aureus*. The highest susceptibility was observed against *E. coli*, while the lowest antibacterial effect was recorded against *K. pneumoniae*. The antibacterial inhibition effect of the crude extract increased with increasing concentration. It is worth noting that the commercial

standard drug, Ofloxacin, showed no inhibitory effect against *K. pneumoniae*. Additionally, the commercial standard drug exhibited a greater inhibitory effect than a concentration of 62.5 µg/ml of the crude extract in *S. aureus*, and a lesser effect in *E. coli.* The positive control for bacteria is Ofloxacin 5 µg/ml. Inhibition zone values above 19 are considered susceptible, values between 15 and 19 are intermediate, while values below 15 are resistant (Table 2).

Table 2: Antibacterial analysis of *P. esculentus.*

Microorganisms	Fraction concentration (µg/ml) Zone of inhibition (mm)* (Mean ± SE)				
	500	250	125	62.5	Control
Staphylococcus aureus	28.0±2.65	20.67±5.86	15.00±2.00	11.00±2.00	14.0±2.00
Klebsella pneumoniae	21.0±5.29	19.0±5.29	15.0±2.65	11.0±4.0	0.00±0.00
Escherichia coli	27.0±7.00	25.0±8.66	20.0±5.00	20.0±6.93	16.7±1.00

* mm = milli meter, µg = micro gram, ml = mil and Control = Ofloxacin

Phytochemical Screening

The phytochemical findings of this study indicate the presence of phenolic compounds, flavonoids, terpenoids, steroids, alkaloids, and tannins in the ethanol tuber extract of *P. esculentus* This is consistent with the previous work of Masimba *et al.* (2019) on the total phenolic content (TPC) determination and phytochemical screening of *P. esculentus* tubers in Rusape, Zimbabwe. The presence of these phytochemicals in the tubers helps explain their medicinal importance. The work of Ezebo *et al.* (2021) on the phytochemical screening and antimicrobial activity of ethanol and methanol extracts of *Lantana camara* leaves also supports the presence of alkaloids, saponins, flavonoids, tannins, and terpenoids. The similarity between these studies may be due to the use of the same plant species in both cases and the use of ethanol as the extraction solvent.

The tuber, which was the plant part used in this study, was found to contain all the tested phytochemicals (phenolic compounds, flavonoids, terpenoids, steroids, alkaloids, and tannins) and exhibited antibacterial effects. This finding contradicts the results of Alkowni *et al.* (2018) in their work on the phytochemical screening and antibacterial activity of *Cyclamon persicum* Mill tuber extracts. Their study revealed the presence of glycosides, saponins, starch, and phenols but did not detect any alkaloids, tannins, or flavonoids. The variation in results may be attributed to differences in the chemical constituents of the plant organs.

Sensitivity test

In the sensitivity test, it is observed that the inhibitory activity of the plant extract increases with an increase in the antibiotic agent concentration. This is in accordance with the work of Jebamalar et al. (2019) with the title "Evaluation of antimicrobial activity and phytochemical analysis of whole plant extract of *Vinca rosea*" who used ethyl acetate, ethanol, and dimethyl sulfoxide (DMSO) whose antibacterial activity was shown to be increasing with an increase in the antibacterial agent concentration. The highest zone of inhibition (susceptibility) was recorded for *E. coli*. This is in accordance with the work of Karthikeyan and Vidya (2019) which the title Phytochemical analysis, antioxidant and antibacterial activity of ethanol extract of pomegranate peel *Punica gratum* where *E. coli* was also recorded to have the highest zone of inhibition among the tested bacterial strains. This also disagrees with the previous report of VAN et al. (2019), who recorded lower values on *E. coli* against different antibiotic concentrations. This variation could be due to the nature of the bioactive compounds present in the plants parts. The cell membrane nature of gram-negative bacteria that forms a protective coat for their microbes could also be responsible for this variation. The gram-positive bacterium used was *S. aureus*, which also showed higher zone of inhibition (susceptibility). This also agrees with the previous report of VAN et al. (2019), who reported higher inhibitory activity (susceptibility) against *S. aureus*. Gram-positive bacteria are known for their susceptibility against different plant extracts.

CONCLUSION

From this study, it can be concluded that various phytochemicals including phenols and flavonoids were tested to be present in the ethanol tuber extract of *P. esculentus*. The presence of phytoconstituents is useful for treating different ailments and it also has the potential of providing useful drugs for human use. The presence of the bioactive compounds presents the plant for its phyto-pharmaceutical usage. This study has also confirmed that the ethanol tuber extract of *P. esculentus* was able to inhibit the growth of the test organisms: *E. coli*, *S. aureus*, and *K. pneumoniae*. The extract of the plant can be used in the preparation of antibiotics against these organisms. The

ability of this extract to highly inhibit the growth of *E. coli* shows its potential in curing illnesses caused by the organism such as gastroenteritis, typhoid fever, dysentery, cholera, and urinary tract infections.

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