# Antimicrobial properties of *Tamarindus indica linn* leaf extracts

**Leratiloe M Ngulube**

# *TAMARINDUS INDICA LINN* LEAVES

**BY**

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## ABSTRACT

*Tamarindus indica l* leaves extract was evaluated against six bacterial strains (2 Gram-positive and 4 Gram-negative) known to cause food spoilage. Functional groups of *Tamarindus indica l* leaves were evaluated using the Fourier transform infrared (FTIR) spectroscopy. The leaves were extracted by the Soxhlet extraction using aqueous and organic solvents (acetone, ethanol and methanol). Stock solution of the extracts was prepared by dissolving 0.8g of each extract in 2ml dimethyl sulphoxide (DMSO) to obtain a concentration of 400mg/ml and the concentration of 200mg/ml was prepared by serial doubling dilution of stock solution and 2ml of DMSO was added. Inoculum suspension was prepared by suspending appropriate colonies of the test microorganisms in sterile distilled water. The 0.5 McFarland of 1% barium chloride and 1% sulphuric acid was used to standardize the inoculum. The antimicrobial activity of the concentrated extracts was evaluated by determination of the zones of inhibition against the test microorganisms using the disc diffusion method. The results of the FTIR revealed the presence of primary and secondary amines and amides, carboxylic acid, alkenes and alkanes, fluorides, amines and aromatics. The extracts were active against both gram-positive and gram-negative bacteria. The activity of the plant extracts could have been affected by different temperature ranges, nature of the tree of freshness and dryness of the leaves. All extracts showed appreciable inhibition on the test microorganisms with the zones of inhibition ranging from 6.4 mm-19.8mm and ethanol extracts showed a broader spectrum of activity against the test microorganisms. Results obtained of this study indicate that *Tamarindus indica l* has broad spectrum antibacterial activity and a potential source of new classes of natural preservatives that could be useful for replacement of synthetic food grade preservatives

**Key words:** *Tamarindus indica l*, Antimicrobial activity, Fourier transform infrared (FTIR) spectroscopy, zone of inhibition.

**DECLARATION**

I, **Leratiloe M Ngulube** (R135995M) sincerely declare that the material in this thesis on; **The antimicrobial properties of *Tamarindus indica linn* leaves."** is my original work that has not been previously submitted to any other institution. Proper citations and acknowledgements in line with copyright laws and ethical requirements have been strictly adhered to in the completion of this dissertation.

**……..** **……/……/…….**

Student Signature Date

**APPROVAL**

This dissertation entitled **"The Antimicrobial Properties of *Tamarindus indica linn* leaves"** by **Leratiloe M Ngulube** meets the regulations governing the award of the Bsc Honours Degree in Food Science and Nutrition of the Midlands State University, and is approved for its contribution to knowledge and literal presentation.

**……** **…../……/.....**

Supervisor Signature Date

**DEDICATION**

I dedicate this research to my parents Mrs S Ngulube and the late Mr S .Ngulube, my grandmother Mrs D.M Mnguni and my aunt Sicelumusa P Ndebele and my family as a whole for your love, support and encouragements.

To my supervisor, Mr D.T.Mugdza, all this would have remained a dream without your inspiration and your guidance.

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# CHAPTER ONE

# INTRODUCTION

## 1.1 INTRODUCTION

Many foods are perishable by nature and require protection from spoilage during their preparation, processing, storage and distribution to give a desired shelf life (Gupta et al., 2014) therefore food safety will remain a critical issue worldwide, with so much outbreaks of foodborne illnesses that are resulting in ample costs to individuals, food industries and the economy in general (Makwanda and Woyo, 2012; Gupta et al., 2014). Amongst other causes bacteria is the major culprit o these illnesses and is said to be responsible for about 76 million illnesses, 323 914 hospitalizations and 5194 deaths per year in the United States of America (Jahan, 2012; Barbudde and Chakraborty, 2009). In a bid, food preservation methods have been implemented to deal with food safety issues, so as to extend product shelf life and prevent food spoilage and these include the use of heat or salt and sugar. These are however not effective enough in some food products thus making it inevitable to use chemical preservatives (Gupta et al., 2014).

The most common classical preservative agents used are weak organic acids like sorbic, lactic and benzoic acid. These chemical preservatives can be harmful due to the chemical residues in foods. An increasing number of consumers prefer minimally processed foods, prepared without chemical preservatives. A renewed interest in 'natural preservation' appears to be stimulated by present food safety concerns, growing problems with microbial resistance and rise in the production of minimal processed food joined with 'green' image polices of food industries (Chen et al., 2013; Gupta et al., 2014). Environmental groups and health conscious people are further putting pressure on governments to reduce and stop the use of chemical preservatives. A number of research studies have also shown that many natural substances of plant origin

such as spices may play a major role in the host-pathogen relationship (Gupta et al., 2008a; Gupta et al, 2010; Gupta et al., 2014).

The global development is now advocating for the use of natural preservatives although some chemical preservatives are still used (Ramachandran, Nivatha and Antony, 2014). Natural preservatives are preferred because they have minimal side effects as compared to chemical preservatives therefore natural preservatives like *Tamarindus indica l can* be used as natural preservative in food industries (Havinga et al., 2010).

Of their properties, the most important is their antimicrobial property as they can be used as natural preservatives as shown in the study of Paul Das et al., (2014) which verified this scientifically by investigating the leaves against gram-negative and gram-positive bacteria and the results were supported by the Stereoscopic Microscope images. El-Siddig et al. (2006) postulates that these leaves also present good levels of protein, fat, fiber and some vitamins such as thiamine, riboflavin, niacin, ascorbic acid and B-carotene.

Despite its wide uses, there is still insufficient literature available that the tree has the potential to be used as an alternative food preservative. The present study aims at determining the plant's antimicrobial potential against 2 gram-positive and 4 gram negative bacterial strains which are known to cause food spoilage so as to help in the development of natural preservatives.

## 1.2 PROBLEM STATEMENT

Utilization of chemical food preservatives gives off an impression of being a significant issue as it is known to have a negative or adverse impact on well-being. Chemicals such as nitrites, sodium benzoate, sodium sorbate, and benzoic acid are used as antimicrobial agents to inhibit the growth of bacteria, moulds and other insects. However they cause allergies, cancer and side effects like headaches and some behavioral changes (Sharma, 2015).

The Tamarind tree has been used for centuries as a medicinal plant, its fruits being of the most value which are often repeated as curative in several pharmaceuticals (Doughari, 2006; Havinga et al., 2010). Tamarind leaves are reported worldwide as antibacterial, antioxidant and antifungal agents and also present good levels of protein, fat, fiber and some vitamins such as thiamine, riboflavin, niacin, ascorbic acid and B-carotene (Escarlonna-Arranz et al., 2010; Abuzied et al., 2014). These compounds have a proven record as antimicrobial agents in many other plants (Escarlonna-Arranz et al., 2010). In Zimbabwe the Tamarind leaves are added to soups and the flowers are an ingredient in salads (Milind and Isha, 2012).

Despite all these properties and health benefits, nothing has been done to perceive the antimicrobial property of the Tamarind leaves such that it can be used as a natural preservative therefore reducing use of chemical preservatives.

## 1.3. OBJECTIVES

### 1.3.1 Main Objective

- To determine the antimicrobial potency of *Tamarindus indica l* leaves.

### 1.3.2 Specific Objectives

- To obtain acetone, ethanol and methanol extracts from dry and fresh leaves of *Tamarindus indica l.*

- To determine the antimicrobial activity of *Tamarindus indica l* leaf extracts using standard pathogenic cultures like Culture *S. aureus, E. coli 0157:H7, Clostridium Perfringens, Salmonella isolate, Salmonella enterica* and *Pseudomonas* spp.
- To test for the antimicrobial activity of *Tamarindus indica l* leaf extracts at (400mg/ml and 200mg/ml) concentrations.

## 1.4 HYPOTHESES

$H_0$1 There is no significant difference between the antimicrobial activity of acetone, ethanol and methanol leaf extracts.

$H_0$2 There is no significant difference of the antimicrobial activity of Tamarind leaf extract at different concentrations.

$H_0$3 There is no significant difference between the activity of fresh leaf extract and dry leaf extract.

## 1.5 DELIMITATIONS

Tamarind leaves were collected from Chegutu and the experiments were carried out in the Midlands State University Food Chemistry, Biological Sciences and Chemical Technology Laboratories.

## 1.6 SIGNIFICANCE OF STUDY

This research will be of use to various food industries in introducing the use of Tamarind leaves as an alternative food preservative. The consumers who prefer natural preservatives as they would be saved from the tremendous side effects of chemical preservatives and hence boost their confidence in buying safe food products. Farmers will also benefit from this research and start practicing farming of this tree. Midlands State University will benefit by the expansion of resources into the library accordingly giving all the more learning zones and bestowing information to different understudies.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 FOOD PRESERVATION

Historically, the most important reason to process or prepare foods has been to make them last longer before spoilage. Early development used techniques like salting meats, fermenting dairy (into cheese or yoghurt) and pickling vegetables (Hopkins, 2016). Food preservation is a critical control point that impacts and determines a whole range of outcomes, ranging from preservation of nutritional quality, food safety, the wholesome nature of foods, texture, taste and organoleptic qualities and consumer appeal, along with conformance to several points in the value chain that include long distance transportation, marketing and long term storage (Bhat, Alias and Paliyath, 2012). Recently, the significance of food preservation methods on the physiology and behavior of microorganisms in foods, i.e. their metabolic pathways, homeostasis and stress reactions, are taken into account, and the novel concept of multi-target food preservation emerged (Leistner, 2000).

## 2.2 FOOD PRESERVATIVES

Food preservatives are additives that play a significant role during food storage and transportation and they preserve the food for a long time by preventing its deterioration. Food preservatives mainly inhibit growth of microorganisms rather than killing them. Almost all the packaged food products have some preservatives and without them the food cannot stay longer (Seetaramaiah, 2011). According to the Food Protection Committee of the Food and Nutrition Board, food additives may be defined as a substance or mixture of substances other than fundamental food staffs, which are present in any food as a result of any aspect of processing, production, packaging or storage (Brannen et al., 2002). Additives can be divided into 6 major categories which are: preservatives, colorizing agents, nutritional additives, texturizing agents,

flavouring agents and miscellaneous additives (Brannen et al., 2002; Bhat, Alias and Paliyath, 2012). Food preservatives are classified into artificial or chemical preservatives and natural preservatives. Both, chemical and natural preservatives which are used in foods are grouped under the International Numbering System (INS) into 3 types which are antimicrobials, antioxidants and anti-browning or anti-enzymatic (Leistner, 2000; Brannen et al., 2002).

Antimicrobials

They are used to check or prevent and to destroy or delay the growth of microorganisms (Leistner, 2000; Brannen et al., 2002). Antimicrobials play a major role in extending shelf life of numerous snacks and convenience foods and have come into even greater use in recent years as microbial food safety concerns have increased (Brannen et al., 2002). For example nitrates and nitrites prevent botulism in meat and meat products. Sulphur dioxide prevents further degradation in fruits, beer and wines and sorbates and benzoates are anti-fungal used in jams, salads, cheese and pickles (Leistner, 2000).

Anti-oxidants

These are used to prevent vitamin or lipid oxidation in food products. They are used primarily to prevent oxidation and immediate development of rancidity and off flavours. They vary from natural substances such as vitamin C and E to synthetic chemicals such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Antioxidants are especially useful in preserving dry and frozen foods for an extended period of time (Brannen et al., 2002)

Anti-browning or Anti-enzymatic preservatives

These are chemicals used to prevent both non-enzymatic and enzymatic browning in food products especially in dried fruits and vegetables, they block the enzymatic processes such as ripening occurring which in foodstuffs even after harvesting (Leistner, 2000; Brannen et al., 2002). For example citric acid and erythorbic acid stop the action the phenolase enzyme which results in brown colour in cut fruits or potatoes surfaces (Leistner, 2000). Brannen et al. (2002) further explains that vitamin C (E300), citric acid (E330) and sodium sulphite (E221) are the most commonly used additives in this category.

### 2.2.1 Natural Preservatives

Natural preservatives are substances that prevent oxidation and rancidity of food and food products. They are extracted and purified from botanical sources (Zegara, 2015). The side effects of chemical compounds have raised consumer concerns. Therefore, leading to consumers demand for natural products in processed foods. These products are either from the plant origin for instance spices or herbs or from the animal origin, for example lysozyme, lactoperoxidase, and also microbial metabolites such as nisin (Altuntas, 2013). These naturally derived preservatives have been evaluated for their efficacy in food preservation. Natural antimicrobials can be combined with other hurdles in multifactor food preservation systems (Leistner, 2002). There are some factors that should be taken into account in the use of natural antimicrobials, these include: physical and chemical properties of the antimicrobial or composition of food as well as the target microorganisms. Amongst other preservatives, the natural preservatives include, plant extracts, natural antimicrobials of plant origin, herbs and spices, essential oils, phenolic compounds, organic acids and Tamarind (Aluntas, 2013).

### 2.2.2 Chemical Preservatives

The federal Food Drug and Cosmetic Act as commended by the food additive Amendment of 1958 defines a chemical preservative as any chemical which tends to prevent or retard deterioration when added to food; but does not include salt, sugar vinegar and spices. Tull (1996) defines chemical preservatives as substances that can be added to food in order to inhibit the action of enzymes, and either to destroy or inactivate microorganisms which may contaminate the food. The purpose of chemical preservatives is generally to preserve the natural characteristics of food and to increase the shelf life of food and inhibit discoloration as well as natural ageing that can occur during food preparation such as the enzymatic browning reaction in apple or potato surfaces after they are cut or bruised (Sharma, 2015) Use of chemical preservatives in manufactured foods is strictly controlled therefore, a permitted list of such chemicals is issued and no other chemical can be used. Stringent safety tests are carried out on these preservatives so as to determine their maximum permitted amounts for use in food products. Preservatives can be used either on their own or combined with other methods of food preservation (Tull, 1996). Aldumumeen et al., (2012) further explains that preservatives can be antimicrobial preservatives, which inhibit the growth of bacteria or fungi, including moulds, or antioxidants such as oxygen absorbers, which inhibit the oxidation of food constituents. Common antimicrobial preservatives include calcium propionate, sodium nitrate, sodium nitrite, sulphites (Sulphur dioxide, sodium bisulfite, potassium hydrogen sulphite), and disodium. Although preservatives are essential for extending the shelf life of the food and food products, they have a possibility of giving rise to certain problems. One of the most harmful effects of chemical preservatives is their potential to cause breathing problems for instance sulphites and benzoates could exacerbate breathing problems in those with asthma and many others (Garg et al., 2010). Another harmful effect of these chemical preservatives is behavioral changes especially in young children such as attention deficit disorder. Some of these preservatives are found to harmfully affect the heart tissues. Regular consumption of such

preservatives lead to weakened heart tissues. One of their most serious harmful effect is their ability to transform carcinogens when digested (Albala, 2015; Kharwar, 2014).

Therefore the safety and benefits of many artificial food additives (including preservatives) remains the subject of debate among regulators and academics specializing in biology, food science and food toxicology (Oladapo et al., 2014) due to their toxicity (cancer causing) when ingested by humans or animals (Russell and Gould, 2012). The uncertainty voiced by consumer organizations and pressure groups over the use of food additives including food preservatives has already been referred to one approach in reassuring the consumer to resorting to natural methods of preservation (Adam and Moss, 2010) like *Tamarindus indica l.*

**2.3 TAMARIND**

Tamarind is a leguminous tree of the *Fabaceae* kingdom that is indigenous to tropical Africa. The Tamarind tree produces edible, pod like fruits which are extensively cuisines around the world. The seeds are traditionally used to treat fever, diabetes and intestinal infections (Gupta et al., 2014). The leaves have a proven hepatoprotective activity which is associated with the presence of polyhydroxylated compounds, with many of them being flavonolic in nature. Due to their antimicrobial, antifungal and antiseptic effects, Tamarind leaves have a greater extent of ethnobotanical use (EscalonaArranz et al., 2010; Lans, 2007).

Tamarind leaves consist of polyphenols, flavonoids and some oils. (Lans, 2007), a research group reported for the first time that a total of B essential oils in which limonene and benzyl benzoate are the major compounds (El-Siddig et al., 2006). Essential oils are one of the plants main secondary metabolites involved in antimicrobial and antiseptic activities. Flavonoids and polyphenols in association with alkoids were linked to the antimicrobial activity of *Tamarindus indica l* leaves (Paul Das et al., 2014).

**Fig 2.1** Picture of *Tamarindus indica l* Leaves. **Source**: (Milind and Isha, 2012).

## 2.4 GENERAL USES OF TAMARIND

*Tamarindus indica l* is a multipurpose tropical tree used primarily for its fruits, which are eaten fresh or processed, it is also used as seasoning or spice, the fruits and seeds are processed for non-food use. The species has a wide geographical distribution in the subtropics and semi-arid tropics and is cultivated in numerous regions (El-Siddig et al., 2006).

Virtually every part of the tree (wood, roots, leaves, bark and fruits) is of a significant value in the subsistence of rural people as well as a number of commercial applications. Tamarind fruits are versatile as they can be used for many purposes. The unique sweet or sour flavour of the pulp is popular in cooking and flavouring (El-Siddig et al., 2006). It is often made into juice, brine/infusion. In Ghana bitter infusion of the pods is usually used for cooking cereals and is often added to the water in which poisonous yams are soaked in order to detoxify them whereas, in India the pulp is used to preserve fish (up to 6 months), when mixed with acetic acid (Paul Das and Banerjee, 2014).

In some African countries the pulp juice is often mixed with wood ash and added to tartaric acid in order to neutralize its sour taste, but the common method is to add sugar to make a pleasant drink. In Ghana the pulp juice is mixed with honey to make a sweet drink 'Jugo' and 'Fresco Tamarindo' in South America. The pulp juice is also an ingredient of several barbecue sauces commonly used in North American and European countries such as the worcestershire sauce (Gupta et al., 2014).

Tamarind leaves are used as a vegetable by indigenous people in producing countries. They contain 4.0 to 5.8% proteins while flowers only contain 2 to 3%. The leaves are a fair source of vitamin C and beta carotene and they have high mineral content, particularly calcium, potassium, magnesium and phosphorus. The leaves contain maleic acid and tartaric acid, the latter is found in excess and increases with the age of the leaves. Oxalic acid is also present and the tender leaves show a good calcium or oxalate ratio of 1:1 at pH of 4.5. This indicates that the leaves are a good source of calcium, however, the presence of oxalic acid may affect their nutritive value (Williams, 2006).

**Table 2.1 Chemical Composition of tender leaves and flowers of *Tamarindus indica l.***

| Constituents | Tender Leaves (%) | Flowers (%) |
|---|---|---|
| Moisture | 70.5-78.0 | 80 |
| Proteins | 4.0-5.8 | 2.8 |
| Fat /Oil | 1.2-2.1 | 1.5 |
| Fiber | 1.9-3.0 | 1.5 |
| CHO (Total) | 16.0-18.0 | |
| Ash /Minerals | 1.0-1.5 | 0.7 |
| Calcium (mg) | 101-250 | 35.5 |
| Magnesium (mg) | 71.0 | |
| Phosphorus (mg) | 14.0 | |
| Iron | 2.0-5.2 | |
| Copper | 2.0 | |
| Chlorine | 94.0 | |
| Sulphur | 63.0 | |
| Thiamine | 0.1-0.2 | 0.07 |
| Riboflavin | 0.1-0.2 | 0.14 |
| Niacin | 1.5-4.1 | 1.14 |
| Vitamin C | 6.0 | 13.80 |
| Carotene | | 0.31 |
| Sodium | | |
| Potassium | 270.0 | |
| B carotene (µm) | 250.0 | |
| Calories (Kal) | 75.0 | |
| Oxialic Acid (mg) | 196.0 | |

**Source: (Lewis and Neelakntan, 1964a; Anon, 1976; Duke, 1981)**

## 2.5 POTENTIAL USES OF TAMARIND

### 2.5.1 Antimicrobial

An antimicrobial is an agent that kills microorganisms or inhibits their growth. The leaves constituents like flavonoids, polyphenols and essential oils are believed to possess antimicrobial properties. *T. indica l* has a wide range of antibacterial activities, as shown in experiment that was carried out by Gupta (2014) where the *T. indica l* leaves were extracted using methanol and the results showed a significant antimicrobial efficiency against *Klebsiella.* The antimicrobial activity was then compared with some standard antimicrobials such as *Piperacillin*. The antimicrobial activity of the concentrated extracts like ethanol and acetone extracts were evaluated by determination of the zone of inhibition using the paper disk diffusion method, against both gram-negative, gram-positive bacteria and fungi and it was observed that although Tamarind is effective against both groups of bacteria but its activity was high in Gram positive bacteria as compared to Gram-negative bacteria. These observations are in accordance with the earlier observations reported by Escalona-Arranz et al. (2010).

Escalona-Arranz et al. (2010) further explains that the *T. indica l* leaves have significant antimicrobial activity against *Salmonella typhi*, *Salmonella paratyphi*, *Bacillus subtilis* and *Staphylococcus aureus* while other studies have suggested that Tamarind has shown great potential of antimicrobial activity; in petroleum ether extract and water extract. *T. indica l* ripe fruits were also evaluated for their possibility of antibacterial activity both against gram-positive and gram-negative bacteria species and the methanol and aqueous extract showed antimicrobial activity (Escalona-Arranz et al., 2010; Lans, 2007). The methanol extracts of the *T. indica l* flower also showed antibacterial activities during this preliminary screening. The results showed that all the extract from *T. indica* exhibited strong *in vitro* antibacterial activity against all the bacteria species tested (Gupta, 2014).

### 2.5.2 Antioxidant

*T. indica l* contains phenolic antioxidant compounds which exhibit good antioxidant activity and maybe an important source of cancer chemo preventive. The solvent extraction of Tamarind seed coat using ethanol was found to be the most active. The ethanolic extract of the *Tamarindus indica l* fruit pulp of has shown significant hypolipidemic and antioxidant activity. The antioxidant activity of the ethanol extract of the *T. indica l* seed coat is determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical testing method using ascorbic acid as a standard (Gupta, 2014). This activity of *Tamarindus Indica l* extract may be attributed to its free radical scavenging ability. The ethanol extract prepared from the seed coat of *T. indica l* usually exhibits an antioxidant activity measured by the thiocyanate and thiobarbituric method (Escalonna-Arranz et al., 2010) whereas, the ethyl acetate extracts prepared from the seed coat also have a strong antioxidant activity. This was confirmed by a study that was carried out by Bhadoriya et al. (2011). *T. indica l* seed coat, a by-product of the Tamarind gum industry, could be used as a safe and low cost source of antioxidant, although other herbals may be more effective.

### 2.5.3 Antidiabetic

The antidiabetic activity of *T. indica l* leaves is attributed to the presence of polyphenols, flavonoids and other constituents which show blood reduction in glucose levels therefore it has a potential to be used in the management of diabetes mellitus (Paul Das et al., 2012). A study by Bhadoriya et al. (2011) showed that aqueous extract of *T. indica l* leaf extracts were given to mild diabetic and severe diabetic rats, and hyperglycemia was significantly reduced. This was measured by fasting blood glucose levels. Similarly, hyperlipidemia was also found to be reduced and it was measured using different contents of blood cholesterol.

### 2.5.4 Anti-inflammatory and analgesic activity

*T. indica l* bark has been used traditionally for the treatment of pain, the present work was undertaken to prove this scientifically by using animal screening models, using animals that are suitable, the models included a hot plate test and acetic acid induced writing test at the dose of 50 mg/kg, Petroleum ether extract showed a significant increase in reaction time as compared to other extracts (Bhadoriya et al., 2011). Preliminary phytochemicals tests showed presence of sterols in the extract, hence these sterols can be responsible for analgesic activity. (Gupta, 2014) Tamarind leaf juice with ginger can be used for the treatment of bronchitis, whereas, dried and pounded bark is used for the treatment of eye inflammation (Gupta, 2014).

### 2.5.5 Hepatoprotective and anti-asthmatic activity

Some experimental studies have predicted that *T. indica l* shows anti-asthmatic and hepatoprotective activity. The methanol extract of *T. indica l* leaves, exhibited significant anti-histaminic, adaptogenic, and mast cell stabilizing activity in the laboratory when it was used specifically on animals (Havinga et al., 2010). The aqueous extracts of different parts of *T. indica l*, such as the leaves, unroasted seeds and fruits were administered and a significant hepatoregenerative effect was observed for the aqueous extracts of Tamarind leaves, fruits, and unroasted seeds and was evaluated using the parameters previously studied ( EscalonaArranz et al., 2010).

## 2.6 HEALTH BENEFITS OF TAMARIND

Medicinal plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80 % of world population in Latin America, Africa and Asia and it is reported to have minimal side effects (Abuzied et al., 2014). *T. indica l* is used in traditional medicine for the treatment of stomach disorders, diarrhea, jaundice, colds, flue as well as for skin cleansing (Doughari, 2006). Tamarind seeds are known to inhibit activities of snake envenomation enzymes which are responsible for inflammation, local or permanent tissue damage and hypotension (Ushanandini et al., 2006). A polysaccharide isolated from Tamarind seeds has biological applications and it has immunomodulatory effect and lacks carcinogenic and cytotoxic activities (Sreelekha et al., 1993).

## 2.7 MICROORGANISMS RELATED TO FOOD

Nature uses microorganisms and their metabolic pathways to carry out fermentation processes, for thousands of years mankind has used bacteria, yeast and moulds in the food production processes such as beer, wines, vinegar, breads, cheese, yoghurt as well as fermented fish, meat and vegetable products. The first realization that microorganisms were involved in food production processes was in 1837, when scientists discovered the role of yeasts in alcoholic fermentation. Later when the world renowned, French chemist and biologist Louis Pasteur was trying to explain what happened during the production of beer and vinegar in the 1680s and he found out that microorganisms were responsible (FDA, 2014). However, it was it was not after the Second World War that the food industries began to develop the biotechnological techniques we rely on today to produce a variety of better and safer foods under controlled conditions (EFFCA, 1992).

## 2.8 FOOD SPOILAGE MICROORGANISMS

Microorganisms capable of spoiling food are available commonly in soil, air and water, on the fruits and vegetables, skin of cattle, on the hulls and shells of nuts, on the feathers of poultry, on the clothing and skin of handling personnel, on processing equipment and within the intestines and body cavities of animal and human bodies. There are three types of microorganisms which spoil food and these are yeasts, moulds and bacteria (Tull, 1996). The spoilage bacteria are microorganisms that can cause the deterioration of food and develop unpleasant tastes, odours and textures. Food spoilage is defined as a complex process and excessive amounts of foods are lost due to microbial spoilage even with modern day preservation techniques (Gram et al., 2002), leading to the loss of the original texture, flavour and nutritional value resulting in the food becoming detrimental to human health and unsuitable to eat. Microbial spoilage causes high economic losses in the food industries especially as a result of improper storage conditions for example improper refrigeration practices, resulting in economic problems (Bohme, 2012).

## 2.9 FOOD PATHOGENS

Foodborne illnesses are triggered by agents that enter the body through the consumption of food and the same food can act as a medium that transmit disease from person to person as well as a bacterial growth medium that can result in food poisoning (Bohme, 2012). Foodborne infections are caused by pathogenic bacteria in food or can either be due to foodborne infection or foodborne intoxication. Pathogenic bacteria normally do not change the taste, colour, odour, or texture of a food product, thus making it difficult for them to be recognized even if the food product is contaminated. If bacteria multiply in the contaminated product and the food is consumed, it may continue to grow in the intestines of the host causing illnesses. Amongst others, most common examples of these pathogens include *Clostridium perfringens,*

*Campylobacter jejuni*, *Staphylococcus aureus* and pathogenic *E. coli* 0157 (FDA, 2014). The consumption of toxins or poisons produced in food as by-products of bacterial growth and multiplication in food results in food intoxication and in this scenario, illness is not caused by bacteria but by the toxins which are produced by the bacteria. For example *Clostridium perfringens* illness is caused when toxins are released in the gut or when large amount amounts of vegetative cells are consumed (Biozaris, 2015).

### 2.9.1 *S. aureus*

*Staphylococcus aureus* are gram positive aerobic or facultatively anaerobic cocci that tends to form "grape like" clusters (Staphle) is Greek for bunch of grapes and coccus means "grain" or "berry" (Bhunia, 2007). They do not form spores, are none motile, they grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Optimal growth temperature range is 35 to 37ºC and can grow over optimal pH of 6 to 7 (Deshpande, 2002; Pannerseelan, 2008). The effect of pH on *S. aureus* growth varies with strain and is affected by the growth medium, inoculum level, sodium chloride concentration, redox potential temperature. An important characteristic of *S aureus* is that it can tolerate high levels of sodium chloride and can as well grow in media containing 5 to 7% sodium chloride and some strains are capable of growth in the presence of 20% sodium chloride (Deshpande, 2002).

*S. aureus* commonly inhabits in warm nasal passages and the skin. It often gains entry into the food systems through food handlers. Foods that require hand preparation such as breads salad spreads and sandwiches are the most susceptible to contamination by *S. aureus* (Pannerseelan, 2008). Food poisoning due to this bacterium is usually rapid within 1 to 6 hours after eating the contaminated food and lasts for 24 to 48 hours. The symptoms include stomach cramps, diarrhea, nausea and vomiting (FDA, 2014). The organism is more resistant to heat than most

gram-positive bacteria and requires exposure to 60ºC for 20 to 30 minutes for destruction. Foods that harbor *S. aureus* should be heated to an internal temperature of 74ºC to ensure its destruction (Deshpande, 2002). Several laboratories in developing countries screen for presumptive *Staphylococcus aureus* based on its growth on mannitol salt agar (MSA) and/or DNase tests and confirmation is done with the TCT (Pannerseelan, 2008).

Recently an outbreak of staphylococcal food poisoning among children and staff at a Swiss boarding school in Switzerland due to soft cheese made from raw milk has been recorded. On October 1, 2014, children and staff members at a Swiss boarding school consumed Tomme, a soft cheese produced from raw cow milk. Within the following 7 hours, all the people who ingested the cheese fell ill (10 children and 4 staff members). Symptoms included abdominal pain and violent vomiting, followed by severe diarrhea and fever. The duration of the incubation period depended on the age of the patient: 2 hours 30minutes in children under 10 years of age, 3hours 30 minutes in older children and teenagers, and 7 hours in adults. The soft cheese exhibited low levels of staphylococcal enterotoxin (Johler et al., 2015).

### 2.9.2 *Clostridium perfringens*

*Clostridium perfringens (C. perfringens)* is one of the most common causes of food poisoning in the United States. According to some estimates, this type of bacteria causes nearly a million illnesses each year. *C. perfringens* types A, B, C, D and E produce at least different types of toxins that may be involved in its pathogenesis. These antigens have been given the names alpha, beta, epilson and iota toxin (major toxins) and delta, theta, kappa (collagenase), lambda (protease) mu (hyluronidase) nu (deoxyribonuclease) (Wijnands and Volksgezondetied en Miliey, 2009). The heat stable food poisoning strains of *C. perfringens* exist water, soils, undercooked foods, spices, dust and the GI tract of humans and other animals. The heat sensitive types are common to the intestinal tract in all humans. *C. perfringens* directly contaminates meat during the slaughtering of animals or by subsequent contamination of

containers used for storing the meat, by the handlers, or dust. *C. perfringens* is a spore former and can with stand the adverse environmental conditions of heat or drying and certain toxic compounds (Jay, 2000). Incubation period is usually 8 to 16 hours after contaminated food is congested and the symptoms of poisoning include abdominal pain, diarrhoea, and sometimes nausea and vomiting which usually last for a day or less (FDA, 2014).

*C. perfringens* is mesophilic, with an optimum growth temperature between 37ºC and 45°C. The growth temperature is approximately 20ºC whereas, the highest is approximately 50ºC. Optimum growth in thioglycollate medium for six strains was found to occur between 30ºC and 40ºC, and the optimum for sporulation in Ellner's medium was 37 to 40ºC. Growth at 45ºC under otherwise optimal conditions leads to generation times as short as 7 minutes. Regarding pH, many strains grow over the range 5.5 to 8.0 but generally not below 5.0 or above 8.5 (Jay, 2000).

### 2.9.3 *Escherichia coli* 0157: H7

*Escherichia coli* is a Gram-negative rod (bacillus) in the family of *enterobacteriaceae*. Most *E. coli* are commensals mainly found in the gastrointestinal tract of healthy people and animals. Most varieties are harmless and cause brief bacteria, but a few particularly nasty strains such as Enterohemorrhagic *E. coli* 0157: H7 (EHEC), which is also known as Shiga Toxin Producing *E. coli* (STEC) can cause bloody diarrhoea severe, abdominal cramps and vomiting. One can be exposed to *E. coli* through contaminated food, water, vegetables and undercooked beef raw hamburgers and unpasteurised juice. (Marisch et al., 2013; FDA, 2014). *E. coli* is usually enumerated on Mckonkey agar or TBX agar and its presence is indicated by blue colonies (Virk et al., 2012). Incubation period is usually 3 to 4 days after ingestion, but may occur from 1 to 10 days after eating contaminated food. Severe stomach cramps, bloody diarrhoea, and nausea usually occur after contaminated food is ingested. It can also manifest as

non-bloody diarrhoea or it can be symptomless. In severe cases, *E. coli* 0157: H7 can cause permanent kidney damage which can lead to death in young children (FDA, 2014).

### 2.9.4 *Pseudomonas* spp

*Pseudomonas* spp. are gram-negative rod bacteria commonly found in soil, ground water, plants and animals. Pseudomonal infection causes a necrotising inflammation, between 2008 and 2012, there was 6% decrease in the number of *Pseudomonas* spp bacteraemias reported to Public Health England (PHE) compared with a 1% decrease for all bacteraemias (Chen, 2016). The overall incidence in 2012 for *Pseudomonas* spp bacteraemias was 6.4 cases over 100 000 population in England, Wales and Northern Ireland and *Pseudomonas aeruginosa* is the most common cause of pseudomonal infection (Public Health England, 2011)

*Pseudomonas* is a clinically significant and opportunistic pathogen, often causing nosocomial infections. In addition to causing serious and often life threatening diseases, these organisms exhibit innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial agents. Some pseudomonal species that previously were considered the causative agents of old diseases now are being re-examined for their potential use as biological warfare agents (FDA, 2014; Chen, 2016). The current classification of the genus *Pseudomonas* is divided into 5 groups based on their DNA homology or ribosomal RNA (rRNA). More than 20 pseudomonal species have been found from human clinical specimens (Chen, 2016). Although *P. aeruginosa* is a common human saprophyte, it rarely causes disease in healthy persons. Most infections with this organism occur in compromised hosts. Examples of compromising conditions include disrupted physical barriers to bacterial invasion (for example, burn injuries, intravenous lines, urinary catheters, dialysis catheters, endotracheal tubes) and dysfunctional immune mechanisms, such as those that occur in neonates and in individuals with cystic fibrosis, acquired immunodeficiency syndrome (AIDS), neutropenia,

complement deficiency, hypogammaglobulinemia, and iatrogenic immunosuppression (Chen, 2016 ; Rodgers and Cafaso, 2016).

### 2.9.5. *Salmonella* spp

*Salmonellae* are gram-negative, flagellated, facultatively anaerobic bacilli possessing three major antigens: H or flagellar antigen; O or somatic antigen; and Vi antigen (possessed by only a few serovars). H antigen may occur in either or both of two forms, called phase 1 and phase 2. The organisms tend to change from one phase to the other. O antigens occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface. Vi antigen is a superficial antigen overlying the O antigen; it is present in a few serovars, the most important being *S typhi* and *S. enterica* (Moraes, 2016; fukushi, 2003).

*Salmonella* is usually found in contaminated water, faeces, contaminated carcases as well as live animals. *Salmonella* can be transferred between humans and animals. Pathogenic salmonellae such as *S. enterica* is ingested in food, survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. Invasion of epithelial cells stimulates the release of pro inflammatory cytokines which induce an inflammatory reaction. The acute inflammatory response causes diarrhoea and may lead to ulceration and destruction of the mucosa. The bacteria can disseminate from the intestines to cause systemic disease (Baron, 1996; Mossel, 1997).

Salmonellosis ranges clinically from the common Salmonella gastroenteritis (diarrhoea, abdominal cramps, and fever) to enteric fevers (including typhoid fever) which are life-threatening febrile systemic illness requiring prompt antibiotic therapy. Focal infections and an asymptomatic carrier state occur. The most common form of salmonellosis is a self-limited, uncomplicated gastroenteritis (Baron, 1996; Mossel, 1997).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 INTRODUCTION

The fundamental aim of this chapter is to review methods that were used in carrying out the research. This chapter is comprised of a research design, population, sample and sampling techniques, data collection procedures, research analysis and data analysis.

## 3.2 RESEARCH DESIGN

The design of the study serves as the framework of any research paper. It can be either a quantitative or qualitative design. In the present research, an experimental design was implemented in order to obtain quantitative data. Dimethyl sulphoxide (DMSO) was used as the negative control.

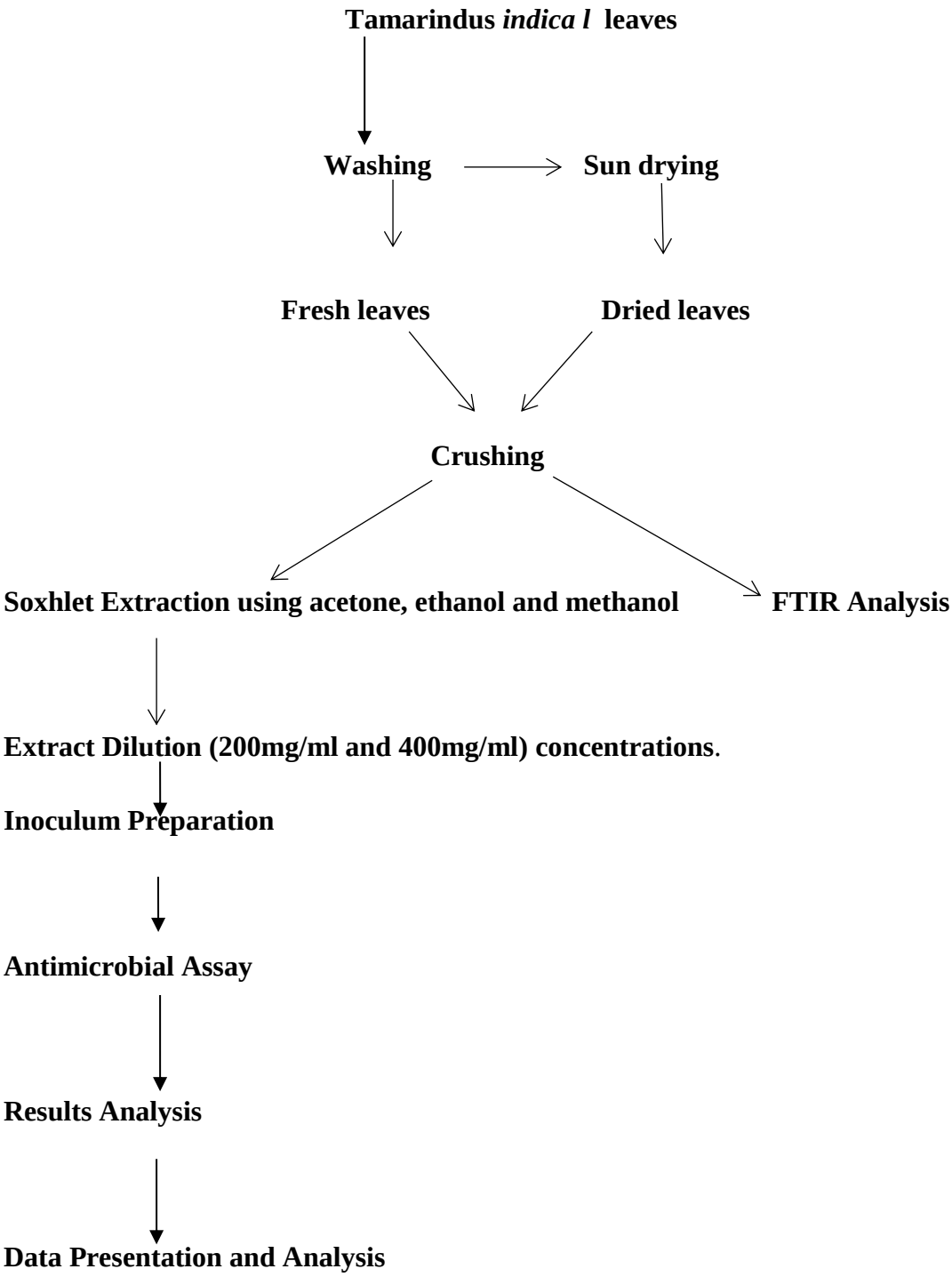


**Fig 3.1** Experimental Design

### 3.3 POPULATION OF STUDY

The population of the study was the *Tamarindus indica l* leaves.

### 3.4 SAMPLING TECHNIQUE

A sample is a representative of a target population to be used in a study. Non probability sampling was used in this study in which purposive or judgmental sampling technique was used whereby the researcher chose a sample based on what they think would be appropriate for the study.

### 3.5 RESEARCH INSTRUMENTS

**Table 3.1: Equipment and reagents used in the study**

| EQUIPMENT | REAGENTS |
|---|---|
| Inco-therm Incubator, Bunsen Burner ,Water Bath, Petri Dishes, High Care Autoclave, Hot Plate, Analytical Balance ,Paper Puncher, Beakers, Durum Bottles ,Spatula , Test Tubes , Permanent Marker, Pestle, Mortar, Sterile Inoculating Loops, FTIR. | Nutrient Agar, Muller Hinton Agar, Mannitol Salt Agar, Eosin Methylene Blue Agar, Acetone, Ethanol, Methanol, Dimethyl Sulphoxide (DMSO), Sulphuric Acid, Barium Chloride, KBr. |

## 3.6 DATA COLLECTION PROCESS

### 3.6.1 Collection of Plant Material

The Researcher handpicked the Tamarind leaves from a Tamarind tree in Chegutu.

### 3.6.2 Preparation of the Extracts

The collected Tamarind leaves were washed with distilled water to remove dirt such as dust, debris and small stones and other impurities. For dried leaf extract, leaves were sun dried until they the attained constant weight.

The extraction method that was used is the Soxhlet extraction method and the solvents used were acetone, ethanol and methanol. 50g fresh and 50g dried Tamarind leaves were used for the reparation of the extracts and, they were crushed using a pestle and motor. The solvents, 250 ml of ethanol, acetone or methanol were added into a round bottom flask, which was attached to a Soxhlet extractor and condenser in a water bath. The crushed plant material was wrapped using a filter paper and then dampened using the solvent (ethanol/acetone/methanol) used. The wrapped plant material was loaded into a thimble, which was placed inside the Soxhlet extractor. The solvent was heated using the water bath at 70°C for ethanol, 50°C for acetone and 60°C for methanol respectively so as to enable the solvent to evaporate, thus moving through the apparatus to the condenser. The solvents evaporated, condensated and then dripped into the reservoir containing the thimble and as the level of solvent reached the siphon it poured back into the flask and the cycle began again and the process was left to run 12 hours (Redfern et al, 2014). The extracts were concentrated by evaporating the solvent using a water bath at 60°C and then left to dry.

### 3.6.3 Determination of functional groups

Fourier Transform Infra-red Spectroscopy (FTIR) was used to detect functional groups of *T. indica l* leaves. FTIR is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterize some inorganics. The FTIR relies on the fact that most molecules absorb light in infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule (Smith, 2011; Alvarez-Ordonez and Prieto, 2012). The frequency range is measured as wave numbers.

KBr discs allow suspension of powders or contaminants in IR transparent KBr so they may be analysed (Smith, 2011).

A ratio of 1mg sample: 100mg KBr were mixed and ground into fine powder using a motor and pestle. The powder was then compressed into a pellet and then analyzed using FTIR and spectrum was recorded in a specified spectral range.

### 3.6.4 Test Microorganisms

The antimicrobial activity of *Tamarindus indica l* was assessed against 6 pathogenic bacterial cultures *Escherichia Coli (0157: H7), Staphylococcus aureus, Clostridium perfringens, Pseudomonas* spp*, Salmonella isolate* and *Salmonella enterica,* were obtained from Midlands State University, Department of Food Science and Nutrition and Parirenyatwa Hospital. The cultures were grown on Eosin Methylene Blue Agar, Mannitol Salt Agar, MacConkey Agar and Nutrient Agar and then refrigerated at a temperature of 4°C.

### 3.6.5 Disc Preparation and Extract Dilution

A paper puncher was used make discs of about 6mm diameter Whitman's No.1 filter paper and the discs were then sterilized at 121°C for 15minutes using an autoclave (Bukar, et al. 2010 ; Gupta, 2014). Two concentrations were made per each extract respectively. The first concentration of 400mg/ml was prepared by dissolving 0.8g of each extract in 2ml dimethyl lsulphoxide (DMSO). The second concentration of 200mg/ml was prepared by serial doubling diluting stock solution by adding 2ml of DMSO.

### 3.6.6 Standardization of Inoculum

Selected colonies for the test microorganisms were suspended in sterile distilled water using a cotton swab. Turbidity of the inoculum was standardized to a turbidity equivalent to a 0.5 McFarland standard of 1% barium chloride and 1% sulphuric acid. The turbidity of the suspensions were compared by placing the tubes in front of a white surface with black horizontal lines (Cavalieri et al., 2005).

### 3.6.7 Antimicrobial Assays

Disc diffusion method was used to test for the antimicrobial activity of the Tamarind leaf extracts. Disc diffusion refers to the ability of an antimicrobial agent to diffuse into a specified concentration from tablets, disks or strips, into the solid culture medium that has been seeded with selected inoculum in a pure culture. (Coyle, 2005) The principle behind this method is to determine a zone of inhibition proportional to the bacterial susceptibility to the antimicrobial present in the disc by placing them on the surface of the Muller Hinton agar medium inoculated with the test organisms. The plates are incubated and the zones of inhibition around each disc are measured.

The prepared Muller Hinton Agar plates were inoculated with test microorganisms using the spread plate method. Sterilized filter paper discs were soaked in 1ml plant extracts and then placed on the surface of seeded agar plate using sterile forceps and pressed down to ensure complete contact with agar under a laminar flow, to provide an aseptic environment. The agar plates were then incubated at 37ºC for 24 hours which was followed by the examination of the plates and measurement of diameters of complete inhibition using a ruler.

## 3.7 DATA PRESENTATION AND ANALYSIS

Data collected was analyzed using the Graph pad statistical analysis package with the variance ($p = 0.05$) or 95% confidence level. Unpaired T-test and One Way ANOVA were used to test the hypothesis. Data was presented in the form of tables and graphs.

## 3.8 RELIABILITY AND VALIDITY

Samples were run in triplicates for accuracy and reproducibility so as to achieve stable and consistent results.

# CHAPTER 4

# RESULTS AND DISCUSSION.

The main aim of the research was to determine the antimicrobial properties of *Tamarindus indica l* leaves on *S. aureus, C. perfringens, E. coli, Pseudomonas* spp, *Salmonella isolate and Salmonella enterica*. The results gathered using the methods described in chapter three are displayed, analysed and discussed in this chapter.

## 4.1 RESULTS

### 4.1.1 Susceptibility of microorganisms to tamarind leaves extracts.

**Table 4.1: Susceptibility of test microorganisms to different concentrations of dried leaf acetone extract.**

| | **Zones of Inhibition (mm)** | |
|---|---|---|
| **Microorganism** | **400mg/ml** | **200mg/ml** |
| *S. aureus* | 16.6 | 14 |
| *Clostridium perfringens* | 8.6 | 7.6 |
| *E. coli* 0157: H7 | 17.3 | 14 |
| *Pseudomonas* spp | 13 | 12 |
| *Salmonella isolate* | 12.6 | 13 |
| *Salmonella enterica* | 13.6 | 13 |

**Table 4.2: Susceptibility of test microorganisms to different concentrations of fresh leaf acetone extract.**

| | **Zones of Inhibition (mm)** | |
|---|---|---|
| **Microorganism** | **400mg/ml** | **200mg/ml** |
| *S. aureus* | 17.3 | 16.6 |
| *Clostridium perfringens* | 11.6 | 10 |
| *E. coli* 0157: H7 | 18.6 | 17 |
| *Pseudomonas* spp | 14.8 | 12 |
| *Salmonella isolate* | 14.5 | 10.6 |
| *Salmonella enterica* | 15.8 | 14 |

**Table 4.3: Susceptibility of test microorganisms to different concentrations of dried leaf ethanol extract.**

| | **Zones of Inhibition (mm)** | |
|---|---|---|
| **Microorganism** | **400mg/ml** | **200mg/ml** |
| *S. aureus* | 19 | 16.3 |
| *Clostridium perfringens* | 14 | 11.3 |
| *E. coli* 0157: H7 | 19.3 | 17.6 |
| *Pseudomonas* spp | 18.6 | 16.6 |
| *Salmonella isolate* | 18.3 | 15.3 |
| *Salmonella enterica* | 15.3 | 13.3 |

**Table 4.4: Susceptibility of test microorganisms to different concentrations of fresh leaf ethanol extract.**

| | **Zones of Inhibition (mm)** | |
|---|---|---|
| **Microorganism** | **400mg/ml** | **200mg/ml** |
| *S. aureus* | 19.8 | 18.6 |
| *Clostridium perfringens* | 16 | 12 |
| *E. coli* 0157: H7 | 19.3 | 17.3 |
| *Pseudomonas* spp | 19.3 | 18.3 |
| *Salmonella isolate* | 19 | 16.3 |
| *Salmonella enterica* | 17 | 14 |

**Table 4.5: Susceptibility of *E.coli, S. aureus, Salmonella isolate, Salmonella enterica, Pseudomonas, Clostridium perfringens* to different concentrations of dried leaf methanol extract.**

| | **Zones of Inhibition (mm)** | |
|---|---|---|
| **Microorganism** | **400mg/ml** | **200mg/ml** |
| *S. aureus* | 18.3 | 16.3 |
| *Clostridium perfringens* | 15 | 11 |
| *E. coli* 0157: H7 | 19 | 14.3 |
| *Pseudomonas* spp | 15.6 | 13 |
| *Salmonella isolate* | 14.6 | 13 |
| *Salmonella enterica* | 16 | 14 |

**Table 4.6: Susceptibility of test microorganisms to different concentrations of fresh leaf methanol extract.**

| | **Zones of Inhibition (mm)** | |
|---|---|---|
| **Microorganism** | **400mg/ml** | **200mg/ml** |
| *S. aureus* | 19.3 | 18 |
| *Clostridium perfringens* | 15.6 | 13.6 |
| *E. coli* 0157: H7 | 19.6 | 16.3 |
| *Pseudomonas* spp | 18.8 | 16.6 |
| *Salmonella isolate* | 18.3 | 15.6 |
| *Salmonella enterica* | 16.3 | 13.6 |

### 4.1.2 Susceptibility of specific microorganisms to *Tamarindus indica l* leaf extracts.

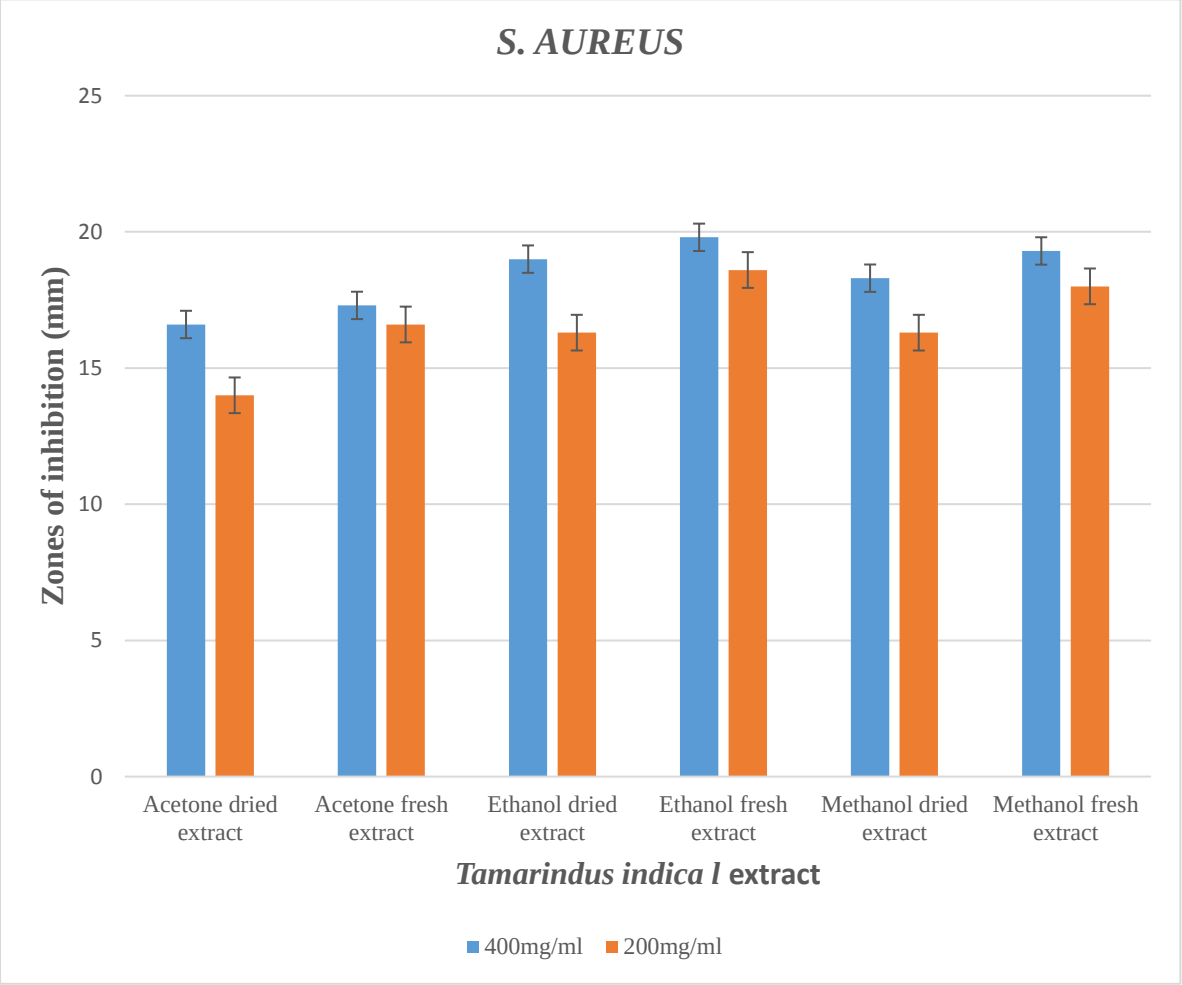


**Figure 4.1: Susceptibility of *S. aureus* to *T. indica l* leaf extracts**

Fig 4.1 illustrates the susceptibility of *S. aureus* to *Tamarindus indica l* extracts. *S. aureus* was highly susceptible to the fresh leaf ethanol extract at 400mg/ml showing an average inhibition zone of 19.8mm and less susceptible to acetone dried leaf extract at 200mg/ml showing an average zone of inhibition of 14mm. However *S. aureus* was less susceptible to the lower concentrations as compared to the higher concentration 400mg/ml concentration.

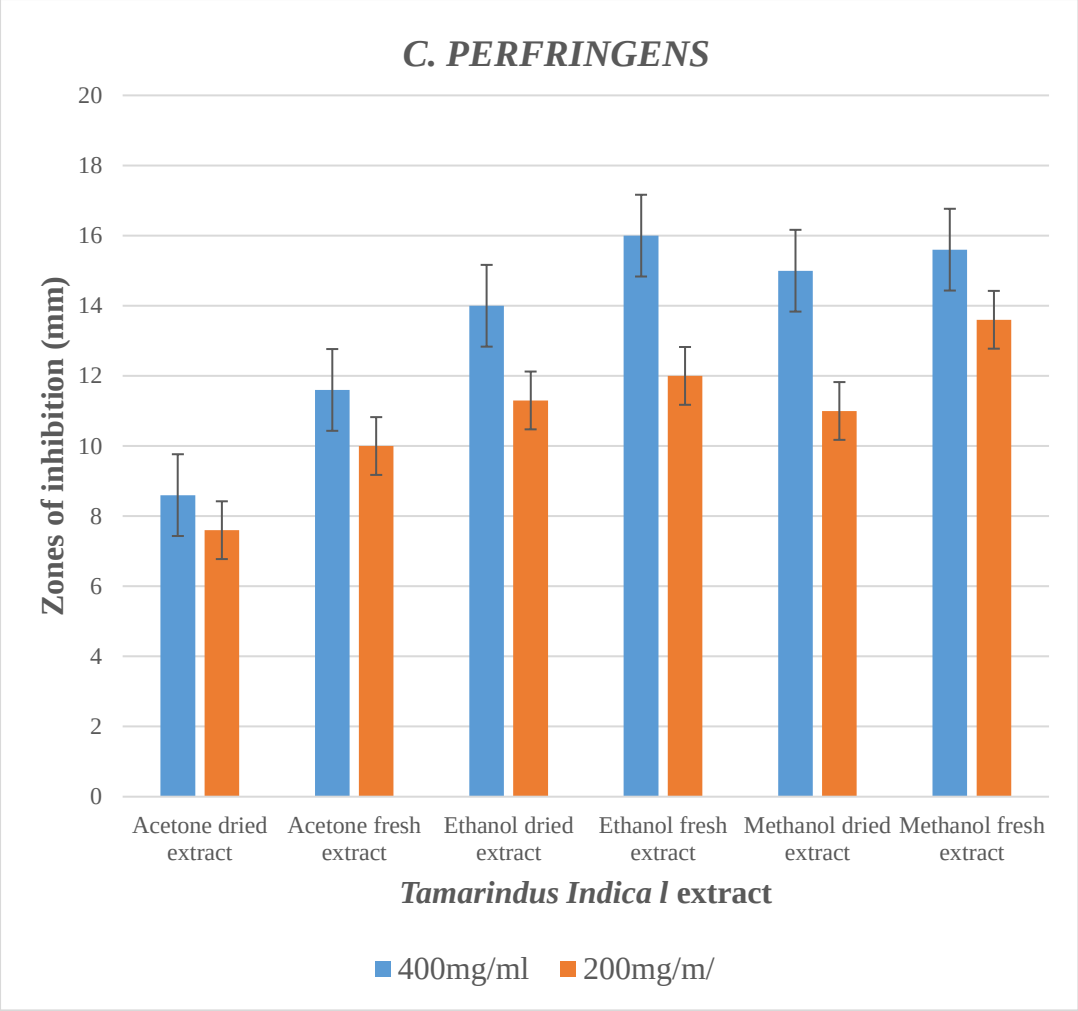


**Figure 4.2: Susceptibility of *C. perfringens* to *T. indica l* leaf extracts**

Fig 4.2 illustrates the susceptibility of *C. perfringens* to *Tamarindus indica l* extracts. The microorganisms were highly susceptible to ethanol leaf extracts and less susceptible to acetone leaf extracts especially the dried leaf extract. High susceptibility of microorganisms is observed at 400mg/ml concentration and lower at 200mg/ml concentration respectively.

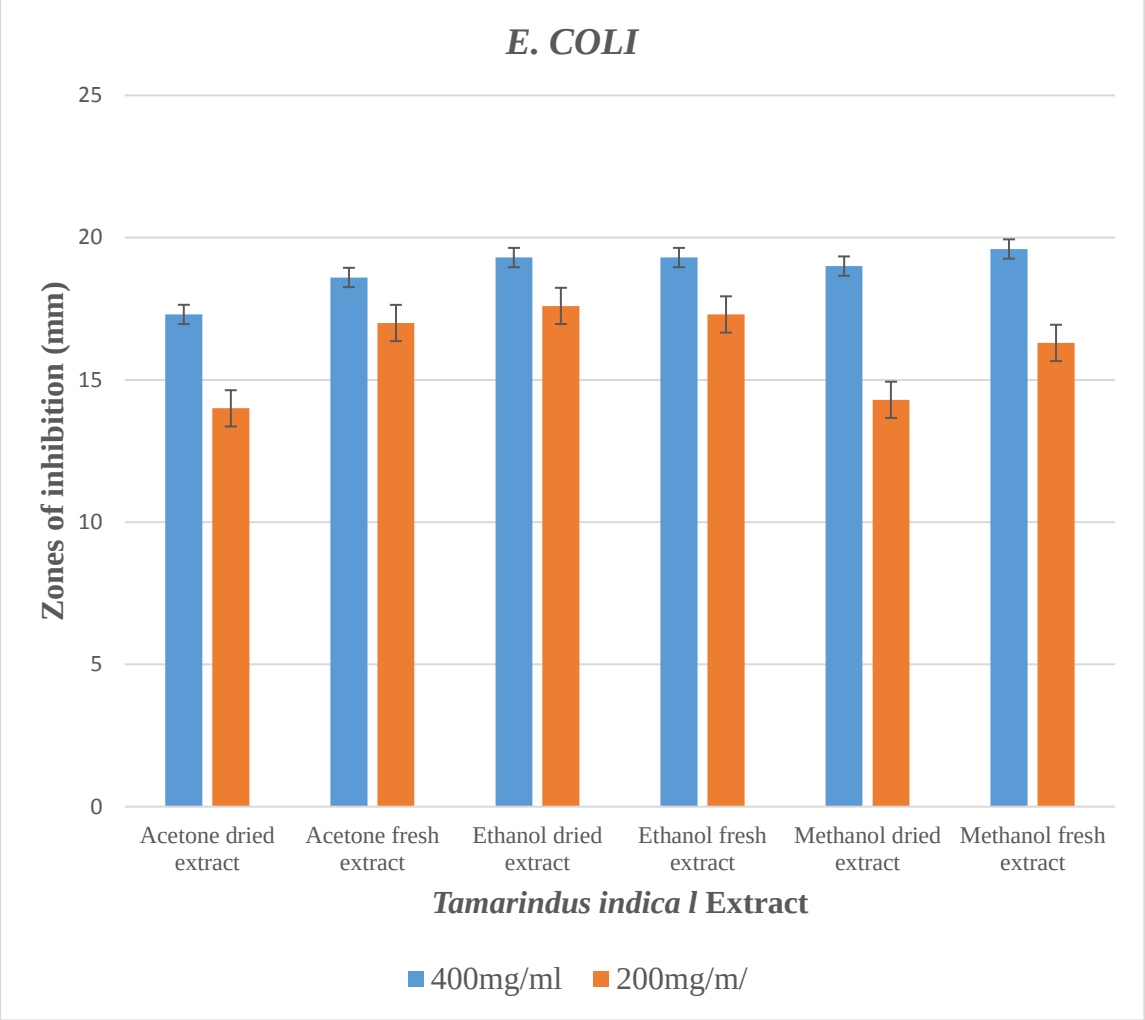


**Figure 4.3: Susceptibility of *E. coli* to *T. indica l* leaf extracts**

Fig 4.3 shows that *E.coli* was highly susceptible to the fresh leaf methanol and ethanol extracts at 400mg/ml showing an average inhibition zone of 19.6mm and 19.3mm respectively. Less susceptibility was observed in the dry leaf extracts especially in the lower concentration which is 200mg/ml. The same pattern is observed on all the extracts. However *E. coli* was less susceptible to the lower concentrations (200mg/ml) when compared to the higher concentration (400mg/ml).

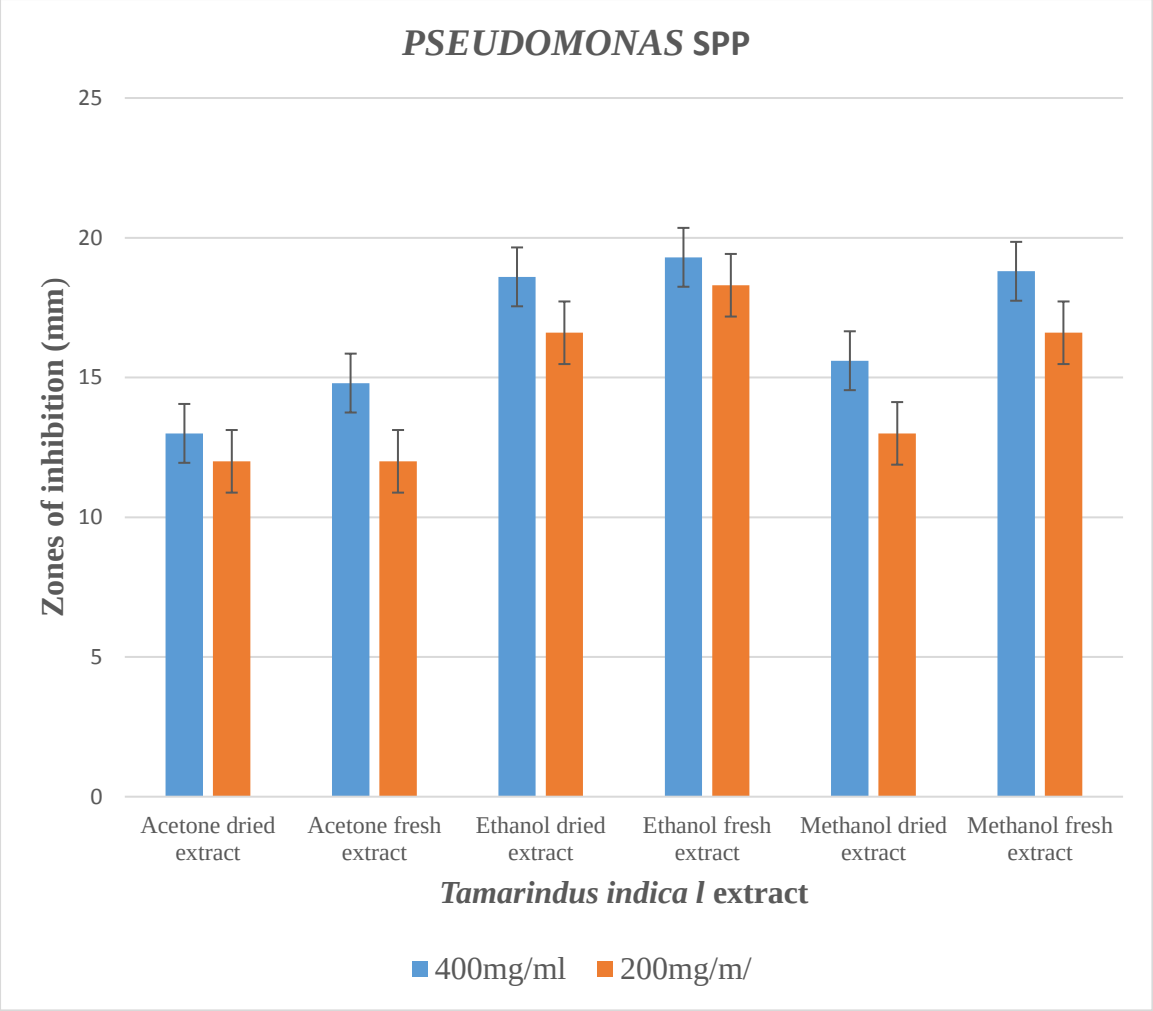


**Figure 4.4: Susceptibility of *pseudomonas spp* to *T. indica l* leaf extracts**

Fig 4.1 illustrates the susceptibility of *Pseudomonas* spp to *Tamarindus indica l* extracts. Susceptibility is high in ethanol fresh and dried leaf extracts at 400mg/ml and less susceptible to acetone dried leaf extracts. *Pseudomonas* spp was highly susceptible to fresh leaf extracts than the dried leaf extracts and less susceptible to the lower concentrations (200mg/ml) when compared to the higher concentration (400mg/ml).

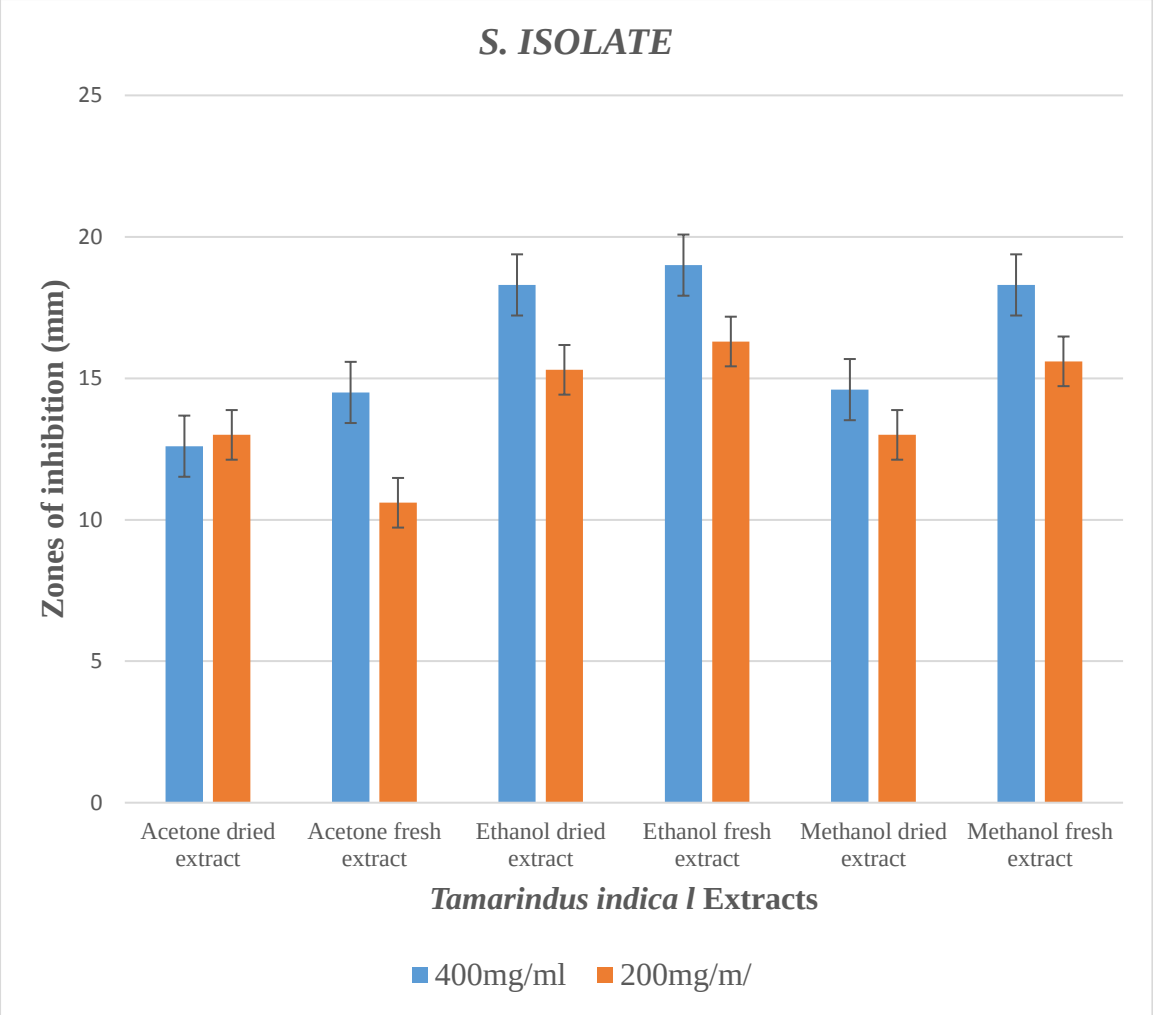


**Figure 4.5: Susceptibility of S. *isolate* to *T. indica l* leaf extracts**

Fig 4.5 illustrates the susceptibility of *Salmonella isolate* to *Tamarindus indica l* extracts. *Salmonella isolate* was highly susceptible to ethanol fresh leaf extracts and less susceptible to acetone dried leaf extracts. However high susceptibility of Salmonella isolate to *Tamarindus indica l* extracts was also observed at 400mg/ml whereas less susceptibility was observed at 200mg/ml.

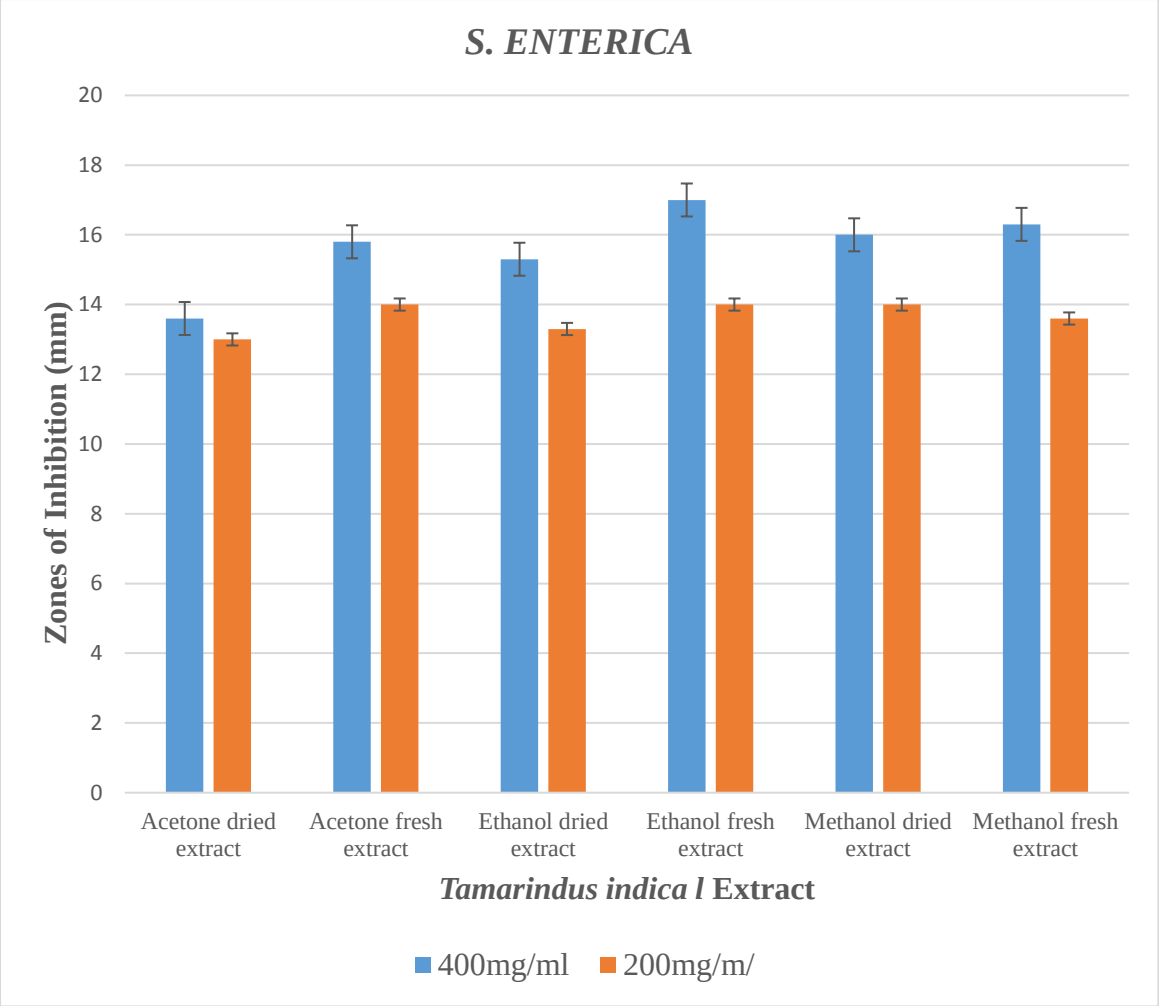


**Figure 4.6: Susceptibility of *S. enterica* to *T. indica l* leaf extracts**

Fig 4.6 illustrates the susceptibility of *S. enterica* to *Tamarindus indica l* extracts. *S. enterica* was highly susceptible to the fresh leaf ethanol extracts at 400mg/ml showing an average inhibition zone of 17mm. Less susceptibility was observed in the dry leaf extracts especially in the lower concentration which is 200mg/ml showing zones of inhibition ranging from 13mm to 14mm respectively. The same pattern is observed on the methanol and acetone extracts.

## 4.2 DETERMINATION OF FUNCTIONAL GROUPS OF *TAMARINDUS INDICA L* LEAVES.

**Table 4.7 FTIR of *Tamarindus indica l* leaves**

| Wave number /cm-1 | Functional groups |
|---|---|
| 3406,28 | N-H Primary and Secondary Amines and Amides<br>O-H Carboxylic acid |
| 2930.44 | C-H Alkanes |
| 1621.24 | C=C Alkene |
| 1415,96 | C-X Fluoride |
| 1086.86 | C-N Amines |
| 668.56 | Aromatics |

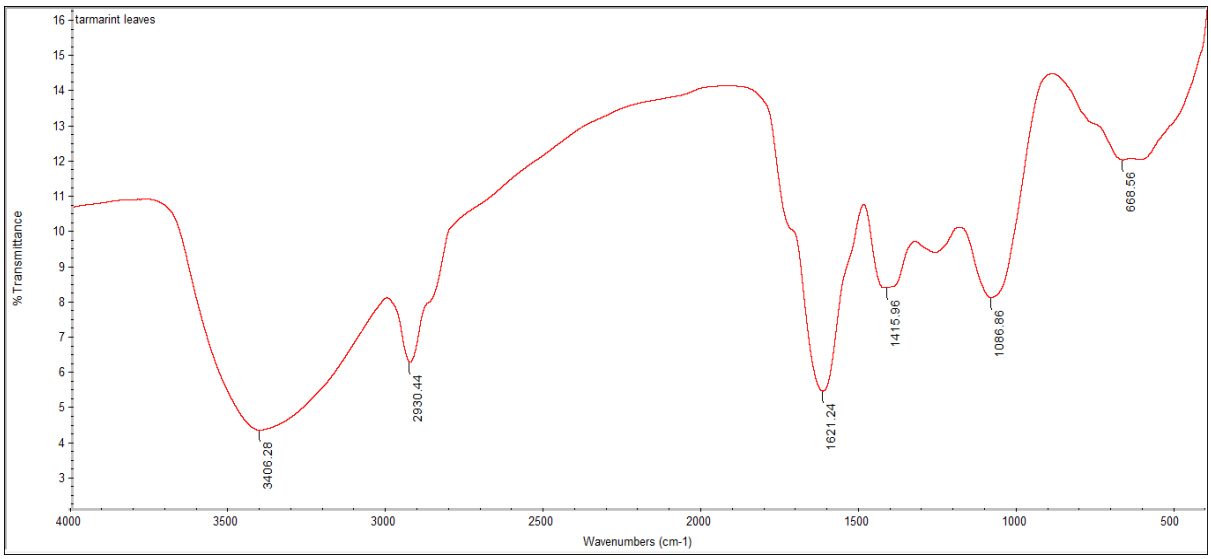


**Fig 4.7: FTIR for tamarind leaves**

Fig 4.7 illustrates the data of IR spectrum of the *T. indica l* sample that was obtained and it showed that *T. indica l* leaves had a peak of Aromatics at 668, 56cm-1 wavelength, followed by Amines which were at 1086.86cm-1, Fluoride at 1415.96cm-1, Alkenes at 1621.24cm-1 and Alkanes at 2930.44cm-1. Lowest peak of wavelengths were observed in Primary and secondary amines and carboxylic acid at 3406.28cm-1 respectively.

## 4.3 HYPOTHESIS TESTING

T-Test and one-way ANOVA were used for hypothesis testing.

### 4.3.1 One way ANOVA of acetone vs ethanol vs methanol extracts on *S. aureus, E. coli* and *S. enterica*

$H_0$1 There is no significant difference between the antimicrobial activity of ethanol, methanol and acetone leaf extracts.

Decision criteria: If $F_{calculated} > F_{tabulated}$ Reject $H_o$

Decision: Fail to Reject $H_0$1

Conclusion: There is no significant difference between the antimicrobial activity of ethanol, methanol and acetone leaf extracts on *S. aureus, E. coli* and *S. enterica.*

### 4.3.2 One way ANOVA of acetone vs ethanol vs methanol extracts on *C. perfringens, Pseudomonas* and *Salmonella isolate.*

$H_0$1 There is no significant difference between the antimicrobial activity of ethanol, methanol and acetone leaf extracts.

Decision criteria: If $F_{calculated} > F_{critical}$ Reject $H_o$

Decision: Reject $H_0$1

Conclusion: There is a significant difference between the antimicrobial activity of ethanol, methanol and acetone leaf extracts on *C. perfringens, Pseudomonas* and *Salmonella isolate.*

### 4.3.3 T-test results of the antimicrobial activity of *Tamarindus indica l* leaf extracts on *S. aureus, C. perfringens, E. coli, Pseudomosas spp, Salmonella isolate* and *Salmonella enterica* at different concentrations

**$H_0$2** There is no significant difference of the antimicrobial activity of Tamarind leaf extract at different concentrations.

Decision Criteria: If $t_{calculated} < t_{tabulated}$ Reject Ho

Decision: fail to Reject $H_0$2

Conclusion: There is no significant difference of the antimicrobial activity of Tamarind leaf extract at different concentrations.

### 4.3.4 T-test results of the antimicrobial activity of dried and fresh leaf extracts on *S. aureus, C. perfringens, E. coli, Pseudomonas* and *Salmonella enterica*

**$H_0$3** There is no significant difference between the activity of fresh leaf extract and dry Tamarind leaf extract.

Decision Criteria: If $t_{calculated} < t_{tabulated}$ Reject Ho

Decision: Fail to reject $H_0$3

Conclusion: There is no significant difference between the activity of fresh leaf extract and dry Tamarind leaf extract on *S. aureus, C. perfringens, E. coli, Pseudomonas* and *Salmonella enterica.*

### 4.3.17 T-test results of the antimicrobial activity of dried and fresh leaf extracts on *Salmonella isolate.*

**Ho3** There is no significant difference between the activity of fresh leaf extract and dry Tamarind leaf extract.

$T_{calculated} = 0.8263$

$T_{tabulated} = 1.812$

Decision: Reject $H_0$3

Conclusion: There is a significant difference between the activity of fresh leaf extract and dry Tamarind leaf extract on *Salmonella isolate*.

## 4.4 DISCUSSION

A total of seven bacterial species were used in the present study to determine the antimicrobial activity of acetone, ethanol and methanol extracts of *Tamarindus indica l* leaves at 400mg/ml and 200mg/ml concentrations. The antimicrobial activity of the extracts was determined by the disc diffusion method. Dimethyl sulphoxide (DMSO) was used as a negative control in the study and it was not effective, producing no inhibition zones respectively. Tables 4.1 to table 4.6 shows the antimicrobial activity of the dried and fresh tamarind extracts against two gram-positive bacteria and four gram-negative bacteria. Both the dried and fresh leaf extracts were effective against both gram-positive and gram-negative bacteria. However out of the three solvents that were used for extraction, ethanol showed the highest activity, followed by methanol and then acetone as shown in fig 4.1 to 4.6, different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent (Doughari, 2006; Yagoub, 2008). These results are in line with the study of Chikwundi and Mohammed (2013) that focused on the 'Phytochemical Screening and Antibacterial Activity of the Fruit and Leaf Extracts of *Tamarindus indica (Linn)'* and had similar results in terms test organisms being highly sensitive to ethanol leaf extracts.

This distinction can be advocated by the report of Kuljanabagavada et al. (2010) who pointed out that polar solvents (ethanol) extracts were more active than extracts of other non-polar or less polar solvents such as acetone, the reason for this being that the active components were principally soluble in ethanol and were stabilized by ethanol or that the compounds that were extracted by ethanol interfered with the antimicrobial activity. In this study however, there was a difference in the antimicrobial activity of *Tamarindus indica l* leaves in all the three solvents. There was a significant difference in the activity of *Tamarindus indica l* leaves in the activity of *C. perfringens* and *Pseudomonas* spp on acetone, ethanol and methanol ($P>0.05$) the reason

being that different solvents are said to be capable of extracting different active components from the leaves.

Figs 4.1 to fig 4.6 shows that all the three extracts had antimicrobial activities against all test microorganisms. The highest zones of inhibition were found on *S. aureus*, this might be because *S. aureus* is a gram-positive bacteria and Escalona-Arranz et al (2010) states that the difference in the susceptibility of test microorganisms may be ascribed to the fact that the cell wall in gram-positive bacteria is single layered, whereas the cell of a gram-negative bacteria is multi layered. So the differences in cell wall will affect the efflux of the bioactive compounds as the intake of plant compounds may be inhibited to pass through the cell wall therefore making gram-negative bacteria less susceptible to any antibacterial agents as compared to gram-positive bacteria. These observations are in accordance with the earlier observations reported by Escalona-Arranz et al. (2010) and Gupta (2014) who also found that gram-negative organisms were less susceptible to the herbal extracts than gram-positive isolates. It may possibly be due to the presence of high lipid content in the cell walls of gram- negative bacteria. gram-positive bacteria like *Staphylococcus epidermidis* and *S. aureus* contains teichoic acid in the peptidoglycan layer and is therefore inhibited by Tamarind extracts (Shan et al., 2007). Furthermore, the outer membrane of gram-negative bacteria is known to present barrier to penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes, which are capable of breaking down foreign molecules introduced from outside thus providing greater resistance to them (Duffy and Power, 2001; Gupta et al.,2014).

From the overall antimicrobial activity of the Tamarind leaves, (table 4.1-4.6), it has been observed that the effects of all the extracts except those resistance, are concentration dependent and this shows that with higher concentrations, more effect (antimicrobial activity) is expected, Abuzied et al., (2014) states that this can be explained by the natural effect of the increase in dose, leading to increase in effect as observed in (Fig 4.5 the activity of *Salmonella isolate* at

different concentration, there was a significant difference between the activity of 400mg/ml leaf extracts and 200mg/ml extracts (P>0.05).

Furthermore, it can be seen on fig 4.2, 4.4 and 4.5 that inhibition zones were higher in fresh leaf extracts as compared to dried leaf extracts. In fig 4.2 *C. perfringens* fresh leaf extracts were ranging from 8.6mm to 16mm whereas dry leaf extracts were ranging from 7.6mm to 13mm, in Fig 4.4, where *Pseudomonas* spp was tested against both dry and fresh leaf extracts, dry leaf extracts exhibited inhibition zones ranging from 12mm to 18mm for dried leaf extracts and 12mm to 19.3mm for fresh leaf extracts and fig 4.5 where inhibition zones of S. isolate against the extracts ranged from 10.6mm to 16.3mm for dry leaf extracts and 12.6 to 19mm for fresh leaf extract. This is due to the fact that in fresh leaf extracts the amount of polyphenols is comparatively higher than in sun dried leaves extracts and is more or less of the same level in the fluid extracts, but with a broad antimicrobial spectrum, looks like these kind of compounds are not the driving force behind the antimicrobial spectrum of Tamarind leaves extracts.

On the other hand, similar flavonoid concentrations in extracts do not expose a clear relevance in the different spectra observed between sun dried and fresh Tamarind leaves' extracts, as well as the similar microbiological spectra of the both fluid extracts, while they have different flavonoid concentrations (Escalona-Arranz, 2010). In this study this is proven by the Fourier transfer infra-red FTIR spectroscopy results that showed the presence of Carboxylic acids with hydrogen bonding at wavelength of 3406.28cm-1, which lead them to having high stabilization and high boiling points. The presence of the OH bond in *Tamarindus indica l* leaves indicates the presence of phenols, which give the *T. indica l* leaves their antioxidant properties and these phenol groups promote their property of being flavonoids. AL-Kahtani & Abou-Arab (1993). Phenolic compounds aid in the maintenance of food, fresh flavor, taste, colour, and prevention of oxidation deterioration. This could be the reason why carboxylic acids are generally used in

the manufactures of pharmaceuticals, polymers, food additives and solvents. Boundless Chemistry (2016).

Moreover, as it is shown in fig 4.2 the Fourier transfer infra-red FTIR Spectroscopy indicated the presence of alkanes in the *T. indica l* leaves at 1621.24cm-1 wavelength. According to Robbins, (2003) alkenes give compounds the ability to be insoluble in water, but however make them soluble in organic solvents. Liquid alkanes tend to be good solvents themselves for other organic molecules. This further explains the reason why the susceptibility of the microorganisms varied according to the solvents that were used for extraction that is acetone, ethanol and methanol. A sharp peak of aromatics at 668.56 wavelength indicated the presence of (C=C) group in the *T. indica l* leaves. This is an indication that *T. indica l* leaves have the ability to resist rancidity due to the presence of the double bonds. This ability to resist rancidity is the proof of the presence of phenols in Tamarind leaves as postulated by the study of Gupta, (2014) on 'The antimicrobial activity of Tamarind (*Tamarindus indica*) and its potential as food bio-preservative".

Lastly, it has been widely observed and accepted that the antimicrobial activity of plants lies in the bioactive phytocomponents present in the plants (Veermuthu et al., 2006) and the components are the products of secondary metabolites of plants that serves as defence mechanisms against predation by microorganisms, insects and herbivores (Nascimento et al., 2000; El-mahmood, 2009). The presence of such phytochemicals in the different extracts of *T. indica l* may be accounted for it antimicrobial activity (Mahajan and Badgujar, 2008; Srinivasan et al., 2001; Doughari, 2006). These differences may also be due to the difference in environmental conditions and geographical locations of the place where the plant materials were obtained or the use of different methods and or procedure. Temperature resistance may be an indication that the phytoconstituents can withstand higher temperatures. This also

explains the traditional usage of these plant parts where a very high temperature is used to boil them and for a longer period of time. A study of Vaghasiya and Chanda (2007) showed that variances in the antimicrobial properties of plant extracts could be ascribed to the physical factors of the plant used like temperature, freshness, water or even its age and contamination of extracts during preparation. These can therefore be other factors that affected this study.

# CHAPTER 5

# SUMMARY, CONCLUSION AND RECOMMENDATIONS

This chapter summarizes the research, conclusions were based on the findings. Recommendations are likewise set up to ensure utilization of the *T. indica l* leaf extract as a natural antimicrobial substitute based on the results obtained in relation to the study.

## 5.1 SUMMARY

This research was focused mainly on determining the antimicrobial potential of *Tamarindus indica l* leaves using standard pathogenic cultures, *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, *Pseudomonas* spp *salmonella* spp and *Salmonela* isolate. The study was carried out at the Food Science Chemistry Laboratory, Biological sciences Laboratory and Chemical Technology Laboratory of Midlands State University. *Tamarindus indica l* leaves were collected from Chegutu. The acetone, ethanol and methanol extracts were obtained using the Soxhlet extraction method. Disc diffusion method was used to test for the antimicrobial activity of the plant extracts and the zones of inhibition were measured using a ruler. The inhibition zones ranged from 7.6mm-19.8mm which therefore indicated that *Tamarindus indica l* possess some antimicrobial properties.

## 5.2 CONCLUSION

From the findings of this study, it was concluded that leaves of *Tamarindus indica l* possess antimicrobial properties as the leaf extracts inhibited the growth of the 2 gram-positive and 4 gram-negative bacteria that were used on this study. These results therefore show that *Tamarindus indica l* leaves are a potential source of natural antimicrobial agents that can be used in food industries as a natural food preservative or used together with minimum doses of artificial preservative in order to reduce the cases of food spoilage and foodborne illnesses

globally. This is also supported by the results of the FTIR, amongst other functional groups the *Tamarindus indica l* leaves showed the highest peak in aromatics. This is an indication of the leaves' ability to resist rancidity due to the presence of the double bonds. It is therefore, possible that it represents a source of food preserving agents that are less costly.

## 5.3 RECOMMENDATIONS

- Further research should be carried out in order to test the effectiveness of the extracts as a preservative on a number of food products, preferably meat and meat products as they are mostly used for spicing food and garnishing salads.
- Further studies on the isolation of the active compounds should be done so that all the active compounds found in the extracts will be further tested and quantified as per their toxicity to human consumption.
- Further research should be done and applied if possible for controlling and inhibiting different pathogenic bacteria for the benefit of the pharmaceutical industry.

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## APPENDICES

### Appendix A: One way ANOVA of acetone vs ethanol vs methanol extracts on *S. aureus.*

| Table Analyzed | | | |
|---|---|---|---|
| acetone vs ethanol vs methanol on *S. aureus* | | | |
| One-way analysis of variance | | | |
| P value | 0.0952 | | |
| P value summary | ns | | |
| Are means signif. different? (P < 0.05) | No | | |
| Number of groups | 3 | | |
| F | 3.088 | | |
| R squared | 0.4070 | | |
| | | | |
| ANOVA Table | SS | df | MS |
| Treatment (between columns) | 11.01 | 2 | 5.507 |
| Residual (within columns) | 16.05 | 9 | 1.783 |
| Total | 27.06 | 11 | |

**Appendix B: One way ANOVA of acetone vs ethanol vs methanol extracts on *C. perfringens.***

| Table Analyzed | | | |
|---|---|---|---|
| acetone vs ethanol vs methanol on *C. perfringens* | | | |
| One-way analysis of variance | | | |
| P value | 0.0245 | | |
| P value summary | * | | |
| Are means signif. different? (P < 0.05) | Yes | | |
| Number of groups | 3 | | |
| F | 5.763 | | |
| R squared | 0.5615 | | |
| | | | |
| ANOVA Table | SS | df | MS |
| Treatment (between columns) | 44.96 | 2 | 22.48 |
| Residual (within columns) | 35.11 | 9 | 3.901 |
| Total | 80.07 | 11 | |

**Appendix C: One way ANOVA of acetone vs ethanol vs methanol extracts on *E. coli***

| Table Analyzed | | | |
|---|---|---|---|
| acetone vs ethanol vs methanol on *E. coli* | | | |
| One-way analysis of variance | | | |
| P value | 0.6378 | | |
| P value summary | ns | | |
| Are means signif. different? (P < 0.05) | No | | |
| Number of groups | 3 | | |
| F | 0.4729 | | |
| R squared | 0.09510 | | |
| | | | |
| ANOVA Table | SS | df | MS |
| Treatment (between columns) | 3.722 | 2 | 1.861 |
| Residual (within columns) | 35.42 | 9 | 3.935 |
| Total | 39.14 | 11 | |

**Appendix D: One way ANOVA of acetone vs ethanol vs methanol extracts on *Pseudomonas* spp.**

| | | | |
|---|---|---|---|
| Table Analyzed | | | |
| acetone vs ethanol vs methanol on *Pseudomonas* spp | | | |
| One-way analysis of variance | | | |
| P value | 0.0053 | | |
| P value summary | ** | | |
| Are means signif. different? (P < 0.05) | Yes | | |
| Number of groups | 3 | | |
| F | 9.926 | | |
| R squared | 0.6881 | | |
| | | | |
| ANOVA Table | SS | df | MS |
| Treatment (between columns) | 50.09 | 2 | 25.04 |
| Residual (within columns) | 22.71 | 9 | 2.523 |
| Total | 72.80 | 11 | |

**Appendix E: One way ANOVA of acetone vs ethanol vs methanol extracts on *Salmonella isolate.***

| | | | |
|---|---|---|---|
| Table Analyzed | | | |
| acetone vs ethanol vs methanol on *Salmonella isolate* | | | |
| One-way analysis of variance | | | |
| P value | 0.0178 | | |
| P value summary | * | | |
| Are means signif. different? (P < 0.05) | Yes | | |
| Number of groups | 3 | | |
| F | 6.515 | | |
| R squared | 0.5915 | | |
| | | | |
| ANOVA Table | SS | df | MS |
| Treatment (between columns) | 40.37 | 2 | 20.18 |
| Residual (within columns) | 27.88 | 9 | 3.098 |
| Total | 68.25 | 11 | |

**Appendix F: One way ANOVA of acetone vs ethanol vs methanol extracts on *S. enterica***

| | | | |
|---|---|---|---|
| Table Analyzed | | | |
| acetone vs ethanol vs methanol on *S. enterica* | | | |
| One-way analysis of variance | | | |
| P value | 0.5928 | | |
| P value summary | ns | | |
| Are means signif. different? (P < 0.05) | No | | |
| Number of groups | 3 | | |
| F | 0.5545 | | |
| R squared | 0.1097 | | |
| | | | |
| ANOVA Table | SS | df | MS |
| Treatment (between columns) | 2.097 | 2 | 1.049 |
| Residual (within columns) | 17.02 | 9 | 1.891 |
| Total | 19.12 | 11 | |

**Appendix G: T-test for *S. aureus* at different concentrations.**

| Table Analyzed | *S. aureus* at different concentrations |
|---|---|
| Column A | 400mg/ml |
| Vs | vs |
| Column B | 200mg/ml |
| Unpaired t test | |
| P value | 0.0602 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=2.118 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 18.38 ± 0.5029 N=6 |
| Mean ± SEM of column B | 16.63 ± 0.6556 N=6 |
| Difference between means | 1.750 ± 0.8263 |
| 95% confidence interval | -0.09093 to 3.591 |
| R squared | 0.3097 |
| F test to compare variances | |
| F,DFn, Dfd | 1.699, 5, 5 |
| P value | 0.2874 |
| P value summary | ns |
| Are variances significantly different? | No |

**Appendix H: T-test of *C. perfringens* at different concentrations.**

| Table Analyzed | *C. perfringens* at different concentrations |
|---|---|
| Column A | 400mg/ml |
| vs | vs |
| Column B | 200mg/ml |
| Unpaired t test | |
| P value | 0.2328 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.270 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 12.70 ± 1.137 N=6 |
| Mean ± SEM of column B | 10.92 ± 0.8240 N=6 |
| Difference between means | 1.783 ± 1.404 |
| 95% confidence interval | -1.345 to 4.912 |
| R squared | 0.1389 |
| F test to compare variances | |
| F,DFn, Dfd | 1.904, 5, 5 |
| P value | 0.2484 |
| P value summary | ns |
| Are variances significantly different? | No |

**Appendix I: T-test of *E. coli* at different concentrations**

| Table Analyzed | *E. coli* at different concentrations |
|---|---|
| Column A | 400mg/ml |
| vs | Vs |
| Column B | 200mg/ml |
| Unpaired t test | |
| P value | 0.0062 |
| P value summary | ** |
| Are means signif. different? (P < 0.05) | Yes |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=3.457 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 18.70 ± 0.4082 N=6 |
| Mean ± SEM of column B | 16.08 ± 0.6374 N=6 |
| Difference between means | 2.617 ± 0.7569 |
| 95% confidence interval | 0.9302 to 4.303 |
| R squared | 0.5444 |
| F test to compare variances | |
| F,DFn, Dfd | 2.438, 5, 5 |
| P value | 0.1752 |
| P value summary | Ns |
| Are variances significantly different? | No |

**Appendix J: T-test for *Pseudomonas* spp at different concentrations.**

| Table Analyzed | *Pseudomonas* spp at diff concentrations |
|---|---|
| Column A | 400mg/ml |
| vs | vs |
| Column B | 200mg/ml |
| Unpaired t test | |
| P value | 0.2372 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.257 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 16.68 ± 1.053 N=6 |
| Mean ± SEM of column B | 14.75 ± 1.120 N=6 |
| Difference between means | 1.933 ± 1.538 |
| 95% confidence interval | -1.492 to 5.359 |
| R squared | 0.1365 |
| F test to compare variances | |
| F,DFn, Dfd | 1.131, 5, 5 |
| P value | 0.4481 |
| P value summary | ns |
| Are variances significantly different? | No |

**Appendix K: T-test for *Salmonella isolate* at different concentrations**.

| Table Analyzed | *Salmonella isolate* at diff concentrations |
|---|---|
| Column A | 400mg/ml |
| vs | vs |
| Column B | 200mg/ml |
| Unpaired t test | |
| P value | 0.1372 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.616 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 16.22 ± 1.081 N=6 |
| Mean ± SEM of column B | 13.97 ± 0.8774 N=6 |
| Difference between means | 2.250 ± 1.392 |
| 95% confidence interval | -0.8522 to 5.352 |
| R squared | 0.2071 |
| F test to compare variances | |
| F,DFn, Dfd | 1.519, 5, 5 |
| P value | 0.3289 |
| P value summary | ns |
| Are variances significantly different? | No |

**Appendix L: T-test of *S. enterica* at different concentrations**

| Table Analyzed | *S. enterica* at different concentrations |
|---|---|
| Column A | 400mg/ml |
| vs | vs |
| Column B | 200mg/ml |
| Unpaired t test | |
| P value | 0.0026 |
| P value summary | ** |
| Are means signif. different? (P < 0.05) | Yes |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=3.991 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 15.70 ± 0.4830 N=6 |
| Mean ± SEM of column B | 13.65 ± 0.1746 N=6 |
| Difference between means | 2.050 ± 0.5136 |
| 95% confidence interval | 0.9056 to 3.194 |
| R squared | 0.6143 |
| F test to compare variances | |
| F,DFn, Dfd | 7.650, 5, 5 |
| P value | 0.0217 |
| P value summary | * |
| Are variances significantly different? | Yes |

**Appendix M: T-test of *S. aureus* dry vs fresh leaf extracts**

| | |
|---|---|
| Table Analyzed | *S. aureus* dry vs fresh |
| Column A | Dry leaf extract |
| vs | vs |
| Column B | Fresh leaf extract |
| Unpaired t test | |
| P value | 0.1123 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.741 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 16.75 ± 0.7178 N=6 |
| Mean ± SEM of column B | 18.27 ± 0.4937 N=6 |
| Difference between means | -1.517 ± 0.8712 |
| 95% confidence interval | -3.458 to 0.4243 |
| R squared | 0.2326 |
| F test to compare variances | |
| F,DFn, Dfd | 2.113, 5, 5 |
| P value | 0.2155 |
| P value summary | Ns |
| Are variances significantly different? | No |

**Appendix N: T-test of *C. perfringens* dry vs fresh leaf extracts**

| | |
|---|---|
| Table Analyzed | *C. perfringens* fresh vs dry |
| Column A | Dry leaf extracts |
| vs | Vs |
| Column B | Fresh leaf extract |
| Unpaired t test | |
| P value | 0.2363 |
| P value summary | Ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.260 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 11.25 ± 1.184 N=6 |
| Mean ± SEM of column B | 13.22 ± 1.017 N=6 |
| Difference between means | -1.967 ± 1.561 |
| 95% confidence interval | -5.444 to 1.511 |
| R squared | 0.1370 |
| F test to compare variances | |
| F,DFn, Dfd | 1.354, 5, 5 |
| P value | 0.3738 |
| P value summary | Ns |
| Are variances significantly different? | No |

**Appendix O: T-test of *E. coli* dry vs fresh leaf extracts**

| | |
|---|---|
| Table Analyzed | *E. coli* dry vs fresh |
| Column A | Dry leaf extracts |
| vs | Vs |
| Column B | Fresh leaf extract |
| Unpaired t test | |
| P value | 0.3281 |
| P value summary | Ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.028 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 16.92 ± 0.9307 N=6 |
| Mean ± SEM of column B | 46.97 ± 29.21 N=6 |
| Difference between means | -30.05 ± 29.23 |
| 95% confidence interval | -95.16 to 35.06 |
| R squared | 0.09561 |
| F test to compare variances | |
| F,DFn, Dfd | 985.0, 5, 5 |
| P value | P<0.0001 |
| P value summary | *** |
| Are variances significantly different? | Yes |

**Appendix P: T-test of *Pseudomonas* spp dry vs fresh leaf extracts**

| | |
|---|---|
| Table Analyzed | *Psedomonas* spp dry vs fresh |
| Column A | Dry leaf extracts |
| vs | vs |
| Column B | Fresh leaf extracts |
| Unpaired t test | |
| P value | 0.2642 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.183 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 14.80 ± 1.043 N=6 |
| Mean ± SEM of column B | 16.63 ± 1.146 N=6 |
| Difference between means | -1.833 ± 1.550 |
| 95% confidence interval | -5.286 to 1.620 |
| R squared | 0.1228 |
| F test to compare variances | |
| F,DFn, Dfd | 1.208, 5, 5 |
| P value | 0.4206 |
| P value summary | ns |
| Are variances significantly different? | No |

**Appendix Q: T-test of *Salmonella* isolate dry vs fresh leaf extracts**

| Table Analyzed | *Salmonella isolate* dry vs fresh |
|---|---|
| Column A | Dry leaf extract |
| vs | vs |
| Column B | Fresh leaf extract |
| Unpaired t test | |
| P value | 0.4279 |
| P value summary | Ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=0.8263 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 14.47 ± 0.8793 N=6 |
| Mean ± SEM of column B | 15.72 ± 1.231 N=6 |
| Difference between means | -1.250 ± 1.513 |
| 95% confidence interval | -4.621 to 2.121 |
| R squared | 0.06391 |
| F test to compare variances | |
| F,DFn, Dfd | 1.960, 5, 5 |
| P value | 0.2389 |
| P value summary | Ns |
| Are variances significantly different? | No |

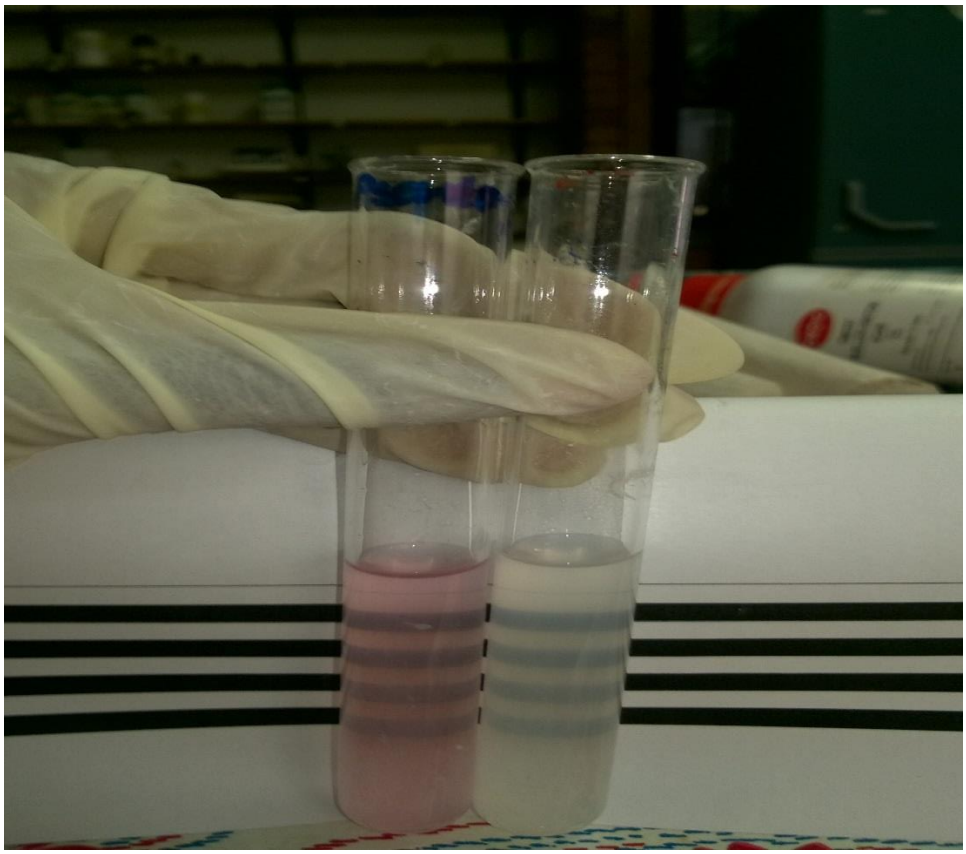
**Appendix R: T-test of *S. enterica* dry vs fresh leaf extracts**

| Table Analyzed | *S. enterica* dry vs fresh |
|---|---|
| Column A | Dry leaf extracts |
| vs | Vs |
| Column B | Fresh leaf extract |
| Unpaired t test | |
| P value | 0.2458 |
| P value summary | Ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.233 df=10 |
| How big is the difference? | |
| Mean ± SEM of column A | 14.20 ± 0.4865 N=6 |
| Mean ± SEM of column B | 15.15 ± 0.5976 N=6 |
| Difference between means | -0.9500 ± 0.7706 |
| 95% confidence interval | -2.667 to 0.7669 |
| R squared | 0.1319 |
| F test to compare variances | |
| F,DFn, Dfd | 1.509, 5, 5 |
| P value | 0.3313 |
| P value summary | ns |
| Are variances significantly different? | No |

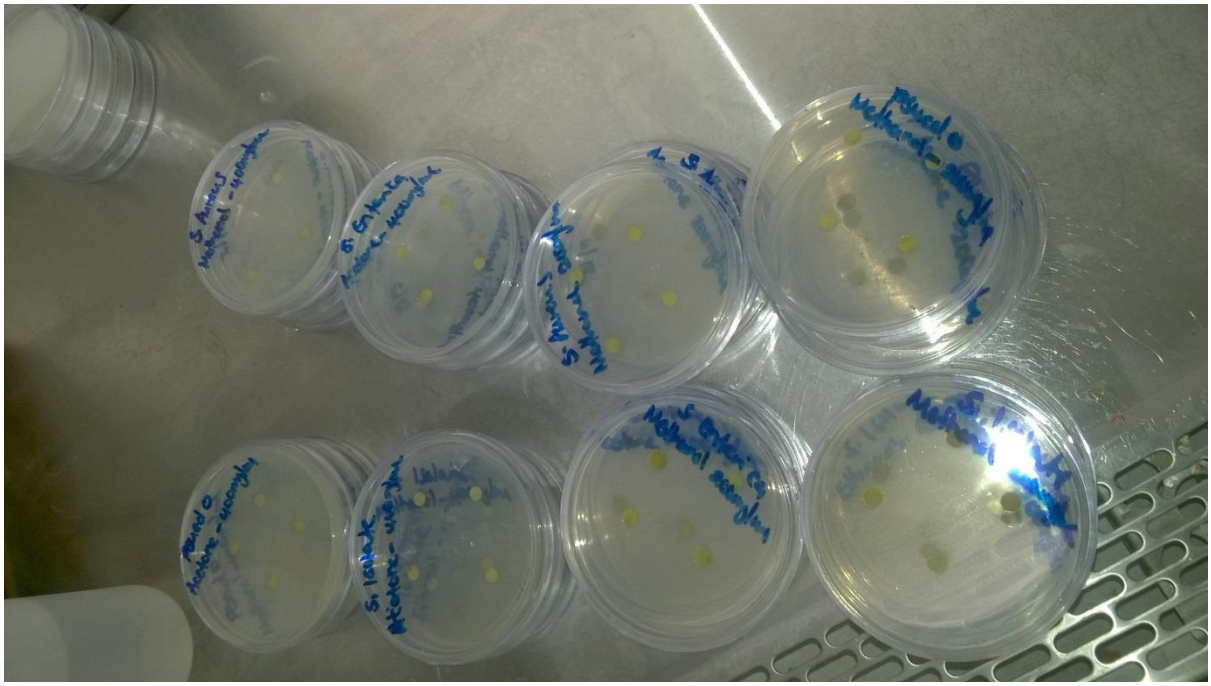

*Salmonella isolate* and *Salmonella enterica* isolates


Media plates setting in the laminar flow

400mg/ml and 200mg/ml extract dilutions



0.5 MacFaland Standardization



Disc diffusion