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Evaluation of Antibacterial Activity of Tanzanian Gooseberry (*Physalis peruviana*) Leaf Extract against Multi-Drug Resistance *Escherichia coli* and *Salmonella typhi*

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ABSTRACT

This study was conducted to evaluate in-vitro antibacterial activities of methanolic leaf extract of *Physalis peruviana* growing in Dodoma, Tanzania, against multidrug-resistant bacteria (*Escherichia coli* and *Salmonella typhi*) using agar well diffusion and standard microdilution methods. Moreover, leaf extract was screened qualitatively for the presence of phytoconstituents using standard methods. Methanolicleaf extract of *P. peruviana* (from 0.2 mg/mL to 0.4 mg/mL concentrations) showed antibacterial activity against tested *E. coli* and *S. typhi* with zones of inhibition ranging from 19 to 21 mm and 17.7 to 22 mm, respectively. The micro-dilution method revealed the minimum inhibitory concentration of methanolic leaf extract of *P. peruviana* to be 10 µg/µL and 5 µg/µL for *E. coli* and *S. typhi*, respectively. Qualitative phytochemical analysis of methanolic leaf extract of *P. peruviana* identified the presence of tannins, cardiac glycosides, alkaloids, terpenoids, steroids, flavonoids, and phenols. Methanolic leaf extract of *P. peruviana* has antibacterial activity against multidrug-resistant bacteria (*E. coli* and *S. typhi*). This plant may be a promising new source of future antibiotics that potentially combat the existing problem of antimicrobial resistance.

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1.0 Introduction

The use of antibiotics without prescriptions from a physician is one of the reasons for the emergence of resistant microbial strains. The emergence of resistant strains has led to the prevalence of multidrug-resistant (MDR) infections, which in turn has led to the failure of infection treatment (Sonola *et al.,* 2021). Antimicrobial resistance (AMR) is responsible for approximately 700,000 deaths worldwide and is expected to increase to 20 million by 2050, costing over $2.9 trillion (Watkins & Bonomo, 2016, 2020; WHO, 2022).

In Tanzania, studies have reported high levels of AMR, including MDR, in *E. coli* isolated from chickens, humans, rodents, and soil samples, depicting MDR as a crisis (Mshana et al., 2013a; Sonola *et al*., 2021). In clinical settings across Tanzania, the prevalence of multidrug-resistant bacteria ranges from 25% to 50% (Moremi *et al*., 2016; Moyo *et al*., 2014; Mshana *et al*., 2013b). Prior research has indicated concerningly elevated levels of bacterial resistance to widely utilised antibiotics, including *E. coli* to cephalosporins and fluoroquinolones, Staphylococcus aureus to methicillin, Streptococcus pneumoniae to penicillin, non-typhoidal Salmonella and Shigella species to fluoroquinolones, Neisseria gonorrhoeae to cephalosporins, and Mycobacterium tuberculosis to rifampicin, isoniazid, and fluoroquinolones (Moremi *et al*., 2016; Mshana *et al*., 2013a; Sonola *et al.,* 2021).

A high level of AMR and MDR drives the need to explore cures from medicinal plants against MDR infections. One of the medicinal plants used by indigenous people in Tanzania for treating dysentery, typhoid, and stomachache is *Physalis peruviana,* commonly known as gooseberry (Mbwambo *et al*., 2007). However, there is little information regarding the phytochemical constituents and antibacterial activity of *P. peruviana* found in Tanzania.

Therefore, the aim of this study was to evaluate the antibacterial activity of leaf extract of *P. peruviana growing* in Tanzania against multidrug-resistant *E. coli* and *S. typhi.* The result of this study contributes to linking traditional and modern medicine and adds information about possible sources of new antibacterial medicines that have the potential to treat infections caused by multidrug-resistant bacteria. Moreover, this study widens the avenue for community understanding and awareness about the potential medicinal benefits of *P. peruviana*. Products of *P. peruviana* may thus serve as the more affordable and widely available MDR bacteria inhibitors, compared to the pricey commercially available medicines that have shown resistance.

2.0 Materials and Methods

2.1 Plant Collection

Leaves of *P. peruviana* were collected from Kikuyu, a local area within the Dodoma City. Briefly, the leaves were carefully hand-plucked, properly examined, and those attacked by insects were removed. The remaining leaves in good condition were placed in ziplock bags and transported to the Biology Laboratory at St. John's University of Tanzania. In the laboratory, the leaves were air dried for one week at room temperature ranging from 25–27°C (Begum *et al.,* 2021). After drying, the leaf specimens were identified by a botanist, authenticated, and preserved in the herbarium of the same laboratory.

2.2 Plant Extraction

Methanolic leaf extract of *P. peruviana* was obtained according to Begum *et al.* (2021). Briefly, air-dried samples were pulverised, weighed, and soaked for 48 hours in absolute methanol. The crude extract was then filtered and concentrated *in vacuo using* a rotary evaporator while maintaining the water bath temperature below 40 °C to avoid thermal decomposition of labile compounds. The extract obtained was taken to carry out experiments on phytochemical screening and antimicrobial activity testing.

2.3 Phytochemical Screening

Phytochemical screenings were carried out using a standard procedure (Begum *et al.,* 2021), as follows:

2.3.1 Test for Alkaloids (Wagner's test)

Methanolic *P. peruviana* leaf extract was treated with two to three drops of Wagner's reagent (0.5 g of iodine and 1.5 g of potassium iodide dissolved in 5 mL of distilled water followed by solution dilution to 20 mL using water). Formation of reddish-brown precipitate indicated the presence of alkaloids; otherwise, the extract test was negative.

2.3.2 Test for Terpenoids (Salkowki's Test)

Methanolic *P. peruviana* leaf extract was dissolved in 2 mL of chloroform and treated with 2 mL of concentrated sulphuric acid to form a layer. Reddish-brown colouration at the interface indicated the

presence of terpenoids; otherwise, the extract test was negative.

2.3.3 Test for Flavonoids (Alkaline Reagent Test)

Methanolic *P. peruviana* leaf extract was treated with two to three drops of 20% sodium hydroxide solution. The formation of an intense yellow colour, which turns colourless on the addition of 5% dilute hydrochloric acid, was indicating the presence of flavonoids; otherwise, the extract test was negative.

2.3.4 Test for Tannins (Braymer's Test)

Methanolic *P. peruviana leaf* extract was boiled in 20 mL of distilled water and then filtered. The filtrate was treated with a 10% alcoholic ferric chloride solution, and the formation of a brownish-green to blue-black colour indicated the presence of tannins; otherwise, the extract test was negative.

2.3.5 Test for Cardiac Glycosides (Kellerkiliani's Test)

Methanolic *P. peruviana* leaf extract was dissolved in 4 mL of distilled water and then treated with 2 mL of glacial acetic acid containing a few drops of ferric chloride solution. Further, 2 mL of concentrated sulphuric acid was added carefully without mixing the solution. Formation of a brown ring at the interface indicated the presence of deoxysugars (characteristics of cardenolides), different from that, the extract test was negative.

2.3.6 Test for Carbohydrates (Benedict's Test)

Methanolic *P. peruviana* leaf extract was treated with Benedict's reagent (cupric citrate complex) and then heated gently. Formation of orange-red/brick red/rusty brown precipitate indicated the presence of reducing sugars. Otherwise, the extract test was negative.

2.3.7 Test for Proteins (Biuret Test)

Methanolic *P. peruviana* leaf extract was dissolved in 4 mL of distilled water and then treated with an equal volume of 1% sodium hydroxide solution followed by 3 drops of aqueous copper (II) sulphate solution. A colour change from blue to purple/violet indicated the presence of proteins; alternatively, the extract test was negative.

2.3.8 Test for Quinones

Methanolic *P. peruviana* leaf extract was treated with concentrated hydrochloric acid. Formation of yellow colouration or precipitate indicated the presence of quinones; otherwise, the extract test was negative.

2.4 Antibacterial Activity Assays

2.4.1 Agar Well Diffusion Method

Investigations of antibacterial activities were done using a standard agar well diffusion method. The zone of inhibition was measured by a ruler and recorded. Zone of inhibition was determined as an average of the zone of inhibition (Bayas-Morejon et al., 2020; Begum et al., 2021; Jamali, 2016; Mayachiew & Devahastin, 2008).

Muller Hinton Agar (MHA) was used as the base medium for screening of antibacterial activity. About 50 mL of the medium was poured into sterile petri dishes and allowed to solidify. Wells of diameter 6 mm were dug out using a sterile cork borer in solidified MHA medium. Using a sterile cotton swab, cultures of MDR *E. coli* and *S. typhi* (obtained from the Biology Laboratory, Faculty of Natural and Applied Sciences, St. John's University of Tanzania) were inoculated evenly on the surface of the MHA plate to make lawn culture. 50 µL of the extract was loaded into three wells at different concentrations, 0.2, 0.3, and 0.4 g/mL, and then plates were incubated at 37˚C for 24 hours. After incubation, the zone of inhibition of growth around the wells was observed.

The experiment was conducted three times, and the zone of inhibition at each concentration for the two MDR bacterial strains was measured. The antibacterial activity of the extract was qualitatively assayed by measuring the diameter of the zone of inhibition around the wells to the nearest mm and related to the susceptibility of the isolate. The zone of inhibition diameter of the disc containing the extract and a standard control drug was interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, 2012).

2.4.2 Micro-Plate Dilution Method

The investigation of minimum inhibitory concentration of the extract was done by the broth micro-dilution technique using sterile flat-bottomed 96-well polystyrene microtitre plates (Begum et al., 2021). Test solutions were prepared by dissolving 20 mg of extracts in 0.1 mL of dimethyl sulfoxide (DMSO) and diluted with 0.9 mL of broth to make a concentration of 20 mg/mL. 50 µL of the test solution was pipetted and added into the first well of each row of plates preloaded with 50 µL of broth. Then serial dilution of concentrations 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 µg/mL was performed by transferring the test sample from the first row well to the next row well, down to the last row.

This was followed by the addition of 50 µL of the solution containing the test organisms (0.5 McFarland dilutions) to each of the wells. This study used three controls: growth controls, positive control (ciprofloxacin (0.5 mg/mL)), and negative control (DMSO).

The microtiter plates were incubated at 37°C for 24 hours. After the incubation period, 30 µL of a 0.2% p-iodonitrotetrazolium chloride (INT) was added to the wells, followed by incubation at 37 °C for 30 min. Presence of microbial growth was indicated by change of INT colour to purple from its yellow colour. Absence of growth was indicated as no colour change (the colourless tetrazolium salt acted as an electron acceptor and reduced to a pink-coloured formazan product by a biologically active organism). The lowest concentration that inhibited microbial growth was recorded as the minimum inhibitory concentration.

3.0 Results and Discussion

3.1 Phytochemical Composition

Phytochemical screening of the methanolic leaf extracts of *P. peruviana* showed the presence of cardiac glycosides, tannins, flavonoids, alkaloids, terpenoids, and phenols when analysed qualitatively (Table 1). These phytochemicals have the ability to act as antioxidants and have antimicrobial activities against both gram-negative and positive bacteria. Phytochemicals identified in this study agreed with previous studies, which found *P. peruviana* extracts to contain biologically active compounds such as tannins, phenols, flavonoids, and alkaloids (El-Beltagi *et al.*, 2019; Kasali *et al.*, 2021; Peter *et al.*, 2020). The absence of some phyto-constituents may be due to the synthesis nature of the constituents or variations in the conditions for sample handling and solvent extraction during the experiment.

Table 1
Qualitative Analysis of Phytochemical Constituents Found in Methanolic Leaf Extract of P.peruviana

Chemical Constituent	Observation
Alkaloids	+
Cardiac glycoside	+
Flavonoids	+
Phenols	+
Proteins	–
Quinones	–
Tannins	+
Terpenoids and steroids	+

Key: (+) Present, (-) Absent

3.2 Antimicrobial Activity of P. peruviana

Assessment of the antimicrobial activity of methanolic leaf extracts of *P. peruviana* was done using agar well diffusion and showed that *P. peruviana* has antibacterial activity against MDR *E. coli* and *S. typhi* as per the observed zone of inhibition in Figures 1 and 2 and the size of the zone of inhibition as shown in Tables 2 and 3, respectively.

Three different tests showed that methanolic leaf extracts of *P. peruviana* could stop *E. coli* from growing in a zone 19 to 21 mm wide, and they could stop *S. typhi* from growing in a zone 17.7 to 22 mm wide at 0.2, 0.3, and 0.4 g/mL of the extract. These findings are comparable to the standard drug, ciprofloxacin, which, at a concentration of 0.5 mg/mL, exhibits a zone of inhibition of 21 mm for *E. coli* and 22 mm for *S. typhi.*

The use of methanol as an extraction medium for *P. peruviana* and 99% DMSO appeared to have no effect on the potency of the active ingredient, leading to wider zones of inhibition. Other factors may come from the synergistic effects between methanol and the active ingredient of the investigated plant, the depth of the wells, or the extract concentrations.

Table 2

Antibacterial Activity Test Results of P. peruviana Leaf Extracts against MDR E. coli Showing Size of Zone of Inhibition

Isolate	Extract conc. (g/ml)	Susceptibility test (S/N)	Zone of inhibition (mm)
E.coli	0.2	1	19
		2	18
		3	20
		Average	19
	0.3	1	19
		2	18
		3	22
		Average	19.7
	0.4	1	21
		2	22
		3	20
		Average	21
Standard drug, Ciprofloxacin (0.5mg/mL)		21	

Well diameter: 6mm

Table 3

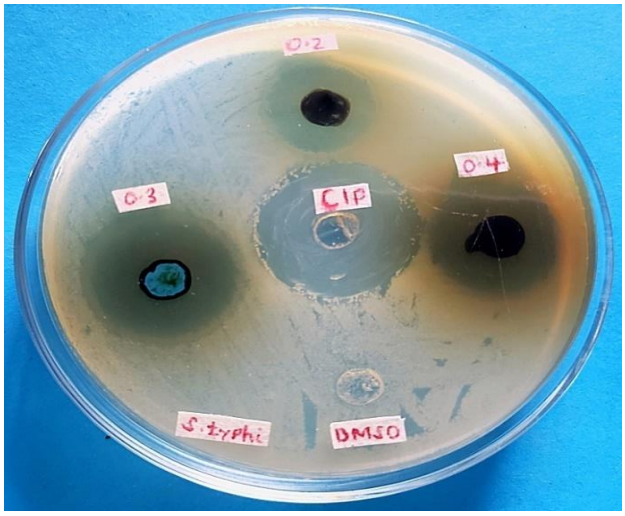
Antibacterial Activity Test Results of Methanolic P. peruviana Leaf Extracts against MDR S. typhi Showing Size of Zone of Inhibition

Strain	Extract conc. (g/ml)	Susceptibility test (S/N)	Zone of inhibition (mm)
S.typhi	0.2	1	17
		2	17
		3	19
		Average	17.7
	0.3	1	20
		2	21
		3	21
		Average	20.7
	0.4	1	21
		2	23
		3	22
		Average	22
Standard drug, ciprofloxacin (0.5mg/ml)		22	

Well diameter: 6mm

Figure 1

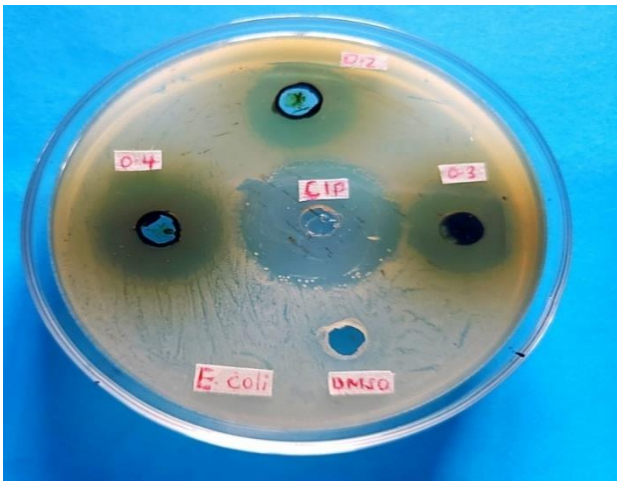
Agar Well Diffusion Results of Antibacterial Activity of P. peruvianaleaf Extracts against MDR S. typhi.



KEY: CIP = ciprofloxacin**,** DMSO =dimethylsulfoxide, 0.2, 0.3 and 0.4 = extract concentrations

Figure 2

Agar Well Diffusion Results of Antibacterial Activity of Methanolic P. peruviana Leaf Extracts against MDR E. coli.



KEY: CIP- ciprofloxacin**,** DMSO- dimethyl sulfoxide, 0.2, 0.3 and 0.4 –extract concentrations

3.3 Minimum Inhibitory Concentration of P. peruviana

The standard microplate dilution method was used to find the minimum inhibitory concentration of methanolic *P. peruviana* leaf extracts against MDR *E. coli* and *S. typhidone*. The minimum inhibitory concentration was 10 and 5 µg/mL, respectively.

The micro-plate row labeled "E" was cultivated with *E. coli,* while the row labelled "S" was cultivated with *S. typhi.* The plate column labeled “A” fed with 50µL of 1% of DMSO as negative control, was not active to inhibit bacterial growth to both strains except in concentrated solutions; well columns labeled “B” at 50µL of 99% DMSO was active to both bacterial strains at the same standard conditions. The microplate labelled “C, fed with only 50 µl of broth, was for a sterility test and showed no bacterial growth that depicted the broth was under well-sterilised conditions. Microplate column labelled “D” fed with bacterial strains with 50 µL of 0.5 mg/mL of CIP held as a positive control as it showed no bacterial growth. For the two resistant bacterial strains, *E. coli* and *S. typhi,* the MIC was 10 µg/µL for *E. coli* and 5 µg/µL for *S. typhi.* This revealed that the crude leaf extract of *P. peruviana is* active against both strains and is more sensitive and active against *S. typhi compared* to the E. *coli* strain.

These study findings support previous studies that reported *P. peruviana to* be an indigenous plant used for treating dysentery, typhoid, and stomachache (Bayas-Morejon *et al.,* 2020; Jamali, 2016; Mayachiew & Devahastin, 2008; Mbwambo et al., 2007).

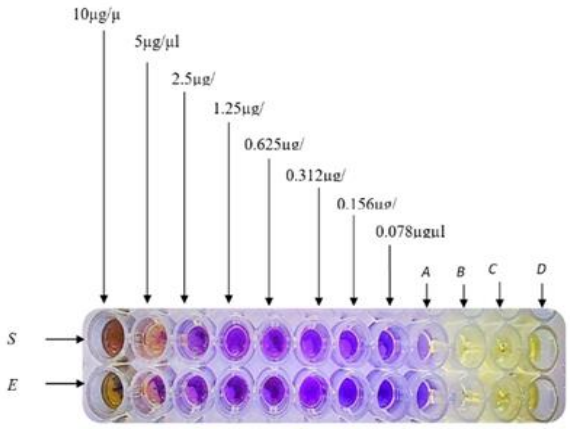
Table 4

Minimum Inhibition Concentrations of Methanolic P. peruviana Leaf Extracts against E. coli and S. typhi Using the Standard Micro-Plate Dilution Method

Leaf extract/ standard drug	MIC value (µg/ml)	
	E. coli	*S. typhi*
leaf	10	5
DMSO (1%)	No activity	No activity
DMSO (99%)	Activity	Activity
CIP (0.5µg/µl)	Activity	Activity

Figure 3

Minimum Inhibitory Concentrations of Methanolic P. peruviana Leaf Extracts against E. coli and S. typhi



Key: A = 1% DMSO, B = Broth, C = 99% DMSO, D = Ciprofloxacin, E - *E. coli,* S *= S. typhi*

4.0 Conclusion

P. peruviana leaves do have antibacterial activity. The findings in the present study support the traditional use of *P. peruviana* and represent an economic and safe alternative drug for the treatment of bacterial infections caused by both E. coli and *S. typhi* at a concentration of 0.4 g/mL for crude leaf extracts.

This study demonstrated that traditional medicine can be as effective as modern medicine to combat diseases caused by both MDR *E. coli* and *S. typhi* and a wide range of pathogenic diseases caused by bacteria. Moreover, isolation of the secondary metabolites from the leaf can be helpful in future research and development of pharmaceutical products in Tanzania.

5.0 Acknowledgements

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6.0 Authors Contributions

BN conceptualized and designed the study, drafted the manuscript, and conducted data analysis. Both BN and JL participated in sample collection,

laboratory analysis, and interpretation of results. All authors reviewed and approved the final manuscript.

7.0 Conflicts of Interests

All authors declare no conflict of interest.

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