MICROBIAL QUALITY AND SAFETY OF SELECTED FRUITS AND VEGETABLES AND ANTIMICROBIAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST ISOLATEDSPOILAGE MICROBES

MSc Thesis;

By

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August, 2019

Jimma, Ethiopia

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JIMMA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY

A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES
JIMMA UNIVERSITY

INPARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (APPLIED MICROBIOLOGY)

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August, 2019
Jimma, Ethiopia

APPROVAL SHEET

SCHOOL OF GRADUATE STUDIES

JIMMA UNIVERSITY

Thesis Submission for Defense Request

Name of Student: RahmetulahDetamAwol

Program of study: MSc. **Applied Microbiology**

Title: **Microbial Quality and Safety of Selected Fruits and Vegetable and Antimicrobial Activity of Some Plant Extracts against Potential Pathogenic Isolates.**

I have incorporated the suggestion and modifications given during the Mock defense and got the approval of my adviser. Hence, I hereby kindly request the Department to allow me to submit my thesis for external thesis defense.

RahmetulahDetamo 

Name signature of student

I, the thesis adviser have verified that the student has incorporated the suggestions and modifications given during the Mock defense and the thesis is ready to be submitted. Hence, I recommended the thesis to be submitted for external defense.

Major Advisor; DelelegnWoyessa (PhD Candidate)_____

Name Signature Date

Decision/suggestion of Department Graduate Council (DGC)

_____

_____ _____ _____

Chair Person, DGC Signature Date

_____ _____ _____

Chair Person**,** CGS Signature

CFU	colony-forming unit
DMSO	Dimethyl sulfoxide
Masl	Mater Above sea level
MSA	Mannitol Salt Agar
PDA	potato dextrose agar
TVC	Total Viable Count
VRBA	Violate red bile agar

LIST OF ABBREVIATIONS AND ACRONYMS

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ACKNOWLEDGEMENTS

At the top of all, I would like to extend my extraordinary thanks to the Almighty Allah for everything of his kindness. Next, I would like to thank my advisor Mr. DelelgnWoyessa for his constructive comments, guidance, encouragement and for his provision of the required materials in all moments beginning from the research proposal preparation. Without exaggeration, the finalization of this Thesis report would have been hardly possible without his guidance and heartily support.

I also extend my special acknowledgements to my friend and sister TsionTefera for her constructive assistance throughout the study period.

Finally, yet importantly, my heartfelt appreciation goes to my family members, especially my father DetamoAwol and my mother NesriyaMudesir, for their endless cares and encouragement. I would also like to thank my husband Fuad Kemal for his never-ending supports, encouragement and responsibility in looking after me and family.

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ABSTRACT

Fruit and vegetables are vital source of nutrients, micronutrients, vitamins and fiber for humans. However, they are easily prone to contamination with diverse microorganisms. Therefore, the current study was conducted to determine the microbial qualityof avocado (Perseaamericana), papaya (Carica papaya) and tomatoes (SolanumlycopersicumL.) and to assess anti-microbial activity of extracts of Curcuma longa (Turmeric), Ocimumbasilicum L. and Ginger (Zingiberofficinale). A total of 75 samples ofavocado, papaya and tomato were purchased from four markets namely Bishishe, Kochi, Ajip and Yebu for microbiological analysis following standard methods. The microbiological analysis indicates that all the samples contained high mean bacterial load ranging between 6.3log cfu/g and 7.4log cfu/g. The presenceof yeast with mean counts ranging between 6.1 log cfu/g and 6.5logcfu/g. The study also detected the presence of mold. Thehighest bacterial counts were recorded in papaya samples obtained from Bishishe market (7.5 log cfu/g) and the highest mean mold and yeast counts of 6.5 log cfu/g were recorded in tomato samples from Yebu market. Among the isolates, the mostprevalent spoilage microbes were coliforms, Staphylococcusand Pseudomonas with decreasing order of frequency of occurrence. All the isolated bacteria showed statistically significant between markets at 1% significant level. Antimicrobial activity evaluation of extracts of Curcuma longa (Turmeric), Basil (Ocimumbasilicum L.)and Ginger (Zingiberofficinale)showed poor activity ranging between 0.6mm to 2.3mm of inhibition zone. Whereas, the combined extracts of Curcuma longa (Turmeric), Basil (Ocimumbasilicum L.) and Ginger (Zingiberofficinale)shown relatively better effect ranging between 1.25 to 2.5 mmdiameters of inhibition zoneagainst isolatedbacterial species. The presence of such spoilage microbes in these fruits and vegetable may cause health risk on the consumers. Therefore, Fruits and vegetable traders and consumers should exercise strict hygienic measures to prevent health risks due to contaminants.

Keywords: *Food spoilage, Fruits and vegetable, Isolation of bacteria and fungi,Plant extracts*

CHAPTER ONE

1. INTRODUCTION

Fruits and vegetables are an extraordinary dietary source of nutrients, micronutrients, vitamins and fiber for humans and are thus vital for health and well-being (Eni*et al.*, 2010). They provide well balanced dietsand prevent vitamin C and vitamin A deficiencies in addition to reduction of risk of several diseases (Kalia and Gupta, 2006). Fruits and vegetables have similar nutritive properties with 70% of their weight water, 3.5% protein and about 1% fat. Consumption of fruits and vegetables can help achieve or maintain a healthy body weight (National Institute of Research on Food and Nutrition Rome, Italy, 1998). Food and Agricultural Organization (FAO) recommends a minimum of 400 g of fruit and vegetables per day for the prevention of chronic diseases, such as heart disease, cancer, diabetes and obesity, and for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries (FAO, 2013).

Fruits and vegetables have living tissues with continuing metabolism, and thus subject to respiration, water loss and cell softening throughout the post-harvest system. Surveys of raw fruits and vegetables demonstrate that there are potentials for wide range of these products becoming contaminated with microorganisms, especially the pathogenic ones (Kader, 1997). Depending on whether the product is a fruit or vegetable, these products can contain different species of microorganisms. The range of microorganisms associated with spoilage of fruits and vegetables include; bacteria, parasites, protozoa and viruses and these are often associated with contaminated water and/or food handlers (Beuchat, 1998).According toSeema (2015), Yeastsoften colonize foods with a high sugar or salt content and contribute to spoilage of maple syrup, pickles, and sauerkraut. Fruits and juices with a low pH are another target. Fresh vegetables can support microbial growth due to their neutral pH and high water and nutrient content. Among all the microorganisms found on these products, about 80-90% of the populations are gram-negative rods, such as *Pseudomonas*, *Enterobacter*, or *Erwinia* species (Francis *et al.*,1999).

In the developing countries at the open markets, tomato, avocado and papaya fruits are often displayed in baskets and on benches for the prospective customers, thereby exposing them to opportunistic microbial infections especially mycotoxins (Baiyewu*et al.*, 2007). Spoilage in

papaya and avocado may be referred to as rot or decay and can be extensive around the surfaces of the fruits. These changes may be accompanied by alterations in taste, smell, appearance or texture. Spoilt papaya and avocado fruits are characterized by tissue softening, formation of rot and mycelia, moisture loss, unpleasant odor, sour taste, and shrinkage. The spoilage may be caused by microbes, attacks by insects and rodents, physical injury such as bruising as well as chemical breakdown of the fruit may also lead to deterioration in fruit quality.

Most of the cases of foodborne illness in Sub-Saharan African countries emanate from poor food supply system and microbiological contamination during production, processing, storage, and handling of several foods. Higher levels of enterotoxigenic*Bacillus cereus* were observed in tested vegetable samples procured from streets of Gabon, Botswana, suggesting risks associated with the consumption of street vended foods in South African regions (Mensah*et al*., 2002). A similar study in Kumasi, Ghana revealed high prevalence of *Staphylococci* (23.7%), *Bacillusspp*. (21.5%), and *S. aureus* (3.7%) in most of the tested foods (Feglo and Saky, 2012).

A study in Jimma, Ethiopia revealed 90% of vegetable samples had aerobic mesophilic count of ≥ 5 $\log^{10}$cfu/g and this could be an indication of poor hygienic practice and frequent hand contact at the time of harvesting and in the market (Dugassa*et al*., 2014). A Similar study indicated aerobic mesophilic flora of the vegetable sample was dominated by *Bacillus spp*(22.3%)followed by*Staphylococcusspp*(17%). In view of the foregoing, there is need to isolate and identify microbes associated with tomato, avocado and papaya fruits spoilage to proffering suitable solutions of controlling them before reaching the final consumers and to safeguard human health.

1.2.Statement of the Problem

Avocado (*Perseaamericana*), papaya (*Carica papaya)* and tomatoes (*Solanumlycopersicum*L.)are an attractive cash crop for small scale farmers and provide potential source of employment to many rural and urban Ethiopia. In the study site, avocado and papaya have been marketed picked from the field whereas; tomato fruits have been purchased from different parts of the country for retailing purposes. Despite the need for the aforementioned fruits and vegetable, damage as a result of post-harvest spoilage micro-organisms has been of serious concern. Within all

postharvest activities, fruits and vegetable can be exposed to microbial contamination through contact with soil, dust, and water. Specific microorganisms affect product safety and quality by increasing product loss and might there for pose a health risk for a consumer. Most spoilage losses are not due to microorganisms that cause plant diseases but rather to bacteria and molds that take advantage of mechanical and chilling damage to plant surfaces (Tournas, 2006).

So, the problem can be enhanced from poor management of fruits and vegetable in the market. In addition, market conditions that favor contamination can be raised from poor hygiene of the venders, using microbially unsafe container, poor handling practice and poor environmental conditions such as sanitarily unsafe marketing environment. Since fruits and vegetables contribute greatly to human health and are often eaten raw (minimally processed products) there is a need to ensure the safety of these produce by addressing common areas of concern by assessing the microbial spectrum of the selected fruits and vegetable. Therefore, this research was aimed at identifying microbiological safety and quality of most frequently used fruits Avocado, papaya and tomatoin Yebu town and JimmaCity and also to explore ways of managingthe potential contaminants using crude plant extracts.

1.3.Objectives

1.3.1. General Objectives

The general objective of the present study was to assess the microbiological safety and quality of some fruits and vegetable and also to determine antimicrobial activity of some plant extracts against isolated spoilage microbes.

1.3.2. Specific Objectives

The specific objectives of the current study were:

- To assess microbial load of avocado, papaya and tomatoes sold inYebu and different Jimmamarkets.
- To isolate and characterize post-harvest spoilage microorganisms fromavocado, papaya and tomatoes.
- To evaluatethe effectiveness of spice extracts of *Curcuma longa(Turmeric*), *Ocimumbasilicum*and *Ginger*(*Zingiberofficinale*)against isolated spoilage microbes.

1.4.Significance of the Study

Avocado, papaya and tomatoes are chosen for this study because they are referred to as ready-to-eat food since they are minimally processed/need not processing and many people take them as a raw directly. Microbial spoilage and contaminating pathogens on these products poses a serious problem in food safety. Individuals of the population especially those in developing countries who usually use spoilt and slightly decaying as a result of their cheaper prices, so knowing the commonest pathogenic bacteria and fungi will help to educate the community to minimize food borne illnesses related with these selected fruit and vegetable spoilage. Thus studying this problem will help us to know the more prevalent microorganism responsible for rotting of avocado, papaya and tomato and help to know the commonest pathogenic bacteria and fungi responsible for spoilage which may result food borne illness in the community. To minimize and/or to inhibit the effect of these microbes, determining anti-microbial activity of some spices like extracts of *Curcuma longa*, *Ocimumbasilicum L. and Zingiberofficinale*will provide paramount important. Additionally, the findings of this study will also be used as base as line data for further investigation.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Microbiological Safety of Fruits andVegetables

The increase in the consumption of fresh fruits and vegetables has been paralleled by an increase in the number of foodborne illnesses attributed to fresh produce (Andrenne*et al*., 2001) and this has made some consumers worry about the safety of eating fresh produce. Raw eaten vegetables and fruits are consumed without enough heating process, and therefore the possibility of foodpoisoning and food-borne infections always exists (Aycicek*et al*., 2006) Fruits and vegetables normally carry nonpathogenic, epiphytic micro flora. During production on the farm and all stages of product handling from harvest to point of sale, produce may be contaminated with pathogens (Beuchat and Ryu, 1997). Possible microbial hazards on the farm include the use of raw manure and contaminated soil amendments, dirty irrigation water, wild animals and birds, and dirty farming equipment. At harvest, employee health and hygiene is critical. In addition, farm tools, utensils, and packaging could possibly contaminate the product. Pack houses pose a risk when using water to wash product or convey product in water flumes. The water quality plays a key role in determining the final quality and safety of the product. Employee hygiene and food contact surfaces have the potential to affect product in the pack house. In addition, transportation and distribution practices determine product quality and safety for future use. When product is displayed at retail and handled in food service operations, there is the potential for contamination. The end user or consumer also plays a critical role in maintaining product safety as produce items are taken from the store, preserved, and prepared in the home.

2.2. Spoilage of Fruits and Vegetables

According to Seema (2015), Food spoilage is invasion by microorganisms such as moulds, yeast and bacteria. In case of mould spoilage a furry growth covers the food and it becomes soft and often smells bad. Bacterial contamination is more dangerous because very often food does not look bad even though severely infected, it may appear quite normal. Many organisms, in particular acid-loving or acid-tolerant bacteria and fungi (yeasts and moulds), can use fruit as substrate and cause spoilage, producing off flavors and odors, discoloration of the product, and if the contaminating micro-organisms are pathogens could also cause illness.

Fresh vegetables are fairly rich in carbohydrates (5% or more), low in proteins (about 1 to 2%), and, except for tomatoes, have high pH. Microorganisms grow more rapidly in damaged or cut vegetables. The presence of air, high humidity, and higher temperature during storage increases the chances of spoilage. The common spoilage defects are caused by molds belonging to genera *Penicillium, Phytophthora, Alternaria, Botrytis,* and *Aspergillus*. Among the bacterial genera, species from *Pseudomonas, Erwinia, Bacillus,* and *Clostridium* are important. Microbial vegetable spoilage is generally described by the common term rot, along with the changes in the appearance, such as black rot, gray rot, pink rot, soft rot, stem-end rot (Hozber*et al*, 2006).

Vegetables are another tempting source of nutrients for spoilage organisms because of their near neutral pH and high water activity. Most spoilage losses are not due to microorganisms that cause plant diseases but rather to bacteria and molds that take advantage of mechanical and chilling damage to plant surfaces. Some microbes are found in only a few types of vegetables while others are widespread. *Erwiniacarotovora*is the most common spoilage bacterium and has been detected in virtually every kind of vegetable. It can even grow at refrigeration temperatures (Tournas, 2005). Bacterial spoilage first causes softening of tissues as pectins are degraded and the whole vegetable may eventually degenerate into a slimy mass. Starches and sugars are metabolized next and unpleasant odors and flavors develop along with lactic acid and ethanol. Besides *E. carotovora*, several *Pseudomonas* spp. and lactic acid bacteria are important spoilage bacteria. Ripening weakens cell walls and decreases the amounts of antifungal chemicals in fruits, and physical damage during harvesting causes breaks in outer protective layers of fruits that spoilage organisms can exploit.

2.2.1. Spoilage of papaya, avocado and tomatoes

Spoilage in papaya may be referred to as rot or decay and can be extensive around the surfaces of the fruits. Spoilt papaya fruits are characterized by tissue softening, formation of rot and mycelia, moisture loss, unpleasant odor, sour taste, and shrinkage. The spoilage may be caused by microbes, attacks by insects and rodents, physical injury such as bruising as well as chemical breakdown of the fruit may also lead to deterioration in fruit quality. The occurrence of spoilage in fruits by microorganisms depends on the types of organisms present and whether the fruit under its existing condition of storage can support the growth of any or all of them. However,

only certain species out of all the organisms present in a fruit will be able to thrive well and spoil it. Susceptibility of fruits is largely due to differential chemical composition such as pH and moisture contents and is associated with greater predisposition to microbial spoilage (Barth *et al*., 2009).

The avocado fruit is vulnerable to bacterial, viral, and fungal diseases which lead to its spoilage. Disease and microorganisms can affect the fruit causing spotting, rotting, cankers, pitting and discoloration (Samson and Van Reenen-Hoekstra, 1988). Numerous species of microorganisms easily attack the fruit. The high spoilage rate of Avocado fruit coupled with its high nutritional contents pre-supposes that an array of microorganisms may be involved in its spoilage of Avocado fruits. The composition of the fruit influences the likely type of spoilage. Thus bacteria soft rot is widespread for the most ones among the fruits are limited to those that are not highly acidic. Because most fruits that are somewhat acidic are fairly dry at the surface, and are deficient in vitamins B, molds are the most common causes of spoilage. The compositions too, must determine the particular kind of molds most likely to grow, thus avocado support a large, variety of fungal spoilage organism and other kinds comparatively few (George *et al*, 1998).

Tomato is a highly perishable crop and it has been shown that as high as 50 % of the produce is lost between rural production and town consumption in the tropical areas (Oyeniran, 1988). Tomato fruit has a short shelf life as well as high vulnerability to postharvest spoilage micro-organisms. During extended storage, tomato fruits are prone to post-harvest spoilage by various pathogens. The growth and subsequent disease development of the different micro-organisms on fruits is of varying degree and rate resulting in deterioration.

Majumder*et al*. (1997) reported that a sizeable portion of the world population in developing and under-developed countries of Africa are poor and suffer from health problems associated with consuming mycotoxin from contaminated grains, cereals, fruits, and vegetables. Stinson *et al*. (1981) reported toxigenic fungi isolated from spoiling fruits. Mechanical injuries that occur during harvesting and handling are good sites for entry of pathogens that cause decay in fruits (Jerry *et al*., 2005).

2.3. Sources and Mechanism of Contamination of Fruits and Vegetables

Fruits and vegetables can become contaminated with microorganisms capable of causing human diseases while still on the plant in fields or orchards, or during harvesting, transport, processing, distribution and marketing, or in the home. Potential pre-harvest sources of contamination include soil, feces, irrigation water, water used to apply fungicides and insecticides, dust, insects, inadequately composted manure, wild and domestic animals, and human handling (Burnett and Beuchat, 2001). Post-harvest sources of contamination include feces, human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dust, rinse water, ice, transport vehicles, and processing equipment (Burnett and Beuchat, 2001). As raw agricultural products, fresh produce should be expected to harbor a wide variety of microorganisms including some pathogenic varieties. Francis *et al*. (1999) stated that out of all the microorganisms found on vegetables; about 80-90% of the populations are gram-negative rods, such as *Pseudomonas, Enterobacter, or Erwinia species* (Francis *et al*., 1999). Yeasts and molds may also be found. Commonly occurring yeasts include *Candida* and *Botrytis cinerea* can cause a gray mold rot on strawberries, onions, garlic, asparagus, potatoes, cabbage, etc. This type of spoilage occurs in warm temperatures and high humidity, and is characterized by production of a gray mycelium on the fruit or vegetable (Jay, 2000).

The microbiological quality of street-sold fruits in San José, Costa Rica, was analyzed over a two-year period from March 1990 to March 1993. Researchers evaluated the presence of *Salmonella spp*., *Shigellaspp*.,*Escherichia coli* and faecal coliforms in several foods. The results showed that *E. coli* was present in more than 10% of the mango and papaya samples, while *Salmonella spp*. and *Shigellaspp*. could not be isolated from these fruits (Monge*et al.,* 1995). Thirty samples of ripe papaya (*Carica papaya*) slices were collected in Calcutta, India, from itinerant roadside vendors over a three-month period. *Salmonella* and *Vibrio cholerae*results were positive in one sample each, and low levels of coagulase-positive *Staphylococcus aureus*were detected in 17% of the samples (Mukhopadhyay*et al.,* 2002). Aworth (1985) also stated that the primary causative agents of microbial postharvest spoilage of tomatoes are molds, bacteria and yeasts. Due to the physiological form of fruits they deteriorate easily in transit and storage, especially under conditions of high temperature and humidity and as a result, heavy losses occur (Idah*et al*., 2007).

2.4. Prevention of contamination of fruits and vegetables

Fresh fruits and vegetables are among the more challenging of food products to commercially produce and distribute. Fresh produce remains metabolically and developmentally active as it proceeds from the commercially appropriate time to harvest (horticultural maturity), to physiological maturity, to senescence and complete deterioration. Prior to ripening, fruits and vegetables are equipped with defensive barriers to infection including active wound healing and the production of phytoalexins which are phenolic substances that are toxic to fungi (Kader, 1992). However, most spoilage microbes infect and initiate decay at punctures and splits in the epidermal layer or, in far fewer cases, through natural openings such as stomata and lenticels

Upon harvest, fresh fruits and vegetables benefit from immediate surface sanitation and rapid cooling to slow product metabolism and growth of spoilage microbes. Reducing the rate of metabolism likewise reduces product respiration which, in turn, reduces the rate of deterioration, or perishability, of the crop (Kader, 1992).

In cooling and storage facilities, contamination can be reduced with the use of ozone; treatment of cold rooms has been reported to be effective in significantly reducing *Listeria monocytogenes*(Suslow, 2004). According to Park *et al*. (2013) microbial contamination of produce is influenced by farm management and environmental factors.

Uses of Plant-Origin Antimicrobials

Food spoilage can occur from raw food materials to the processing and distribution. Spoilage sources might be chemical, physical and microbiological. Preservation techniques for microbiological spoilage have been dramatically improved in recent years to minimize any growth of micro-organisms including pathogenic micro-organisms (Gould, 1996). Research concerning plant-origin food-preservative EOs has increased since the 1990s, with more utilization of spices and their EOs as natural bio-preservatives, to increase shelf life and overall quality of food products and reduce or eliminate pathogenic micro-organisms (Simitzis*et al*., 2008). Application of *Thymus eigii* showed stronger antimicrobial activity compared to vancomycin (30 mcg) and erythromycin (15 mcg) (Toroglu, 2007). Ginger (*Zingiberofficinale*), galangal (*Alpiniagalanga*), turmeric (*Curcuma longa*), and finger root (*Boesenbergiapandurata*) extracts against Gram-positive and Gram-negative pathogenic bacteria at 0.2–0.4% (v/v) for finger root and 8–10% (v/v) for all of the spices (Pattaratanawadee*et al.,* 2006).

The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a burning predicament (Abimbola*et al*., 1993). As a result there is an urgent need to find the alternative of chemotherapeutic drugs in diseases treatment particularly those of plants origin which are easily available and have considerably less side effects (Khulbe and Sati, 2009). Ginger (*Zingiberofficinale*) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (Ali, 2008). Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections (Tan and Vanitha, 2004). The Zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes.

Comparison of published data regarding different methods is complicated: according to some investigators there is increased variation based on extraction method, culture medium, size of inoculum, choice of emulsifier and basic test method (Sofia *et al*., 2007)

Ginger has also been shown to be effective against the growth of both gram-positive and gram-negative bacteria. The main active phytochemicals present in ginger are gingerols, shogoals and paradols. Antimicrobial potency of cumin and ginger has also been reported against different types of microorganisms (Das *et al.*, 2012)

2.5. Medicinal importance of *Curcuma longa*, *Ocimumbasilicum L and Zingiberofficinale*

2.5.1. *Curcuma longa*

*Curcuma longa L.*is a medicinal plant that botanically is related to *Zingiberaceae* family (Chattopadhyay*et al*., 2004). Turmeric powder, derived from the rhizome of *Curcuma longa*, is commonly used as a spice, food preservative, and food-coloring agent (Aggarwal*et al*., 2007). Inhibitory effect of turmeric oil and ethanolic extracts against bacteria and fungi has been reported (Wuthi-udomlert, 2000). Turmeric oil was tested for antibacterial activity against *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Turmeric oil was also tested for its antifungal activity against *Aspergillusflavus,AspergillusparasiticusFusariummoniliforme*and*Penicilliumdigitatum* (Jayaprakasha, and Negi, 2001).

Mandeel*et al*. (2003) evaluated the antibacterial activity of 17 selected spices including turmeric. Ethanol extracts were evaluated against six Gram positive and Gram negative bacteria using a well-diffusion assay. Goel (2005) also evaluated the antimicrobial properties of turmeric. Turmeric showed considerable amount of antimicrobial activity measured by a standard MIC assay. Pathogens were inhibited at 20-100 µg/mL. Kim *et al*. (2005) investigated the antimicrobial activity of ethyl acetate, methanol and water extracts of *Curcuma longa L*. (*C. longa)* against methicillin-resistant *Staphylococcus aureus*. Ethyl acetate extract of *C. longa* demonstrated highest antibacterial activity than that of methanol or water extracts.

2.5.2. *Ocimumbasilicum L.*

Ocimumbasilicum L. commonly called as Sweet Basil belongs to family *Lamiaceace* is native plant of Indo-Malayan region. It is called the "king of herbs" which contains plenty of phytochemicals with significant nutritional as well as antioxidant capabilities and health benefits (Yayasinghe*et al*., 2003). The major components in basil oil, linalool and methylchavicol, have shown anti-inflammatory activities (Moretti*et al*., 1997), supporting the rationale for the basil traditional use in inflammatory diseases of the upper respiratory tract. Linalool has also shown antibacterial and antiviral activities (Bassole*et al*., 2010).

Essential oil of sweet basil, obtained from its leaves, has demonstrated the ability to inhibit several species of pathogenic bacteria that have become resistant to commonly used antibiotic drugs (Opalachenoiva*et al*., 2003) Due to its antimicrobial, insecticidal activity and very pleasant aroma, basil essential oil is widely used in the food, pharmaceutical, cosmetic, and aromatherapy industries.

The antimicrobial activities of *O. basilicum* (acetone, methanol, and chloroform) extracts against the microorganisms were assessed by the presence or absence of inhibition zones and zone diameter. The methanol extract suspended with 10 ml deionized water exhibited inhibition effects against 2 bacterial strains; namely *P. aeruginosa and Shigella spp.* The methanol extract suspended with 5 ml deionized water show inhibitory effects against 6 bacterial strains; namely *P. aeruginosa, Shigella spp., L. monocytogenes, S. aureus*and two different strains of *E. coli.* Besides, the results showed that three different extracts exhibited inhibitory effects against bacterial strains but had no effect against yeast strains. (Adiguzel*et al*., 2005) showed that none of Ethanol, methanol, and hexane extracts from *Ocimumbasilicum L.* tested have antifungal

activities, but these extracts have anti-candidal and antibacterial effects. Among these three extracts, while hexane was observed to be more effective in a wider spectrum when compared to methanol, no effects were observed in the extracts prepared with ethanol. Whereas the study by Nascimento*et al*., (2000) showed methanol extracts have the most effective result among the extracts tested. In another study, the extract of *O. basilicum* had antimicrobial properties against *E. coli, Salmonella paratyphi*and*Shigelladysenterea* (Omoregbe*et al*., 1996)

2.5.3. *Zingiberofficinale*

*Zingiberofficinale*has been traditionally exploited for having broad range of antimicrobial activity against both gram positive and gram negative bacteria and fungi. In vitro studies have shown that active constituents of ginger inhibit multiplication of colon bacteria, these bacteria ferment undigested carbohydrates causing flatulence, this can be counteracted with ginger. It inhibits the growth of *Escherichia coli, Proteus spp., Staphylococci, Streptococci*and*Salmonella*.

Shivani and Ravishankar (2005) studied the antimicrobial action of garlic, ginger, carrot, and turmeric pastes against *Escherichia coliO157:H7* in laboratory buffer and a model food system. Commercial ginger paste and fresh garlic paste showed the strongest antimicrobial activity with complete inhibition of *E. coli O157:H7* in the paste at 3 days fort 4 and 8°C. However, fresh ginger paste showed antimicrobial activity only at 8°C. Only commercial ginger paste had antimicrobial activity in BPW at 4°C for 2 weeks. Oonmetta-aree*et al.* (2006) evaluated ethanol extracts of *Zingiberaceae* family (galangal, ginger, turmeric and krachai) for antimicrobial action on *Staphylococcus aureus*and*Escherichia coli* NIHJ JC-2 by using agar disc diffusion method.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Yebutown and JimmaCity. JimmaCity has a total area of 220 km^2. Jimma is located within $07^0 40^0$ latitude and 36 0 50 0 longitude in an altitude range of 1700-1750 masl and 353 km south west of the capital city, Addis Ababa (Nata, 1994). Based on the 2005 national censes, the total population was 151,679 (CSA, 2005). On the other hand, Yebu town lies between 1,470 and 2,610 masl and is classified in to dega (12%), woinadega (63%) and kolla (25%) agro-climatic zones. Average rainfall is 1,467 mm. The mean minimum and maximum temperatures are 13.0 ^{0}C and 24.8 ^{0}C, respectively (ARDO, 2012).

Jimma city is well known for its vibrant economic activities, and is the capital center of Jimma zone. Whereas, the livelihood of Yebuis based on mixed farming and the main economic activities are crop production and livestock production. Maize, *teff*, sorghum, barley, wheat, coffee and horse bean are the most widely cultivated crops. Fruits and vegetables production of the area contributes significantly to the economic and social development including job opportunities for the people of the area and neighbor region.

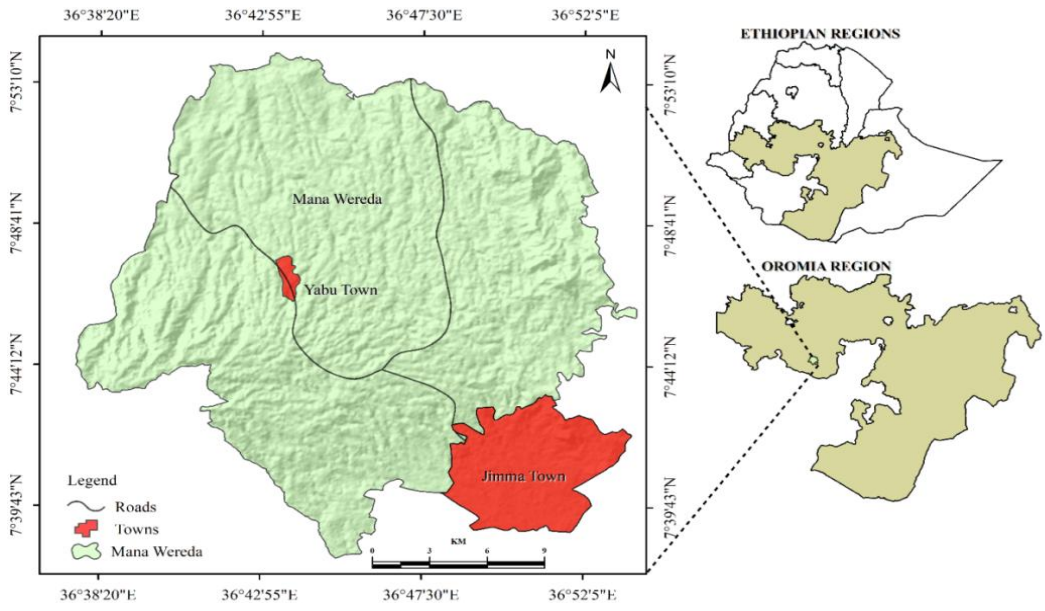

Fig1. Map of the study area
Source: Own computation, 2016/17

Study Design

Cross sectional study was carried out to survey on microbiological quality and safety of avocado and papaya, and tomato sold in Jimma and Yebu town.

3.2. Sampling Technique and Sample Size

Sample size ofeachfruits and vegetable was determined by the following two formula (Cochran, 1977) and (Cochran, 1963).The first equation was used to determine sample size (n) and the second equation was correctional formula used to determine the final sample size of the study.

$$n = \frac{N}{1 + N(e)^2}$$

$$n' = \frac{n}{(1+(n-1)/N}$$

N: the population size, n: sample size, n': corrected sample size and e: the level of precision (95%; e = 0.05).

A total of75 samples comprising of 25 papayas and 25 avocado from"Bishishe" ," Ajip" and "kochi" Jimmalocalmarket and 25 tomato samples from Yebu town market was used for this study. Both purposive and simple random sampling techniques were employed. Purposive sampling was used to select the market center where as simple random sampling was used to select the sample traders of avocado, papaya and tomato. The market center was chosen based on volumes of fruits and vegetables marketed whereas, the type fruits and vegetables considered, have potential source of income and employment for small scale farmers.

3.2.1. Sample Collection

For this study a sample of avocado and papaya were collected from Bishishe, Ajip and Kochi local markets of Jimmacity where as the tomato samples were collectedfrom Yebulocal markets of Yebu town using sterile plastic bagsand then transported to JimmaUniversity,Department of

Biology,Postgraduateand Research Laboratory for further analysis. The sample names were coded for simplicity according to the type of sample and type of market.

Table 1: Distribution of sample traders across market and selected sample traders

Fruits & vegetable	Number of traders by market center				Selected sample traders				Total samples
	Bishishe	Kochi	Ajip	Yebu	Bishishe	Kochi	Ajip	Yebu	
Papaya	33	15	12	_	14	6	5	_	25
Avocado	35	17	13	_	13	7	5	_	25
Tomato	_	_	_	25	_	_	_	25	25

Source: Own computation (2016/17)

"_"; no sample taken

3.2.2. Sample Processing and Plating

For isolation of bacteria and fungi, all fresh samples were chopped upinto smaller pieces using a sterile stainless steel knife prior to weighing. A25g from each sample of avocado, papaya and tomatoes was placed into 225ml of sterile 0.1% (w/v) bacteriological (Buffered) peptone water for 3min separately, to homogenize the sample (Mathur *et al*., 2014). Serial dilution and inoculation was separately used on nutrient agar and potato dextrose agar for bacterial and fungal enumeration, respectively.

3.2.3. Enumeration of Microorganisms

Some microbes were enumerated by using spread plate technique as described by Kim *et al*. (2002). Precisely, lml of the aliquot (supernatant) was pipetted and mixed in another 9ml of sterile distilled water in a test-tube. The test-tube was shakeusing shaker and a tenfold serial dilution was made. Aseptically, from appropriate dilution factor, a 0.1ml of aliquots were transferred to pre-solidified plates of nutrient and potato dextrose agar in duplicate for enumeration of mesophilic aerobic bacteria and fungi, , respectively. The spread plates were incubated at 37^0C for 24-48hours for bacteria andat25^0C± 28^0C for 3-5 days for fungi. Discrete colonies that were developed after incubation were counted and enumerated as colony forming unit (cfu/g).

3.2.4. Enumeration of Specific Bacteria

According to Rahman et al (2011), (Tekorienė ,2008) and (FDA, 2001) From the dilution of 10^{-2} of each sample 0.1 ml of suspension was spread on to a medium VRBA agar, Mannitol Agar, andCentrimide, for enumeration of coliforms, *Staphylococcus* and *Pseudomonas spp*. respectively.All the plates were incubated at 37 ^{o}C for 48 hours.Confirmatory biochemical tests were carried out for further identification.

3.3. Microbes Analysis

The isolates were subjected to morphological, microscopic and biochemical tests and identified by comparing their characteristics with those of known taxa as described by Holt *et al* (1994).Gram staining,catalase test, KOH test, motility test, citrate utilization test, urease test and fermentation of carbohydrate test were used to characterize the spoilage bacteria.

3.3.1. Microscopic Identification

3.3.1.1.Gram Staining

The pure bacterial isolates were stained according to Gram's techniques as described by Baker (1967). Briefly, a thin smear was prepared on clean glass slide, air dried, and heat fixed by placing the slide gently over the flame of the spirit lamp. The smear was stained with crystal violet for 1 minute, and then gently rinsed with tap water. The smear was then covered with Lugol's iodine for 60 seconds and washed off under gentle running tap water. The slide was then decolorized using 95% ethanol after which it was washed under tap water and then counterstained with safranin for 30 seconds. Again it was rinsed with tap water and the slide blotted dry with a piece of filter paper. The stained cells were then examined with the oil immersion objective lens of the light microscope.The discoverer of the stain was Hans Christian Joachim Gram, who was born in Denmark in 1853(Sandle, 2004). The gram positive organism was characterized by a purple color while a gram negative organism picked a pink color as well as the shape of the cells was examined.The Gram stain procedure enables bacteria to retain color of the stains, based on the differences in the chemical and physical properties of the cell wall.

3.3.2. Isolation of fungi (yeasts/moulds)

The pour-plate method also was used for the isolation of fungi following the method of Barnett and Hunter (1987). Potato dextrose agar were used. The diluents from the 4th test-tube were aseptically transferred to sterile Petri dishes and about 15 to 20ml of sterile PDA was poured into the plate, allowed to set and incubated at room temperature (28±20C). Colonies that developed after incubation were counted, enumerated in colony forming unit per gram (Cfu/g) samples.

3.3.3. Biochemical character

Catalase Test

Catalase test was undertaken by mixing a loopful of fresh bacterial culture with 2 drops of solution ($3\%H_2O_2$) on the microscope slide according to method described by He *et al*. (1993).Presence of bubble indicated catalase positive responses.

KOH Test

The test were done by adding a heavy inoculum of a pure culture of bacteria grown on a solid medium to a drop of 3% Potassium hydroxide (KOH) solution (3 grams KOH per 100 mL distilled water) on a clean glass slide. Stir for about one minute, occasionally lifting the loop to look for thickening and "stringing" of the slurry. Gram-Positive Bacteria were not appeared to change the viscosity of the KOH solution. Gram-Negative Bacteria were cause the KOH solution to become stringy or mucoid in appearance and consistency.

Motility Test

Motility by bacterium is mostly demonstrated in a semi solid agar medium. In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye. The medium mainly used for this purpose is SIM medium (SulphideIndole Motility medium) which is a combination differential medium that tests three different parameters, Sulfur Reduction, Indole Production and Motility. This media has a very soft consistency that allows motile bacteria to migrate readily through them causing cloudiness. The inoculum is stabbed into the center of a semisolid agar deep. Bacterial motility is evident by a diffuse zone of growth extending out from the line of inoculation. Some organisms grow throughout the entire medium, whereas others show small areas or nodules that grow out from

the line of inoculation. The non-motile bacteria will only grow in the soft agar tube and only the area where they are inoculated.

Citrate Utilization Test

Citrate agar is used to test an organism's ability to utilize citrate as a source of energy. The medium contains citrate as the sole carbon source and inorganic ammonium salts ($NH_4H_2PO_4$) as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme, citrate-permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism's metabolic cycle for the production of energy. Growth is indicative of utilization of citrate, an intermediate metabolite in the Krebs cycle.When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the bromthymol blue indicator in the medium from green to blue above pH 7.6.Streak the slant back and forth with a light inoculum picked from the center of a well-isolated colony. Incubate aerobically at 35 to 37C for up to 4-7 days.Observe a color change from green to blue along the slant.

Urease Test

Urea is the product of decarboxylation of amino acids. Hydrolysisof urea produces ammonia and CO_2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the color change of phenol red from light orange at pH6.8to magenta (pink) at pH 8.1. Rapid urease-positive organisms turn the entire medium pink within 24 hours.Weakly positive organisms may take several days, and negative organisms produce no color change or yellow as a result of acid production.

The broth medium is inoculated with a loopful of a pure culture of the test organism; the surface of the agar slant is streaked with the test organism. Leave the cap on loosely and incubate the test tube at 35 °C in ambient air for 18 to 24 hours; unless specified for longer incubation. Organisms that hydrolyze urea rapidly (e.g. Proteus spp) may produce positive reactions within 1 or 2 hours; less active species (e.g. Klebsiellaspp) may require 3 or more days. In routine diagnostic laboratories the urease test result is read within 24 hours. If organism produces urease enzyme, the color of the slant changes from light orange to magenta. If organism do not produce urease the agar slant and butt remain light orange (medium retains original color).

Fermentation of Carbohydrate

Some bacteria have the ability to ferment carbohydrates, particularly sugars. Among them, each bacterium can ferment only some of the sugars, while it cannot ferment the others. Thus, the sugars, which a bacteria can ferment and the sugars, which it cannot is the characteristic of the bacteria.The carbohydrate fermentation test is performed to test, separately, the ability of bacteria to ferment the sugars like glucose, sucrose, lactose, maltose and xylose as well as their alcoholic derivatives like aesculin, salicin, adonitol, dulcitol and sorbitol. If the bacteria can ferment a sugar or sugar derivative, acid is produced, which reduces the pH changing the colour of bromocresol purple from purple to yellow.Moreover, if it is an 'aerogenic bacteria', both acid and gas ($C0_2$) are produced, while if it is anaerogenic bacteria', only acid is produced without any gas. Production of gas is indicated by its accumulation as a bubble in an inverted Durham tube (a miniature test tube).In the carbohydrate fermentation test, the test bacteria is grown in a broth medium containing one of the sugars or sugar derivatives and bromocresol purple. An inverted Durham tube is kept submerged in it.If the bacteria "has the ability to ferment the sugar or sugar derivative,the colour of the broth changes from purple to yellow. If gas accumulation is seen as a bubble in the Durham tube, it is an acrogenic bacteria, while if no gas accumulation is seen, it is an anaerogenic bacteria.

3.4. Extraction of Plant Materials

3.4.1. Preparation of Aqueous and Ethanolic Extracts

The spices including*Zingiberofficinale, Curcuma longa* and *OcimumbasilicumL.*around 0.5kg waspurchased from local market of Jimma. Rhizomes of *ginger,turmeric* and leaf of *Basil*were washed with distilled water thoroughly and then dried at room temperature and ground using mortar and pestle for extraction. Approximately 0.1 kgof each ground plant material was separately extracted by maceration with chloroform and methanol (1:1v/v) for 24 hours. This process was repeated by the same solvent system at least three times. Each step was followed by decanting and concentration. Concentration was done using Rotary evaporator operating at 50°Coverwater bath. The dried crude extract was stored in the refrigerator at 4°C until use.

3.5.2. Antibacterial Activity Assay

An adopted agar well diffusion method (Perez *et al*., 1990) was used. A1 ml of diluted inoculum of test organism was spread on Muller-Hinton agar plates .The dried extracts were dissolved in dimethyl sulfoxide (DMSO) with a concentration of 0.1 g/mL. Paper discs (6 mm in diameter) were impregnated with 0.25mL of spice extracts and placed on Mueller Hinton agar plates, which were inoculated with test organisms according to the standard protocol described by the National Committee of Clinical Laboratory Standards (2003). The plates were incubated at 37°C and the diameters of the inhibition zones were measured by measuring scale in millimeter after 18 h. Filter paper discs containing DMSO without any test compounds served as a control and no inhibition was observed. Gentamicin was used as a positive control for antibacterial tests.Antimicrobial activity was evaluated by measuring the diameter of circular inhibition zone around the paper discs. Tests were performed two times.

3.4.2. Data quality management

The sterility of the media used was checked by incubating the media overnight before its use. And regular checking of incubation temperature, careful measurement of inhibition zone diameters were done as part of the data quality management.

3.5. Statistical data analysis

Colony counts were converted into log10 CFU/g. The mean values obtained from the microbiological evaluation of fruits and vegetables were analysed by independent samples *t*-test and to determine any statistically significant difference ($P<0.05$) among the all commodities means by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test using SPSS 17.0 software (SPSS Inc. Chicago, IL, USA) (Kemal*et al*., 2015).

CHAPTER FOUR

4. RESULTS

4.1. Microbial load of papaya, avocado, and tomato

The microbial load of the fruits and vegetables varied with type and market (Table 2). The result showed that all the samples of fruits and vegetable exhibited high load of bacteria and yeast. The mean TVC of bacteria in papaya, avocado and tomato were 6.3, 6.4 and 7.4 log cfu/g, respectively. Similarly, the mean yeast counts of papaya, avocado and tomato were 6.1, 6.2 and 6.5 log cfu/g, respectively.

Table 2: Total viable bacterial and yeast counts (log cfu/g) of papaya, avocado, and tomatoes

Fruits & vegetable	Average TVC of Bacteria	Source			Average TVC Yeast	Source		
		Bishishe	*Ajip*	*Kochi*		*Bishishe*	*Ajip*	*Kochi*
Papaya	6.3	7.5	7.2	7.23	6.1	6.1	7	7.12
Avocado	6.4	6.5	7.17	7.12	6.2	6.3	6.94	7.2
Tomato	7.4	*Yebu*				*Yebu*		
		7.4			6.5	6.5l		

As indicated in Table 3 below, counts of *coliforms were* appeared to be the highest (4.4 log cfu/g) followed by *staphylococci*with 4.3log cfu/g. The maximum *Coliform*countswere recorded in tomato (4.4 log cfu/g), while the minimum was in avocado (4.12 log cfu/g) samples.

Table 3: Mean counts ofbacteria fromavocado, papayaand tomatoes (log cfu/g).

Samples	Microbial Isolates		
	Staphylococcus	***Coliforms***	***Pseudomonas***
Papaya	4.1	4.3	4.1
Tomato	4.3	4.4	4.2
Avocado	4	4.12	4.1

4.2. Dominant microbial isolates from papaya, avocado and tomato

Staphylococcus species, *Pseudomonas*and*Coliforms* were among the dominant microbial species and group frequently identified from most of the fruits & vegetable evaluated in the current study.

Table 4: Dominantmicrobial isolates from papaya, avocado and tomato, 2016/17.

Fruits & Vegetable	Sample size	Isolated bacterial genera (group)	Positive sample	Sources			
				Ajip	*Kochi*	*Bishishe*	Yebu
Papaya	25 (14, 6,5 from *Bishishe, Kochi and Ajip*, respectively)	*Staphylococcus*	12	0	3	9	_
		Pseudomonas	9	0	2	7	_
		Coliform	11	2	2	7	_
Avocado	25 (13, 7,5 from *Bishishe, Kochi and Ajip*, respectively)	*Staphylococcus*	9	0	1	8	_
		Pseudomonas	8	1	1	6	_
		Coliform	11	1	3	7	_
Tomato	25 all from Yebu town market	*Staphylococcus*	5	_	_	_	5
		Pseudomonas	4	_	_	_	4
		Coliform	14	_	_	_	14

"-" indicates no sample was taken from specific sources

As summarized in table 4**,** out of samples of papaya fruit, 12 were found contaminated with *Staphylococcus spp.*, 9of which were purchased from *Bishishe*. Furthermore, 10 samples of avocado fruitwas also shows presence of*Coliforms*, 7 of which werepurchased from *Bishishe market*. About 56%of tomatosamples all of which were purchased from Yebu town market were found harboring*Coliforms*.

4.3. Antimicrobial Activity of Plant Extracts

In the present study, the inhibitory effect of different extracts (*Zingiberofficinale, Curcuma longa and Ocimumbasilicum*) were evaluated separately (individually) and their combination for exploration of their antimicrobial activity against isolated spoilage microorganisms.

Table5: Mean antimicrobial activities of selected spice extracts against isolated spoilage microorganisms in mm.

Dry oils	Microbial Isolates	
	Staphylococcus spp.	*Pseudomonas spp.*
Zingiberofficinale	2	2
Curcuma longa	1.8	2.3
Ocimumbasilicum L.	0.6	0.75
Gentamicin	6.5	8.8
DMSO	1	0.6

As showed in table 5, bacterial isolates did show different pattern of susceptibility to extract from *Zingiberofficinale, Curcuma longa and Ocimumbasilicum L*. The entire individual extracts showed narrow antimicrobial activity as these were less inhibitory against bacteria.

Table6: Mean antimicrobial activities of combined spice extracts against isolated spoilage microorganisms in mm.

Dry oils	Microbial Isolates	
	Staphylococcus	*Pseudomonas*
Z+C+O	2.5	1.75
O+Z	1.25	1.25
C+O	1.25	2
C+Z	2.5	2.5
Gentamicin	5.5	6.5
DMSO	0.75	1

Z+C+O= combined extracts of *Zingiberofficinale, Curcuma longa andOcimumbasilicum***; O+Z**= combined extracts of *Ocimumbasilicum and Zingiberofficinale*, **C+O**= combined extracts of *Curcuma longa and Ocimumbasilicum*, **C+Z**= combined extracts of *Curcuma longa andZingiberofficinale*.

Similarly, the inhibitory effect of combinations of *Zingiberofficinale, Curcuma longa and Ocimumbasilicum L.* against *E.coli, Staphylococcus, Salmonella* and *Pseudomonas* have shown less antimicrobial activity against these bacteria.

CHAPTER FIVE

5. DISCUSSION

The result of this study indicates that all the samples contained high bacterial and fungus were exhibited on all examined fruits and vegetable. The bacterial load ranges between 6.3 log cfu/g and 7.4 log cfu/g. similarly, the total mean count of yeast was between 6.1 log cfu/g and 6.5 log cfu/g. The results of this study is contrary to the works of Uze*et al.*(2009) and Bukar*et al.*(2010) which both of them reported higher bacterial count of 10^8 –10^9cfu/ml found in commonly consumed freshly fruits and vegetables. The probable reason may be geographical variation, seasonal variation and hygiene. In contrary, the result of this study is close to the works of Ogbogu*et al*. (2014) who found the bacterial counts ranging from 2.5 x 10^5 to 3.6 x 10^5cfu/ml.

In this study mold and yeast were detected from all fruits and vegetable under investigation. The numbers of sample contaminated with mold were very low. The total mean count of yeast was between 6.1 log cfu/g and 6.5 log cfu/g which is equivalent to 1.2x10^6 cfu/g and 3.2x10^6cfu/g, respectively. Ethiopia has no standard guideline for comparing the microbiological safety of fresh fruits. However different countries give more concern for the microbiological safety of fruits. For instance South Africa has proposed a standard guideline for raw fruits and vegetables. According to South Africa department of health (1997) guideline, yeast and mold count greater than 1x10^5 cfu / g in any raw fruits and vegetables has no acceptance. The average yeast count from the study area was greater than 10^5cfu / g.

All the bacteria isolated in this study have previously been isolated from fruits and vegetables in other studies (Akhtar*et al*. (2016), Gulti (2013), Tango*et al.* (2018), S.E. Obetta*et al*. (2011), Wogu*et al.* (2014)). Isolated bacteria were *Coliform* (26%),*Staphylococcus* (18%),and *Pseudomonas* (15%). As it clearly shows *Coliform* were the most predominant contaminant followed byand*Staphylococcus*. The highest *coliform* count may be attributed from different variables such as the sanitary condition of the market area and handling practice of different vendors.

The presence of *Staphylococcus aureus*, which are known to be associated with fecal matter, showed that the fruit samples were contaminated through poor human handling processes. This

observation is similar with the earlier report by different scholars like Gulti (2013) who reported that *Staphylococcus, E. coli and Salmonella* were the most common bacterial contaminants of fruits in Gonder town. Similarly Jushi and Patel (2008) analyzed microbiology of fresh fruit and vegetable and found the presence of *Escherichia coli, Staphylococcus aureus, and Pseudomonas spp*. A similar study by Kumar *et al*. (2011) isolated and characterized seven bacterial isolates viz. *Bacillus, Klebsiella, Pseudomonas, E.coli, Lactobacillus, Staphylococcus and Micrococcus* on the basis of morphology and biochemical reactions. The bacteriological study of fresh fruit in Nigeria had shown that among 10 fruit samples 7 fruits were contaminated by *Staphylococcus aureus* (Angela *et al.*, 2010). The presence of *Staphylococcus* in fruits could cause gastroenteritis in the individual who consume fruits without proper washing. Its isolation is also an indicative of poor handling and hygienic problem of handlers at different stage of fruit handling.

The processes such as packing and transporting, fruits and vegetable may encounter physical injury that increases the possibility of contamination. In addition, the problem can be enhanced from poor management at market center. According to Gultie*et al. (*2013) Market conditions that favor contamination can be worsened by poor hygiene of the vendors, using microbial unsafe container poor handling practice and poor environmental conditions such as sanitarily unsafe marketing environment. The consequence of the problems could be increased loss of fruit due to microbial spoilage (Okojie and Isah, 2014).

The organism isolated in the presence study agrees with previous work of (Chukwu*et al.,* 2003) the presence of these organisms in spoilt tomato is an indication that tomato fruits were exposed to fecal contamination water or organic manure (De Rover, 1998).

Microbiological qualities and prevalence of pathogens in fresh produce can vary significantly due to different major factors that can affect microbial safety. Such factors include the location of samples, growth conditions, harvesting season and method, and the microbiological analysis method used (Cardamone*et al.,* 2015). In present study the prevalence of bacterial isolates across different markets indicates that, 47% of bacteria were isolated from sample obtained from *Bishishe* market while only 6% of bacteria were isolated from *Ajip* market. This observation is similar with the earlier report by Gulti (2013) that shows significance difference between the mean AMC of fruits from four marketing areas found in Gonder town. Tango*et al.* (2018) also

reported that microbial contaminations of fruits and vegetables in different market types showed great variability among the samples. Assessment on the management of fruits and vegetable in these marketing areas indicated that there were different conditions such as poor handling of fruits and vegetable, sanitary problem of some marketing areas and also the area were crowded by vehicles that emit dust particles; there were donkey and horse feces which are the source of different contaminant. More over the fruit handlers put fruits on ground without using covering material and used the measuring balance for different commodities such as onion, tomato, different vegetables and cereal crops. These factors could increase the microbial load of fruit.With regard to market area and fungal contamination the highest yeast count (1.4×10^7cfu/g ≈ 7.12 log cfu/g) for papaya was found in sample collected from *kochi* market. Similarly, the highest yeast count (1.6×10^7cfu/g ≈ 7.2 log cfu/g) for avocado also found in sample collected from Kochi market. The difference might be from the handling and sanitary differences in the market around Kochi.

In line with searching for antimicrobial agents from three selected different spices, namely *Zingiberofficinale, Curcuma longa and Ocimumbasilicum L* were extracted with chloroform/methanol (1:1) using maceration technique, and the resulting extracts were subjected to antimicrobial screening. The antimicrobial activity of individual extracts and their combinations against the selected microorganisms was assessed. The entire individual extracts and their different combinations showed less antimicrobial activity against isolated bacteria.

Antimicrobial activity of *Zingiberofficinale, Curcuma longa and Ocimumbasilicum L* extract were evaluated against isolated bacterial based on inhibition zone. The study revealed that isolates did show different but less susceptibility against the extracts. The study also demonstrated the effect of combination of the above mentioned extracts on isolated bacteria species and showed relatively better activity (table 6).

The extracts of *Ocimumbasilicum L* shows very low activity (<1mm) on *Staphylococcus* and *Pseudomonas.*Kaya *et al* (2008) reported contradictory findings with the present study and they found that the methanol extracts of *O. basilicum* showed broader microbial spectrum against strains of *Pseudomonas aeruginosa, Shigella spp., Listeria monocytogenes, and Staphylococcusaureus*and *Escherichia coli*.

The present study revealed that *Zingiberofficinale* extract shows better activity but still very low compared to other finding. Islam *et al.* (2014) in their study showed the potent antimicrobial activity of the ginger extract against the all tested bacterial pathogens including *Escherichia coli, Pseudomonas aruginosa, Staphylococcus aureus, Vibrio cholerae, Klebsiella spp.* and *Salmonella spp*. Sebiomo*et al*. (2011) also reported that ginger extract showed the highest antibacterial activity against *Staphylococcus aureus and Streptococcus pyogenes*.

The current study also shows the combined extract's inhibition zones were ranging from 1.25 to 2.5 mm in diameter against bacteria. The combined extract from the three spices of *Zingiberofficinale. Curcuma longa and Ocimumbasilicum L. likewise* the combined extract from the two spices of*Curcuma longa and**Zingiberofficinale*was found to be highest in inhibiting the microbial growth followed by the combined extract from *Curcuma longa and OcimumbasilicumL*with inhibition zone of 2.5 and 2 respectively. Among the three isolated bacteria, the highest inhibition zone of the combined extract was found on *staphylococcus.*

Skrinjar and Nemet (2009) tested the antimicrobial activity of essential oils of garlic, cumin and ginger against the most common bacteria and fungi that contaminate food including *Listeria spp., Staphylococcus, Salmonella spp., Escherichia spp., Pseudomonas spp., Aspergillus spp., Cladosporium spp*. and concluded that ginger had very weak inhibitory effect. A study by Ponmurugan and Shyamkumar (2012), have shown that extracts of ginger rhizomes demonstrated antibacterial activity against *E.coli* with 15.5 mm zone of growth inhibition.

Differential antimicrobial activity of extracts against different microbes might be due to the presence of different phyto-compounds (Das *et al*., 2012), which may include terpenoides, alkaloids and phenolic compounds (Rios and Recio, 2005).

CHAPTER SIX

6. CONCLUSION

On the basis of the findings of this study, it was concluded that fruits and vegetable sold at the study area possess high bacterial and yeast load. The study revealed the presence of significance difference in TVC of bacteria and yeast count between different fruits and vegetable. Tomato was found to be the most contaminated by bacteria,yeastand mold. In contrast, papaya was the least to be contaminated. Similarly significance difference was observed in TVC of bacteria and yeast count between the different marketing areas and accordingly the highest bacterial count was recorded sample taken from Bishishe market. The highest yeast count was recorded on sample taken from Kochi market. The study also revealed that *Staphylococcus*, *Coliform and Pseudomonas* were isolated from the samples. C*oliform* were the dominant contaminant following *Staphylococcus*. The study had also demonstrated that the identified isolates were more sensitive when *Zingiberofficinale, Curcuma longa and Ocimumbasilicum L* extract used together than separately (individually). However, comparing with other finding still the combination showed very less sensitivity.

CHAPTER SEVEN

7. RECOMMENDATIONS

- Fruits and vegetable are commonly eaten in a raw state, and the possible presence of spoiling microbes on their surface or inside the fruits can be problematic during the manipulation process. Therefore, consumer should have to make due consideration before utilization by washing gently
- Market wastes should be properly disposed of at designated sites to reduce microbial contaminations.
- The use of poor microbiological quality of irrigation water should be avoided.
- The combined extract of *Zingiberofficinale*, *Curcuma longa* and*Ocimumbasilicum L* were more effective to the spoilage microbes. So it is recommended in the future studies that should focus more on different combination of extracts to observe their inhibitory effect.

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List of appendix

Annex 1: Characteristics of isolated bacterial genus

Isolated bacteria genus	Colony morphology on selected	KOH	Catalase	Shape/ Gram rxn	Citrate	Urease	Fermentation	Gas production	Motility

	media								
Staphylococcus	Rarely light gold	-	+	Blue Cocci	+	+	+	+	Non Motile
Coliform	Pink	+	+	Red rod	_	+	+	+	Non Motile/ Motile
Pseudomonas	Green	+	+	Red rod	+	_	+	+	Motile

Annex 2: Microbial genera isolated and their frequency distribution avocado, papaya and tomatoes samples.

Bacterial Isolates	**Isolated Number**	**Frequency of Occurrence (%)**
Coliform	36	26
Staphylococcus	26	18
Pseudomonas	21	15
Total	141	100

Annex 3: Distribution of bacterial isolates from Avocado, papaya and tomatoes purchased from *Bishishe*, *Kochi*, *Ajip* and *Yebu* markets.

Bacterial species	**Market center**				**P-value**
	Bishishe	Kochi	Ajip	Yebu	
Coliform	14	5	3	14	0.173
Staphylococcus	17	4	0	5	0.000
Pseudomonas	13	3	1	4	0.001
Total	44	12	4	23	

Annex 4: Frequency of bacterial isolates between papaya, avocado and tomato sold in Jimma and Yebu town.

Type of bacteria	Type of fruits and vegetable			P-value
	Papaya	Avocado	Tomato	
Staphylococcus	7	14	5	0.056
Coliform	9	8	19	0.030
Pseudomonas	8	9	4	0.249