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Ethnobotany and antibacterial effects of golden berry (physalis peruviana l.) on Salmonella typhi in Mbeya rural district, Tanzania

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ETHNOBOTANY AND ANTIBACTERIAL EFFECTS OF GOLDEN BERRY (*Physalis peruviana* L.) ON *Salmonella typhi* IN MBEYA RURAL DISTRICT, TANZANIA

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Health and Biomedical Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Medicinal plants have been used worldwide in managing human and animal diseases. However, their use and community-based formulation methods in many places, including Mbeya Rural District in Tanzania, are rarely described or documented. This study assessed the ethnobotany and antibacterial effects of *Physalis peruviana* in managing typhoid fever infections in Mbeya Rural District. The research was conducted on five villages;108 key informants, including 93 household members and 15 traditional healers, were involved in the study. The agar diffusion method evaluated the in vitro antibacterial activity of aqueous leaf extract of *Physalis peruviana*. LC-MS/MS was used to identify the phytochemical compounds in the *Physalis peruviana* leaf. Findings from this study indicate that all informants (100%) know the *Physalis peruviana* leaves as a medicine for treating typhoid fever. Boiling and soaking of leaves showed efficacy against the antibacterial activity of *Salmonella typhi*. The inhibitory activities of soaking were 15.16 mm and 14.33 mm for dry and fresh leaf extracts respectively, while that of boiled leaves was 3.66 mm. Phytochemical analysis revealed the presence of eight compounds namely; quinaldic acid, 6-O-malonylglycitin, 4-hydroxyd-2,3,4,6-tetramethoxychalcone,9,10-anthracenedione,1,4-diamino 5nitro, jatrorrhizine cation, 7-hydroxycoumarin-3- carboxylic acid, isovitexin, and nicergoline. Out of these eight compounds, four were identified to have antibacterial activity. This research adds to our knowledge of the antibacterial effects of *Physalis peruviana* leaf. In addition, it provides further studies on the isolation of compounds that can be used to develop useful antibiotics against *Salmonella typhi*.

DECLARATION

I, Charles Chekecha, hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and has never been submitted for a degree or other award in any academic or research institution.

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Prof. Ernest Mbega		12/06/2023
Name of Supervisor 1	**Signature**	**Date**

Dr. Sr. John -Mary Vianney		12-06-2023
Name of Supervisor 2	**Signature**	**Date**

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CERTIFICATION

The undersigned certify that they have read the dissertation titled **"Ethnobotany and antibacterial effects of goldenberry (*Physalis peruviana* L.) on *Salmonella typhi* in Mbeya Rural District, Tanzania"**, and it is recommended for examination in the fulfilment of the requirements for the degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology (NM-AIST).

Prof. Ernest Mbega		12/06/2023
Name of Supervisor 1	**Signature**	**Date**

Dr. Sr. John-Mary Vianney		12.06.2023
Name of Supervisor 2	**Signature**	**Date**

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DEDICATION

This work honours my mother, Editha Kanja; my wife, Lucy G. Cheka; my kids, Aloyce, Gladness, and Gracious; my late father, Eliud Chekecha (May his soul rest in peace, Amen).

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LIST OF ABBREVIATIONS

ABC	ATP-binding Cassette
ABMFL	Aqueous Boil Extracts of Mature *Physalis peruviana* Fresh Leaf
ABSMDL	Aqueous Boil Extracts of Semimature *Physalis peruviana* Dry Leaf
ABSMFL	Aqueous Boil Extracts of Semimature *Physalis peruviana* Fresh Leaf
ACMDL	Aqueous Cold Extracts of Mature *Physalis peruviana* Dry Leaf
ACMFL	Aqueous Cold Extracts of Mature *Physalis peruviana* Fresh Leaf
ACSMDL	Aqueous Extracts of Semimature *Physalis peruviana* Dry Leaf
ACSMDL	Aqueous Extracts of Semimature *Physalis peruviana* Dry Leaf
ACSMFL	Aqueous Extracts of Semimature *Physalis peruviana* Fresh Leaf
AHMDL	Aqueous Hot Extracts of Mature *Physalis peruviana* Dry Leaf
AHMFL	Aqueous Hot Extracts of Mature *Physalis peruviana* Fresh Leaf
AHSMFL	Aqueous Hot Extracts of Semimature *Physalis peruviana* Fresh Leaf
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
DRC	Democratic Republic of Congo
GBD	Global Burden of Disease
GCLA	Government Chemist Laboratory Authority
GID	Gastrointestinal Diseases
HH	Household
MATE	Multidrug and Toxic compound Extrusion
MC	Mature *P. peruviana* Leaf Extract in Cold Water
MC10	Mature *P. peruviana* Leaf Extract in Cold water for 10 Minutes
MC30	Mature *P. peruviana* Leaf Extract in Cold water for 30 Minutes
MDRT	Multidrug-Resistant Typhoid Fever

MFS	Major Facilitator Superfamily
MH	Mature *P. peruviana* Leaf Extract in Cold Water
MH	Mature *P. peruviana* Leaf Extract in Cold Water
MH10	Mature *P. peruviana* Leaf Extract in Hot Water for 10 Minutes
MH30	Mature *P. peruviana* Leaf Extract in Hot Water for 30 Minutes
MUST	Mbeya University of Science and Technology
NM-AIST	The Nelson Mandela African Institution of Science and Technology
QRDR	Quinolone Resistance Determining Region
RND	Resistance Nodulation Division
SMC	Semi-mature *P. peruviana* Leaf Extract in Cold Water
SMC	Semi-mature *P. peruviana* Leaf Extract in Cold Water
SMC10	Semi-mature *P. peruviana* Leaf Extract in Cold Water for 10 Minutes
SMC30	Semi-mature *P. peruviana* Leaf Extract in Cold Water for 30 Minutes
SMH	Semi-mature *P. peruviana* Leaf Extract in Hot Water
SMH	Semi-mature *P. peruviana* Leaf Extract in Hot Water
SMH10	Semi-mature *P. peruviana* Leaf Extract in Hot water for 10 Minutes
SMH30	Semi-mature *P. peruviana* Leaf Extract in Hold water for 30 Minutes
SMR	Small Multidrug Resistance
SPSS	Statistical Package for the Social Sciences
TCV	Typhoid Conjugate Vaccine
TF	Typhoid Fever
TH	Traditional Healers
TPHPA	Tanzania Plant Health and Pesticides Authority
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Typhoid fever is a febrile, acute illness that affects humans and poultry worldwide **(**Shakya *et al.,* 2019; Mulu *et al.,* 2021). The disease is caused by a bacteria, namely *Salmonella typhi* (Zaki & Karande, 2011; Wain *et al.,* 2014; Fazal *et al.,* 2020; Arunkumar *et al.,* 2022). Consuming food and water contaminated by infected human and animal faeces leads to disease transmission (Gomes, 2022). Poor sanitation and hygiene are associated risk factors for the disease, which is more prevalent in developing countries than in developed countries (Havelaar *et al.,* 2015; Ingle *et al.,* 2020; Muresu *et al*., 2020; Antillón *et al.,* 2017; Yemata *et al.,* 2021). For instance, in developing countries, typhoid fever has been cited as a major health-threatening problem **(**Saxena *et al.,* 2021; Yemata *et al.,* 2021). Typhoid fever accounts for more than 21 million illnesses and 220 000 fatalities annually (Arunkumar *et al.,* 2022; Muresu *et al.,* 2020). In Tanzania, drug-resistant typhoid strains are a growing problem in different regions. The Global Burden of Disease (GBG) study estimates that in 2019, Tanzania had 79 334 typhoid cases and 1671 typhoid deaths per year (TyVAC, 2017).

Typhoid fever is treated using Ampicicillin, Amoxicillin, Chloramphenicol, and Ciprofloxacin (Ugboko & De, 2014). However, scientists have reported drug resistance to the antibiotics used to treat *S. typhi* (Wasfy *et al.,* 2002; Browne *et al.,* 2020; Butt *et al.,* 2021). Even worse, multi-drug-resistant and drug-resistant strains have been developed due to the powerful *S. typhi* pathogen **(**Batool *et al.,* 2021; Butt *et al.,* 2021). The advancement of *S. typhi* resistance might be due to the frequent use of synthetic drugs, which might also result in high treatment costs, a poor treatment prognosis, and pollution of the environment. Also, clinically, useful antibiotics are facing major setbacks, which include nephrotoxicity, ototoxicity, neurotoxicity, narrow spectrum of activity, bone marrow depression, and damage to the liver (Braga *et al.,* 2005; Granowitz & Brown, 2008; Rubaka *et al.,* 2014). To manage the challenge, some alternatives are currently proposed. One of the proposed methods includes exploring medicines from plants and biological resources. The viability of the method examined in this study comes from 2/3 of the world's population relying on medicinal plants for primary health care (Chhikara *et al.,* 2021; Kathare *et al.,* 2021). The use of medicinal plants has been partly due to the unaffordability of synthetic medicines (Runyoro *et al.,* 2006). Because of the bioactive

phytochemical compounds found in medicinal plants, various communities around the world have chosen them as an important alternative therapeutic agent (Ogutu *et al.*, 2019; Mag *et al.*, 2019; Mondal, 2021; Sapkota *et al.*, 2021). The bioactive phytochemical compounds have made the plants possess different mechanisms of action against pathogens, which include membrane disruption, complex formation with the cell wall, inactivation of enzymes, and interaction with DNA (Aiyegoro & Okoh, 2009; AL Masaudi & AlBureikan, 2012; Rubaka *et al*., 2014).

Plants from the genus *Physalis,* especially *Physalis peruviana*, have been reported to have a number of bioactive phytochemicals that can be explored against animal and human diseases (El-gengaihi *et al.*, 2013; Petkova & Popova, 2021). To describe: *Physalis peruviana* is an annual, short-lived perennial plant belonging to the family *Solanaceae* (Petkova & Popova, 2021; Ramadan, 2011; Yehia *et al*., 2021). The plant is adapted to a wide range of altitudes, soils, and climatic conditions, making it the most widely distributed plant species of the genus *Physalis* (Gulesci *et al.,* 2021; Kasali *et al.,* 2021; Mazova *et al.,* 2020; Shah & Bora, 2019). *Physalis peruviana* is found in different places, including India, Egypt, Ecuador, Nigeria, Nepal, Uganda, Kenya, China, Atacama Desert etc. *Physalis peruviana* has been used to cure a wide range of illnesses in the aforementioned locations, the diseases cured includes malaria, hepatitis, dermatitis, cancer, rheumatism, asthma, and fungal and bacterial infections (Wu *et al.,* 2004; Puente *et al.,* 2011; Fokunang *et al.,* 2017; Shah & Bora, 2019; Gulesci *et al.,* 2021; Kasali *et al.,* 2021; Yu *et al.,* 2021).

According to Nondo, Moshi, and Runyoro, *Physalis peruviana* has been utilized to treat a number of ailments in the Tanzanian regions of Kagera, Lindi, and Tanga. Nondo (2015) reported that the leaves of *Physalis peruviana* had been used by the communities of Kagera and Lindi in treating malaria; Moshi (2012) reported that the fruit of *Physalis peruviana* is used in treating typhoid fever by the people of Kikulu village in Muleba, Kagera, and also, Runyoro (2006) reported that the leaves of *Physalis peruviana* have been used in treating fungal infections by the communities of Lushoto in Tanga. Thus, in all places in Tanzania where *Physalis peruviana* has been reported to be used for managing various diseases, the leaves have not been reported to be used in treating typhoid fever.

Apart from *Physalis peruviana* being used in various areas of Tanzania, an ethnobotanical survey conducted in Mbeya Rural District revealed that the plant's leaves treat typhoid and other diseases (Personal communication, 2021). Locally, in Mbeya Rural Districts, the plant

can be prepared in two methods: extracting concoctions by boiling fresh leaves to the boiling point (100^0C) or soaking fresh ground leaves and dry powder in water for 10 minutes, 30 minutes, 1 hour and 24 hours. However, the amount of use (dosage) has not been established so far. Furthermore, the existing preparatory methods and used concentrations have not been established and documented. Therefore, this study wants to evaluate the existing indigenous preparatory methods to understand the concentration used in treating typhoid fever and validate the phytochemical compounds present in the plant. Therefore, to optimize the use of *Physalis peruviana*, the current study aims to generate the scientific information to validate the antibacterial effects of *Physalis peruviana,* which will further serve as a potential herbal plant in developing antibiotics against *Salmonella typhi* and other diseases in Mbeya Rural District.

1.2 Statement of the problem

Medicinal plants have been used by different communities worldwide in managing a diversity of illnesses (Berhanu *et al.*, 2020; Mailu *et al.*, 2020; Maroyi, 2013; Nortaa *et al.*, 2019; Nyakudya *et al.*, 2020; Offiah *et al.*, 2011; Okoli *et al.*, 2007; Araya, 2015). However, their use in many places, including Mbeya Rural District, Tanzania, has not been scientifically studied. In addition, the community-based methods used in preparing the plant extracts (*P. peruviana*), like in the case of Mbeya, are rarely described and optimized. Therefore, this study aims to fill this gap by providing a scientific analysis of the plant, optimizing preparation methods of *P. peruviana* extract, and identifying medicinal phytocompounds of *P. peruviana* for treating *S. typhi* using plant samples collected from the study.

1.3 Rationale of the study

This work was proposed to study the ethnobotany of *P. peruviana* efficacy of community-based preparatory methods for extracts of phytochemical compounds present in *P. peruviana* to manage *Salmonella typhi* in Mbeya Rural District. The leaf extracts from *P. peruviana* obtained by boiling and soaking was tested for their effectiveness against *Salmonella typhi*. Also, the plant extracts were analyzed by LC-MS/MS and confirmed for the presence of important compounds in the extracts resulting from soaking with antibacterial properties, which could help manage *Salmonella typhi*. Leaf extracts obtained by soaking were found to have a better impact on managing typhoid fever than leaf extracts obtained by boiling. Therefore, validating community-based preparatory methods can help manage typhoid fever.

1.4 Research objectives

1.4.1 General objective

To assess the ethnobotany and antibacterial effects of *P. peruviana* in managing typhoid fever infections in Mbeya Rural District.

1.4.2 Specific objectives

(i) To assess the ethnobotany value of *P. peruviana* in communities in Mbeya Rural District.

(ii) To determine the efficacy of *P. peruviana* leaf extracts against *Salmonella typhi* from the existing community-based preparatory methods.

(iii) To analyse the potential phytochemical compounds in *P. peruviana* leaf collected from Mbeya Rural District.

1.5 Research questions

(i) What is the ethnobotany value of *P. peruviana* in communities in Mbeya Rural District?

(ii) What is the efficacy of *P. peruviana* leaf extracts against *Salmonella typhi* obtained from the existing community-based preparatory methods?

(iii) What are the potential phytochemical compounds present in the *P. peruviana* leaf?

1.6 Significance of the study

The results of this study will lay the foundation for optimizing the indigenous methods and knowledge for integrated disease management in the study area and the country at large. Also, this study will enable traditional healers and household family members to decide on the best methods to treat typhoid fever. The study will also inform policymakers on the significance of promoting medicinal plants such as *P. peruviana*, which was used in this study against typhoid fever and other diseases. Furthermore, the findings from this study will lead other researchers to study parts of *P. peruviana* other than the leaf, including the diseases that can be treated using those plant parts. Moreover, this study will inform the respective organizations involved in herbal medicine to come up with the specific compounds to be used in treating typhoid fever.

1.7 Delineation of the study

This study assesses the ethnobotany and antibacterial effects of *P. peruviana* on *S. typhi* among the communities in Mbeya Rural District, Tanzania, through studying:

(i) Ethnobotany of *P. peruviana* plant,

(ii) In vitro study of community-based *P. peruviana* leaf extract formulations on the *S. typhi* bacterium.

(iii) Analyses of phytochemical compounds present in *P. peruviana* leaf extract conducted in two laboratories of two different institutions, namely Mbeya University of Science and Technology and the Tanzania Government Chemist Laboratory Authority.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of typhoid fever

Typhoid fever (TF) is still a major public health issue worldwide, particularly in impoverished areas of developing countries (Mukta, 2021). Although TF is one of the most common etiological sources of bacteremia in developing countries, poor hygiene and sanitation areas are more likely to have a high disease burden (Butt *et al.,* 2021). In developing countries (low- and lower-middle-income), over ten million infections from *S. typhi* occur annually, and three million infections occur in Africa only (Kim *et al.,* 2019).

According to scientific reports from different literature, the TF burden in Africa is higher than in other developing countries. This report on the burden of diseases is based on surveillance conducted in sub-Saharan Africa from 13 sites in 10 countries between 2010 and 2014 (Kim *et al.,* 2019).

In East Africa, outbreaks of TF have recently been reported in Kigoma, Tanzania; Kirehe, Rwanda; Kampala, Uganda; and Moyale, Kenya (Kim *et al.,* 2019).

2.2 Typhoid fever burden in Tanzania

According to Typhoid Vaccine Acceleration Consortium [TyVAC] (2017), there are 79 334 typhoid cases, 53% of which were children under 15 years of age; and 1671 typhoid deaths, 73% being children under 15 years of age. Besides the disease burden on Tanzania's mainland, other studies were conducted in Zanzibar the same year, and it was discovered that typhoid was the most commonly occurring disease. Furthermore, the disease is observed to be among the economic threats in Tanzania. According to the update on the Potential of typhoid conjugate vaccines in Tanzania (TyVAC, 2017), each typhoid case costs families an average of TZS 360 532.98, nearly two months of average family income.

Morbidity and mortality have been reported in individuals suffering from typhoid fever. This is due to complications in terms of treatments due to related symptoms of TF and malaria (Malisa & Nyaki, 2014). Moreover, other related studies were conducted in Pemba, Zanzibar, where the disease trend shows that the burden is still triggering and incurring high treatment

costs for the communities (Riewpaiboon *et al.,* 2014). In the Mbeya region, there is still no scientific report regarding the burden of typhoid fever.

2.3 Causative agent of typhoid fever

Typhoid fever is caused by *S. typhi*, a bacterium with the morphological characteristics rod-shaped (Malisa & Nyaki, 2014; Wain, 2014; Fazal, 2020; Arunkumar, 2022). Typhoid fever is transmitted by consuming or drinking contaminated food or water with the faeces of infected people (Gomes, 2022). Upon the entrance of *S. typhi* via food or drinks, the pathogen begins to multiply and overspread in an infected person's body. The exposure after infestation takes 30 days, and the symptoms may vary from mild to severe (Radhakrishnan *et al.,* 2018). The observed symptoms after infections include headaches, nausea, fatigue, constipation, and prolonged fever (Gomes, 2022; Zhang *et al.*, 2022).

2.4 Treatment

Typhoid fever is treated using antibiotics such as fluoroquinolones, i.e., ciprofloxacin, where antibiotic resistance is uncommon (Parry *et al.,* 2009). Otherwise, a cephalosporin such as ceftriaxone or cefotaxime is used (Phan & Wain, 2008). Ampicillin, amoxicillin, chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole are commonly used antibiotics (Amsalu *et al.*, 2021; Manchanda *et al.*, 2006; Ugboko & De, 2014). Antibiotic treatment usually reduces fatality by 1% (Jeon *et al.,* 2019). People may still carry typhoid bacteria in their faeces even though the symptoms have disappeared, meaning they can spread it to others. Because of the pathogen's persistence in the biliary tract, approximately 2% to 5% of people infected with typhoid fever develop chronic disease (WHO, 2018).

2.5 Prevention

Poor sanitation and a lack of access to safe drinking water are likely associated with typhoid fever infestations (Butt *et al.*, 2021). Therefore, good sanitation and access to safe water are essential for effectively preventing the spread of typhoid fever. Three types of vaccines are available globally, namely: oral live attenuated (Ty21a), a Vi-capsular antigen-based unconjugated polysaccharide (Vi-PS), and typhoid conjugate (TCVs) (Butt *et al.*, 2021. The first two vaccines, i.e., Ty21a and Vi-PS, were already licensed and recommended by the World Health Organization (WHO, 2018).

Usually, upon attaining the preliminary qualification by WHO, the typhoid conjugate is now approved for use (Vashishtha & Kalra, 2020). Two formulations of Ty21a, namely liquid and enteric-coated capsules for children above two years and all ages, are available (Organization *et al.*, 2018; Vashishtha & Kalra, 2020). The Typhoid conjugate vaccine is preferred for all ages (WHO, 2018). Generally, for preventing typhoid fever, sanitation, hygiene, and vaccination are of great importance (Im *et al.*, 2022). Also, individuals travelling to typhoid-endemic areas should adhere to precautionary hygienic practices to reduce their risk of infection (WHO, 2018; Batool *et al.*, 2021).

2.6 Multi-drug resistant typhoid fever

Multi-drug resistance is the lack of susceptibility to at least one antimicrobial agent among the three or more antimicrobial categories: beta-lactam, aminoglycoside, and macrolide (Batool *et al.*, 2021). For example, multidrug-resistant typhoid fever (MDRT) is a typhoid fever caused by *S. typhi* strains resistant to all three of the first-line recommended drugs for treatment, namely, chloramphenicol, ampicillin, and co-trimoxazole (Zaki & Karande, 2011).

There are two main mechanisms of drug resistance development in *S. typhi*, i.e., plasmid-mediated and chromosomal DNA-mediated mechanisms (Zaki & Karande, 2011). Plasmids are extrachromosomal, self-replicating circular pieces of DNA that can carry and transfer multiple resistance genes between bacteria (Hawkey, 1998). In *S. typhi*, the antibiotic resistance within the pathogen is associated with IncHI1 (incompatibility group H, subgroup 1) plasmids (Taylor *et al.*, 1985; Zaki & Karande, 2011). The resistance *S. typhi* was first reported in Mexico in 1972, whereby the pathogen was found to be resistant to streptomycin, tetracycline, sulphonamides, and chloramphenicol (Olarte & Galindo, 1973; Cooke & Wain, 2004; Zaki & Karande, 2011; Ugboko & De, 2014; Chauhan & Farooq, 2021). Subsequently, MDR *S. typhi* spread globally, and by 1998, IncHI1 plasmids could be isolated from MDR *S. typhi* worldwide (Phan & Wain, 2008; Zaki & Karande, 2011; Klemm *et al.*, 2016; Argimón *et al.*, 2021; Kumar, 2021). The drug resistance in the chromosome of the pathogen, especially for fluoroquinolones, has been facilitated by selective pressure on the bacterial population, hence resulting in a single point mutation in the quinolone resistance determining region (QRDR) of the topoisomerase gene GyrA, encoding DNA gyrase (Turner *et al.*, 2006; Qian *et al.*, 2020). DNA gyrase is an ATP-dependent enzyme required for DNA transcription, replication, and chromosome segregation (Barkume *et al.*, 2018). Structural DNA gyrase comprises two GyrA and two GyrB subunits (Barkume *et al.*, 2018), where DNA cleavage and reunion are formed.

Therefore, in multidrug resistance, DNA gyrase is a good target for antibacterial agents (Barkume *et al.,* 2018).

Moreover, the permeability decrease and the active efflux of the antimicrobial agent contribute to multidrug resistance (Turner *et al.,* 2006). Microbial permeability is the uptake of antibiotics into bacterial cell membranes. A decrease in membrane permeability in the pathogen is associated with the drug's failure to enter the pathogen membrane due to membrane alterations in the protein membrane, specifically during genetic transformation (Ugboko & De, 2014). The changes in the membrane alter the protein transport pores, leading to the failure of tetracycline, quinolones, and aminoglycosides to cross the pathogen's membrane (Ugboko & De, 2014). Microbial active efflux (MAE) is a transport protein involved in extruding toxic substrates, such as antibiotics, from within cells into the external environment (Webber & Piddock, 2003). These proteins are found in gram-positive and gram-negative bacteria (Webber & Piddock, 2003). One efflux pump can recognise a variety of pathogens, though sometimes one pathogen can be recognized by different efflux pumps (Bhardwaj & Mohanty, 2012). Different efflux pumps are found in the membrane of a pathogen. These include MATE (multidrug and toxic compound extrusion), ABC (ATP-binding cassette), SMR (small multidrug resistance), RND (resistance nodulation division), and MFS (major facilitator superfamily) (Bhardwaj & Mohanty, 2012). The survival of the pathogen in the host as a result of bacteria bile tolerance, hence leading to colonization and invasion, has been associated with efflux pumps (Turner *et al.,* 2006; Bhardwaj & Mohanty, 2012). Therefore, as multidrug resistance has been an important public health issue, it is necessary to look for folk medicine. Medicinal plants usually contain diverse bioactive compounds that can act against different microorganisms (Chauhan & Farooq, 2021).

2.7 Medicinal plants

Medicinal plants are a chief source of secondary metabolites used for therapeutic purposes (Ogutu *et al.,* 2019). Usually, a plant becomes medicinal when its biological activities have been reported scientifically (Ogutu *et al.,* 2019). Medicinal plants have been used in healthcare since ancient times (Sofowora *et al.,* 2013; Woldemariam & Demissew, 2021). About 80% of people in developing countries use traditional medicines for their health care (Kim, 2005). The important advantages of medicinal plants for therapeutic uses in various ailments are their safety and being inexpensive, effective, and easily accessible (Haq, 2004). *Physalis peruviana* is among the medicinal herbs used to treat different diseases (Cueva *et al.,* 2017; El-beltagi *et*

al., 2019; Shah & Bora, 2019; Abou-Baker & Rady, 2020). The medicinal uses, including the part of the plant used, traditional uses, and formulation, are summarized in Table 1.

2.8 *Physalis peruviana*

Physalis peruviana is a plant of the Solanaceae family, native to South America in the Andes region (Kathare *et al.,* 2021). The plant (*P. peruviana*) is among the 120 species of the genus *Physalis* spread worldwide (Shah & Bora, 2019; Chhikara *et al.,* 202; Kathare *et al.,* 2021). *Physalis peruviana* grows perennially in the tropics and is an annual fruit in temperate regions (Shah & Bora, 2019). The yearly average temperature of the plant is between 13–18 °C, tolerating temperatures of 30 °C or above. The amount of growth rainfall for the plant ranges from 800 mm to 4300 mm for well-drained soil; also, the plant prefers full sunlight or partial shade sun, and the favourable soil for plant growth is sandy loam (Namrta Singh *et al.,* 2019). *Physalis peruviana* is an important plant, and it has been widely used for centuries, mainly in folk medicine (Franco *et al.*, 2007; Gulesci *et al*., 2021; Toro *et al*., 2014). Other medicinal properties associated with *P. peruviana* include antispasmodic, diuretic, antimicrobial, antiseptic, antioxidant, sedative, analgesic, helping to fortify the optic nerve, throat trouble relief, elimination of intestinal parasites and amoeba (El-beltagi *et al.,* 2019; Hassan *et al.,* 2017; Huang *et al.,* 2020; Perk *et al.,* 2013; Namrata Singh *et al.,* 2019).

Other medicinal properties of *P. peruviana* worldwide are indicated in Table 1. The health benefits of *P. peruviana* include purifying the blood, decreasing albumin in the kidneys, reconstructing and fortifying the optic nerves, alleviating throat infections, eliminating intestinal parasites, immune system support, digestive health, diabetes management, and digestive health (El-beltagi *et al.,* 2019; S. Huang *et al.,* 2020). *Physalis peruviana* possesses different phytochemical compounds: phenolics, terpenes, tannins, flavonoids, saponins, withanolides, carotenoids, and alkaloids (Mazova *et al.,* 2020; Muñoz *et al.,* 2021). All the mentioned phytochemical compounds are distributed in different parts of plants, such as the root, stem, bark, leaves, flowers, fruits, and seeds. Research indicates *P. peruviana* harbours a diversity of phytochemical compounds that have medicinal properties; this plant has been considered beneficial in treating different bacterial diseases, including TF, as indicated in Table 1.

Typhoid fever continues to be a public health concern worldwide due to an increase in the resistance of antibiotics used to treat bacterial infections diseases (Abiduzzaman *et al.,* 2023;

Mohsin *et al.,* 2021). Therefore, since *P. peruviana* has medicinal properties against microorganisms, including *S. typhi*, the plant can be used as an alternative folk medicine (Kasali *et al.,* 2021; Ramos-Sotelo & Rojas-Rojas, 2022). Also, it is important to investigate further existing community-based formulations of *P. peruviana* against *S. typhi* for documentation and further research. Studying indigenous formulations has become crucial due to the fact that many communities worldwide have been depending on folk medicine to cure different diseases (Chhikara *et al.,* 2021; Resmi & Anju, 2023). One factor that has led different communities to overdependence on medicinal plants, including *P. peruviana,* is the unavailability of health facilities such as hospitals in their homes.

For this reason, many people have been sharing their knowledge of different medicinal plants, including diseases treated using particular plants. Moreover, different preparatory methods, such as decoction (boiling) and maceration (soaking), have been used in different communities. Therefore, as long as the users, such as traditional healers and household members, have reported good results from the medicinal plants, preparatory methods, and different formulations, they will have an impact in treating different diseases, including typhoid fever. Among the advantages of medicinal plants, including *P. peruviana,* having a good impact on different diseases is that they contain a diversity of phytochemical compounds that act synergistically against microbes (Huang *et al.,* 2020; Korany *et al.,* 2022). Therefore, studying on standardization of dosages and determining the chemical composition is important for developing corners (Matovu *et al.,* 2020; Mutai *et al.,* 2021). Therefore, based on the evidence from the literature, it is important to validate community-based preparatory methods as well as the concentrations being used and identify the phytochemical compounds present in *P. peruviana* for further research, especially the development of herbal medicine that can be used to manage typhoid fever in Mbeya Rural District and Tanzania in general.

Table 1: Ethnobotanical uses of *Physalis peruviana* in different countries

Vernacular name		Part(s) used	Traditional uses	Formulations	References
Cameroon		Twigs	Cancer	-	Mbaveng *et al*. (2018)
	Ajijieuh	Leaf and stem	Bile, swelling of legs for pregnant women	Maceration	Yemele *et al*. (2015)
	Mapepie	Leaf and Stem	Fungal infections	Maceration	Tchuenguem *et al*. (2017)
Colombia	Uchuva	Fruit	Ear pain and diabetes		Khalaf-allah *et al*. (2015)
DRC	Mbuma	Leaf	Malaria, intestinal worms,	Decoction and infusion	Mboni *et al*. (2019)
	Mbuma, Mbupuru	Aerial part	Diabetes mellitus, spleen, and malaria	Decoction	Kasali *et al*. (2021)
	Donamas	Fruit	Gastric	Mastication	Murtem (2016)
	Fatki	Leaf and root	Leucorrhea and hydrocele	Decoction	
	Kopalphoota	Whole plant	Jaundice		Barua (2014)
India	Phakephake	Ripe fruit	Throat sore	Mastication	Chettri *et al*. (2014); Sarvalingam *et al*. (2017)
	Pottipalam	Leaf and dried seed	Jaundice and glaucoma		
		Leave	Jaundice	Decoction	Thomas *et al*. (2013)
		Whole plant	Gout		Kapoor *et al*. (2017)

Vernacular name		Part(s) used	Traditional uses	Formulations	References
Indonesia	Depuk-depuk	Fruit and whole plant	Smallpox	Decoction	Silalahi (2018)
	Pultak-pulta	All parts of the plant	Stomache	Decoction	Simbolon (1994)
Kenya	Embunwe, Emiilwa	Stem, root, fruit, and leaf	Inflammation and abdominal ailments	Infusion	Wanzala *et al*. (2012)
	Mayengo	Leaf	Malaria	Decoction	Mukungu *et al*. (2016)
	Munathi	Leaf	Postpartum pain	Decoction	Njoroge and Bussmann (2009)
	Munathi	Leaf	Anthelmintic and typhoid		Njoroge and Kibunga (2007)
	Munathi	Seed, bulb, fruit, leaf and root	Diarrhea	-	Njoroge and Kibunga (2007)
		Leaf	Diabetes, and malaria	Decoction	Maobe *et al*. (2013)
	Gangathopa	Root	Jaundice	Maceration	Rai (2004)
Nepal	Ram bhutka, Jangali mewa	Root	Piles	-	Acharya and Pokhrel (2006)
		Leaf	Sore throat and abdominal pain	-	Paudel *et al*. (2018)
New guinea	Mondon	Leaf	Boils and ulcers	Heating	Wilhelm *et al*. (1992)

Vernacular name		Part(s) used	Traditional uses	Formulations	References
Rwanda	Umuhuhu	Leaf	Facilitate the issuance of the placenta and abortifacient	-	Chagnon (1984)
South Africa	Igquzu	Leaf	Diarrhea	-	Bisi-Johnson (2010)
	Igquzu	Leaf	Diarrhea	Decoction	Madikizela *et al*. (2012)
		Whole plant and leaf	Cancer	Decoction	Fouche *et al*. (2008)
Tanzania	Kitutun kikubwa	Leaf	Malaria	Maceration	Nondo *et al*. (2015)
	Msupu	Leaf	Skin fungal	-	Runyoro *et al*. (2006)
	Ntuntunu	Fruit	Typhoid fever	-	Moshi *et al*. (2012)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The research was conducted in five villages in Mbeya Rural District: Iwindi, Utengule Usongwe, Swaya, Horongo, and Mpinduzi. Mbeya Rural District was selected because there is high practice of using *P. peruviana* as a medicinal plant for treating variety of diseases. The district is located at 7° and 9° Southern Equatorial Latitude and 33° and 35° Eastern Prime Longitude (Fig. 1). Within Mbeya Mountain ranges, the district is situated between 1600 and 2400 meters above sea level. The district receives 1200 mm of rain each year, with an average temperature of 25 °C. The average lowest and maximum temperature ranges are 11 °C and 28 °C, respectively.

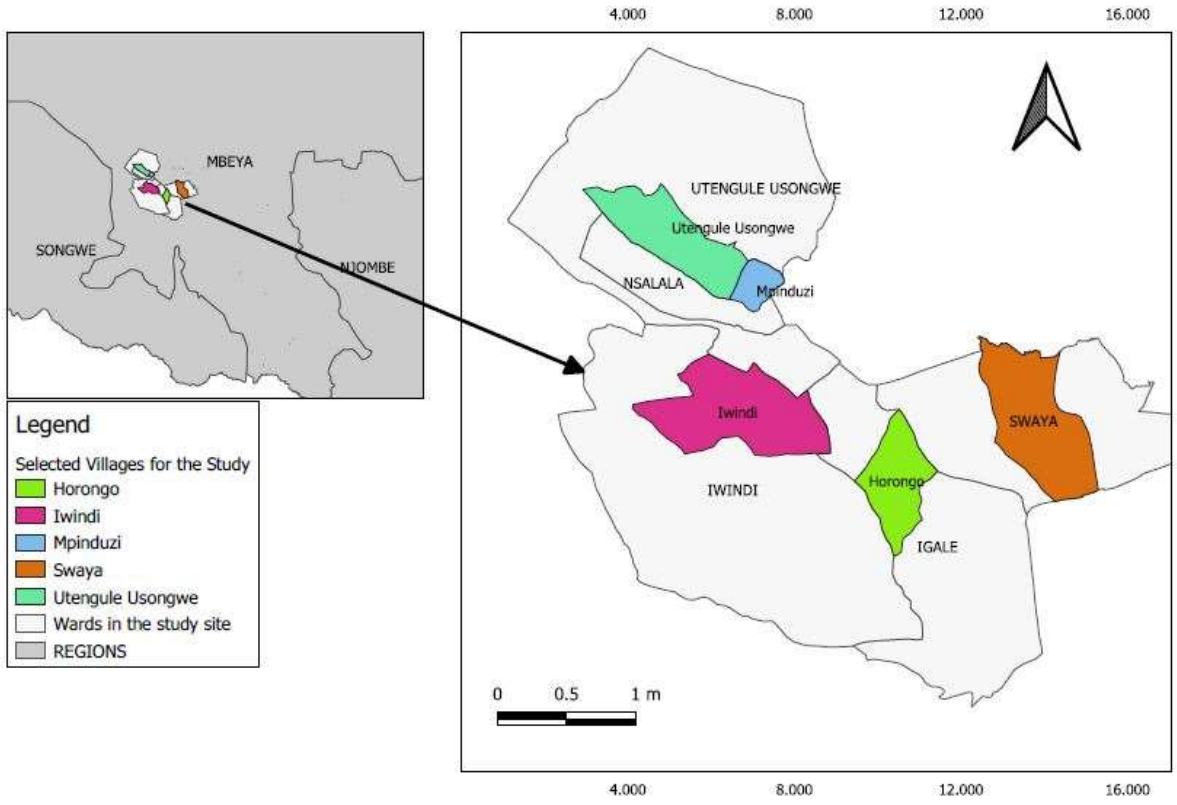

Figure 1: Study sites location in Mbeya Rural district, Tanzania

3.2 Study design

A community-based cross-sectional research approach was used to determine the knowledge, attitude, and practice of using *P. peruviana* in managing typhoid fever within the community in Mbeya Rural District between April and June 2021.

3.3 Study population

Family members and traditional healers served as the study's source population. Key informants were included in the research population if they were eighteen years or older since this age can provide good information regarding the use of medicinal plants. The selected populations were based on their knowledge of *P. peruviana* and herbal medicine preparation methods from *P. peruviana* in managing typhoid fever.

3.4 Sample size estimation

About 108 participants were selected using the method described by Angelsen *et al*. (2011). So, in this study, 25% of 60 registered traditional healers in the district and 25% of the 74 known household members in each village were chosen to represent the district whole population.

3.5 Sampling techniques

Purposive and snowball sampling was used to select key informants. The district traditional healer chairman purposefully selected the traditional healers since the government registers them; therefore, they can be easily identified. The snowball sampling techniques were used to identify the household family members through village leaders. The identified household family member is the one who will help to identify another household family member.

3.6 Data collection

During data collection in all villages between April and June 2021, 108 key informants were interviewed, including 93 household members and 15 traditional healers. The Focus Group Discussion (FGD) was used to collect the data from traditional healers, whereby for household family members, a structured questionnaire was used to collect data. Researchers visited household members at their homes for interviews. The checklist of structured questions and structured questionnaire were tools used to collect data from both traditional healers and

household family members respectively. The method used to gather information from household family members and traditional healers is discussed below.

(i) Focus group discussion

There were 15 participants, which included two groups for the focus group discussion, each with eight and seven people. The day, place, and meeting time between researchers and traditional healers were arranged. When the time came to meet the chosen group of traditional healers, they were first shown the aim of the study, then asked to sign a consent form (Appendix 3), and lastly, the structured questionnaire was used to gather the data (Appendix 2). Two focus group discussion, which took place on April 20 and 28, 2021, was centred on participatory interaction between the researcher and traditional healers. All the information collected was recorded in the notebook for further steps, especially information compilation and data analysis.

Plate 1: Focus Group Discussion between the researcher and traditional healers in study villages

(ii) Questionnaire survey

A structured questionnaire was used to collect the data from household family members. The interviews involved ninety-three (93) participants. The day, date, and time to meet with the household family members were communicated between the researcher and the village leaders in each village so that an arrangement could be made. When the visitation day, date, and time were reached in each village, each visiting household member was individually visited in their homes. The research's goal was first explained to each member of the chosen home family, who then signed the consent form (Appendix 3) before the structured questionnaire's questions were used to gather the data (Appendix 2). Finally, the information collected was recorded on the questionnaire for further analysis and documentation.

Plate 2: Questionnaire survey to the household family members

3.7 Whole plant and leaves collection

Leaves from semi-mature and mature *P. peruviana* were collected from Swaya village, where there is a forest. All five villages depend on this forest to collect medicinal plants. First, the collected leaves were washed with tape water, distilled water and air-dried under the shed for one month. Then, the dried leaves were ground into powder using hot water and cleaned local motor and pestle. The powder was stored in sterile nylon bags for transportation to Arusha for further laboratory analysis. When the ground of the air-dried samples was complete, the fresh leaves from semi-mature and mature *P. peruviana* were collected and packed in sterile nylon bags and kept in a cool box.

Furthermore, one whole plant was collected from Swaya village and packed in a paper bag. The dried powder, fresh leaves, and the entire plant were transported to Arusha to the National Herbarium at Tanzania Plant Health and Pesticides (TPHPA) for identification. It was given a

collection number of JE 1563. After plant identification, analysis was conducted at the NM-AIST Microbiology, GCLA, and MUST laboratories. The analysis was conducted in three different laboratories depending on the availability of materials and equipment.

Plate 3: Under shed air-drying of semi-mature and mature *Physalis peruviana* leaves

3.8 Determination of the efficacy of different extracts from the existing community-based preparatory methods against *Salmonella typhi*

3.8.1 Preparation of *Physalis peruviana* leaf extracts

The methods described by household members and traditional healers were applied while preparing *P. peruviana* leaf extracts. Briefly, to obtain the extracts, a hand full of fresh leaves from semi-mature and mature *P. peruviana* (equivalent to ten and twelve grams of leaves from each plant) were boiled with 100 ml of water in the pan. For soaking of fresh and dry ground leaves, one, two, three, and four food spoons equivalent to 11 g, 22 g, 33 g, and 44 g of fresh leaves and 5 g, 10 g, 15 g, and 20 g of dry leaves respectively were measured and soaked in 100 ml of cold and hot water at different time intervals (10 minutes, 30 minutes, 1 hour, and 24 hours, respectively). The extracts from boiling and soaking were kept in falcon tubes and stored in a refrigerator at 4 °C, ready for use.

The assigned codes hereunder were used for the identification of leaf extracts; (Aqueous Boil Extracts of Semi-mature *P. peruviana* Leaf (ABSML), Aqueous Cold Extracts of Semi-mature *P. peruviana* leaf (ACSML), Aqueous Hot Extracts of Semi-mature *P. peruviana* Leaf (AHSML), Aqueous Boil Extracts of Mature *P. peruviana* Leaf (ABML), Aqueous Cold Extracts of Mature *P. peruviana* Leaf (ACML), and Aqueous Hot Extracts of Mature *P. peruviana* Leaf (AHML).

3.8.2 Evaluation of aqueous *Physalis peruviana* leaf extracts against the *Salmonella typhi*

Salmonella typhi (ATCC 13062) pathogen collected from Mount Meru Referral Hospital in Arusha was used to test the antibacterial activity of *P. peruviana* leaf extracts. Disc diffusion methods described by Kamau *et al*. (2020) were used to evaluate the antibacterial activities of leaf extracts. Briefly, to obtain active culture, a loopful of *S. typhi* pathogen from stock cultures was cultured in Muller-Hinton petri dish agar, followed by incubation overnight at 37 ^{0}C. To get results equal to 2 x 10^{6} CFU/ml of bacteria, dilutions of multiple colonies were produced in 2 ml of normal saline by comparing their turbidity to the 0.5 McFarland standard. Sterile filter paper discs (Whatman No. 1, GE Healthcare Company, UK) of 6 mm in diameter were soaked in plant extracts having different concentrations. Ciprofloxacin 30 µg/ml was used as a positive control, and discs were impregnated with sterile distilled water as a negative control. The extract-containing discs were put together on inoculated plates with positive and negative controls. The plates were let to stand for 5–10 minutes for the extract to diffuse. A 24-hour incubation period at 37 °C was used to test the antibacterial activity of the plates. The antibacterial activity was determined by forming inhibition zones surrounding the disc containing the extract, measured in millimeters using the meter rule. The mean zone of inhibition (mm) and standard error mean of three triplicate measurements were used to express the results because the experiment was done in triplicate for each extract.

3.9 Handling of the *Salmonella typhi* pathogen in the laboratory

The *Salmonella typhi* (ATCC 13062) isolates were collected from Mount Meru Referral Hospital and taken to the NM-AIST microbiology laboratory. It was kept in a cryo vial and stored in a -20 ^{0}C freezer to prevent the infection from spreading to laboratory personnel. The inoculation procedures were carried out at the NM-AIST microbiology laboratory using a biosafety cabinet to avoid risk exposure to the laboratory personnel working with the pathogen. At the end of the inoculation process, the used apparatuses were sterilized by framing and

autoclaving, while the used disposable materials were sterilized by spraying with 70% alcohol, followed by disposing of them in a container labelled "highly infectious materials."

3.10 Phytochemical analysis of *Physalis peruviana* leaf collected from Mbeya, Tanzania

3.10.1 Qualitative phytochemical screening of *Physalis peruviana* leaf

The standard qualitative procedures described by Akpor *et al.* (2021); Ayoola *et al.* (2008); Cheikhyoussef *et al.* (2015) and Wadood *et al.* (2013) were carried out at MUST laboratory. As stated below, the carried-out techniques were utilized to determine whether phytochemical substances were present in P. peruviana leaf tissue.

(i) Test for alkaloids

Two milliliters of the leaf extract were dissolved in 1% HCl and added a few drops of Wagner's reagent. The color change into a cream/brown precipitate indicates the presence of alkaloids (Akpor *et al.,* 2021).

(ii) Test for saponins

The 0.5 ml of the leaf extract was added to 5 ml of distilled water, followed by vigorous shaking. The presence of persistent foaming indicated the presence of saponins (Akpor *et al.,* 2021).

(iii) Test for steroids

To 2 ml of the extract, 1 ml of chloroform was added, followed by the addition of concentrated H_2SO_4 along the side of a vessel containing the extract; a color change to a cream/red upper layer confirms the presence of steroids (Akpor *et al.,* 2021.

(iv) Test for terpenoids

To the test tube containing 5 ml of extract, 2 ml of chloroform was added, followed by the addition of a few drops of concentrated H_2SO_4 along the side of the test tube containing the extract. The presence of terpenoids was indicated by the formation of a grey color (Cheikhyoussef *et al.,* 2015; Akpor *et al.,* 2021).

(v) Test for anthraquinone

To 2 ml of the leaf extract, 1 ml of 10% ammonia was added. The change of color into red/violet/pink confirms the presence of anthraquinone (Akpor *et al.,* 2021).

(vi) Test for flavonoid

To 0.5ml of the leaf extract, 1ml of dilute NaOH was added. The formation of a yellow color/precipitate soluble in dilute mineral acids indicates the presence of flavonoids (Akpor *et al.,* 2021).

(vii) Test for phlobatannins

To 2 ml of the leaf extract, a few drops of 1% dilute hydrochloric acid was added and then boiled. The colour change of the red precipitate indicates the presence of phlobatannins (Wadood *et al.,* 2013; Akpor *et al.,* 2021).

(viii) Test for phenols

To 1ml of the leaf extract, 5% ferric chloride was added. The formation of a blue color confirmed the presence of phenols (Ayoola *et al.,* 2008; Akpor *et al.,* 2021).

(ix) Discarding chemicals used during phytochemical screening

At the end of the phytochemical screening processes, all used chemical solutions were discarded following the disposal methods specified in the material safety data sheet (MSDS) provided by the chemical manufacturer.

3.10.2 Liquid Chromatography-Mass Spectroscopy (LC-MS/MS) analysis

Liquid chromatography-mass spectroscopy (LC-MS/MS) was used to identify phytochemical compounds in *P. peruviana* leaf. Liquid chromatography-mass spectroscopy was analyzed at the Government Chemist Laboratory Authority, Dar es Salaam. During this process, different procedures were carried out as described below:

(i) Preparation of *Physalis peruviana* leaf extracts

One gram of each of ground fresh and powdered semi-mature and mature *P. peruviana* was weighed and placed into a centrifuge tube. The tube was filled with 10 ml of the extracting solvent (water), which was then warmed in a 100^{o}C water bath for 5 minutes before being sonicated for 30 minutes. The mixture was decanted, and the extraction was repeated twice. The resulting extracts were concentrated under nitrogen to dryness. For each extract, 1 ml of acetonitrile water with a ratio of 1:1 was added and centrifuged at 13 000 rpm for 10 minutes. The supernatant was collected for LC-MS/MS analysis.

Liquid chromatography (LC) (Ultimate 3000) coupled with a tandem mass spectrometer was used for the analysis of phytochemical compounds present in the sample. An aliquot of 5l for each extracted sample was injected into a column (Hypersil GOLD aQ) of C18 with a particle size of 1.9l and a 100 x 2.1 mm dimension size. Three solutions, namely, solution A: 0.1% of formic acid in the water, B: 0.1% in acetonitrile, and C: 1:1 methanol to mil Q water, were present in the mobile phase. The oven temperature was set at 40^{o}C while the running time for each sample was 30 minutes. With a collision energy of 45 v, the mass spectrometry (Q-Exactive, Orbitrap) ran in the electron ionization energy mode. The mass scan range was from 150-2000 m/z with a resolution of 140 000. The compounds in the mixture were identified by comparing the mass spectra with those in the National Institute of Standards and Technology (NIST) library.

3.11 Statistical analysis

The collected data on demographic information and knowledge of *P. peruviana* were subjected to descriptive statistics and analyzed into frequencies using Excel and Statistical Package for the Social Sciences (SPSS) version 20 to determine their percentages. The chi-square statistical test was also used to determine the association between the demographic information. The concentrations of the extracts were subjected to an ANOVA using R programming software to determine whether there were statistical differences between their means.

3.12 Ethical clearance

Permission to carry out the study was granted by the KNCHREC (Kibong'oto Infectious Diseases Hospital, Nelson Mandela African Institution of Science and Technology, and the Centre for Educational Development in Health), Arusha, Tanzania, whereby an ethical number

of KNCHREC 00052/08/2021 was given. Permission to collect the data was further given by Mbeya region and Mbeya District offices.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographic information

Among 108 interviewed key informants, 13.9% (15/108) were traditional healers, and 86.1% (93/108) were household members; from 93 household members, 52% (52/93) were male, and 48% (45/93) were female; more than half of household members (53%; 49/93) were over 50 years old. For education, the majority of participants (73%; 68/93) attended primary school, and a few (5%; 5/93) attended tertiary education. While most traditional healers (66.7%; 10/15) were male and more than half of the traditional healers were above 50 years old, about traditional healers' education, the majority (66.7%; 10/15) attended primary school. All the information is summarized in Table 2. The characteristics of household members in different villages are presented in Table 3.

Table 2: Information on the demographics of both household members and traditional healers

Variable	Category	HH, n (%)	TH, n (%)
Gender	Male	48(52)	10 (66.7)
	Female	45(48)	5(33.3)
Age	<18	0 (0)	0 (0)
	18-30	6 (6)	0 (0)
	31-40	17(18)	1(6.7)
	41-50	21(23)	6 (40)
	>50	49 (53)	8 (53.3)
Marital status	Single	2(2)	0(0)
	Married	90(97)	15(100)
	Divorced	0(0)	0(0)
	Widow/widower	1(1)	0(0)
Education level	Primary school	68(73)	10(66.7)
	Secondary school	7(8)	0(0)
	Tertiary	5(5)	0(0)
	Didn't attend school	13(14)	5(33.3)

HH= Household, TH= Traditional healers, n= number, %= percentage

Table 3: Characteristics of household members in selected villages

Variables	Villages						Chi-square	*p*-value
	Swaya	U/Usongwe	Mpinduzi	Iwindi	Horongo	Overall		
Gender								
Males	47.4	42.1	58.8	47.4	63.1	51.8	12.39	0.014
Females	52.6	57.9	41.2	52.6	36.9	48.2		
Age								
18-30	10.5	10.5	0	10.5	0	6.3	124.36	<0.001
31-40	36.8	5.3	29.4	15.8	5.3	18.5		
41-50	21	21	29.4	31.6	5.3	21.7		
>50	32.6	63.2	35.3	42.1	89.5	52.5		
Marital status								
Single	0	5.3	5.9	0	0	2.2	14.37	0.006
Married	100	94.7	94.1	100	100	97.8		
Education level								
Primary school	73.7	68.4	76.5	68.4	78.9	73.2	41.99	<0.001
Secondary school	0	15.8	5.9	10.5	5.3	7.5		
Tertiary	5.3	5.3	5.9	10.5	0	5.4		
Didn’t attend school	21.1	10.6	11.8	10.5	15.8	13.96		

U/Usongwe – Utengule/Usongwe

4.1.2 Knowledge of *Physalis peruviana*

The section presents information concerning *P. peruviana* and how it is used in the community to manage typhoid fever and other diseases. The summary of how the plant is used is presented in Table 4.

Table 4: Linkages between key informants and information associated with *Physalis peruviana*

Variable	HH, n (%)	TH, n (%)
Awareness on *P.peruviana*	Yes-93(100)	Yes-15(100)
P. peruviana and typhoid fever	Yes-93(100)	Yes-15(100)
P. peruviana and other diseases	Yes-59(63.4) No-11(11.8)	Yes-15(100)
Other diseases	I don't know-23(24.7) Fever-4(4.3) GID-8(8.6) Hemorrhoids-1(1.1) Intestinal worms-2(2.2) Malaria-6(6.5) Peptic ulcers-16(17.2) Stomach ache-9(9.7) Typhoid in chicken-2(2.2) Urinary tract infection-11(11.8)	Malaria-3(20.0) Peptic ulcers-4(26.7) Stomach ache-5(33.3) Urinary tract infection-3(20.0)
Part of plant used as herbal medicine	Root-0(0) Stem-0(0) Leaf-93(100) Flower-0(0) Fruit-0(0)	Root-0(0) Stem-0(0) Leaf-15(100) Flower-0(0) Fruit-0(0)
Herbal medicine traditional preparation methods	Boiling-28(30.1) Soaking -65(69.9)	Boiling-5(33.3) Soaking -10(66.7)
Herbal medicine administration	Oral-93(100)	Oral-15(100)
Dissolving time	Less than one day-64(68.8) One day-29(31.2)	Less than one day-9(60) One day-6(40)
Measurements of volume of water and amount of leaves	Yes-54(58.1) No-39(41.9)	Yes-15(100)
Amount of ground/powder leaves	Approximated-40(40.3) Glass full of powder leaves-2(2.2) Half glass of grinded fresh leaves-7(7.5)	One food spoon of powder leaves-2(13.3) Quarter a glass of grinded fresh leaves-4(26.7) Two food spoon of powder leaves-6(40.0)

Variable	HH, n (%)	TH, n (%)
	Half glass of powder leaves-1(1.1) Half tea cup of grinded fresh leaves-4(4.3) Half tea cup of powder leaves-2(2.2) Hand full of grinded fresh leaves-2(2.2) One food spoon of grinded fresh leaves-4(4.3) One food spoon of powder leaves-5(5.4) Quarter a glass of grinded fresh leaves-1(1.1) Quarter glass of powder leaves-2(2.2) Tea cup full of grinded fresh leaves-4(4.3) Tea cup full of leaves-2(2.2) Three food spoon of powder leaves-4(4.3) Two food spoons of grinded fresh leaves-2(2.2) Two food spoon of powder leaves-11(11.8)	Glass full of grinded fresh leaves-1(6.7) Half glass of grinded fresh leaves-2(13.3)
Amount of water	Approximated-40(43.0) Glass full of cold water-4(4.3) Glass full of hot water-7(7.5) Half a liter of cold water-1(1.1) Half glass of hot water-1(1.1) Half glass of hot water-1(1.1) Half tea cup of cold water-1(1.1) One liter of cold water-2(2.2) Quarter a glass of cold water-2(2.2) Quarter a glass of hot water-17(18.3) Quarter a liter of hot water-2(2.2)	Glass full of hot water-5(33.3) Half glass of cold water-1(6.7) One liter of hot water-2(13.3) Quarter a glass of hot water-6(40.0) Quarter a liter of hot water-1(6.7)

Variable	HH, n (%)	TH, n (%)
	Quarter tea cup of hot water-5(5.4)	
	Tea cup full of cold water-2(2.2) Tea cup full of hot water-5(5.4) Three-quarters a glass of cold water-3(3.2) Tea cup full of cold water-2(2.2) Tea cup full of hot water-5(5.4) Three-quarters a glass of cold water-3(3.2)	
Duration of the dose	Less than a week-51(54.8) More than a week-3(3.2)	Less than a week-6(40.0) More than a week-3(20.0)
Dose per day	One week-39(41.9) Once per day-17(18.3) Thrice per day-56(60.2) Twice per day-20(21.5)	One week-6(40.0) Once per day-6(40.0) Thrice per day-5(33.3) Twice per day-4(26.7)
Dose repetition	Yes-63(67.7) No-30(32.3)	Yes-15(100)
Efficacy btn industrial medicine and herbal medicine	Yes-55(59.1) No-10(10.8) I don't know-28(30.1)	Yes-15(100)
Side effects associated by herbal medicine	No-84(90.3) I don't know-9(9.7)	No-15(100)
Any diagnostic test before herbal medicine use	Yes-43(46.2) No-50(53.8)	Yes-7(46.7) No-8(53.8)
Plant type used as herbal medicine	Semi-mature-60(64.5) Mature-33(35.5)	Semi-mature-9(60.0) Mature-6(40.0)

(iii) Awareness of the *Physalis peruviana*

It was observed that 100% of the key informants (household family members and traditional healers) population were familiar with *P. peruviana* (Table 4).

(iv) *Physalis peruviana* and typhoid fever

According to the survey data, 100% of key informants (household family members and traditional healers) said that the leaf from *P. peruviana* is used to treat typhoid fever (Table 4).

(v) Other diseases treated by *Physalis peruviana* leaf

The survey's findings revealed that *P. peruviana* leaf is also used to treat other diseases, as stated by household members, such as intestinal worms; (2.2%; 2/93); peptic ulcers (17.2%; 16/93); and (26.7%; 4/15) by traditional healers. In addition, the household members also mentioned gastrointestinal diseases (8.6%; 8/93); urinary tract infections (11.8%; 11/93); and traditional healers (20%; 3/15). Furthermore, both household members and traditional healers mentioned malaria (6.5%; 6/93); 20%; 3/15); stomach aches (9.7%; 9/93); and 33.3%; 5/15). Not only that, but also household members mentioned fever (4.3%; 4/93), hemorrhoids (1.1%; 1/93; 2/93), and typhoid in chicken (2.2%; 2/93) (Table 4).

(vi) Part of *Physalis peruviana* used to treat typhoid fever

The results from the survey showed that 100% of the key informants (household family members and traditional healers) use the leaf and no other parts from *P. peruviana* in treating typhoid fever (Table 4).

(vii) Herbal medicine preparation

The findings showed that boiling was stated by 30.1% of home family members and 33.3% of traditional healers, whereas soaking was reported by 69.9% of home family members and 66.7% of traditional healers (Table 4).

(viii) Application of measurements (estimation) in preparing herbal medicine

According to the findings, 58.1% of home family members and 100% of traditional healers use measurements during herbal preparation, whereas 41.9% do not (Table 4).

(ix) Amount of ground or powder leaves used for the preparation of herbal medicine

The findings revealed that 43% of home family members use estimation, whereas 57% of home family members and 100% of traditional healers utilize measurement (Table 4).

(x) The amount of water used for the preparation of herbal medicines

Based on the data, it was noted that whereas 57% of home family members and 100% of traditional healers utilize measures, only 43% use approximations (Table 4).

(xi) Dissolving time of ground or powder leaves

The survey revealed that while 67.7% of home family members and 60% of traditional healers often dissolve the ground or powder leaves for less than one day, 32.3% of household family members and 40% of traditional healers typically do so for one day (Table 4).

(xii) Herbal medicine administration

According to 100% of the key informants (household family members and traditional healers), the findings showed that the oral route is the sole way to administer herbal medicines (Table 4).

(xiii) Herbal medicine dose use

18.3% of household family members and 20% of traditional healers reported using herbal medicine once per day, 60.2% of household family members and 26.7% of traditional healers reported using herbal medicine twice per day, and 21.5% of household family members and 46.7% of traditional healers reported using herbal medicine three times per day, per the results (Table 4).

(xiv) The herbal medicine dose duration

Results showed that 40.9% of household family members and 40% of traditional healers use the dose for one week, 40.9% of household family members and 40% of traditional healers use the dose for more than one week, and 5.4% of household family members and 20% of traditional healers stated that they usually use the dose for less than a week (Table 4).

(xv) Herbal medicine dose repetition

According to the findings, 67.7% of home family members and 100% of traditional healers claimed that herbal medication is repeated, whereas 32.3% of household members claimed that the dose is never repeated (Table 4).

(xvi) Side effects associated with herbal medicine

The findings showed that 90.3% of home family members and 100% of traditional healers said there were no ill effects from herbal medication, while 9.7% of household members said they were unsure (Table 4).

(xvii) Diagnostic test prior to the use of herbal medicine dose

The findings found that whereas 53.8% of household members and 53.3% of traditional healers do not undertake diagnostic testing, 46.2% of domestic family members and 46.7% of traditional healers do (Table 4).

(xviii) Type of *Physalis peruviana* used as herbal medicine

According to the findings, semimature *P. peruviana* is used by 64.5% of home family members and 60% of traditional healers, whereas mature *P. peruviana* is preferred by 35.5% of household family members and 40% of traditional healers (Table 4).

4.1.3 Antibacterial activity of aqueous *Physalis peruviana* extracts by agar diffusion method

The results of the agar diffusion test against *S. typhi* are presented in Tables 5, 6, and 7. The results showed that ABSMFL had inhibition of (3.66 ± 2.98) mm while ABMFL did not inhibit bacterial growth. For semi-mature *P. peruviana* fresh leaf extracts, the highest inhibitory activity was that of ACMFL (0.44 g/ml) with inhibition of (14.33±0.27) mm in 1 hour, followed by AHSMFL (0.44 g/ml) with inhibition of (13±0.98) mm in 30 minutes, and that of ACMFL (0.44 g/ml) with inhibition of (12.33±0.54) mm in 30 minutes while the least inhibition of (2.66±2.17) mm was that of ACMFL (0.22 g/ml) in 24 hours. Also, for dry leaf extract, AHMDL had high inhibition of (15.16±0.36) mm in 24 hours, followed by ACMDL (0.20 g/ml) with inhibition of (12.83±1.06) mm in 24 hours, and AHSMDL (0.05 g/ml) with inhibition of (9.66±1.63) mm in 24 hours while the least inhibition of (2.63±1.63) mm was that of ACMDL (0.05 g/ml) and AHMDL (0.05 g/ml) in 24 hours. The in vitro analysis indicated statistically significant differences ($p<0.05$) for the extracts of ACSMFL, AHSMFL, AHMFL, ACSMDL, AHSMDL, ACMDL, and AHMDL (Table 5, 6 & 7).

Table 5: Antimicrobial activity of aqueous semi-mature and mature *Physalis peruviana* boiled leaf extracts by agar diffusion method

Plant	Concentration(g/ml)	Mean inhibition zone(mm)	*p*-value
ABSMFL	0.1	3.66±2.98	
	0.12	-	
ABMFL	0.1	-	0.216
	0.12	-	
Water	-	-	NA
Ciprofloxacin	30 µg/ml	39.66±0.27	NA

ABSMFL=Aqueous Boil Extracts of Semi-mature *P. peruviana* Fresh Leaf; ABMFL= Aqueous Boil Extracts of Mature *P. peruviana* Fresh Leaf.

From the table, the mean zone of inhibition observed was that of ABSML, with a concentration of 0.10 g/ml. Hence there was no statistical significance for ABSMFL and ABMFL ($p>0.05$). NA = Not Applicable, and "-" means no zone of inhibition.

Table 6: Antimicrobial activity of aqueous *Physalis peruviana* fresh leaf extracts by agar diffusion method

Plant part	Conc.(g/ml)	Time				*p* -value
		10 min	30 min	1 hr	24 hrs	
ACSMFL	0.11	-	-	4±1.63	7±0.81	
	0.22	-	-	8.33±1.51	5.33±2.37	.00288*
	0.33	-	9±0.94	3.66±2.99	5.33±4.35	
	0.44	4.33±3.53	11.66±1.78	9±0.55	10±1.24	
AHSMFL	0.11	-	-	8.66±0.94	8±6.53	
	0.22	-	2.66±2.17	6.66±0.27	3±2.44	
	0.33	2.66±2.17	9.33±3.83	7±2.44	10.66±4.45	.0282*
	0.44	3±2.44	13±0.98	7.66±0.27	8±6.53	
ACMFL	0.11	-	-	4± 1.63	4.66±3.81	
	0.22	-	-	6±0.0	2.66±2.17	0.128
	0.33	-	10±4.18	7.66±0.72	4.66±3.81	
	0.44	2.66±2.17	12±1.24	14.33±0.27	9±2.94	
AHMFL	0.11	-	-	7±0.81	5±4.08	
	0.22	-	-	6±0.0	6±2.49	.0168 *
	0.33	-	9.33±3.83	6.66±3.31	12±5.08	
	0.44	3±2.44	12.33±0.54	9.21±0.27	8.66±0.36	
Water	NA	-	-	-	-	NA
Ciprofloxacin	30 µg/ml	39.66±0.27	39.66±0.72	38.33±0.72	39.66±0.27	NA

ACSMFL = Aqueous Cold Extracts of Semi-mature *P. peruviana* Fresh Leaf; AHSMFL = Aqueous Hot Extracts of Semi-mature *P. peruviana* Fresh Leaf; ACMFL= Aqueous Cold Extracts of Mature *P. peruviana* Fresh Leaf; and AHMFL = Aqueous Hot Extracts of Mature *P. peruviana* Fresh Leaf; NA = Not Applicable; and "-" means no zone of inhibition.

The values are expressed as the mean zone of inhibition (mm) ± SEM of three triplicate readings with ciprofloxacin as reference standard, (-) sign indicates the extract had no antimicrobial activity against the test organism; NA = Not Applicable for the *p*-value. From the table, AH

SMFL (0.44 g/ml) appears to perform well in 30 minutes compared to other concentrations. T he *p*-value for ACSMFL and AHSMFL indicated statistically significant differences ($p<0.05$. For mature *P. peruviana* fresh leaf extracts, AHMFL (0.44 g/ml) appears to perform well in 1 hour compared to other concentrations. The *p*-value of AHMFL showed statistically significa nt differences ($p<0.05$), while there was no statistically significant difference for ACMFL.

From the Table, 0.33 g/ml of all extracts in 30 minutes and 0.44 g/ml of all extracts in 10 and 30 minutes of semi-mature and mature *P. peruviana* fresh leaf have shown effects on the growth of *S. typhi* compared to other concentrations that didn't show the same growth.

Table 7: Antimicrobial activity of aqueous *Physalis peruviana* dry leaf extracts by agar diffusion method

Plant part	Conc.(g/ml)	time				*p*-value
		10min	30min	1hr	24hrs	
ACSMDL	0.05	-	-	-	7.33±3.88	
	0.1	-	-	-	6.40±4.06	.000721 ***
	0.15	-	-	5.33±4.35	5.33±2.37	
	0.2	2.83±2.31	8.33±0.27	5.33±4.35	8.66±0.27	
AHSMDL	0.05	-	-	4.66±1.90	9.66±0.28	
	0.1	-	-	7.66±0.27	8.33±0.51	.001326 **
	0.15	-	9.16±0.36	5.66±4.62	7±3.55	
	0.2	4.66±3.81	5±0.40	8±0.0	9.33±0.27	
ACMDL	0.05	-	-	-	2±1.63	
	0.1	-	-	-	4.33±8.99	
	0.15	-	-	5±4.08	7.66±4.06	.018 *
	0.2	-	8.67±0.54	8.33±6.8	12.83±1.06	
AHMDL	0.05	-	-	7.66±0.27	2±1.63	
	0.1	-	-	8±0.0	10±8.16	
	0.15	3.33±2.72	5.66±4.62	4.66±3.81	9.5±0.62	00543 **
	0.2	5±4.08	8.67±0.27	8.33±0.27	15.16±0.36	
Water	NA	-	-	-	-	NA
Ciprofloxacin	30 µg/ml	39.66±0.	39.66±0.72	38.33±0.72	39.66±0.27	NA

ACSMDL = Aqueous Cold Extracts of Semi-mature *P. peruviana* Dry Leaf; AHSMDL = Aqueous Hot Extracts of Semi-mature *P. peruviana* Dry Leaf ACMDL= Aqueous Cold Extracts of Mature *P. peruviana* Dry Leaf; and AHMDL = Aqueous Hot Extracts of Mature *P. peruviana* Dry Leaf; NA = Not Applicable; and "-" means no zone of inhibition.

From the Table, AHSMDL (0.05 g/ml) appears to perform well in 24 hours compared to other concentrations. The *p*-value in both semi-mature *P. peruviana* dry leaf extracts in cold and hot water shows a statistically significant difference in all concentrations ($p<0.05$).

Also, for mature *P. peruviana* dry leaf extracts in cold and hot water, AHMDL (0.20 g/ml) appears to perform well in 24 hours compared to other concentrations. The concentrations' *p*-value shows a statistically significant difference ($p<0.05$). From the table, 0.15 g/ml of AHMFL in 10 minutes, AHSMFL and AHMFL in 30 minutes, 0.20 g/ml of ACSMDL, AHSMDL, and AHMDL in 10 minutes, and all extracts in 30 minutes of semi-mature and mature *P. peruviana* dry leaf have shown effects on the growth of *S. typhi* compared to the rest of the concentrations. The petri dish results for some of the *Physalis peruviana* extracts.

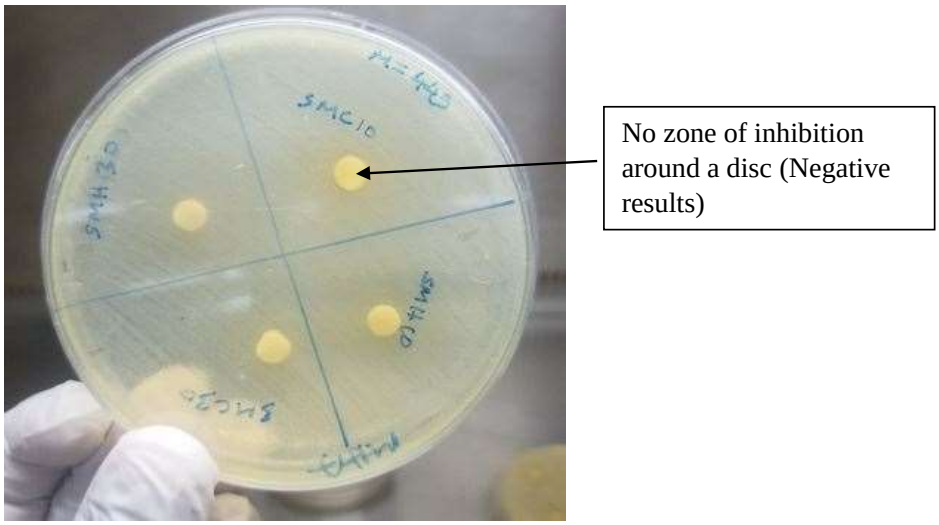

Plate 4: A petri dish showing negative results for all semi-mature *Physalis peruviana* fresh leaf extracts

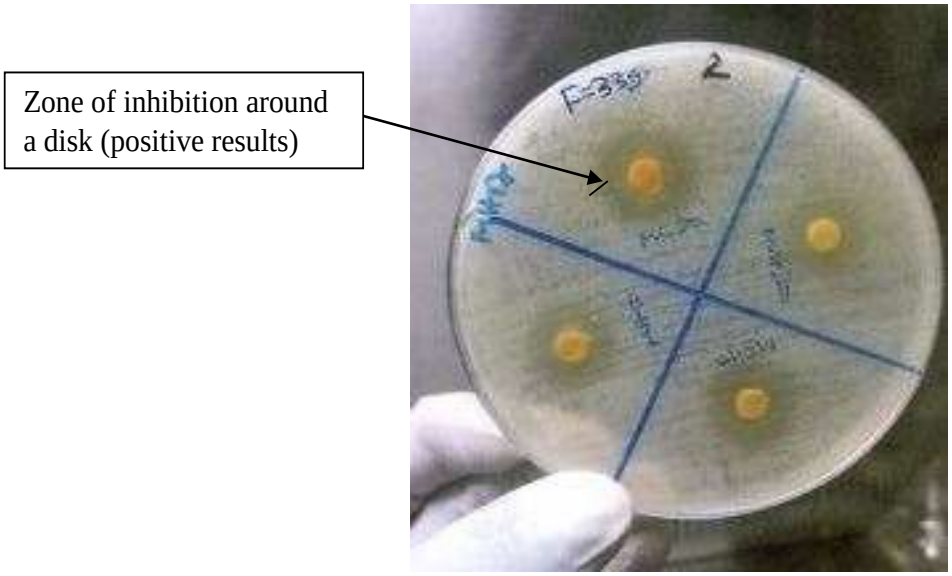

Plate 5: A petri dish showing the zone of inhibition for semi-mature and mature *Physalis peruviana* fresh leaf extracts

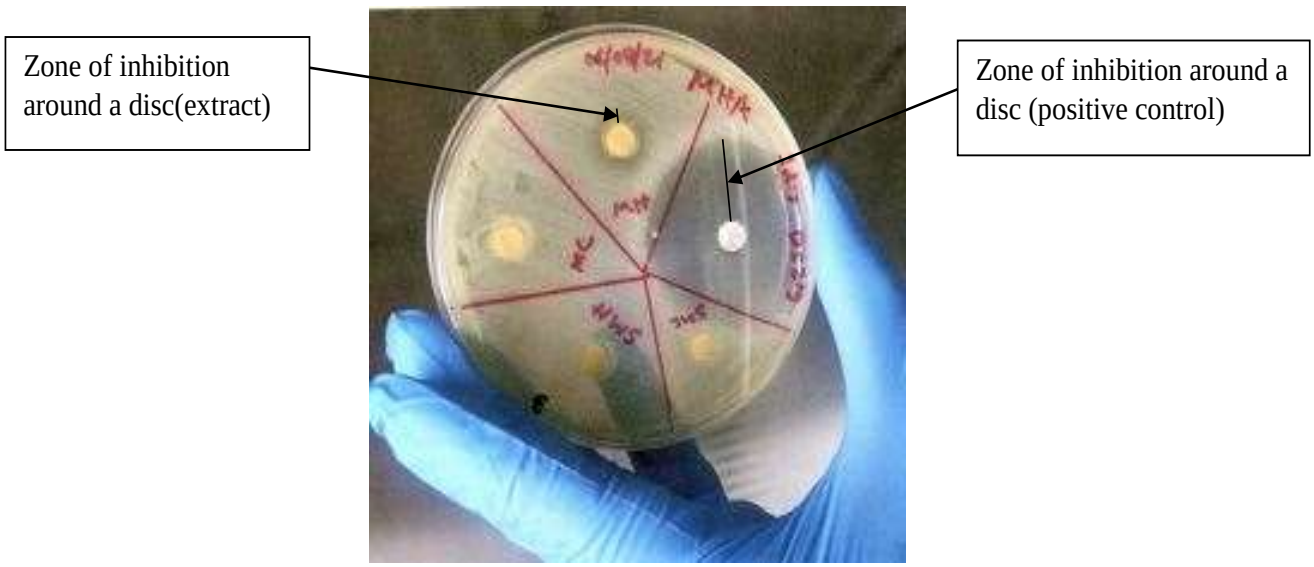

Plate 6: A petri dish showing the zone of inhibition for ciprofloxacin and mature *Physalis peruviana* dry leaf extract in hot water

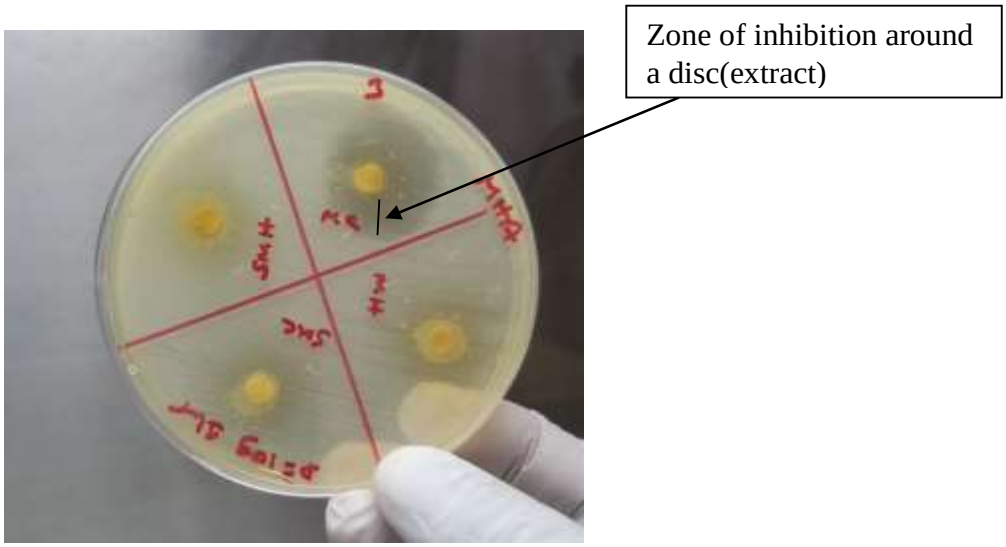

Plate 7: A petri dish showing the inhibition zone of mature *Physalis peruviana* dry leaf extract in cold water

4.1.4 Qualitative phytochemical screening of *Physalis peruviana* leaf extracts

The phytochemical analysis of aqueous *P. peruviana* leaf extracts revealed the presence of different phytochemical compounds present in leaf extracts, as indicated in Table 8.

Table 8: Phytochemical analysis of semi-mature and mature *Physalis peruviana* fresh leaf

Plant extract	Phytochemical components							
	Alkaloids	**Saponins**	**Steroids**	**Terpenoid**	**Anthraquinone**	**Flavonoids**	**Phlobatannins**	**Phenols**
ABSMFL	-	+	+	-	-	-	-	-
ABMFL	-	+	+	-	-	-	-	-
ACSMFL	-	+	+	-	-	+	-	-
AHSMFL	-	+	-	-	-	+	-	+
ACMFL	-	+	-	-	-	+	-	+
AHMFL	-	+	+	-	+	+	-	+

ABSMFL = Aqueous Boil Extracts of Semi-mature *P. peruviana* Fresh Leaf; ACSMFL = Aqueous Cold Extracts of Semi-mature *P. peruviana* Fresh Leaf; AHSMFL = Aqueous Hot Extracts of Semi-mature *P. peruviana* Fresh Leaf; ABMFL = Aqueous Boil Extracts of Mature *P. peruviana* Fresh Leaf; ACMFL = Aqueous Cold Extracts of Mature *P. peruviana* Fresh Leaf; and AHMFL = Aqueous Hot Extracts of Mature *P. peruviana* Fresh Leaf.

From the Table, ‘+’ indicate presence while ‘–’ indicate absent. From the Table; saponins, and flavonoids were abundant in all semi-mature and mature fresh leaf extracts compared to the rest of phytochemical compounds

Table 9: Phytochemical analysis of semi-mature and mature *Physalis peruviana* dry leaf

Plant extract	Phytochemical components							
	Alkaloids	**Saponins**	**Steroids**	**Terpenoid**	**Anthraquinone**	**Flavonoids**	**Phlobatannins**	**Phenols**
ACSMDL	+	+	+	-	-	+	-	+
AHSMDL	+	+	+	-	-	+	-	+
ACMDL	+	+	+	-	-	+	-	+
AHMDL	+	+	+	-	-	+	-	+

ACSMDL = Aqueous Cold Extracts of Semi-mature *P. peruviana* Dry Leaf; AHSMDL = Aqueous Hot Extracts of Semi-mature *P. peruviana* Dry Leaf; ACMDL = Aqueous Cold Extracts of Mature *P. peruviana* Dry Leaf; and AHMDL = Aqueous Hot Extracts of Mature *P. peruviana* Dry Leaf. '+' indicate presence while '–' indicate absent.

From the Table; alkaloids, saponins, steroids, and phenols were abundant in semi-mature and mature *P. peruviana* dry leaf extracts.

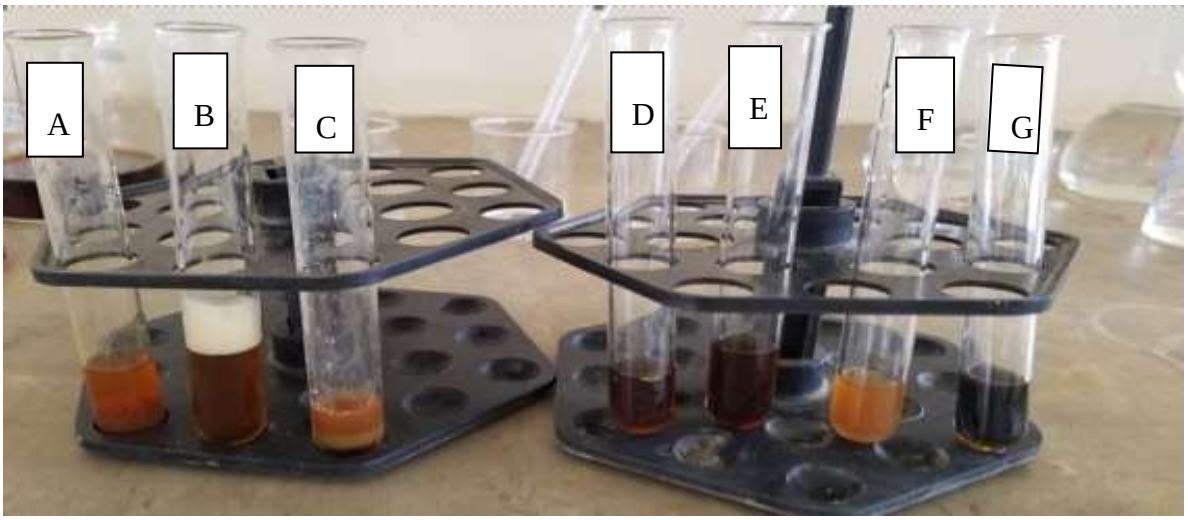

Plate 8: Test tube representing different colors of groups of phytochemical compounds present in *P. peruviana*: A represent brown precipitate for alkaloids; B represent foaming for saponins; C represent cream for steroids; D & E represent deep red for absence of anthraquinone; F represent yellow for flavonoids; G represent blue precipitate for phenols.

4.1.5 Compounds present in *Physalis peruviana* leaf following interpretations of LC-MS/MS spectra

According to LC-MS/MS spectra, eight compounds were identified, among which four have been previously reported for antibacterial activity. The identified compounds with antibacterial activities include quinaldic acid, isovitexin, jatrorrhizine cation, and nicergoline.

Table 10: Aqueous boil, cold and hot extract of semi-mature and mature *Physalis peruviana* fresh and dry leaf

S/N	Extract	Retention time (min)	Name	Molecular formula	Molecular weight
1	ACSMDL	0.75	Quinaldic acid	$C_{10}H_7NO_2$	173.05
2	AHMDL	6.27	6-O-Malonylglycitin	$C_{25}H_{24}O_{13}$	532.4
3	AHSMDL	7.78	4-hydroxy-2,3,4,6-tetramethoxychalcone	$C_{19}H_{20}O_6$	344.13
4	ACMFL	8.38	9,10-anthracenedione,1,4-diamino-5nitro	$C_{14}H_9N_3O_4$	283.06
5	ACMDL	11.50	Jatrorrhizine cation	$C_{20}H_{20}NO^{4+}$	338.14
6	ACSMFL	14.5	7-Hydroxycoumarin-3-carboxylic acid	$C_{10}H_6O_5$	206.02
7	ACSMDL	14.51	Isovitexin	$C_{21}H_{20}O_{10}$	432.11
8	AHSMFL	15.44	Nicergoline	$C_{24}H_{26}BrN_3O_3$	483.12

ACSMDL- Aqueous Cold Semi-mature *P. peruviana* Dry Leaf; AHMDL- Aqueous Hot Mature *P. peruviana* Dry Leaf; AHSMFL- Aqueous Hot Semi-mature *P. peruviana* Fresh Leaf; ACMFL- Aqueous Cold Mature *P. peruviana* Fresh Leaf; ACMDL- Aqueous Cold Mature *P. peruviana* Dry Leaf; ACSMFL- Aqueous Cold Semi-mature *P. peruviana* Fresh Leaf; ACSMDL- Aqueous Cold Semi-mature *P. peruviana* Dry Leaf; AHSMFL- Aqueous Hot Semi-mature *P. peruviana* Fresh Leaf

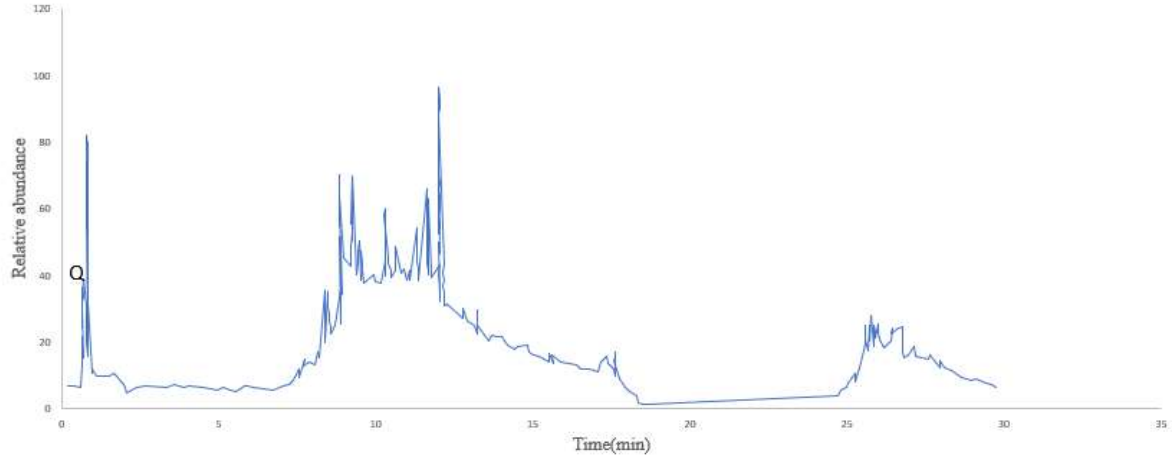

Figure 2: Chromatogram showing quinaldic acid represented by Q

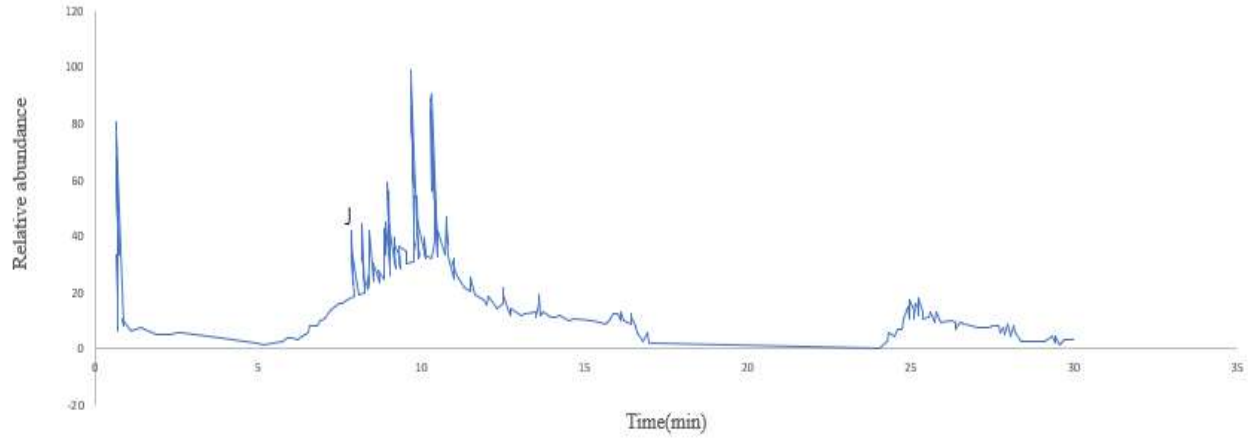

Figure 3: Chromatogram showing jatrorrhizine represented by J

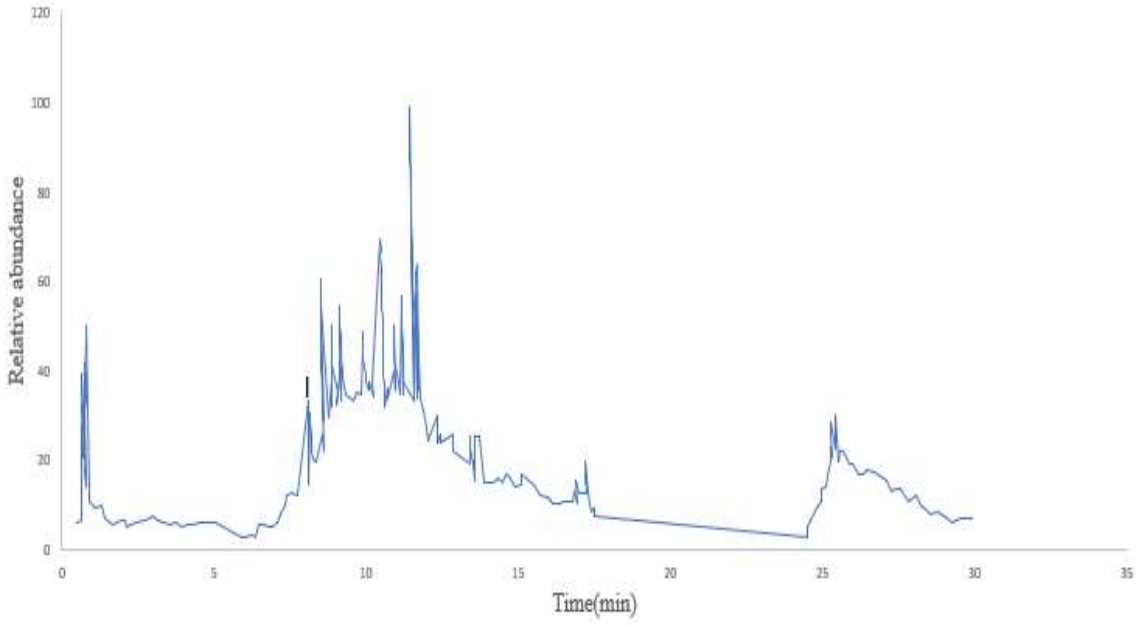

Figure 4: Chromatogram showing isovitexin represented by I

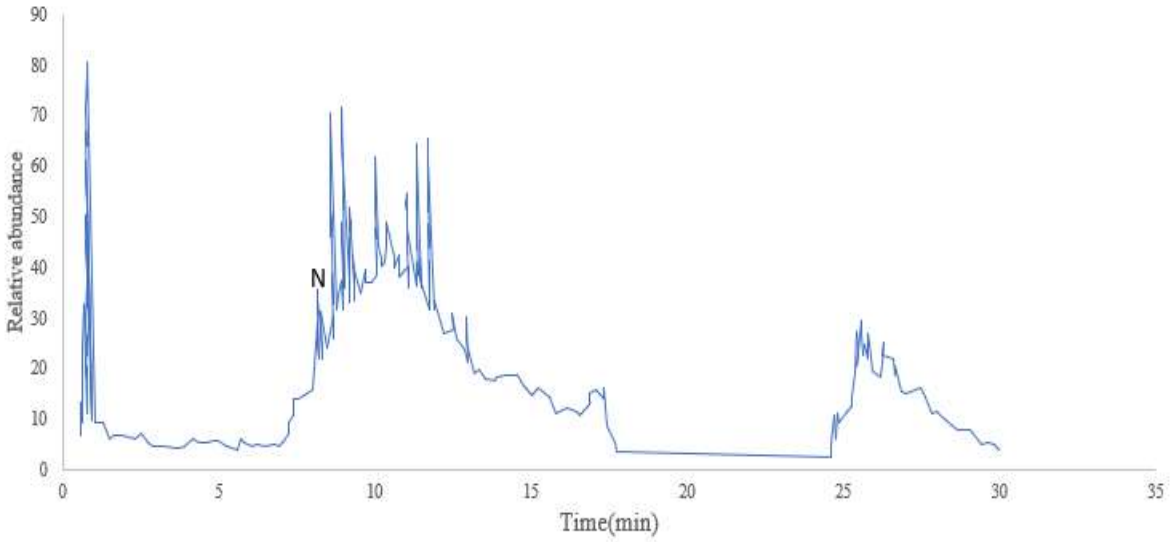

Figure 5: Chromatogram showing nicergoline represented by N

4.2 Discussion

This study aimed to evaluate the ethnobotany value of *P. peruviana* in Mbeya Rural District, findings have demonstrated that people in Rural Mbeya are aware of *P. peruviana* as a medicinal plant (*P. peruviana*) and use the leaf extracts in managing typhoid fever. Furthermore, in vitro investigation in this study has demonstrated the efficacies among the two main different community-based preparatory methods which support the existing indigenous

knowledge informing the effectiveness of the *P. peruviana* leaf in managing *S. typhi* infections. Similarly, in this study, relevant compounds phytochemical were revealed to be the main bioactive phytochemical compounds for *P. peruviana*.

4.2.1 Ethnobotany assessment of *Physalis peruviana* for Mbeya rural communities

This study reported that there was an association between demographic information and knowledge of the ethnobotany of *P. peruviana* in treating typhoid fever. As the results indicated, the majority of key informants were married; therefore, marriage and medicinal use might correlate in such a way that there might be knowledge sharing among family members. Buwa (2019) from South Africa's Eastern Cape province reported the equal responsibility of mother and father within the family regarding knowledge sharing related to family care and medicinal use. The involvement of family members in medicinal use is also related to age; the results of this study revealed that the majority of key informants were above 50 years old, indicating that the elder group within communities has more knowledge on medicinal use than the young people. Also, Eastern Brazil and Aziz *et al.* (2018) from Pakistan all stated that ethnomedical knowledge appears to be positively linked with age and that older community members are connected with medicinal knowledge and use. Based on the study's findings and the literature, it is probably true that in Mbeya Rural District, the usage of *P. peruviana* leaf in treating typhoid fever is associated with the knowledge of the plant among the old people within the community. Also, plant use is associated with the ease of availability and accessibility of the plant in the area, the cost of the plant relative to modern medicine and facilities, and the belief that natural remedies are safer than synthetic ones. This implies that *P. peruviana* leaves have been used in treating typhoid fever in the study area.

Since the leaves of *P. peruviana* are used for treating typhoid fever, as indicated in the survey results. Findings from this study agree with those of Jaca and Kambizi (2011) from South Africa, Maobe *et al.* (2013) and Kamau *et al.* (2020), all from Kenya, that the leaves of *P. peruviana* have antibacterial activity. Therefore, the use of *P. peruviana* leaf in preparing the herbal medicine used in treating typhoid fever might be associated with the belief in the community that only the leaf of *P. peruviana* performs better compared to the rest of the plant. Also, there is a knowledge sharing about medicinal plants, including different diseases treated using specific plants in the study area.

According to the findings from interviews with key informants, it was noticed that apart from *P. peruviana* leaf being used in treating typhoid fever, it is also used to treat other diseases such as peptic ulcers, stomach aches, and gastrointestinal tract diseases, typhoid in chickens, fever, and malaria. The result agrees with other studies that have indicated that *P. peruviana* leaf is used to treat other diseases. For example, Runyoro (2006) from Tanzania reported that the juice extract of the leaf is used to treat skin fungal infection; Also Nondo (2015) from Tanzania reported that maceration of *P. peruviana* leaf is used to treat Malaria; also, Maobe (2013) from Kenya stated that leaf decoction of *P. peruviana* can be used to treat diabetes, malaria, and pneumonia; so far Mboni (2019) from Democratic Republic of Congo reported that an extract resulted by decoction of *P. peruviana* leaf is used to treat malaria, intestinal worms, and diabetes mellitus. The ability of this plant to treat various diseases may be related to its ability of having a diversity of phytochemical compounds as identified in this study.

Additionally, key informants in this study revealed that two preparatory medicinal methods, i.e., boiling and soaking, are the most common preparation techniques used for *P. peruviana* medicinal leaves. According to the survey results, the herbal medicine resulting from boiling and soaking preparatory methods is associated with estimating certain amounts of leaves and water. For estimation, most societies use tea or food spoons to estimate the fresh ground and dried leaves, whereas estimation of water involves the use of a cup of tea, glasses, and a calibrated jar. Likewise, according to Mag *et al.* (2019), boiling and soaking have been used in preparing herbal medicine from different medicinal plants by the Seymour Society in South Africa and other places, as reported by Erhabor *et al.* (2013) from Nigeria, Kiringe (2006) from Kenya, Kitula (2007) from Tanzania, and Rahmatullah *et al.* (2012) from India; the estimation of water and leaves is being done using spoons, cups of tea, and glasses as well. This indicates that the use of *P. peruviana* leaf in Mbeya Rural District in treating diseases has been used in different concentrations.

In addition, the results from the survey showed that the herbal medicine resulting from boiling and soaking is administered orally. Also, Offiah *et al.* (2011) and Mag *et al.* (2019), both from South Africa, Mohan *et al.* (2008) and Savithramma *et al.* (2016),), from India, Sintayehu Tamene *et al.* (2020), and Tolossa *et al.* (2013) from Ethiopia reported that the herbal medicine resulting either by boiling or soaking is administered orally. This demonstrated that herbal medicine preparation methods and modes of administration are similar in different societies

from different locations, which might have resulted from knowledge sharing within the society or community.

In this study it was found that communities had a variety of measures of *P. peruviana* as they take a range of one to three teaspoons, others a half cup of tea, a half glass, etc., of herbal medicine once daily, twice daily, or three times daily. In addition, studies indicate that the dose used for herbal medicine differs from one family to another within the same or different localities (Adia *et al*., 2014; Borokini *et al*., 2013; Korkmaz *et al*., 2016; Nakaziba *et al*., 2021). Also, according to Mag *et al.* (2019) from South Africa, herbal medicines derived from boiling leaves, such as half a cup, one cup, and so on, are taken orally three times a day. So far, Kimutai (2019) from Kenya reported that people from Nand County, Kenya, take three-quarters of a cup of herbal medicine prepared by boiling fresh leaves. Therefore, according to the aforementioned literature, herbal medicine dose use among societies differs, probably due to knowledge and perception of medicinal plants. Further, the results showed that the dose repetition of herbal medicine used by different societies among the family members differs. For example, some family members tend to repeat the dose if recovery does not persist, but others do not repeat the dose even if there is no recovery. Because there is no information or the information might be present but not documented in their respective country, the associated knowledge for drug repetition has not been practised in other communities worldwide. Therefore, it is important to know whether there is a repetition of herbal medicine in different communities so that further studies can be conducted to know the efficacy of the doses taken.

Findings regarding side effects associated with the use of *P. peruviana* leaf showed no side effects from the plant. These findings agree with those of Kamau *et al.* (2020) from Kenya, who reported that the aerial parts of *P. peruviana,* i.e., stem, leaves, and fruits, have no toxicity. Likewise, Kathare *et al.* (2021) from Kenya reported that the stem back of *P. peruviana* is not associated with toxicity. Therefore, the herbal drug from *P. peruviana* leaf might be health-friendly to users. Also, the results from the survey showed that diagnostic tests are either undertaken or not by people in the study area before the use of herbal medicine dose resulted by *P. peruviana* leaf. This information lacks other evidence from other literature that might be present but has not been published. A diagnostic test before herbal medicine use is of great importance to a sick person since it enables an individual to know what type of disease, he or she has for better selection of herbal medicine to be used.

Furthermore, an interview found that some family members use semi-mature leaves of *P. peruviana* while others prefer mature ones for treating typhoid fever. Unfortunately, there is no documented information in the current literature regarding using semi-mature, mature *P. peruviana* leaves. Therefore, the use of semi-mature or mature *P. peruviana* in the studied community is due to either the choice or interest of particular family members, and this is because the plant is readily available in their homes.

4.2.2 Antibacterial activity of aqueous *Physalis peruviana* extracts by agar diffusion method

This study found that the boiling and soaking methods of semi-mature and mature *P. peruviana* leaves indicated antimicrobial activity against *S. typhi*. This is consistent with the findings of Cueva *et al.* (2017); El-Beltagi *et al.* (2019); Ertürk *et al.* (2017), and Çakir *et al.* (2014) that the leaf of *P. peruviana* has antibacterial activity. Furthermore, for soaking, the increase in concentrations of extracts was observed to affect the growth of *S. typhi,* whereby the higher zone of inhibition was observed at a concentration of 0.44 g/ml for fresh leaf and 0.20 g/ml for dry leaf, with zones of inhibition of 15.16 mm and 14.33 mm, respectively. Furthermore, the increase in concentrations of extracts corresponds with the study carried out by Kamau *et al.* (2020) from Kenya, who reported that extracts of 125 mg/ml and 250 mg/ml of the leaf of *P. peruviana* against *S. typhi* resulted in inhibition zones of 3.33 mm and 4.67 mm, respectively.

Moreover, different concentrations resulting from soaking both ground and powdered leaves have shown different zones of inhibition, whereby all of the extracts exposed for 1 hour and 24 hours showed zones of inhibition against *S. typhi,* indicating that as the extracts are exposed to the solvent for a long time, there is a possibility of phytochemical compounds being well dissolved and released, resulting in the effects on the growth of *S. typhi*. Exposure of extracts at different time intervals has also been reported by Lean-teik *et al.* (2005) from China, who reported the extraction of secondary metabolites in 1 hour; however, Anooj *et al.* (2019) from India, Bhandari *et al*. (2021) from Nepal reported the extraction of plant materials at 24 hours. As long as all concentrations of semi-mature and mature *P. peruviana* fresh and dry leaf showed effects on *S. typhi* growth in 1 hour and 24 hours, some concentrations in 10 minutes and 30 minutes, as described, showed the zone of inhibition against *S. typhi*. Therefore, according to the results, 0.33 g/ml of all extracts in 30 minutes; 0.44 g/ml of all extracts in 10 and 30 minutes; 0.15 g/ml of AHMFL in 10 minutes; AHSMFL and AHMFL in 30 minutes; 0.20 g/ml of ACSMDL, AHSMDL, and AHMDL in 10 minutes; and all extracts in 30 minutes

of semi-mature and mature *P. peruviana* fresh and dry leaf have shown effects on the growth of *S. typhi*. The antibacterial activity of *P. peruviana* leaf extracts in 10 minutes and 30 minutes agrees with the study carried out by Benmehdi *et al.* (2012); Santos *et al.* (2018); Yin *et al.* (2013); Zandoná *et al.* (2020) from Brazil whose reported extraction was carried out in 10 minutes, and (Shaheen & Ahmad, 2020) from Pakistan, whose reported extraction was carried out in 30 minutes. Therefore, the observed antibacterial activity resulting from boiling and soaking preparatory methods is associated with the presence of a diversity of phytochemical compounds present in the leaves of *P. peruviana*. Also, the differences in efficacies between boiling and soaking might be associated with the loss of active phytochemical compounds present in the leaf of *P. peruviana* that might have been lost during the boiling process (Monalisa *et al.*, 2020; Podsędek *et al.*, 2008). As higher inhibitory activity has been observed in concentrations resulting from soaking compared to concentration resulting from boiling, soaking leaves is proposed to be used in this community for preparing leaf extracts from *P. peruviana* for typhoid management.

4.2.3 Qualitative and LC-MS/MS screening of *Physalis peruviana* leaf extracts

(i) Qualitative screening of *Physalis peruviana* leaf extracts

The phytochemical analysis of *P. peruviana* leaf extract revealed the presence of alkaloids, saponins, steroids, anthraquinones, flavonoids, and phenols. The identified phytochemical compounds in *P. peruviana* have also been reported by Kamau *et al.* (2020) from Kenya, who also reported the presence of saponins, flavonoids, and alkaloids in leaf extracts of *P. peruviana*; Khalaf-allah *et al.*, 2015) from Egypt reported the presence of flavonoids in the leaf extracts of *P. peruviana.* In addition, Bayas-Morejon *et al.* (2020) from Ecuador reported the presence of phenols in the leaf extracts of *P. peruviana.* This study's laboratory findings and results from other studies on P. peruviana indicate that *P. peruviana* leaf harbours a diversity of bioactive phytochemical compounds. Therefore, the presence of the reported phytochemical compounds in the leaves of *P. peruviana* plays a major role in antibacterial activity.

(ii) LC-MS/MS analysis of *Physalis peruviana* leaf

Different sub-groups of phytochemical compounds were identified using LC-MS/MS. Of the identified compounds, only four have antibacterial activity. The phytochemical compounds with antibacterial properties were identified in different plants, as described hereunder. According to Nocetti *et al.* (2020) from Egypt, isovitexin has been identified in the calyces of *P. peruviana*. Also, Qian *et al.* (2020) from China isolated jatrorrhizine from various plant families such as Annonaceae, Berberidaceae, Menispermaceae, Papaveraceae, Ranunculaceae, and Rutaceae. Additionally, quinaldic acid has been isolated in *Ephedra pachyclada* in the Republic of Korea (Lee, 2009).

Moreover, nicergoline has been extracted from sesame oil in Egypt Abourehab *et al.*, (2021). Only three of the four antibacterial phytochemical compounds identified, i.e., quinaldic acid, jatrorrhizine, and nicergoline, were found in plant species other than *P. peruviana*. The presence of these compounds in the leaf of *P. peruviana* might be due to the solvent used during extraction or the plant's geographical location. Therefore, LC-MS/MS results confirm that the *P. peruviana* leaf is associated with antibacterial activity.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study's findings clearly indicate that people in Mbeya Rural District are aware of *P. peruviana* leaf extracts for treating typhoid fever. Laboratory findings have demonstrated the existence of different relevant phytocompounds present in the leaf of *P. peruviana*, which seems to be the source of the plant's antibacterial activity for the plant (*P. peruviana*). Boiling and soaking leaves from *P. peruviana* were identified as the most common preparatory methods for herbal medicine from the plant. In vitro, studies of the different extracts resulting from boiling and soaking methods have shown different activities against *S. typhi*. By comparing concentrations produced by boiling and soaking, it has been found that soaking produces concentrations with higher inhibitory antibacterial activity. Furthermore, the analysis of phytochemical compounds in *P. peruviana* leaf revealed the presence of quinaldic acid, jatrorrhizine, isovitexin, and nicergoline, which in other ways could have contributed to the antibacterial activity of the plant. Therefore, validating the community-based methods used to prepare extracts from *P. peruviana* leaf can help enhance the treatment of typhoid fever in Mbeya Rural District.

5.2 Recommendations

Taking into account the study's findings, the following suggestions are now put forth:

(i) Comparing the boiling and soaking methods, soaking was more effective against *S. typhi*. Therefore, the soaking method is proposed to be used in the community to prepare herbal medicine from *P. peruviana*.

(ii) This study recommends a pharmacological and toxicological investigation of *P. peruviana* leaf extracts so their safety level is known for public health importance.

(iii) Further studies on *P. peruviana* leaf extracts resulting from soaking, specifically in isolating and purifying a natural compound that can be used to treat typhoid fever, are highly emphasized.

(iv) Further studies on *P. peruviana* leaf extract resulting from soaking, which is used in treating other diseases apart from typhoid fever, are required.

(v) To better understand the medicinal effect of the whole plant (*P. peruviana*), further investigation is suggested for extracts of the plant roots, stems and its fruits against *S. typhi*.

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APPENDICES

Appendix 1: Materials, reagents, and equipment's including their sources

S/n	Materials/equipment's	Source
1	*Physalis peruviana*	Swaya village-Mbeya
2	*Salmonella typhi* pathogen	Mount Meru Referral Hospital-Arusha
3	Ciprofloxacin disc (30µg)	Mount Meru Referral Hospital-Arusha
4	Mueller Hinton agar	Volt laboratory equipment distributors-Dar Es Salaam
5	Laminar flow	NM-AIST laboratory-Arusha
6	Incubator	NM-AIST laboratory-Arusha
7	Freezer	NM-AIST laboratory-Arusha
8	Refrigerator	NM-AIST laboratory-Arusha
9	Autoclave	NM-AIST laboratory-Arusha
10	LC-MS/MS	GCLA-Dar es Salaam
11	Ethanol (96%)	GCS MED-EQUIPMENT SOLUTION-Arusha
12	Sterile cotton swab	Mount Meru Referral Hospital-Arusha
13	Sterile disposable petri dishes	GCS MED-EQUIPMENT SOLUTION-Arusha & Volt laboratory equipment distributors-Dar Es Salaam
14	Falcon tubes (15ml)	Volt laboratory equipment distributors-Dar Es Salam
15	Sterile disposable wire loop(10µl)	Volt laboratory equipment distributors-Dar Es Salaam
16	British filter paper (9cm)	Volt laboratory equipment distributors-Dar Es Salaam
17	Spirit burner	GCS MED-EQUIPMENT SOLUTION-Arusha
18	Gloves	GCS MED-EQUIPMENT SOLUTION-Arusha
19	Cool box (14 liter)	Geomat laboratory equipment's-Mbeya
20	Chemicals (Barium Chloride, Conc. Nitric acid, conc. Sulphuric acid, conc hydrochloric acid, sodium hydroxide, crystal iodine, potassium iodide, Ammonium hydroxide)	Geomat laboratory equipment's-Mbeya
21	Distilled water	NM-AIST laboratory
22	Weighing balance	MUST laboratory-Mbeya & NM-AIST laboratory-Arusha
23	0.5 McFarland	Mount Meru Referral Hospital-Arusha

Appendix 2: Questionnaire

Respondent No:……. Date of interview: …… Ward:…….. Time: ……

Demographic information

1. Gender: Male ☐ Female: ☐

2. Age:

<18	18 – 30	31 – 40	41 – 50	>50

3. Marital status:

Single	Married	Divorced	widow/widower

4. Education level:

Primary school	Secondary school	Tertiary	Didn't attend school

Knowledge on *P. peruviana*

5. Do you know *P. peruviana* plant?

Yes	No

6. Is *P. peruviana* plant used for the treatment of typhoid fever?

Yes	No

7. Apart from typhoid fever do *P. peruviana* plant used for the treatment of other diseases?

Yes	No	I don't know

8. Other diseases apart from typhoid fever treated by *P. peruviana*

   i)……

   ii)……...

   iii)……..

   iv)……..

9. Part of *P. peruviana* plant used for the treatment of typhoid fever:

Root	Stem	Leaf	Flower	Fruit

10. What are the methods used for preparation of herbal drug?

Boiling	Soaking

11. Mode of drug administration:

Oral	Other

12. Dissolving time of fresh or powdered part of *P. peruviana* plant:

Less than a day	One day	More than one day

13. How often does the medicine apply per day?

Once per day	Twice per day	Thrice per day

14. Are measurements used in preparing *P. peruviana* drug?

Yes	No

15. Amount of *P. peruviana* plant part used to make herbal drug:

    i)…….

    ii)…….

16. Amount of water used for preparing herbal drug:

    i)……

    ii)……

17. How long is the dose of the herbal drug?

Less than a week	One week	More than a week

18. Is there any repetition of herbal drug in case of unrecovered?

Yes	No

19. Are there any side effects associated with herbal drug?

Yes	No	I don’t know

20. Is there any diagnostic test undertaken before taking medicine prepared from *P. peruviana* plant?

Yes	No

21. What age of *P. peruviana* is used to make herbal drug?

Young	Semi-mature	Mature

Appendix 3: Participant Information sheet

Introduction

The research aim is to assess the ethnobotany and antibacterial effects of *P. peruviana* and determine its efficacy in managing typhoid fever in Mbeya, Tanzania. Therefore, to accomplish my study you are requested to participate fully in this study. The information about my study have been provided in this document. Thus, participation for the study is accompanied with consent form which should be signed by participants.

Reasons for undertaking the study?

According to different studies which have been conducted worldwide on medicinal plants it has been shown the beneficial of medicinal plants especially for the disease treatments. In managing the disease different methods are being used for the preparation of the herbal drugs by different communities however the differences in effectiveness among the methods is not documented internationally. Thus, this study aims to look on the effects of community-based goldenberry plant formulations in managing typhoid fever. Hence by conducting this study will bring contribution to the community especially pointing out the good method useful for management of typhoid fever.

Why have I been chosen?

Being part of the study means you are the one whom could bring the right information's useful for the study. So as to be familiar with this study you are asked to pass through the document to understand on what is required from you without forgetting to ask if there is any cruel.

Do I have to take part?

Being part in this study is obligation of you since as the human rights law states you are not forced to do anything unwillingly. Thus, you are free to decide to be part or not in the study.

What will happen to me if I take part?

Once you will be part of this study means many information associated with goldenberry plant will be known. Such information's will be what is this goldenberry, how the plant can be obtained, the diseases which can be treated using the plant, part of the plant used and the preparation methods.

What are the possible disadvantages and risks of taking part?

No any risk once you will be involved in this study.

Shall my information be kept confidential if I participate in the study?

As ethical research states in terms of confidentiality, all information collected/used from the participant during the study will be kept confidential. During data collection the participant information is mainly indicated with letter and the date/year of birth but only the name of participant will appear on consent form. The letter which represents the participant will be used during data analyses from the collected data.

Who is organizing and funding this research?

This study is funded by Higher education student loan board (HESLB).

Who has reviewed the study?

This study was reviewed and approved by KIDH, NM-AIST, CEDHA HEALTH RESEARCH ETHICAL COMMITTEE (KNCHREC) and approved by Nelson Mandela Institution of Science and Technology (NM-AIST).

Who shall I contact at any time regarding this research?

For any concern, views and anything regarding this study don't hesitate to contact Charles Chekecha through the following address:

Charles Chekecha

The Nelson Mandela African Institution of Science and Technology (NM-AIST).

School of life science and Bioengineering

P.O. Box 447, Tengeru, Tanzania

Telephone: +255 762 016 624

Thank you in advance

Consent Form

Participant Identification information:

Name of principal Investigator: Charles Chekecha

1. I confirm that I have read and understood the participant information sheet dated March 23, 2021, for the intended study and I have had the opportunity to ask for clarifications.

2. I understand that my participation is my willing thus I am free to withdraw consent without providing any reason.

3. I understand that sections of any of my herbal records available at this village office may be looked at by responsible researchers where it is relevant to my taking part in the research. I give permission for these researchers to have access to my records.

4. I agree to take part in the aforementioned study.

…….	……	……
Name of participant	Signature	Date
……..	…….	……..
Name of Researcher	Signature	Date

Appendix 4: Elaborated recruitment procedure

Title: Ethnobotany and antibacterial effects of goldenberry (*Physalis peruviana* L.) on *Salmonella typhi* in Mbeya Rural District, Tanzania

Objective

To assess the ethnobotany and antibacterial effects of *P. peruviana* and determine its efficacy in managing typhoid fever in Mbeya Rural District, Tanzania

Specific objectives

i. To assess the ethnobotany of *P. peruviana* in communities living in Mbeya Rural District, Tanzania

ii. To analyze the phytochemical compounds in *Physalis peruviana* leaf collected from Mbeya Rural District, Tanzania

iii. To determine the efficacy of different *P. peruviana* leaf extracts from existing community -based methods against *S. typhi*

Population: household family members and traditional healers

Inclusion criteria

i. Willingness to participate by signing written informed consent

ii. Aged 18 years and above

iii. With the knowledge on *P. peruviana*

Exclusion criteria

None

If the Potential Participant met the Inclusion Criteria, follow the following procedure

Informed consenting ⟶ interview (questionnaire) ⟶ recording in the questionnaire or note book

RESEARCH OUTPUTS

Publication paper

Chekecha, C.,Vianney, J., & Mbega, E., 2022. "Community-based methods of using goldenberry (*Physalis peruviana* L.) for managing *Salmonella typhi*," *Int. J. Biosci.*, vol. 6655, pp. 340–351. https:// doi: 10.12692/ijb/21.2.340-351.

Poster presentation

Ethnobotany and antibacterial effects of goldenberry (*Physalis peruviana* L.) on *Salmonella typhi* in Mbeya Rural District, Tanzania