Thesis Ref. No:……

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF BIOMEDICAL SCIENCES

MSC IN VETERINARY PHARMACOLOGY PROGRAM

PHARMACOLOGICAL ACTIVITY OF SELECTED MEDICINAL PLANTS EXTRACT ON *PASTEURELLA MULTOCIDA* AND *MANNHEIMIA HAEMOLYTICA* ISOLATED FROM SMALL RUMINANTS

MSc THESIS

BY

BESHADA ASFA JIRU

JUNE, 2023

BISHOFTU, ETHIOPIA

PHARMACOLOGICAL ACTIVITY OF SELECTED MEDICINAL PLANTS EXTRACT ON *PASTEURELLA MULTOCIDA* AND *MANNHEIMIA HAEMOLYTICA* ISOLATED FROM SMALL RUMINANTS

BY

BESHADA ASFA JIRU

MSc THESIS SUMBITTED TO DEPARTMENT OF BIOMEDICAL SCIENCES, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, ADDIS ABABA UNIVERSITY IN THE FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCES IN VETERINARY PHARMACOLOGY

ADVISOR

TAKELE BEYENE TUFA (ASSOCIATE PROFESSOR)

CO-ADVISOR

Dr. DEREJE NIGUSSIE (PHD, SENIOR RESEARCHER)

JUNE, 2023

BISHOFTU, ETHIOPIA

PHARMACOLOGICAL ACTIVITY OF SELECTED MEDICINAL PLANTS EXTRACT ON PASTEURELLA MULTOCIDA AND MANNHEIMIA HAEMOLYTICA ISOLATED FROM SMALL RUMINANTS

Submitted by: Beshada Asfa Jiru _____ _____

Name of Student Signature Date

Approved for submittal to thesis assessment committee:

1. Takele Beyene (Associate Professor) _____ _____

Advisor Signature Date

2. Dr. Dereje Nigussie (PHD, Senior Researcher) _____ _____

Co- Advisor Signature Date

3. Yoseph Cherinet (Associate Professor) _____ _____

Department chairperson Signature Date

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF BIOMEDICAL SCIENCES

This is to certify that the thesis prepared by Beshada Asfa, entitled **"Pharmacological activity of selected medicinal plants extract on *Pasteurella multocida* and *Mannheimia haemolytica* isolated from small ruminants"** and submitted in partial fulfilment of requirement for the degree of master of sciences in veterinary pharmacology complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

Approved by examining committee:

Name	Signature	Date
1. Dr. Worku Bedada (PhD) External examiner	_____	_____
2. Dr. Getachew Tadesse Internal examiner	_____	_____
3. Takele Beyene Tufa (Associate Professor) Advisor	_____	_____
4. Yoseph Cherinet (Associate Professor) Department Chairperson	_____	_____

TABLE OF CONTENTS

PAGE

DEDICATION

I would like to dedicate my work to my family. They instilled in me a desire to learn and made sacrifices so I would have access to high quality education from an early age. Also, this is dedicated to Addis Ababa University, College of Veterinary Medicine and Agriculture for their great opportunity of scholarships awarded to me and overall support throughout my years of studies.

DECLARATION

I, hereby, declare that this submitted thesis manuscript for the degree of Master of Sciences in Veterinary Pharmacology at Addis Ababa University, College of Veterinary Medicine and Agriculture, is my bona fide work and that it has not been submitted previously to any institution or higher education. All sources of materials used in this work have been duly acknowledged.

This work was done from December 2022 to May 2023 at College of Veterinary Medicine and Agriculture of the Addis Ababa University, National Veterinary Institute and Ethiopian Public Health Institute.

ACKNOWLEDGEMENTS

First of all, I would like to thank Almighty God for the eternal activities, unconditional love, and the strength he gives me in every moment of my life. My overflowing heartfelt extends to my family for their unconditional love and support throughout my life. My parents, Asfa Jiru and Midagdu Bikila, instilled in me the values of education, determination and hard work; without these traits, I would not be where I am today. Dad and Mom, you have always encouraged me to achieve my dreams and you've always stood by me as I've made my way through life.

I am sincerely obliged to many worthy persons, Dr. Dereje Nigussie, Takele Beyene (Associate Professor) and Professor Teshale Sori, without them I could never have had the opportunity of conducting research with great interest. These influential instructors have occupied my life joyfully day and night until really my engagement with my research has wholly come to its end. Again, my work without their help is incomplete work. Still, I am truly indebted and thankful too.

First and foremost, my advisor, Takele Beyene (Associate Professor), whose guidance has been present every day in my memory. Being also the best and kindest of all people. Co- advisor, Dr. Dereje Nigussie, who has softly encouraged me with technical support and unreserved comments during the thesis writing and Mr. Melaku Wendaferash (botanist) for verification of the scientific name of a medicinal plant.

Also, I would like to thank Addis Ababa University College of Veterinary Medicine and Agriculture, RDC-TR project, National Veterinary Institute and Ethiopian Public Health Institute for their overall support. This study was supported by the thematic research project funded by Addis Ababa University "RDC-TR".

Lastly, my faithful friend who's excellent and perfect companionship I might never forget; my friends and classmates for their sympathy and passionate encouragement to complete my work successfully.

LIST OF ABBREVIATIONS AND ACRONYMS

AAU	Addis Ababa University
ANOVA	Analysis of Variance
CFU	Colony forming unit
CME	Crude methanol extract
CN	Gentamicin
CVMA	College of Veterinary Medicine and Agriculture
DMSO	Di-Methyl-Sulfoxide
Eos	Essential Oils
EPHI	Ethiopian Public Health Institute
EVM	Ethnoveterinary medicine
HSD	Honestly Significant Difference
MBC	Minimum Bactericidal Concentration
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory concentration
NHE	National Herbarium of Ethiopia
NI	No Inhibition
NMSA	National Meteorological Service Agent
NVI	National Veterinary Institute
OIE	World Organization for Animal Health
OT	Oxytetracycline
RDC-TR	Respiratory Disease Complex-Thematic Research project
Spp	Species
SR	Small Ruminants
TM	Traditional Medicine
ZI	Zone of Inhibition

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

LIST OF ANNEXES

PAGE

ABSTRACT

Small ruminants (SR), namely sheep and goats, are playing key roles in supporting the livelihood of poor farmers particularly in the developing country due to their potential to replicate and rapid growth, and valuable commodities where they are raised for meat, milk, and wool. Despite the economic and sociocultural significance of SR in Ethiopia, the occurrence of diseases and other factors cause their productivity to be very low. Pneumonic pasteurellosis is one of the most economically important infectious diseases of SR, with a global distribution. Antibiotics may be effective in treating the disease. However, farmers residing in remote areas of Ethiopia prefer to practice herbal medicines to manage respiratory infections in SR. Hence, the aim of the current study was to evaluate the antibacterial activity of the crude extracts of selected medicinal plants. The medicinal plants, namely *Nicotiana tabacum, Psidium guajava* and *Solanum incanum* were selected based on a literature review of previous studies that showed promising effects on respiratory diseases, namely *P. multocida* and *M. haemolytica* strains. Agar well diffusion method was used to determine the antibacterial activity of methanol and chloroform extracts of the three selected medicinal plants against *P. multocida* and *M. haemolytica* strains. The phytochemical constituents of the extracts of the three medicinal plants were also investigated. The antibacterial activity evaluation results showed the methanol extracts of the three medicinal plants had good activity against the two strains at 200mg/ml concentration and was comparable to gentamicin and streptomycin. From the three selected medicinal plants *S. incanum* showed higher zone of inhibition (26.3mm) as compared to that of *N. tabacum* (19.8mm) and *P. guajava* (19.6mm). Similarly, the chloroform extracts showed good activity against the two strains. However, the chloroform extracts of *P. guajava* showed the highest activity (30.2mm) on *P. multocida* at 200mg/ml. The results of the phytochemical screening showed various levels of alkaloids, flavonoids, tannins, saponins and terpenoids. The results of the antibacterial activity investigation showed that the crude extracts of all tested plants inhibit the growth of the tested bacterial strains. The current findings support the traditional use of these plants against major respiratory diseases causing two bacterial strains in SR.

Keywords: Antibacterial activity; Medicinal plants; *Mannheimia haemolytica; Pasteurella multocida*; phytochemical screening; Small ruminants

1. INTRODUCTION

1.1. Background

Sheep and goats are small ruminants with widespread distributions, particularly in developing countries, due to their potential to replicate and grow rapidly. Small ruminants (SR) contribute significantly to the nutritional security of millions of rural people, particularly landless smallholder farmers in tropical countries (Chakraborty *et al*., 2014). The goat is a valuable commodity in many parts of the world, where it is raised for meat, milk and wool. At the same time, sheep husbandry has significant contributions to rural households by providing mutton, wool, manure and skin (Hakim *et al*., 2014).

Small ruminants are important in Ethiopia, as they are in other developing countries. Despite the presence of large animal populations and their economic significance, the occurrence of diseases, malnutrition, poor management systems and poor performance of the local breed cause animal productivity to be very low. Disease constraints, such as respiratory disease, contribute to significant financial losses and the socioeconomic development of poor farmers (Tewodros & Annania, 2016).

Respiratory tract infection is a common occurrence in small ruminants. However, pneumonic pasteurellosis is one of the most economically important infectious diseases of sheep and goats, with a global distribution. Aerosol spread is the primary mode of transmission for respiratory diseases. The major etiological agents of the disease are *Mannheimia haemolytica* and *Pasteurella multocida*. Both *Mannheimia* and *Pasteurella* species are commensally resident in the upper respiratory tract of healthy ruminants and are capable of causing infection in animals with a compromised pulmonary defence system. This led to great economic losses due to mortality and morbidity, decreased food availability for human use, reduced quality of the final product, and the high cost of treatment (Mohamed & Abdelsalam, 2008).

Antibiotics may be effective in treating the disease. Oxytetracycline is the antibiotic of choice for pasteurellosis because, unlike cattle, there are few antibiotic-resistant strains in sheep and goats (Scott, 2011).

Other antibiotics such as penicillin-streptomycin, tilmicosin and florfenicol can also be used. There is also the use of antibiotics as supplements in animal feed for both prophylaxis and growth promotion, which causes drug resistance. As a result of antibiotic resistance and the high cost of treatment, efforts have been made to discover new sources of active antimicrobials derived from plants to combat infectious diseases (Upadhyay, 2014).

Traditional medicine refers to any ancient, culturally-based healthcare practice that differs from scientific medicine. It is commonly regarded as an indigenous, alternative, or folk medicine that is largely orally transmitted and used by communities of various cultures (Lulekal *et al.*, 2008). Medicinal plants are mostly used in traditional medicines. Ethiopia is thought to be the home of approximately 6500-7000 species, with approximately 12% of these being endemic (Banchiamlak and Young-dong Kim, 2019). The long history of using traditional medicinal plants for treating various ailments in Ethiopia can be confirmed by consulting the country's medico-religious manuscripts. Plant remedies remain the most important, and sometimes the only, source of therapeutics for nearly 80% of humans and 90% of livestock in Ethiopia (Mesfin *et al.*, 2014).

The country has a high level of use and interest in medicinal plants, owing to its cultural acceptability of healers, accessibility, and the low cost of traditional medicine. The majority of Ethiopian farmers, mainly in remote areas of the country, continue to rely on traditional medicine due to the high prices of modern drugs. Other reasons, such as a lack of pharmaceuticals and insufficient coverage by the modern medical system, could also play a role in the use of ethnoveterinary medicines (Yirga *et al.*, 2011).

Phytochemicals are biologically active compounds present in plants that are direct medicinal agents derived from the seed coat, flowers, roots, leaves, barks, seeds, and pulp of plants. Secondary metabolites such as alkaloids, glycosides, phenolics, tannins, saponins etc., are extracted using various solvent systems and extraction techniques. Pharmacologically, secondary metabolites have antiepileptic, anti-inflammatory, anticancer, antipyretic, and analgesic, immunomodulatory, and antimicrobial properties (Reduction *et al.*, 2021).

1.2. Statement of the Problems

In Ethiopia, respiratory diseases, particularly pneumonic pasteurellosis, are causing serious problems in small ruminants. Antimicrobials are the primary tools for preventing and controlling *Pasteurella* and *Mannheimia* infections. However, indiscriminate antimicrobial use increases the risk of selecting resistant bacteria, encourages the spread of resistance genes, and consequently decreases the effectiveness of antimicrobial agents currently available for the treatment of animals used for food production (Kehrenberg *et al*., 2001).

Moreover, antibiotics are less available and very expensive in resource-poor countries. As a result, it would be appealing to develop a socially acceptable and effective remedy from low-cost resources that can supplement modern medicine. Communities in Ethiopia use alternative medicines to treat both human and livestock diseases (Gomaa *et al*., 2019).

Research conducted by Nascimento *et al*., (2000), Kama-Kama *et al*., (2016), Koné and Kamanzi Atindehou (2008) and others has documented plants of medicinal importance against livestock ailments. However, few of the plants being used to treat SR pasteurellosis were documented by El-hamid *et al*., (2019) and Hemeg *et al*., (2020). Furthermore, substantial scientific research to determine the phytochemical composition and pharmacological effects, if any, of many plants with therapeutic promise is still lacking (Godswill, 2019). Hence, further detail investigating the antimicrobial effects of solvent extracts of selected herbal plants that were reported by previous studies in Ethiopia to be used in treating pasteurellosis in SR has paramount importance.

The literature search reveals that still no work has been done on the absolute solvent extracts of *Nicotiana tabacum*, *Psidium guajava*, and *Solanum incanum* plants. Accordingly, the activities of three medicinal plants, against the strains of *Pasteurella multocida* and *Mannheimia haemolytica* were evaluated in this investigation. The aim of this study was to take into account the pharmacological activity of selected medicinal plant crude extracts on pasteurellosis in SR.

Therefore, the specific objectives of this study were:

- To evaluate the in vitro antibacterial activities of crude extracts of *N. tabacum*, *P. guajava* and *S. incanum* compared to commercially available antibiotics (gentamicin, oxytetracycline and streptomycin) against *P. multocida* and *M. haemolytica*.
- To determine bioactive components and the antibacterial activity variation between crude methanol and chloroform extracts of selected medicinal plants.

2. LITERATURE REVIEW

2.1. Pasteurellosis of Small Ruminants in Ethiopia

Pasteurellosis is a multifactorial respiratory disease of ruminants. Pneumonic pasteurellosis is a common respiratory infection in Ethiopia that causes outbreaks of acute pneumonia in sheep and goats of all ages (Abera & Mossie, 2023). It is important to note that *Mannheimia haemolytica* and *Pasteurella multocida* are the most important members of the family *Pasteurellaceae* that pose serious risks in the livestock industry (Tadesse *et al.*, 2017). Pasteurella serotypes are endemic in Ethiopia, posing a significant threat to farm animal production that is difficult to control; however, good management, chemoprophylaxis, and early immunization are recommended control and preventive measures (Jilo *et al.*, 2020).

2.2. Classification of *Pasteurella Multocida* and *Mannheimia Haemolytica*

Bacteria within the family *Pasteurellaceae* play a major role in the final progression to severe pleuropneumonia in cattle, sheep and goats, where "pasteurellosis" is often synonymous with "respiratory disease." Predisposing environmental conditions and/or management conditions that cause stresses in the animals, such as transport (shipping fever), marketing, change of feed, climate, and ventilation are determining factors in the onset of a pathological process that is commonly accompanied by high morbidity but low mortality. The affected animals' slower growth rate causes significant economic losses (Kehrenberg *et al.*, 2001).

Pasteurella and *Mannheimia* species are generally intracellular organisms that elicit many humoral immune responses. *Pasteurella* spp. measures 0.2- 2.0µm in length and are non- motile, non-spore forming, facultative an aerobic, Gram-negative coccobacilli or small rods in the family *Pasteurellaceae*. They are characterized by bipolarity, which is the staining of only the tips of cells, which is demonstrable with a polychrome stain like the Giemsa stain (Tadesse *et al.*, 2017).

Pasteurella multocida subspecies may also be divided into five capsular serogroups (A, B, D, E and F) and *Mannheimia haemolytica* is also classified into 12 serotypes (A1, A2, A5-A9, A12-A14, A16, and A17). *Pasteurella multocida* serotype A and D usually associated with pasteurellosis. *Mannheimia haemolytica* serotype A_1 and A_2 are the most predominant and robust in its ability to resist nutrient deprivation for long period (OIE, 2018).

Despite the fact that antimicrobial therapy is the most effective tool for controlling infectious diseases caused by *Pasteurella multocida*. Antibiotic agents are failing to end many infections due to the emergence of drug resistant pathogens, which is recognized as a serious threat to the effective treatment and prevention of bacterial infections in animals. As a result, effective management is necessary to lessen psychological and physical stressors, reliable diagnostic techniques are required for effective antimicrobial therapy to reduce the development of drug resistance, and the country's available circulating serotypes of the disease agent should be taken into account when producing vaccines (Sarangi *et al.*, 2015).

2.3. Economic Significance of Pneumonic Pasteurellosis

Small ruminants play an important role in the Ethiopian livestock economy because of their remarkable adaptability to harsh environments. They supply more than 30% of all domestic meat consumption and generate revenue from live animal, meat, and skin exports. But disease constraints like respiratory disease contribute to great economic losses for poor farmers (Abera & Mossie, 2023). Diseases causing respiratory problems in sheep and goats have a significant economic impact in Ethiopia's central highlands, with frequent outbreaks and fatalities. Pasteurellosis is a ruminant disease that causes high mortality and morbidity, high treatment costs, reduced weight gain, delayed marketing, and thriftiness among flock survivors (Jilo *et al.*, 2020).

Vaccination is the best alternative practical control strategy to reduce the incidence and burden of the disease and minimize antimicrobial use. Several vaccines against pasteurellosis are currently available worldwide. The presence of multiple serotypes of *M. haemolytica* and *P. multocida* without cross-protection makes the development of a vaccine that is effective globally and regionally challenging (Berhe *et al.*, 2017).

The unfavourable effects and high costs of antimicrobial administration must still be considered in the control of pasteurellosis. As a result, there is an urgent need to develop alternative prophylactic strategies to combat infections caused by these strains. Currently, numerous researchers have rekindled their interest in the medicinal power of some higher plant as a viable source for discovering novel antimicrobial compounds, which has resulted in the development of alternative plant-based antimicrobial drugs with the least side effects as compared to commercial antibiotics (El-hamid *et al*., 2019).

2.4. Ethnoveterinary Medicine (EVM)

In Ethiopia, ethnoveterinary medicine (EVM), the scientific term for traditional animal health care, encompasses a community's knowledge, skills, methods, practices, and beliefs about animal health care. Traditional treatment procedures play an important role in complementary or alternative medicine (Assefa & Bahiru, 2018). While their effectiveness and mechanisms of action have not been scientifically tested in most cases, these simple medicinal formulations frequently mediate beneficial responses due to their active chemical constituents. For disease prevention and control, EVM is one of the alternatives and possibly the most sustainable method easily adaptable to rural livestock farming (Raja Priya *et al*., 2020).

Plant-based remedies are still the most important, and sometimes the only, source of therapeutics for nearly 90% of the livestock population, in the absence of modern veterinary services. It provides medicines that are less expensive and more widely available than pharmacotherapy. Farmers can prepare and use homemade remedies without any expenditure. The knowledge is transferred from generation to generation by word of mouth (oral tales) with greater secrecy than in written form (Tamiru *et al*., 2013).

Prior to the introduction of modern medicine, traditional medicine was the only option for healthcare for the prevention, diagnosis, and treatment of social, mental, and physical illnesses. Because of its long history of practice and existence, it is an essential part of

Ethiopian culture. Traditional medical knowledge of medicinal plants as well as indigenous cultures' use of them are important not only for the preservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future (Yirga *et al.*, 2011).

There is a lot of interest in the ethnoveterinary applications of local plants. This is due to the fact that chemical treatments on animals are suspected of leaving residues in animal products; a number of drugs once thought essential to breeding animals, such as antibiotics used as growth promoters or to prevent ailments such as coccidiosis and respiratory problems, are being phased out; and parasites develop global resistance to chemicals, rendering the treatment ineffective (Landau *et al.*, 2014).

2.4.1. Economic significance of EVM in comparison with modern medicine

Like in other developing and least developed countries, the country's modern health care services are not only insufficient but also inaccessible and unaffordable to the majority. Ethiopians have used traditional medicines (TMs) for many centuries, and their use has become an integral part of Ethiopia's various cultures due to cultural acceptability, efficacy against certain diseases, and economic affordability. EVMs are widely available and simple to prepare and administer, with little or no cost to the farmer. It offers TM treatments, which are locally available and usually less expensive than standard treatments. Homemade remedies can be prepared and used by livestock owners at a low cost (Temeche & Asnakew, 2020).

In Ethiopia, conventional veterinary services have played a critical role in the control and prevention of livestock diseases over the last three decades. However, due to insufficient labour, logistical issues, an erratic supply of drugs, and the high cost of drugs and equipment, they are unable to provide complete coverage in preventive and curative health care practices. As a result, the majority of those raising livestock in rural areas are far from the locations of veterinary stations, and those who do have access to veterinary services may be unable to pay for them. A practical solution to this problem is to create socially acceptable and effective remedies from relatively inexpensive sources that can supplement modern medicine. The practice of traditional veterinary medicine provides such a shortcut (Oyda, 2017).

2.4.2. Description and antimicrobial activities of the selected plants

Tobacco is a genus of plants in the Solanaceae family, which is locally called (“Tambo’’ in Afan Oromo, “Timbeho’’ in Amharic). *Nicotiana* originated from Australia, South West Africa, America, and the South Pacific region. *Nicotiana* species contain a variety of phytochemical metabolites, including pyridine alkaloids, nicotine, isoprenoids, aromatic compounds, and flavonoids. *Nicotiana* species grow naturally in various parts of the world and are used by humans for treatment and recreation (Zou *et al*., 2021). *Nicotiana tabacum* leaf extract exhibited antibacterial activity against Gram-positive and Gram-negative bacte rial species like *Klebsiella pneumoniae, E. coli, Streptococcus, Bacillus subtilis, S. aureus, Pseudomonas aeruginosa,* and *Salmonella typhimurium* at different concentrations in different polar solvents (ethanol, ethyl acetate, butanol and water). The ethyl acetate extract showed a good range of inhibitory effects against Bacillus cereus at a high concentration of 24 mg/mL (Bakht *et al*., 2012).

The *Psidium guajava* (‘’Zayituna’’ in Afan Oromo, ‘’Zeituna’’ in Amharic) well known by a common name ‘’Guava’’ belongs to the Myrtaceae family and is a potentially medicinal plant that is native to South America but is now widely cultivated in tropical and subtropical regions of many countries around the world. Guava has a high antibacterial activity against gram-positive bacteria and a moderate antibacterial activity against gram- negative bacterial strains. Guava leaves, fruits, roots, and barks have traditionally been used to treat diarrhea in various countries. Leaf extracts have anti-inflammatory, analgesic, antioxidant, and antimicrobial properties. Chloroform and ethyl acetate extracts of *P. guajava* demonstrated more sensitivity towards the growth of *B. subtilis* and *P. multocida,* with MICs of 230±3.02, 316.±6.2, 237±5.09 and 288±1.55 µg/ml, respectively (Afzal *et al*., 2019). Guava's therapeutic potential is due to the presence of alkaloids, flavonoids, glycosides, steroids, saponins, terpenes, tannins, vitamin C, and xanthine. The water and methanol extracts of guava leaves showed the same minimum inhibitory concentration (MIC) values against *P. multocida* (0.156 mg/ml) and *Escherichia coli* (5 mg/ml), while the acetone extract showed the highest antibacterial activity against *Streptococcus suis* and *P. multocida* at a MIC of 0.312 mg/ml (Puntawong *et al*., 2012).

The *Solanum incanum* (''Hiddii loonii'' in Afan Oromo, ''Embouy'' in Amharic) is the largest *Solanaceae* (nightshade) family, with several species found in tropical and subtropical areas and used in folk medicine and dietary supplements. The plant is a good source of numerous phytochemicals used against predators and pathogens as well as to treat a variety of illnesses in both humans and animals (Ullah *et al.*, 2016). The fruits of *S. incanum* have been reported to have a marked antibacterial effect against several Gram-positive and Gram-negative bacteria, such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus anthracis*, and *Salmonella* species. Similarly, the leaf extracts showed antibacterial activity against *Escherichia coli*, *S. pyogenes*, *S. aureus* and *P. aeruginosa* (Ayodele *et al.*, 2019). The images of medicinal plants were taken during sample collection indicated in Figure 1.

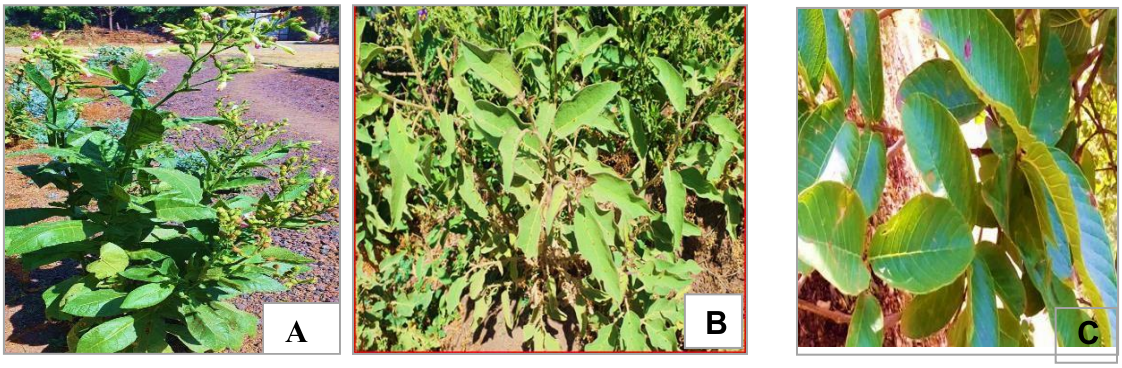


Figure 1. Selected medicinal plants image taken during sample collection. A) *Nicotiana tabacum*; B) *Solanum incanum*; C) *Psidium guajava*

2.4.3. Antimicrobial activity of other herbal extracts

Plant extracts and phytochemicals, both of which possess antimicrobial properties, can be very useful in treating infections. Many plants have been used for their antimicrobial properties, which are due to compounds synthesized in the plant's secondary metabolism (Nascimento *et al.*, 2000). For instance, crude aqueous and hydro-alcoholic extracts of the leaves of *Foeniculum vulgare* ("Insilal"), *Lyonites ocymifolia* ("Ras kimir"), *Jasminum abysinicum* ("Tembelel"), and *Myrsine africana* ("Kechemo") have significant antibacterial activity against some veterinary pathogenic bacterial species (Tesfamariam *et al.*, 2011).

Medicinal plants containing essential oils (EOs) bear great potential for combined or alternative therapy of respiratory conditions. EOs like thyme, eucalyptus, tea tree, cinnamon cassia, winter savoury, coriander wintergreen, peppermint and clove oil are already being used by some practising veterinarians for the treatment of respiratory infections and have effectively inhibited the growth of *P. multocida* and *M. haemolytica* (Bismarck *et al.*, 2022).

Previous research conducted in Ethiopia has also indicated that different medicinal plants differ in the values of the zone of inhibition (ZI) diameter and MIC (Table 1). *Marjoram*, followed by *cinnamon*, has the largest ZI diameters (up to 35 and 33 mm, respectively) with very low-recorded MICs (up to 2 µg/ml each). The antibacterial activities of black seed and onion oil extracts against *P. multocida* isolates were moderate, with ZI diameters and MICs of up to 29 mm and 16 µg/ml, respectively. Garlic oil extract, on the other hand, demonstrated limited antibacterial activity, with the smallest ZI diameters and highest MIC values (up to 15 mm and 64 µg/ml) (El-hamid *et al.*, 2019). Other medicinal plants such as guava (*Psidium guajava L*), mulberry (*Morus alba L.*), and olive (*Olea europaea L*) leaves were tested against several microbial populations representing Gram positive and Gram negative, with MICs and maximum bactericidal concentrations (MBCs) ranging from 625 to 5000µg/ml (Hemeg *et al.*, 2020).

Table 1. The zone of inhibition and MIC values of different medicinal plants against respiratory disease-causing bacteria in livestock

Plant name	Extract	Solvent	Conc.	Bacteria	ZI (mm)	MIC (µg /ml)
Marjoram	crude	DMSO	2 µ g/mL	*PM*	26.3 ± 2.7	8 ± 4.8
Cinnamon	‘’	‘’	2 µ g/mL	‘’	23.3 ± 2.9	36.5 ±14
Garlic	‘’	‘’	2 µ g/mL	‘’	13.3± 0.88	96±14.3
Guava	’’	Ethanol	100%	‘’	18 ± 0.95	5000
Olive	‘’	‘’	100%	‘’	9.12± 0.05	625
Mulberry	‘’	‘’	100%	‘’	5.4 ± 0.15	1250
A. majus	‘’	Methanol	10	‘’	29.40	----
Guava	‘’	‘’	40	‘’	30.6 ± 1.2	0.16
Guava	‘’	Aqueous	40	‘’	29.7 ± 2.2	0.16
S. nigrum	‘’	Methanol	15	‘’	10.7 ± 1.3	----
S. xanthocarpum	‘’	‘’	15	‘’	14.9 ± 3.3	----
F. vulgare	‘’	Aqueous	250	*MH*	11.5	100
L.ocymifolia	‘’	HA	250	‘’	15.5	1000
J.abycynicum	‘’	HA	250	‘’	11	1000
M.africana	‘’	HA	250	‘’	15	1000

DMSO, Dimethyl sulfoxide; HA, hydro-alcoholic extract; MIC, minimum inhibitory concentration; PM, *P. multocida*; MH, *M. haemolytica*; ZI, zone of inhibition. *Source*: (Al-snafi, 2019), (Afzal *et al.*, 2019), (El-hamid *et al.*, 2019), (Hemeg *et al.*, 2020), (Puntawong *et al.*, 2012), and (Tesfamariam *et al.*, 2011).

3. MATERIALS AND METHODS

3.1. Chemicals and Instruments

All chemicals namely 99.8% chloroform (Daryaganj, New Delhi-110002, India) and 99.8% methanol (Tarapur MIDC, Boisar, Palghar, Maharashtra, India) were purchased from chemical importers at Addis Ababa. Antibiotic disks of gentamicin, oxytetracycline and streptomycin (Oxiod Ltd., Basingstoke, Hampshire England), dimethyl sulfoxide (DMSO, Tarapur MIDC, Boisar, Palghar, Maharashtra India), and Mueller Hinton Agar (MHA, D-88/2, MIDC, Turbhe-400705, New Mumbai. India) were also used. Instruments, such as electrical powder grinder, electronic analytical balance, universal bottle, filter paper, rotary evaporator, round flask, Petri dish, Bunsen burner, were available in Addis Ababa University College of Veterinary Medicine and Agriculture (AAU-CVMA), National Veterinary Institute (NVI) and Ethiopian Public Health Institute (EPHI).

3.2. Description of the Study Area

The research was carried out in Bishoftu town, central Oromia region, Ethiopia (Figure 2). Bishoftu is located in the East Shoa Zone of the Oromia regional state. The area is located 47 kilometres southeast of Addis Ababa and at 8°45′ N, 38°59′ E / 8.75°N, 38.983°E with an elevation of 1920 m a.s.l in the central highland of Ethiopia. It has an average annual rainfall of 1150 mm, with 84% falling during the long rainy season from June to September and the remainder falling during the short rainy season from March to May. The mean annual minimum and maximum temperatures are 8.5 and 30.7 °C, respectively, and the mean relative humidity is 61.3% (NMSA, 2005).

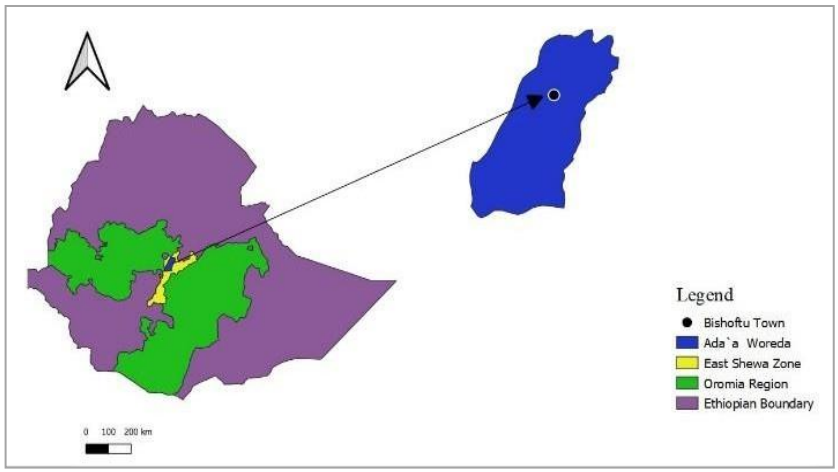


Figure 2. Map of Ethiopia showing the current study area.

3.3. Study Design and Sampling Methods

A laboratory-based experimental study design was used to assess the phytochemical screening test and the in vitro antibacterial activity of the medicinal plants. A literature review based on previous research outputs, traditional uses reported in the available literature, information from society while collecting the medicinal plants and laboratory-based crude extract activity evaluation of medicinal plants with high use value against respiratory problems in livestock or small ruminants (SR) is used as a basis for plant selection. Society informed us that, they have been using these medicinal plants to treat their ruminants when they show a clinical sign of coughing. Three different plants (*Nicotiana tabacum, Psidium guajava* and *Solanum incanum)* were collected to identify the pharmacological activity of selected medicinal plant extracts. An in vitro experimental study of the antibacterial activity of crude extracts of selected plants was done on reference bacteria using the agar well diffusion method.

3.4. Plant Collection and Preparation

In this investigation, the leaves of *N. tabacum*, *P. guajava* and *S. incanum* plants were harvested based on different types of literature reviews and information from the society. During the month of January, plant samples were collected and packed in a polyethylene bag from Bishoftu town on the way of Babogaya Road beside the highway (approximately 8.78 latitudes, 39.00 longitudes), 50kms from Addis Ababa, Oromia, Ethiopia. Since the plants are widely grown abundantly, no access permit was required

for the collection of these plants. Specimens were transported to Addis Ababa University, College of Natural Science herbarium for future reference, and taxonomically identified and authenticated by botanist with voucher specimen numbers BA001, BA002, and BA003, respectively. After collection, plant materials were washed with running tap water to remove the soil and dust particles. Plant materials were dried under shade for three weeks at room temperature, and ground into a fine powder. Finally, the specimens were preserved in a closed container at 4°C for further use at AAU-CVMA. A summary of the methodology is shown in Figure 3.

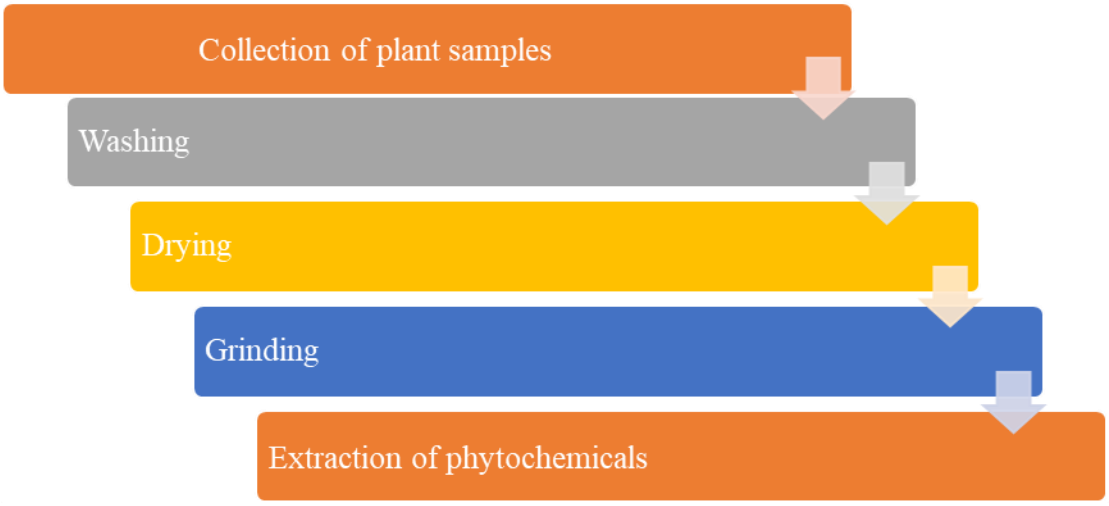


Figure 3. Methodology summary

3.5. Extraction of Medicinal Plants

Extraction of medicinal plants is the process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from the inert or inactive material using an appropriate solvent and a standard extraction procedure (Poojar *et al.*, 2017). The extraction was performed using a maceration technique (Bibi *et al.*, 2016). A 100-gram powdered leaf sample of each medicinal plant was placed in different round extraction flask and macerated with 400 ml of extracting solvents (99.8% methanol and chloroform) at room temperature. This procedure was repeated three times and the mixture was allowed to stand for 72 hours and stirred intermittently to facilitate extraction. The mixture was filtered through Whitman paper. The resulting volume of filtration was reduced using the rotary evaporator (Rotavapor R-200, Buchi) water bath temperature initially adjusted at 60°C

but not exceeding 40ºC when the extract was nearly dry to obtain a crude residue. Finally extracts allowed to air in Petri dish and drying were done using a hot air oven at 40ºC. The dried concentrate was weighted, percentage yield determined and scooped into well labelled sterile screwed bottle and stored in refrigerator at 4ºC until tested for antibacterial activity and phytochemical screening for identification of secondary metabolite. The summary of phytochemical extraction procedure of *P. guajava* shown on Annex 1. The same procedure applied for all selected medicinal plants.

3.6. Phytochemical Screening

In order to identify different classes of phytoconstituents present in different parts of the base for the discovery of drugs, a scientific process known as phytochemical screening involves analyzing, inspecting, extracting, and experimenting (Sharma *et al.*, 2020). Various chemical tests were carried out in order to create a profile of the three extracts' chemical composition. The presence of alkaloids, glycosides, saponins, flavonoids, steroids, tannins were analyzed by standard phytochemical tests. Different chemical reagents were prepared and specific test, for specific phytochemicals was done.

Alkaloids: To 2ml of extract, 1ml of Mayer's reagent was added. The formation of a brown/ reddish colour precipitate indicates the presence of alkaloids (Siddiqui, 2021).

Flavonoids: 2 ml of 2% sodium hydroxide solution was added to 1 ml of each extract. A yellow coloration was observed indicating the presence of flavonoids (Abdalla *et al.*, 2020).

Phenols: plant extract was dissolved in 5ml distilled water and 3 ml of 10% lead acetate solution was added. The formation of white precipitate colour indicates the presence of phenols (Pandey & Tripathi, 2014).

Saponins: 2 g of powdered sample was boiled in 20 mL of distilled water. 10 mL of filtrate, 5 mL of distilled water were quivered vigorously. The appearance of frothing indicated the presence of saponins (Abdalla *et al.*, 2020).

Steroids: 1g of plant extract was dissolved in distilled water and filtrated. A few drops of concentrated sulfuric acid was added. The appearance of red colour in the lower layer indicates the presence of steroids (T. Sharma *et al.*, 2020).

Tannins: To the extract, 4 ml of 10% sodium hydroxide was added. The formation of emulsion indicates the presence of tannins (Pandey & Tripathi, 2014).

Terpenoids: 0.2g of crude extract was dissolved in 2 ml of chloroform. To this, 2 ml of concentrated H_2SO_4 was added. An interface with a reddish-brown colour indicates the presence of terpenoids (Siddiqui, 2021).

3.7. Identification of Bioactive Constituents from Medicinal Plants

Medicinal plants contain various types of bioactive compounds or phytochemicals with different polarities, their separation remains as big challenge for the process of identification and characterization of them. They have been discovered to be precursors for the semi-synthesis of chemotherapeutics. Several secondary metabolites or natural products isolated from various medicinal plants have clinically active properties or can be used as drug leads (Macdonald *et al.*, 2016).

3.8. Source of Bacterial Strains

Two lyophilized reference bacterial strains, specifically *M. haemolytica* (serotype A_2) and *P. multocida* (serotype A) isolated from small ruminants, were provided by National Veterinary Institute's (NVI) Microbiology Laboratory in Bishoftu, Oromia. In collaboration with the institute, further laboratory procedures were carried out to evaluate the in vitro efficacy of plant extracts.

3.9. Preparation of Inoculum

Mueller Hinton Agar (MHA) medium was prepared by adding 38g in 1 litre of distilled water and boiled to dissolve the powder completely. This procedure was repeated four times (total 171g in 4.5 litre). After dissolving, sterilized with the help of an autoclave at

pressure of 151Lbs and temperature of 121^0c for 15 minutes. Then, all Petri dishes were filled with an equal volume of 25ml. The bacterial strains were prepared from frozen stock, streaked on MHA plates and incubated at 37^0C for 24hrs. After incubation, a single colony of each organism was picked and inoculated into 5ml of distilled water and the tube was shaken vigorously using a vortex shaker to obtain homogeneity of the solution. Each cultured isolate was compared with 0.5 McFarland standard, creating 10^8 colony forming units (CFU)/ml for turbidity standards as described by Remel *et al.*, (2020). Distilled water was used to standardize the turbidity of the isolates.

3.10. Preparation of Stock Solution and Serial Dilution

This was carried out using the procedures outlined by Charles *et al.*, (2012). Solutions 400mg/ml were prepared by reconstituting 800mg of each of the dried crude powder in 2ml of 5% dimethyl sulfoxide (DMSO) solution. From this stock solution, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml working solutions were prepared and used for susceptibility testing. Five sterile vial bottles for each extract were arranged on a table and 1ml of sterile solvent solution was dispensed into them. From the stock solution, 1ml of each extract was transferred into the first vial bottle and agitated by vortex and then successive two-fold serial dilutions of each extract were carried out. The resultant concentrations of *N. tabacum*, *P. guajava* and *S. incanum* in the vial bottles were 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml as shown in Annex 2 (A).

3.11. Screening for Antibacterial Activities

The antibacterial activities of the extracts against reference bacterial strains of *Mannheimia haemolytica* (Serotype A_2) and *Pasteurella multocida* (Serotype A) were determined using the agar-well diffusion method, as described by Romha *et al.*, (2018). The inoculum of each of the test organisms (prepared in sterile test tube) were inoculated on the surface of Mueller Hinton agar plates with sterile swabs and evenly distributed by rocking the plate. The plates were allowed to dry at room temperature but covered to prevent atmospheric contamination. After that, using a circular 6 mm diameter cork borer, two holes (2 for plant extracts at concentrations of 200mg/ml, 100mg/ml and two antibiotic disks on the first petri dish) and four holes (3 for plant extracts at

concentrations of 50mg/ml 25mg/ml and 12.5 mg/ml and 1 for negative control (DMSO)) on the second petri dishes were made on the surface of the Mueller Hinton agar plate at equal distances apart. The holes were filled with 100µL methanol and chloroform extracts of *N. tabacum*, *S. Incanum and* P. *guajava* leaves (at concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5 mg/ml), as well as the negative control, dimethyl sulfoxide (DMSO) as indicated on Annex 2 (B). As a positive control, an antibiotic disc containing Gentamicin (10 µg), Oxytetracycline (30µg) and Streptomycin (10 µg) was placed using forceps on the surface of the agar plate. Petri dishes were pre-incubated at 4˚C for 2 hours to allow uniform diffusion of extracts into the agar. After pre-incubation, these plates were incubated at 37˚C and examined after 24hrs of growth. For each of methanol and chloroform extracts of all plants, the tests were performed three times. The antibacterial agent diffuses in the agar medium and inhibits the growth of bacterial strains tested. The antibacterial activities were evaluated by measuring the zone of inhibition in millimeters (mm) using a digital caliper as shown on Annex 2 (C). Each zone of inhibition was tested twice (vertically and horizontally), and the average value has been calculated to ensure that readings were accurate. The results were noted as a mean of three independent replicates for each extract. The effect was compared to those of antibiotic discs. A stock solution and working solutions of plant extracts were prepared in 5% dimethyl sulfoxide (DMSO) in distilled water. The whole experiment were performed under strict aseptic conditions.

3.12. Data Analysis

The raw data was stored in the Microsoft Excel database system used for data management. Statistical analysis was performed using R-Studio software. Statistical differences in the mean ZI for two bacterial strains *P. multocida* (Serotype A) and *M. haemolytica* (serotype A_2) and differences in susceptibility of bacteria between three selected medicinal plants were determined by one-way analysis of variance (ANOVA) followed by multiple comparison tests (Post Hoc/Tukey's test/HSD) to compare parameters within and between groups at a significance level of $P < 0.05$. The p-value was considered significant and all data were expressed as mean ± standard deviation of the mean.

4. RESULTS

4.1. Yields of Extracts

The percentage yield of absolute methanol and chloroform extracts of *N. tabacum*, *S. incanum* and *P. guajava* are given in Table 2. The maximum yield was obtained from the methanol extract of *S. incanum* (13.5%), followed by *N. tabacum* (8.5%) and *P. guajava* (3.3%). In contrast, the maximum yield was obtained from the absolute chloroform extracts of *N. tabacum* (4 %), *P.guajava* (2.1%) and *S.incanum* (1.46%) respectively. A marked contrast was observed between methanol and chloroform extracts of *S. incanum*. The percentage yield obtained from methanol and chloroform extracts of *N. tabacum* and *P. guajava* is almost half of each other (Table 2). From this investigation, methanol extract of these three medicinal plants showed significantly ($P<0.05$) a higher percentage yield than that of chloroform extract. The percentage yield was calculated as weight of extract obtained/weight of plant sample X100 (Duniya *et al.*, 2018).

Table 2. The weight in gram (g) and percentage yield of crude methanol and chloroform extracts of *N. tabacum*, *P. guajava* and *S. incanum*

Sample	**Weight of sample in g**	**Methanol extract**		**Chloroform extract**	
		Weight (g)	Yield (%)	Weight (g)	Yield (%)
N. tabacum	300	25.5	8.5	12.2	4.0
P. guajava	300	9.9	3.3	6.3	2.1
S. incanum	300	40.5	13.5	4.4	1.46

4.2. Results of the Phytochemical Screening

The tests were done on crude methanol and chloroform extracts to assure the presence of bioactive components in the leaves of *N. tabacum*, *S. incanum* and *P. guajava*. The presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids were determined using the standard method lined by Abdalla *et al.*, (2020), Siddiqui, (2021) and Pandey and Tripathi, (2014) (Table 3).

Table 3. Qualitative phytochemical analysis of leaves extract of *N. tabacum*, *P. guajava* and *S. incanum*

Phytochemical	*N. tabacum*		*P. guajava*		*S. incanum*	
	Methanol	chloroform	Methanol	Chloroform	Methanol	Chloroform
Alkaloids	+	+	+	-	+	-
Flavonoids	-	+	-	+	-	-
Phenols	+	+	+	+	+	+
Saponins	+	+	+	±	+	±
Steroids	+	-	+	-	+	-
Tannin	+	+	+	+	-	+
Terpenoids	+	-	+	+	+	-

+ means presence of phytochemicals; - means absence of phytochemicals; ± found in lower

4.3. Results of Antibacterial Activity of Plant Extracts

The antibacterial activity of plant extracts was evaluated using the agar well diffusion method. The findings of the assessment showed various degrees of efficacy against *P. multocida* and *M. haemolytica*. The in vitro antibacterial activity was tested in the presence or absence of ZI, in comparison with reference drugs. The reference antibiotics (gentamicin, oxytetracycline and streptomycin) were chosen because they are often used as first line antibiotics for this bacterial infection. The observed ZI in diameter from the edge of each well varied according to their increasing order of concentration, between solvents and individual bacteria (Annex 3, 4 and 5). The mean ZI of triplicate experiments for the five different concentrations of extracts are summarized in (Table 4).

The absolute concentration (99.8%) of methanol and chloroform extracts of *N. tabacum*, *P. guajava* and *S. incanum* showed high ZI against both tested bacterial strains of *P. multocida* and *M. haemolytica* serotypes at concentrations of 200mg/ml (Table 4). The methanol and chloroform extracts of *N. tabacum* showed (19.8, 15.34mm) and (19.06,

15.29mm) zone of inhibition on *P. multocida* and *M. haemolytica* respectively at a concentration of 200mg/ml. In contrast methanol extracts of *N. tabacum* has no ZI at a concentration of 12.5mg/ml, but chloroform extracts has 6mm ZI. At the same time the methanol and chloroform extracts of *P. guajava* against *P. multocida* with (19.6, 30.2mm) inhibition zone at concentrations of 200mg/ml and *M. haemolytica* with the ZI being (18.6, 15mm) respectively. Methanol and chloroform extracts of *S. incanum* showed (26.34, 16.08mm) ZI on *P. multocida* and (22.22, 14.77mm) zone of inhibition on *M. haemolytica* at concentration of 200mg/ml.

There was a significant difference in ZI between the three selected medicinal plant extracts at different concentration as compared to reference drugs. All five concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of chloroform extracts of the three selected medicinal plants showed significant activity against two bacterial strains, as compared to the standard drugs. The highest ZI was recorded by methanol extracts of S. *incanum* (26.34mm) and chloroform extracts of *P. guajava* (30.2mm) on *Pasteurella multocida*. The methanol and chloroform extracts of *N. tabacum* and *P. guajava* showed low activity at concentration of 25mg/ml. The variability of the M. *haemolytica and P. multocida* susceptibility to the extracts was tested using pairwise comparison of mean ANOVA ($p>0.05$).

There was no significant difference between the two strains in terms of susceptibility. The result indicated that *P. multocida* has more susceptibility to all extracts at different concentrations and standard drugs as compared to *M. haemolytica*. The methanol extracts of *S. incanum*, *N. tabacum* and *P. guajava* showed a high ZI (26.3, 19.8 and 19.6mm) compared with reference drugs on *P. multocida* and *M. haemolytica* with a fairly good ZI (22.2, 19 and 18.6mm) at a concentration of 200mg/ml respectively.

The methanol and chloroform extracts of the three plant leaf extracts showed different levels of ZI and no ZI at different concentrations. All of the standard drugs (antibiotic discs) used in the test (gentamicin 10µg, oxytetracycline 30µg and streptomycin 10µg) showed varied zone of inhibition (22.3, 35.2 and 16.6mm) and (19.5, 31 and 13mm) on *P. multocida* and *M. haemolytica,* respectively. Oxytetracycline showed the highest zone of inhibition (53.2mm), followed by gentamicin (22.3mm) and streptomycin (16.6mm).

There was no ZI in the case of negative control (DMSO). In general, it was found that bacterial growth inhibition, as indicated by the diameter of ZI, became increased as the concentration of plant extract increased (Table 4).

Table 4. Antibacterial test results of methanol and chloroform extracts of *N. tabacum*, *P. guajava* and *S. incanum* with mean zone of inhibition (mm) (Mean ± Standard deviation)

Bacterial strains	**Conc. (mg/ml)**	**Zone of inhibition (mm) (Mean ± Standard deviation)**					
		N. tabacum		*P. guajava*		*S. incanum*	
		Methanol	Chloroform	Methanol	chloroform	Methanol	Chloroform
P.multocida (Serotype A)	200	19.8±0.49	15.3±0.61	19.6±0.28	30.2±0.41	26.3±0.24	16.0±0.14
	100	15.3±0.57	13.3±0.63	13.6±0.31	28.5±0.33	24.3±0.22	14.6±0.17
	50	12.2±0.35	12.1±0.2	12.6±0.12	19.4±0.43	20.6±0.3	13.4±0.14
	25	6	10.9±0.27	6	15.7±0.85	19.3±0.49	12.0±0.43
	12.5	NI	6	NI	12.9±0.26	17.3±0.26	10.4±0.23
	10µg CN	22.3±0.37	22.3±0.37	22.3±0.37	22.3±0.37	22.3±0.37	22.3±0.37
	30µg OT	35.2±0.5	35.2±0.5	35.2±0.5	35.2±0.5	35.2±0.5	35.2±0.5
	10µg S	16.6±0.1	16.6±0.1	16.6±0.1	16.6±0.1	16.6±0.1	16.6±0.1
	DMSO	NI	NI	NI	NI	NI	NI
M. haemolytica (serotype A_2)	200	19.0±0.34	15.3±0.55	18.6±0.35	15.0±0.5	22.2±0.49	14.7±0.42
	100	15.4±0.62	12.2±0.11	13.6±.36	13.3±0.6	20.5±0.19	12.8±0.23
	50	13.4±0.5	11.2±0.14	10.3±0.32	12.3±0.46	18.9±0.06	12.1±0.31
	25	6	10.4±0.17	8.7±.29	10.8±0.23	16.9±0.16	10.7±0.05
	12.5	NI	6	6	8.6±0.23	14.7±0.15	9.4±0.2
	CN	19.5±0.55	19.5±0.55	19.5±0.55	19.5±0.55	19.5±0.55	19.5±0.55
	OT	31.0±0.5	31.0±0.5	31.0±0.5	31.0±0.5	31.0±0.5	31.0±0.5
	S	13.0±0.1	13.0±0.1	13.0±0.1	13.0±0.1	13.0±0.1	13.0±0.1
	DMSO	NI	NI	NI	NI	NI	NI

Keys; CN: Gentamicin; NI: No inhibition; OT: Oxytetracycline; S: Streptomycin

5. DISCUSSION

The use of antimicrobial agents to treat infectious diseases reveals numerous issues that result in bacterial resistance to various antibiotics. As a consequence of the emergence of resistance, conventional antimicrobial drugs are becoming ineffective. Natural products contain a variety of lead compounds that may facilitate the development of novel antimicrobial agents. A medicinal plants secondary metabolites may act against bacteria in various ways that prevent the emergence of resistance (Nigussie *et al.*, 2021).

A wide range of these bioactive substances, which are found in different plant parts, can play complementary roles in the regulation of various mechanisms, including immune system stimulation, the control of gene expression in cell proliferation and apoptosis, the metabolism of hormones, and the effects of antioxidant, antibacterial, and antiviral agents (Afzal *et al.*, 2019). For many years, various animal and human diseases have been treated with medicinal plants and herbs. More and more reports of medicinal plants' antimicrobial properties are coming in from all over the world. The antimicrobial substances found in plants might inhibit bacterial growth through methods other than those currently in use (Yavuz *et al.*, 2017).

The pharmacological activity evaluation study result of the three selected medicinal plants was based on literatures review and information from the society during collection. The finding of the present study indicated that the absolute methanol extraction yields of *N. tabacum*, *P. guajava* and *S. incanum* were significantly ($P<0.05$) higher when compared to absolute chloroform extract. The highest yield was obtained with the most polar solvent (methanol) next to the water and the lowest yield was obtained with lower polarity (chloroform). This shows that the difference in extraction yield is due to the polarities of different compounds found in the plants. When the three plants were compared for their yield, the absolute methanol extracts of *S. incanum* was higher than that of two medicinal plants (Table 2). Hence, the current study found that the three selected medicinal plants contain more polar bioactive ingredients rather than low polarity bioactive compounds which showed that solvent influences the extraction index of bioactive compounds. The bioactive ingredients are not found uniformly between plants and some plants tend to have more bioactive compounds (Duistermaat & Kolk, 2000).

A comparison of the antibacterial activity of methanol and chloroform extracts was done per each medicinal plants against the tested bacterial strains. According to the results of the agar well diffusion method, all plants tested in this study showed good antibacterial activity against the two strains, with ZI that ranged from 6 to 30.2mm. The absolute chloroform extracts of *P. guajava* showed the highest ZI (30.2mm) on *P.multocida* at a concentration of 200mg/ml as compared to that of all selected medicinal plant extracts which is in agreement with the previous findings (Afzal *et al*., 2019) with the exception of concentration difference. When compared to reference drugs, it has a comparable antibacterial activity to oxytetracycline against *M. haemolytica*. Also better activity than gentamicin and streptomycin against *P.multocida* and *M. haemolytica* (Table 4). The observation from this study is in line with the previous report describing the antibacterial activity of *P. guajava* (Puntawong *et al*., 2012).

Similarly, methanol extracts of *Solanum incanum* showed a higher zone of inhibition at all concentrations as compared to *N. tabacum* and *P. guajava* and better activity than Gentamicin and Streptomycin against *P. multocida* and *M. haemolytica* at 200mg/ml and 100mg/ml concentrations. According to the previous studies reported methanol extracts of leaves of *Solanum incanum* (*Solanaceae*) have an antibacterial activity against multidrug resistance bacteria (Ayodele *et al*., 2019). Methanol extracts of *N. tabacum* and *P. guajava* were found to have the lowest activity against the two strains at 25mg/ml and did not inhibit the growth of the two strains at 12.5mg/ml concentrations. The result suggested that 25mg/ml is the minimum inhibitory concentration against the two strains. The chloroform extracts of the three selected medicinal plant showed less activity against the two strains at 12.5mg/ml concentration (Table 4). The mean zone of inhibition values obtained from the present study indicated that the methanol extracts of the three selected medicinal plant leaves were more potent against *P. multocida* than *M. haemolytica* as compared to reference drugs. In comparison with the standard (reference) drug streptomycin, the highest activity of three selected medicinal plants was recorded against the two bacterial strains. Based on the results of this study, almost all crude extracts of each selected medicinal plant showed good antibacterial activity at high concentrations.

According to performance standards for antimicrobial susceptibility testing, the organisms are susceptible (≥15mm), intermediate (12-14mm) and resistant (≤11mm) to antibiotics based on their zone diameters (CLSI, 2020). Based on this criteria the current

study showed that *P. multocida* was susceptible to the three selected reference drugs (gentamicin, oxytetracycline and streptomycin). While *Mannheimia haemolytica* was susceptible to gentamicin and oxytetracycline and intermediate to streptomycin antibiotics. However, there isn't a set formula for determining whether a zone of inhibition is resistant, intermediate, or susceptible to plant extracts.

To a large extent, phytochemical screening of medicinal plants is necessary for the discovery of new sources of essential metabolites for both industrial and therapeutic purposes. Plants have the potential to be underutilized sources of powerful antibacterial agents because they are a significant source of chemical diversity and secondary metabolites. The majority of secondary metabolites are inherently antibacterial. Plants use phytochemicals for a variety of secondary purposes, such as promoting plant growth, protecting them by triggering defence mechanisms and providing colour, flavour, and odour (WHO, 2004).

For instance, phenol derivatives reduce pH, increase membrane permeability, or alter efflux pumping to inhibit bacterial growth. Phenolic compounds have an impact on a variety of bacterial targets, such as cytoplasmic membrane damage, topoisomerase inhibition, NADH-reductase, and ATP synthase inhibition. Additionally, tannins cause bacterial membrane damage and metabolism inactivation. In turn, flavonoids may encourage the production of extracellular complex soluble proteins and inhibit DNA synthesis, metabolism, and proteins found in cell walls. Plant secondary metabolic compounds are promising candidates to be used in the development of new drugs to address the escalating issue of antimicrobial resistance due to their mechanisms of action (Keita *et al.*, 2022).

Saponins may have a toxic effect on all organized tissues by altering the permeability of cell walls. By combining with cell membranes to cause changes in cell morphology that result in cell lysis, they are able to exert some antibacterial activity. Different plant-based components may act against bacterial strains in different ways, including by interfering with phospholipid cell membranes, which increases permeability and results in the loss of cellular components; harming the enzymes responsible for cellular energy production and structural component synthesis; and destroying or inactivating genetic material. In general, disruption of the cytoplasmic membrane, disruption of the proton motive force,

electron flow, active transport mechanisms, and coagulation of cell composition are thought to be the mechanisms of action (C *et al.*, 2014). Secondary metabolites in plants are typically produced as protective mechanisms against animals, insects, pathogens, and predators (B *et al.*, 2006).

Alkaloids, flavonoids, saponins, tannins, terpenoids, phenolics, and steroids were detected in *N. tabacum*, *P. guajava*, and *S. incanum* leaf extracts through qualitative phytochemical screening. Phytochemical studies showed a remarkable presence of Alkaloids, saponins, tannins, terpenoids, phenolic, and steroids in methanol extracts of *N. tabacum* and *P. guajava*. Flavonoids were found to be absent. Flavonoids and tannins were found to be absent in methanol extracts of *S. incanum* (Table 3 and Annex 6). The results of this investigation agree with those of the previous study reports (Naseer *et al.*, 2018; Sbhatu & Abraha, 2020 and Séré *et al.*, 2022).

Flavonoids found to be present in the methanol extract of the stem of *N. tabacum* as reported by Y. Sharma *et al.*, (2016). When compared to methanol, chloroform extracts where the bioactive compounds were only present in low concentrations. Low concentrations of secondary metabolites were found in each extract. Chloroform extracts of *N. tabacum* leaves have been positively tested for alkaloids, steroids and terpenoids and negatively tested for flavonoids, phenolics, tannin and saponins (Oeung & Yin, 2017) which is in contrast to our investigation showing the presence of Alkaloids, flavonoids, phenolic, tannins and saponins in the chloroform extracts of *N. tabacum* whereas steroids and terpenoids were found to be absent. Flavonoids, phenolic, tannins, terpenoids and saponins found to be present in chloroform extracts of *P. guajava*; however, alkaloids and steroids were found absent. Phytochemical studies undertaken by previous researcher reported that only phenol and terpenoids were present in chloroform extracts of *P. guajava* (Fitokimia, 2020). Phenolic, tannins and saponins were present in the chloroform extracts of *S. incanum* whereas alkaloids, flavonoids, steroids, tannins and terpenoids were found absent (Table 3 and Annex 7). This is in agreement with the study reported by Hamida *et al.*, (2022).

Generally, this study was based on a literature review and information from society while collecting medicinal plants. However, most of the previous researchers reported the antibacterial activity of the three selected medicinal plants on other bacterial diseases rather than pasteurellosis in small ruminants. Hence, this investigation served as a significant starting point for additional research into the isolation and characterization of phytoconstituents from the chosen plants for drug development.

Study limitations

This research was carried out with the greatest care of all concerned bodies to finalize this work at the AAU-CVMA and in collaboration with the National Veterinary Institute and the Ethiopian Public Health Institute. It was also accompanied by a few unfortunate circumstances in the meantime. *N. tabacum*, *P. guajava* and *S. incanum* macerated extracts were used in this study, which was conducted using only 99.8% methanol and chloroform. Due to the high cost of solvents and the lack of resources, other alternative extraction and fractionation techniques were not conducted. While conducting this research, there is also a lack of strong and in-depth investigation of the pharmacological properties and its relationship to structural elucidation

6. CONCLUSION AND RECOMMENDATIONS

With the help of this work, we were able to extract materials from the leaves of *N. tabacum*, *P. guajava* and *S. incanum*. When comparing the methanol and the chloroform macerated extracts, the extraction yield reveals a predominance of polar compounds. The main goal of this study was to assess the antibacterial activity of the three selected medicinal plants' crude extracts. Taking this into consideration, this study showed the antibacterial activity of *N. tabacum*, *P. guajava* and *S. incanum* against the two bacterial strains causing respiratory disease in small ruminants. Medicinally effective bioactive compounds (secondary metabolites) found in the three selected medicinal plants were detected using phytochemical screening. Qualitative analysis reveals the presence of secondary metabolites, including flavonoids, terpenoids, steroids, saponin, alkaloids, and tannins. These phytochemicals could render the medicinal values of the studied medicinal plants. The above study provided a strong evidence that the absolute methanol extracts of *N. tabacum*, *P. guajava* and *S. incanum* contains high contents of medicinally important secondary metabolites as compared to absolute chloroform extract. Based on the above conclusions the following recommendations are forwarded;

- Future studies should strongly advocate for the bioassay guided fractionation, purification, and characterization of the bioactive compounds to determine antibacterial effect of specific compounds.
- It is necessary to conduct additional scientific studies to assess the pharmacological (mechanism of action, MBC and MIC) and toxicological (in vivo toxicity), safety and standardize dose of these medicinal plants.

7. REFERENCES

Abdalla, A. A., Mustafa, I., Makhawi, A. M., & Mustafa, M. I. (2020). Phytochemical screening and antimicrobial activities studies of Acacia nilotica fruit cover. *BioRxiv*, *Mic*, 2020.02.11.943456.

Abera, D., & Mossie, T. (2023). A review on pneumonic pasteurellosis in small ruminants. *Journal of Applied Animal Research*, ***51***(1), 1–10.

Afzal, M., Iqbal, R., Mahmood, Z., Zeshan, B., & Wattoo, J. I. (2019). Study of gc-ms and hplc characterized metabolic compounds in guava (Psidium guajavaL.) LEAVES. *Pakistan Journal of Agricultural Sciences*, ***56***(3), 709–713.

Al-snafi, A. E. (2019). *Iraqi Medicinal Plants with Antibacterial Effect- A Review*. ***9***(8), 22–103.

Assefa, A., & Bahiru, A. (2018). Ethnoveterinary botanical survey of medicinal plants in Abergelle, Sekota and Lalibela districts of Amhara region, Northern Ethiopia. *Journal of Ethnopharmacology*, *213*, 340–349.

Ayodele, O. A., Yakaka, A. B., Sulayman, T. B., & Samaila, M. (2019). Antibacterial activities of aqueous and methanol leaf extracts of Solanum incanum Linn. (Solanaceae) against multi-drug resistant bacterial isolates. *African Journal of Microbiology Research*, ***13***(4), 70–76.

B., Zipfel, C., Rowland, O., Keller, I., Robatzek, S., & Jones, J. D. G. (2006). *Plant Response to Bacterial Pathogens. Overlap between Innate and Gene-for-Gene Defense Response*. ***142***(November), 809–811.

Bakht, J., Azra, & Shafi, M. (2012). Antimicrobial activity of Nicotiana Tabacum using different solvents extracts. *Pakistan Journal of Botany*, ***44***(1), 459–463.

Banchiamlak, N. T., & Young-dongKim. (2019). Ethnobotanical study of medicinal plants in the Hawassa Zuria District, Sidama zone, Southern Ethiopia. *Journal of ethnobiology and Ethnomedicine*, *7*, 1–21.

Berhe, K., Weldeselassie, G., Bettridge, J., Christley, R. M., & Abdi, R. D. (2017). Small ruminant pasteurellosis in Tigray region, Ethiopia: Marked serotype diversity may affect vaccine efficacy. *Epidemiology and Infection*, ***145***(7), 1326–1338.

Bibi, Y., Qayyum, A., Nisa, S., Waheed, A., & Chaudhary, M. F. (2016). Isolation studies from stem extract of Pistacia Integerrima Stew. Ex Brand. *Journal of the Chilean Chemical Society*, ***61***(2), 2916–2920.

Bismarck, D., Becker, J., Becher, V., Nau, L., Mayer, P., Kissingen, B., & Waldsee, B. (2022). *Screening of Antimicrobial Activity of Essential Oils against Bovine Respiratory Pathogens – Focusing on Pasteurella multocida Authors*. 274–281.

C, O. G., O, E. F., O, J. A., & O, E. C. (2014). *Mechanisms of Antimicrobial Actions of Phytochemicals against Enteric Pathogens – A Review*. ***2***(August), 77–85.

Chakraborty, S., Kumar, A., Tiwari, R., Rahal, A., Malik, Y., Dhama, K., Pal, A., & Prasad, M. (2014). *Small Ruminants*. *2014*.

Charles, O. E., Anthony, A. A., Kwaliafon, S. M., Nneka, N. I., & Kennedy, F. C. (2012). Antimicrobial activity of Psidium guajava Linn. Stem extracts against methicillin-resistant Staphylococcus aureus. *African Journal of Biotechnology*, ***11***(89), 15556–15559.

CLSI. (2020). CLSI M100-ED29: 2020 Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition. In *Clsi* (Vol. **40**, Issue 1).

Duistermaat, J. J., & Kolk, J. A. C. (2000). *Proper Actions*. ***8***, 93–130.

Duniya, S. V., Ojonugwa, M. C., Adamu, A. D., John, O., Eleojo, S. I., & Salifu, U. O. (2018). Phytochemical constituent, percentage yield and phenolic content estimation of different solvent system of Caricapapaya leave. *Int. J. Chem, Pharm, Sci.*, ***6***(7), 201–205.

El-hamid, M. I. A., El-sayed, M. E., Ali, A. R., Abdallah, H. M., & Arnaout, M. I. (2019). Comparative Immunology, Microbiology and Infectious Diseases Marjoram extract down-regulates the expression of Pasteurella multocida adhesion, colonization and toxin genes: A potential mechanism for its antimicrobial activity. *Comparative Immunology, Microbiology and Infectious Diseases*, ***62***(February 2019), 101–108.

Fitokimia, S. (2020). *Phytochemical screening and antioxidant activity of Psidium guajava*. ***24***(2), 173–178.

Godswill, C. (2019). *Medicinal plants: the medical, food, and*. *5*(11).

Gomaa, S. E., Yahayu, M. A., Nurjayadi, M., Dailin, D. J., & El Enshasy, H. A. (2019). Antimicrobial compounds from Catharanthus roseus- A review. *International Journal of Scientific and Technology Research*, ***8***(10), 113–121.

Hakim, A. S., Bakry, M. A., Nagwa, S. S. A., & Zaki, M. S. (2014). Role of molecular techniques in characterization of bacteria causing pneumonia in small ruminants. *Life Science Journal*, ***11***(6), 147–153.

Hamida, M. E., Ateshim, B., Tekle, F., kahsay, M. T., Tekleab, M., Tekie, R., Weldemariam, S., Achila, O. O., Mengistu, S. T., & Ghebremeskel, G. G. (2022). Evaluation of antimicrobial activity and phytochemical screening of Silene

macrosolen and Solanum incanum: A common medicinal plant in Eritrea. *Journal of Bacteriology & Mycology: Open Access*, ***10***(1), 15–20.

Hemeg, H. A., Moussa, I. M., Ibrahim, S., Dawoud, T. M., Alhaji, J. H., Mubarak, A. S., Kabli, S. A., Alsubki, R. A., Tawfik, A. M., & Marouf, S. A. (2020). Saudi Journal of Biological Sciences Antimicrobial effect of different herbal plant extracts against different microbial population.

Jilo, K., Belachew, T., Birhanu, W., Habte, D., Yadeta, W., & Giro, A. (2020). *Journal of Tropical Diseases and Public Health Pasteurellosis Status in Ethiopia : A Comprehensive Review*. 1–5.

Kama-Kama, F., Midiwo, J., Nganga, J., Maina, N., Schiek, E., Omosa, L. K., Osanjo, G., & Naessens, J. (2016). Selected ethno-medicinal plants from Kenya with in vitro activity against major African livestock pathogens belonging to the "Mycoplasma mycoides cluster." *Journal of Ethnopharmacology*, *192*, 524–534.

Kehrenberg, C., Schulze-Tanzil, G., Martel, J. L., Chaslus-Dancla, E., & Schwarz, S. (2001). Antimicrobial resistance in Pasteurella and Mannheimia: Epidemiology and genetic basis. *Veterinary Research*, ***32***(3–4), 323–339.

Keita, K., Darkoh, C., & Okafor, F. (2022). Secondary plant metabolites as potent drug candidates against antimicrobial - resistant pathogens. *SN Applied Sciences*, *March*.

Koné, W. M., & Kamanzi Atindehou, K. (2008). Ethnobotanical inventory of medicinal plants used in traditional veterinary medicine in Northern Côte d'Ivoire (West Africa). *South African Journal of Botany*, ***74***(1), 76–84.

Landau, S. Y., Muklada, H., Abu-Rabia, A., Kaadan, S., & Azaizeh, H. (2014). Traditional Arab ethno-veterinary practices in small ruminant breeding in Israel. *Small Ruminant Research*, ***119***(1–3), 161–171.

Lulekal, E., Kelbessa, E., Bekele, T., & Yineger, H. (2008). An ethnobotanical study of medicinal plants in Mana Angetu District, southeastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *4*, 1–10.

Macdonald, O., Adeboye, O., Ngozi, R., Olatunbosun, S., & Author, C. (2016). Fractionation and Identification of Bioactive Constituents From Sapium Ellipticum (Hochst) Leaf Extract. *Animal Research International*, ***13***(3), 2492–2503.

Mesfin, F., Seta, T., & Assefa, A. (2014). An ethnobotanical study of medicinal plants in Amaro Woreda, Ethiopia. *Ethnobotany Research and Applications*, ***12***(September), 341–354.

Mohamed, R. a, & Abdelsalam, E. B. (2008). A Review on Pneumonic Pasteurellosis with Emphasis on Pathogenesis, Virulence Mechanisms and Predisposing Factors. *Bulgarian Journal of Veterinary Medicine*, ***11***(3), 139–160.

Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, ***31***(4), 247–256.

Naseer, S., Hussain, S., Naeem, N., Pervaiz, M., & Rahman, M. (2018). *The phytochemistry and medicinal value of Psidium guajava (guava).*

Nigussie, D., Davey, G., Legesse, B. A., Fekadu, A., & Makonnen, E. (2021). Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. *BMC Complementary Medicine and Therapies*, ***21***(1), 1–11.

Oeung, S., & Yin, V. (2017). *Phytochemical analysis of different extracts of leaves of Nicotiana tabacum L. of Cambodia Data collector View project First author View project*. *January*.

OIE. (2018). Pasteurella spp. *Potential Impacts of Disease Agent Beyond Clinical Illness References Articles*.

Oyda, S. (2017). Review on Traditional Ethno-Veterinary Medicine and Medicinal Plants Used By Indigenous People in Ethiopia: Practice and Application System. *International Journal of Research -GRANTHAALAYAH*, ***5***(8), 109–119.

Pandey, A., & Tripathi, S. (2014). *Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug*. ***2***(5), 115–119.

Poojar, B., Ommurugan, B., Adiga, S., Thomas, H., Sori, R. K., Poojar, B., Hodlur, N.,Tilak, A., Korde, R., Gandigawad, P., In, M., Sleep, R., Albino, D., Rats, W., Article,O., Schedule, P., Injury, C. C., Sori, R. K., Poojar, Ba. … Gandigawad, P. (2017). Methodology Used in the Study. *Asian Journal of Pharmaceutical and Clinical Research*, ***7***(10), 1–5.

Puntawong, S., Okonogi, S., & Pringproa, K. (2012). In vitro antibacterial activity of Psidium guajava Linn. Leaf extracts against pathogenic bacteria in pigs. *Chiang Mai University Journal of Natural Sciences*, ***11***(2), 127–134.

Raja Priya, R., Bhaduhsha, N., Manivannan, V., & Gunasekaran, T. (2020). Extraction and Isolation of Bioactive Compounds from a Therapeutic Medicinal Plant-Sennaalata (L.) Roxby, Palarch's. *Journal of Archaeology of Egypt/Egyptology*,

17(12), 1569–1577.

Reduction, D., Of, A., Reducing, D., In, T., & Animals, O. F. D. (2021). *Plant Archives* ***21***(table 1), 2184–2185.

Remel: Análisis Avanzados. (2020). *McFarland Equivalence Turbity Standards*. www.remel.com

Romha, G., Admasu, B., Hiwot Gebrekidan, T., Aleme, H., & Gebru, G. (2018). Antibacterial Activities of Five Medicinal Plants in Ethiopia against Some Human and Animal Pathogens. *Evidence-Based Complementary and Alternative Medicine*, *2018*.

Sarangi, L. N., Thomas, P., Gupta, S. K., Priyadarshini, A., Kumar, S., Nagaleekar, V. K., Kumar, A., & Singh, V. P. (2015). Virulence gene profiling and antibiotic resistance pattern of Indian isolates of Pasteurella multocida of small ruminant origin. *Comparative Immunology, Microbiology and Infectious Diseases*, ***38***, 33–39.

Sbhatu, D. B., & Abraha, H. B. (2020). *Preliminary Antimicrobial Profile of Solanum incanum L.: A Common Medicinal Plant*. *2020*.

Scott, P. R. (2011). Treatment and Control of Respiratory Disease in Sheep. *Veterinary Clinics of North America - Food Animal Practice*, ***27***(1), 175–186.

Séré, M., Konaté, K., Santara, B., Sanou, D. O., & Belem, A. M. G. (2022). *Study of the antibacterial capacity of extracts of Solanum incanum L., (Solanaceae) traditionally used for the management of avian cholera in rural areas of Burkina Faso*.

Sharma, T., Pandey, B., Shrestha, B. K., Koju, G. M., Thusa, R., & Karki, N. (2020). Phytochemical Screening of Medicinal Plants and Study of the Effect of Phytoconstituents in Seed Germination. *Tribhuvan University Journal*, ***35***(2), 1–11.

Sharma, Y., Srivastava, N., & Dua, D. (2016). *Antibacterial activity, phytochemical screening and antioxidant activity of stem of nicotiana tabacum introduction: Natural bioactive compounds have shown various anti-bacterial, anti-fungal, and described in Ayurveda and other alternative the medicine*. *7*(March).

Siddiqui, M. (2021). Phytochemical Analysis of Some Medicinal Plants. *Liaquat Medical Research Journal*, ***3***(8), 1–5.

Tadesse, B., Alamirew, K., Ketema, A., Kiflie, W., & Endashaw, M. (2017). Ruminant

Pneumonic Pasteurellosis: Review on Epidemiology, Pathogenesis and Virulence Mechanism. *Academic Journal of Animal Diseases*, ***6***(2), 30–39.

Tamiru, F., Terfa, W., Kebede, E., Dabessa, G., & Roy, R. K. (2013). Ethnoknowledge of plants used in veterinary practices in Dabo Hana District, West Ethiopia. *Journal of Medicinal Plants Research*, ***7***(40), 2960–2971.

Temeche, M. A., & Asnakew, A. T. (2020). A review on status of ethnoveterinary medicine and challenges it faces in Ethiopia. *International Journal of Veterinary Sciences and Animal Husbandry*, ***5***(5), 39–48.

Tesfamariam, G., Wubete, A., & Eguale, T. (2011). *In vitro antibacterial activity of four Ethiopian medicinal plants against some bacteria of veterinary and public health importance*. ***4***(12), 1230–1234.

Tewodros, A., & Annania, T. (2016). Sheep and goats pasteurellosis: Isolation, identification, biochemical characterization and prevalence determination in Fogera Woreda, Ethiopia. *Journal of Cell and Animal Biology*, ***10***(4), 22–29.

Ullah, M., Murtaza, G., & Hussain, I. (2016). Relative bioavailability study of succinic acid cocrystal tablet and marketed conventional immediate release tablet formulation of carbamazepine 200 mg in rabbits. *Acta Poloniae Pharmaceutica - Drug Research*, ***73***(4), 1023–1027.

Upadhyay, R. K. (2014). *R. K. Upadhyay (2011). Plant natural products: Their pharmaceutical potential against diseases and drug resistant microbial pathogens. Journal of Pharmacy Available online through Plant natural products: Their pharmaceutical potential against disease*. ***4***(January 2011), 1179–1185.

World Health Organization (WHO). (2004). WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. *World Health Organisation, Geneva*, 82.

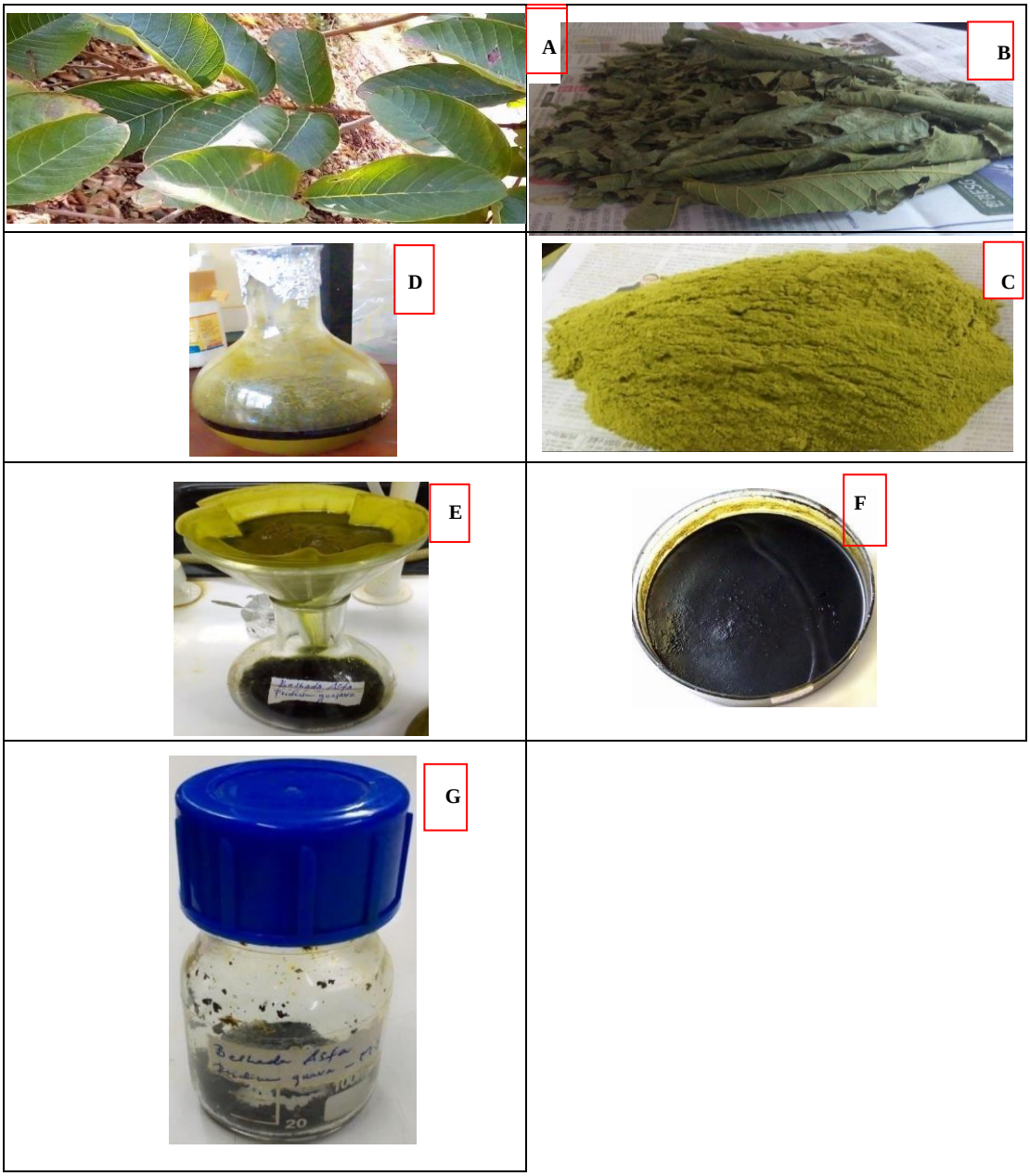
Yavuz, C., Dereli Kılıç, D. D., Ayar, A., & Yıldırım, T. (2017). Antibacterial Effects of Methanol Extracts of Some Plant Species Belonging to Lamiaceae Family. *International Journal of Secondary Metabolite*, ***4***(2), 429–433.

Yirga, G., Teferi, M., & Kasaye, M. (2011). Survey of medicinal plants used to treat human ailments in Hawzen District, Northern Ethiopia. *International Journal of Biodiversity and Conservation*, ***3***(13), 709–714.

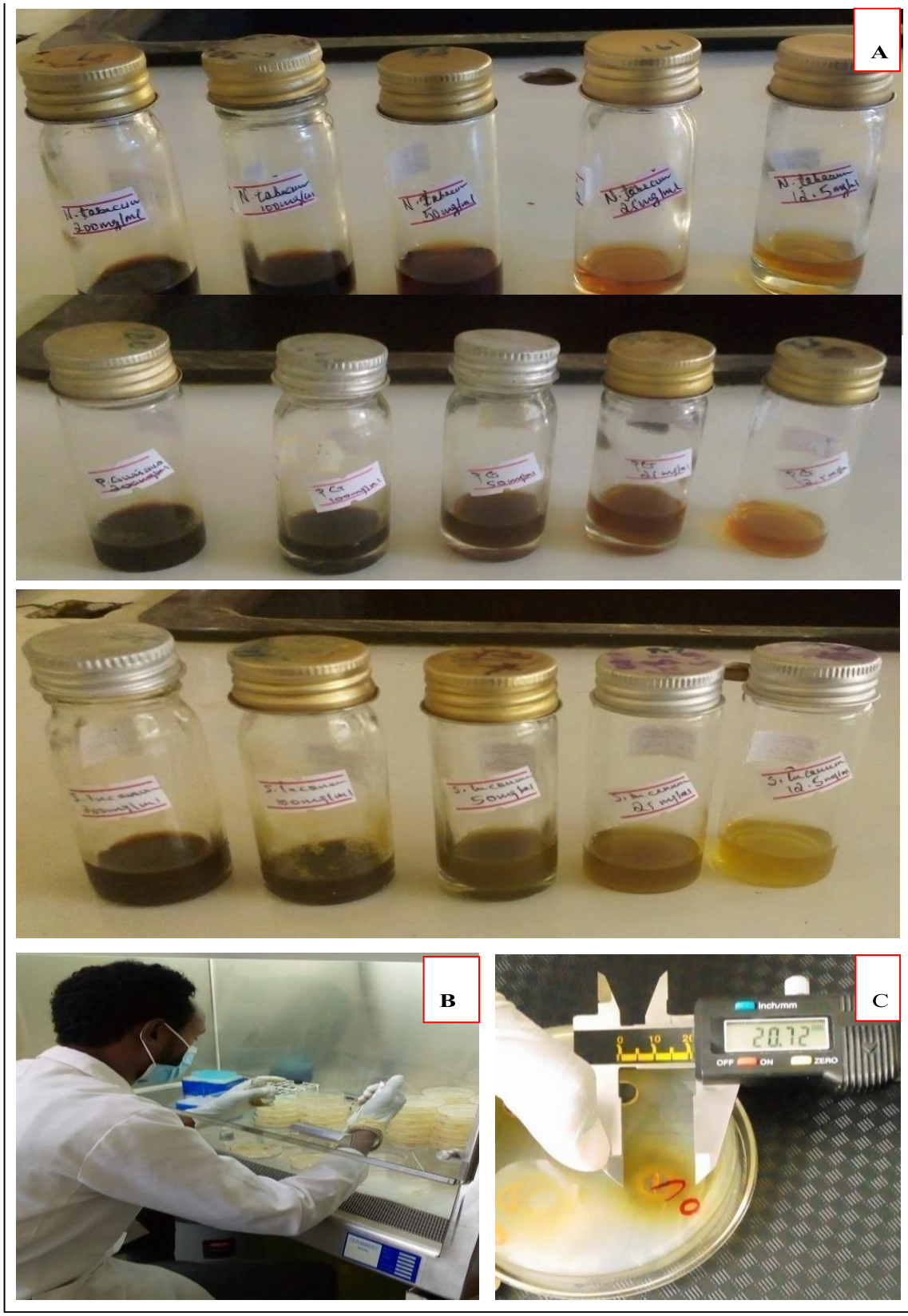
Zou, X., BK, A., Abu-Izneid, T., Aziz, A., Devnath, P., Rauf, A., Mitra, S., Emran, T.

Bin, Mujawah, A. A. H., Lorenzo, J. M., Mubarak, M. S., Wilairatana, P., & Suleria, H. A.R. (2021). Current advances of functional phytochemicals in Nicotiana plant and related potential value of tobacco processing waste: A review. *Biomedicine and Pharmacotherapy*, ***143***(September), 112-191.

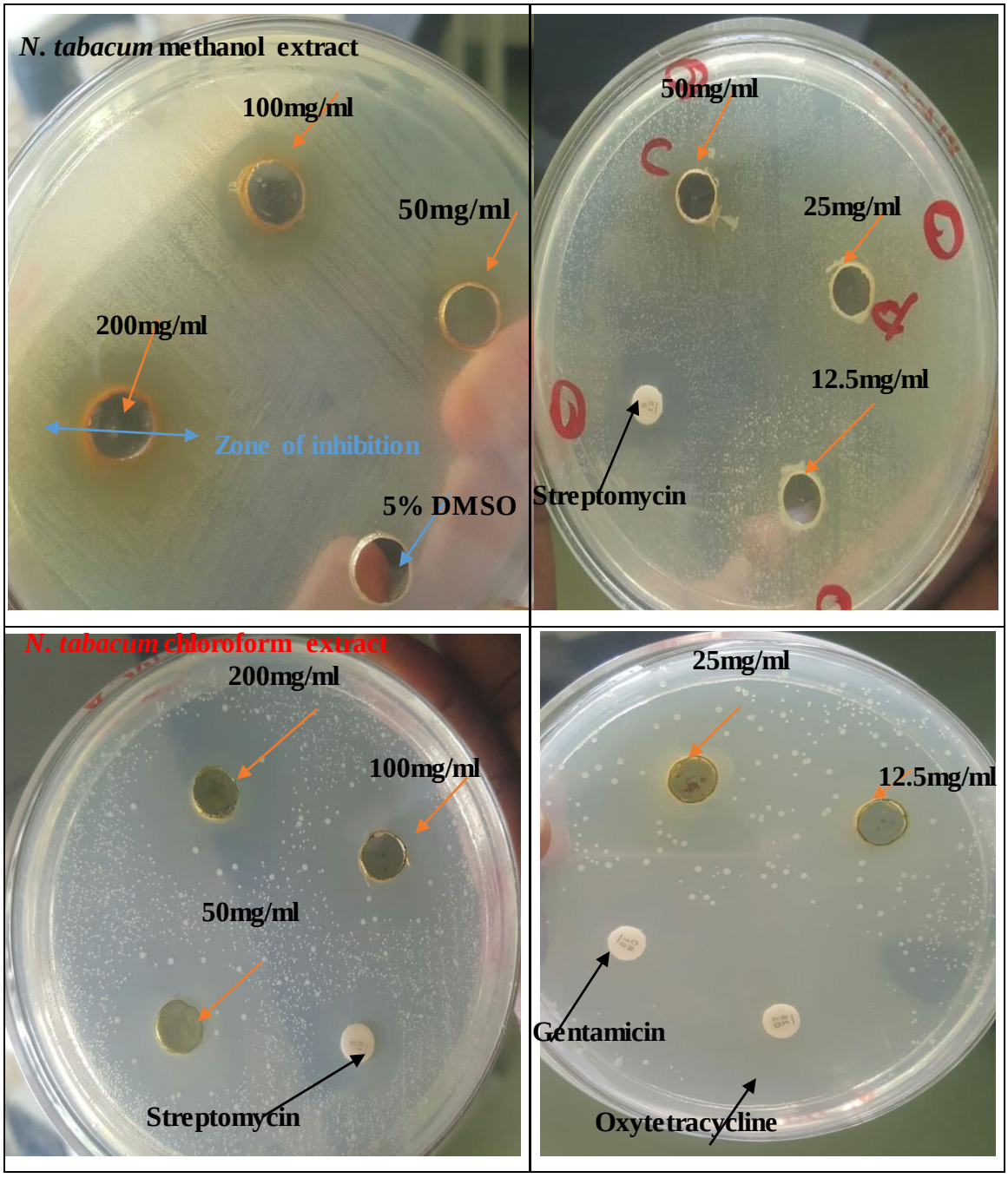
8. ANNEXES



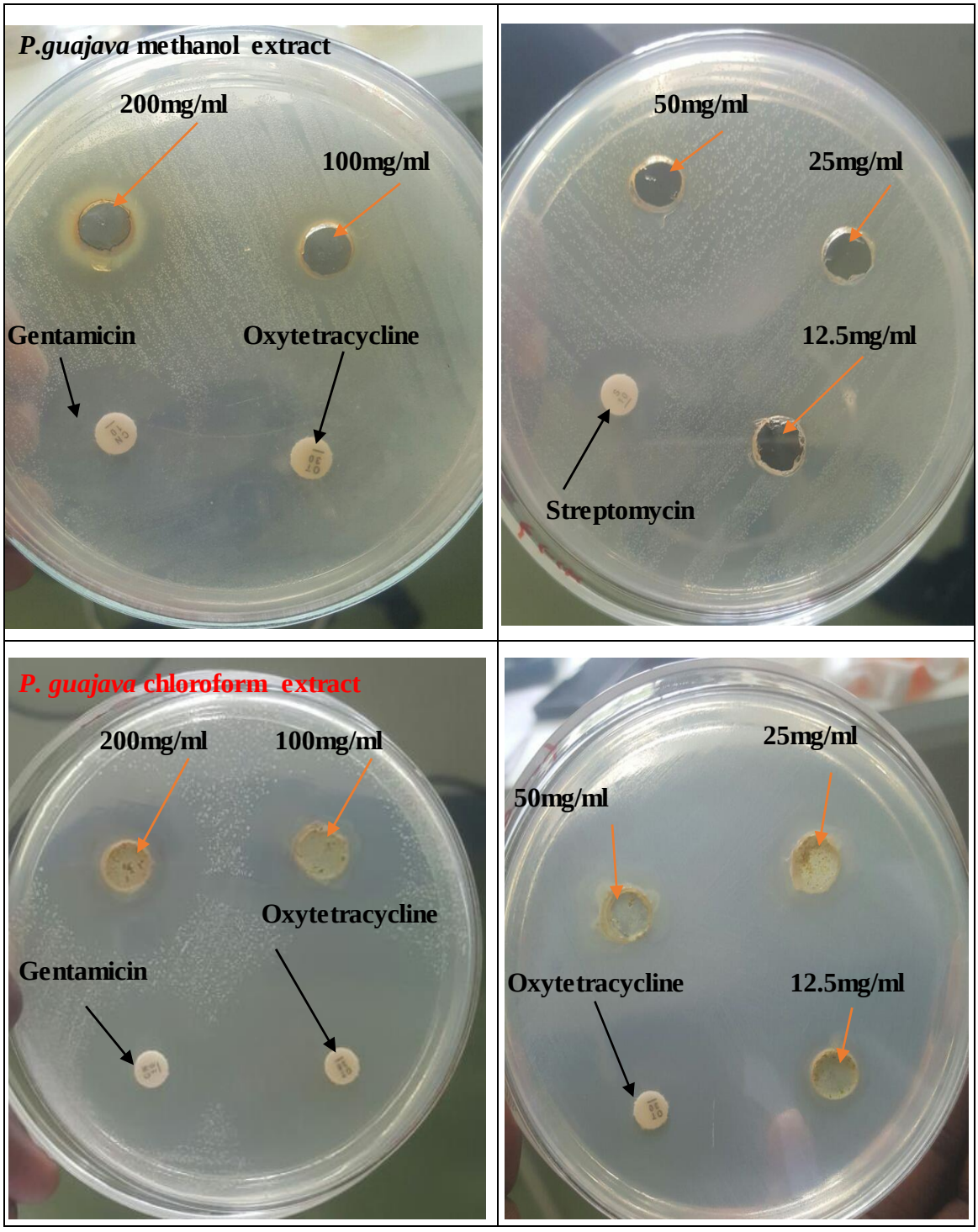
Annex 1. The summary of phytochemical extraction procedure: **A**) sample (leave of Psidium guajava); **B**) washed and dried leave; **C**) powder (grinded by electrical grinder); **D**) maceration (100g with in 400ml); **E**) filtration; **F**) crude**; G**) stored in screwed bottle



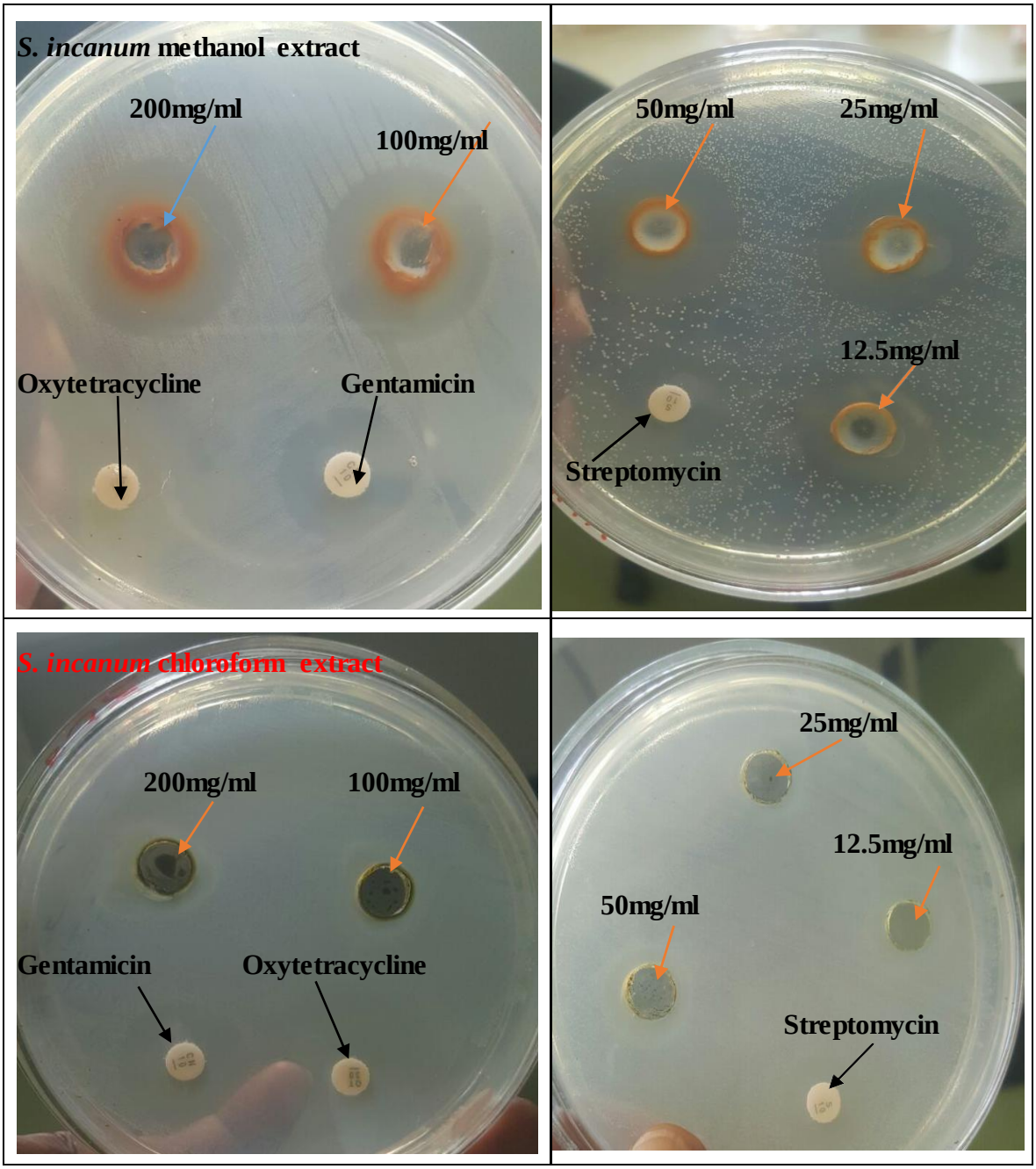
Annex 2. A) Serial dilution of the working solution prepared; **B**) dispensing; **C**) measuring zone of inhibition



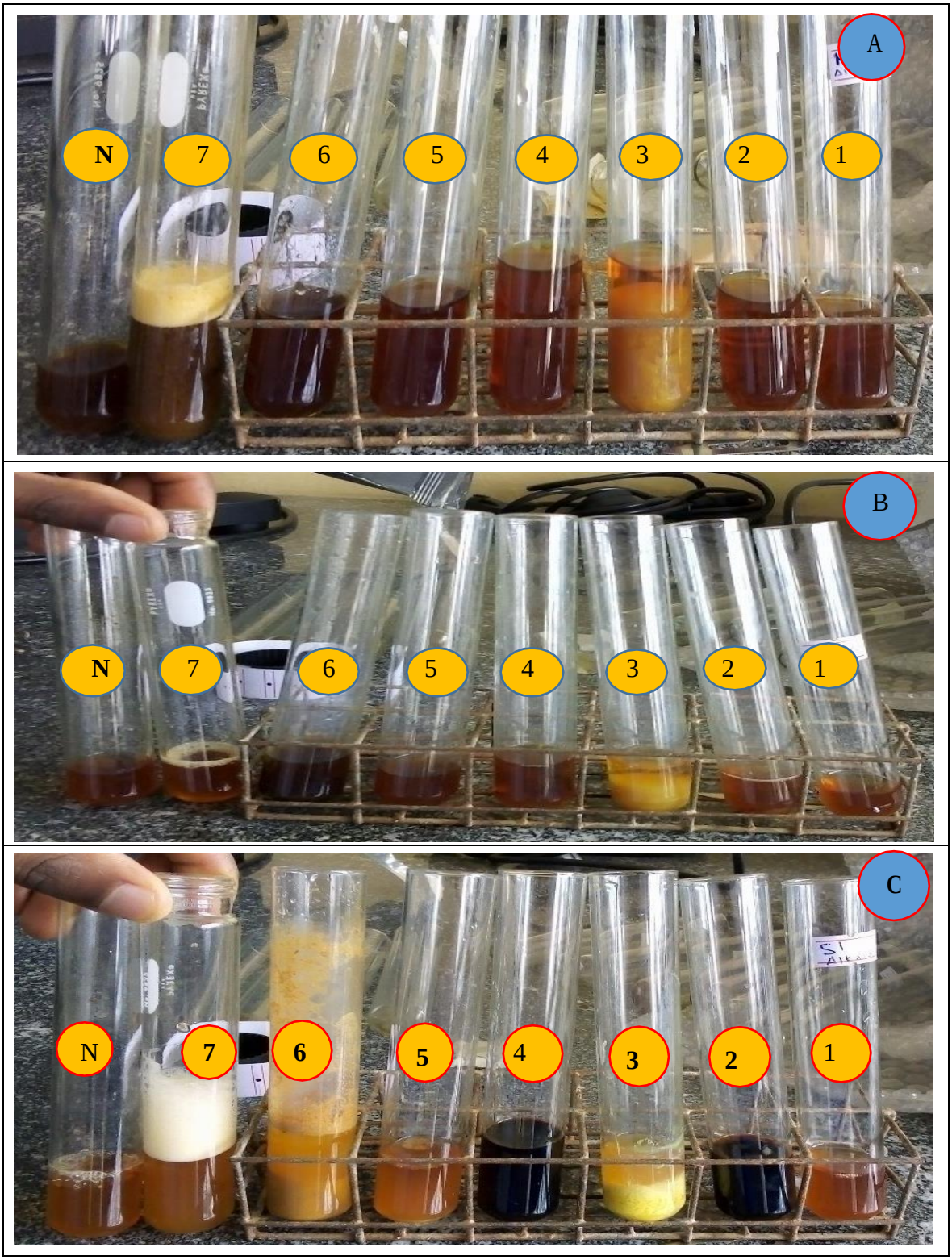
Annex 3. Zone of inhibition of methanol and chloroform extracts of *N. tabacum* against *P.multocida* and *M. haemolytica*



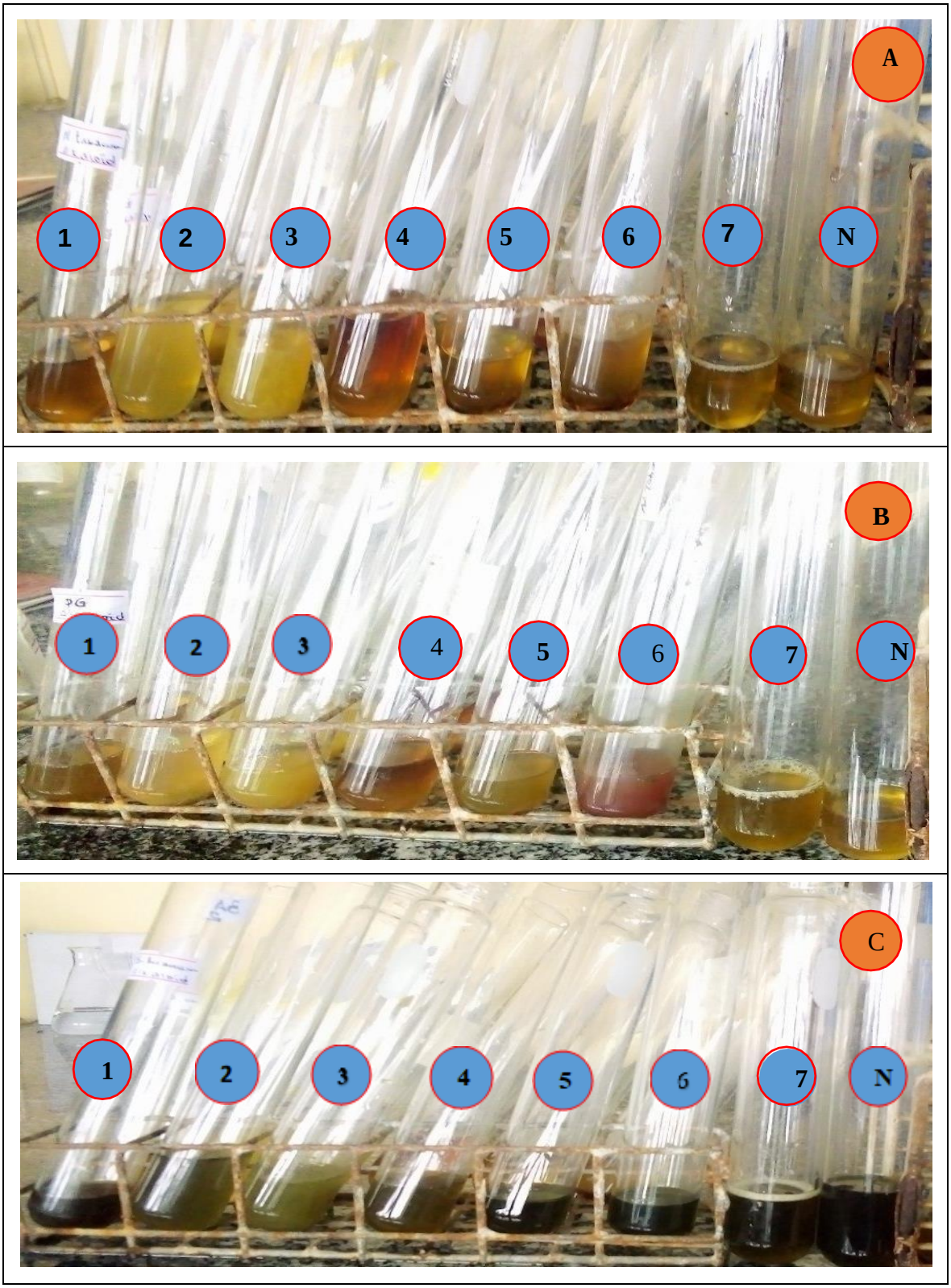
Annex 4. Zone of inhibition of methanol and chloroform extracts of *P.guajava* against *P. multocida* and *M. haemolytica*



Annex 5. Zone of inhibition of methanol and chloroform extracts of *S. incanum* against *P. multocida* and *M. haemolytica*



Annex 6. Phytochemical screening test of methanol extracts of *N. tabacum* (**A**); *P.guajava* (**B**); *S. incanum* (**C**): 1) Alkaloids; 2) Flavonoids; 3) phenolic; 4) Tannin; 5) Steroids; 6) Terpenoids; 7) Saponin; N) Negative control



Annex 7. Phytochemical screening test of chloroform extracts of *Nicotiana tabacum* (**A**); *Psidium guajava* (**B**); *Solanum incanum* (**C**): 1) Alkaloids; 2) Flavonoids; 3) phenolic; 4) Tannin; 5) Steroids; 6) Terpenoids; 7) Saponin; N) Negative control