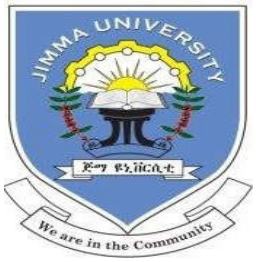

JIMMA UNIVERSITY
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MICROBIOLOGICAL QUALITY AND SAFETY OF SELECTED SPICES, AND THEIR ANTI-MICROBIAL ACTIVITY ON FOOD BORNE PATHOGENS IN HAWA GALAN DISTRICT, OROMIA REGION.

BY: SHUMA ABDISA YIGEZU

A THESIS PRESENTED TO THE SCHOOL OF GRADUATE STUDIES OF JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN BIOLOGY

ADVISOR: KETEMA BACHA (PHD, PROF.)
CO-ADVISOR: SHIFERAW DEMISSIE (MS.C)

OCTOBER, 2019
JIMMA, ETHIOPIA

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APPROVAL SHEET

This is to certify that the thesis prepared by Shuma Abdisa entitled: Microbiological quality and safety of selected spices and antimicrobial activity of their extracts against food borne pathogens in Hawa Gelan District, Kelem Welega Zone of Oromia Region, Western Ethiopia submitted to Department of biology in partial fulfillment of the requirements for the degree of Master of Science in (Biology) complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner (inter.) _____Signature_____ Date___/___/_____

Examiner (exter.)_____ Signature_____ Date____/___/_____

Main Advisor _____Signature _____ Date____ /____ /____

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Department Head _____ Signature_____ Date____/___/_____

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Abstract

Spices are essential components of cuisines since ancient times. These are used in minute amounts to impart flavor, and aroma in food preparation to improve their palatability. The present study was performed to detect the presence of contaminating microorganisms in three commonly available spices samples (*Red pepper, Curcuma longa* and *Ginger*) collected from different areas of Hawa Galan district, Ethiopia and to assess their antimicrobial activity of their extract against selected food borne pathogens. Cross sectional and experimental study design were used. Sixty (60) samples of three different spices were collected. This study took the time from August 2017 to September 2019. The mean microbial count of Staphylococcus was detected as 4.03±0.011logcfu/g. *Staphylococcus* was found in ginger samples only. the mean microbial count of Coliform showed as 4.07±0.15log cfu/g. the mean microbial count of *Enterobacteriaceae spp* was 6.29±0.014logcfu/g. The range of mean total aerobic mesophilic bacterial count was 6.03±0.014log cfu/g to 6.32±0.56log cfu/g. all samples showed a high fungal load that was ranged from 6.02±0.03logcfu to 6.36±0.01log cfu/g. Antibacterial activity of *Red pepper, Curcuma longa and Ginger* samples was demonstrated against three bacterial references (*Escherichia coli*, *Salmonella typhimurium*, *staphylococcus aureus)* and one fungi (yeast *Candida albican*). Different extraction solvents (methanol, Chloroform, and Petroleum ether) were used and extracts were examined against the strains. The % extract yield of the spices ranged from 1.6gm to 9.2gm per 100gm of spices dry weight. Agar disc diffusion assay for antimicrobial activity yielded the inhibitory zone of 7.2 to 23 mm diameter for Red pepper, 8 to 18.5 mm diameter for ginger and 8 to 19 mm diameter for Turmeric extract indicating that Red pepper was the most effective spice in inhibiting the microbial growth. The MIC of individual extracts was 25 mg/ml against most of the tested microorganisms.

Keywords: Antimicrobial activity, quality, safety, spices.

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List of Acronomy

ATCC American Type Culture Collection

DAEC Diffuse Adhering *Escherichia coli.*

EAggEC Entero Aggregative *Escherichia coli.*

EIEC Entero Invesive *Escherichia coli*.

EPEC- Entero Pathogenic *Escherichia coli*.

ESA- European Spices Association

ETEC- Entero Toxigenic *Escherichia coli*

HC- Haemorrhagic Colitis.

HGWANRO- Hawa Gelan Woreda Agricultural and Natural Resource Office

HUS- Hemolytic Uremic Syndrome.

ICMSF- International Commision on Microbilogical Specification for Food.

LMFs- Low Moisture Foods.

LPS- lipopolysaccharide

MIC- Minimum Inhibitory Concentration.

PCA- Plate Count Agar.

STEC- Shiga Toxin producing *Escherichia coli*.

TAMC- Total Aerobic Mesophilic Count.

TCC- Total Coliform Count.

TTP- Thrombo cytopaenicpurpura.

VRBA- Violet Red Bile Agar.

VRBGA- Violet Red Bile Glucose Agar.

VTEC- VeroToxin producing *Escherichia coli*.

1. Introduction

Spices are non timber forest products extracted or obtained from vigor such as seeds, fruits, flowers, rhizomes, bulbs, barks, leaves and stems that are used as foods additives in order to provide odor and flavor (Leek *et al*., 2004). It encompasses different chemical nutrients, minerals, including water, protein, fat, carbohydrates, ash, calcium, potassium, sodium, phosphorus, iron and various essential vitamins (Singh *et al*., 2002).

Spices are essential components of cuisines since ancient times and are used in minute amounts to impart flavour and aroma in food preparation to improve their palatability (Rahman and Gul, 2002; Nair and Chanda, 2006). They are also to stabilize several food items from deterioration (Mandeel, 2005). It has also been prescribed for aiding digestion, raising sexual potency, decreasing blood pressure, controlling metabolism and delaying the onset of degenerative disease (Jayaprakasha *et al.*, 2001). On the other hand spices are used as refreshing food, drink and daily meal components (Andersson *et al*., 2000).

The value of spices also reported by many scientists (Leite de Souza *et al*,, 2006). Spices have been used for rituals, cosmetics and perfumery, their coloring, flavoring, preservatives and antimicrobial activity against some microbes that affect the quality of food and their shelf life (Tajkarimi *et al*., 2010). In addition to making food taste good, culinary spices have been used as food preservatives and for their health-enhancing properties for centuries (Kaefer and Milner, 2011).

Spices are functional foods; these are foods that can be demonstrated to have a beneficial effect on certain target functions in the body beyond basic nutritional requirements (Lobo *et al.*, 2010). Spices occur in a variety of flavor, color, and aroma contributing a wide range of nutrients to foods (Mann, 2011). They enhance and complement flavor in foods with no detrimental effect on the organoleptic quality of the food (Kaefer and Milner, 2011). The combined values of spices also generally show synergetic ant-microbial effect especially on fungus (Das, 2002). This is due to the presence of different phyto-chemicals or compounds like alkaloids and terpenoids (Jackson, *et al*., 1995).

Even if spices are multi-purpose non timber forest product, they could be spoiled and contaminated by diverse microorganisms during and post harvest in poor hygiene condition (Gurbuz,*et al*., 2000). Accordingly, production, processing, packaging, transport and storage of spices should be geared towards minimizing microbial food safety hazards (CAC/RCP, 1995).

The level of contamination of spices depends mainly on microbes present naturally on plants, epiphytic micro biota and secondary contamination with water, soil or airborne microbes during harvest, drying, transport and storage (Kunicka-Styczynsk and Smigielski, 2011). Spices have been regarded as highly contaminated food additives due to their natural origins in soil-grown plants (Tajkarimi *et al*., 2010, IFT, 2002). Spices can also be contaminated with sewage, animal or human fecal matters and dust (Benerjee and Sarkar, 2003).

Recently, the prevalence of pathogenic microbes in spices, such as bacteria and toxigenic fungi has been reported in several research works (Kong *et al*, Little *et al.,* 2003). Non-hygienic and improper conditions in production, processing, distribution and storage of spices enhance the risk of microbial contamination (Elmali and Yaman, 2005; Stankovich *et al*., 2006). The risk of the growth of the pathogens is also elevated when spices are added in foodstuffs that are not subjected to thermal treatment (Little *et al*., 2003). Most spices are significantly contaminated with bacteria like bacilli of the family Enterobacteriaceae and fungi (Banerjee and Sarkar, 2004, Garcia *et al*., 2001, Witkowska *et al*., 2011).

Keeping quality and safety of spices is directly related to the condition of their product at harvest, post-harvest processing and properly storage. Most harvested spices require no further drying; where roots, barks and certain berries may require various drying time. When properly dried and stored spices are generally resistant to microbial spoilage. Food borne disease is an increasingly serious public health problem all over the world. The main cause is determined to be microorganisms. The control of pathogens may significantly reduce the food borne disease outbreaks (Ravikiran *et al.*, 2008).

Spices and aromatic vegetable materials have long been used in food not only for their flavor and fragrance qualities and appetizing effects but also for their preservative and medicinal properties. Since the ancient times, they have been used for preventing food spoilage and deterioration and also for extending the shelf life of foods (Shan *et al.*, 2007).

It has been extensively reported that the essential oils of spices have shown antimicrobial functions against food borne pathogens (Reichling *et al.*, 2009). Interest in the antimicrobial properties of active compounds is strengthened by the findings that they affect the behavior of pathogenic bacteria or fungi of agro-food or medical field. Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial function and could serve as a source of antimicrobial agents against food pathogens (Lai and Joy, 2004).

Plants that are traditionally used for medicinal purpose in different parts of the world have been screened for possible anti-microbial action by several works (Bonjar, 2004). Although many scientific reports are already made on the medicinal value of several medicinal plants, the current research was designed to fill the knowledge gap that the former research report didn't address in the study area.

1.2. Statement of the problem.

Spices and herbs have been used in food preparation for centuries to provide a characteristic aroma and flavour. These spices are transported as fresh or dried products, and the production (growth, harvesting, drying, storage, transportation, grinding and handling) occurs in many countries. However, dried herbs can be an important vehicle for microbiological hazards, especially because they are often added to foods with minimal processing prior to consumption (Sospedra *et al*., 2010). Several micro-organisms detected in herbs have the potential to cause human illness, including bacteria *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus and* aflatoxin producing fungi (e.g. *Aspergillus* spp.) (Cho *et al*. 2008; Cosano *et al.,* 2009).

Outbreaks of salmonellosis associated with the consumption of contaminated spices or herbs have been reported (Vibha *et al*., 2006). However, the most frequent food borne pathogens previously reported, come from spoilage and contamination of spices by pathogenic microbes during and post harvesting in poor hygiene condition (Gurbuz *et al.*, 2000). Hence they are considered as significant carriers of microbial contamination primary molds and some bacteria (Dimic *et al*., 2000; Romagnoli *et al.*, 2007).

Mostly spices are also known to have some ethno-medicinal or anti- microbial properties (Singh *et al.*, 2002). Active compounds of spices have been included in class of naturally occurring food preservatives (Brull and Coote, 1999). Inhibitory effects of spices on a variety of microorganisms have been reported, although considerable variation for resistance of different microorganisms to a given spice and of the same microorganisms to different spices has also been observed (Akgul and Kivanç, 1988). In recent time food borne illness were observed on some peoples; especially on childrens around Hawa Galan district. To these effects the present study was designed to assess the microbiological quality and safety of selected spices and ant-microbial activity of their extracts against some selected food borne pathogens in Hawa Gelan Wereda.

1.2. Objectives of the study

General objective:

- The general objective of the study was:

To evaluate microbiological quality and safety of selected spices and to assess the anti-microbial activity of their extracts against some selected food borne pathogens.

Specific objectives:

- The specific objectives of the study were to:-

Assess the microbial load of three selected spices.

Identify the isolated fungi to genus level

Evaluate Antimicrobial activity of spice extracts against some food borne pathogens

Determine the minimal inhibitory concentration of the screened extract on selected food borne pathogens.

2. Literature review

2.1. Historical View of Traditional Spices.

Generally, plants have been indispensable and the most important source of both preventative and curative traditional preparations for human beings and live stock. Spices are derived from different parts of the plants, such as cardamom from seed, bay leaf from leaf, clove from flower bud, pepper from fruit, cinnamon from bark or ginger from rhizome. Furthermore, there is no a common method to classify spices. They can be classified by their flavor and color, i.e., hot (pepper), pungent (garlic), aromatic (cinnamon, clove), coloring (turmeric) and herbaceous (rosemary, sage), or according to their taste, such as sweet, spicy, sour, bitter and astringent (Ceylan and Fung, 2004).

Despite Western medicine becoming more widespread in Ethiopia, Ethiopians tend to rely more on traditional medicine. Conventional medical services remain concentrated in urban areas and have failed to keep pace with the growing population, keeping health care access out of reach for most Ethiopians living in Ethiopia. Because traditional medicine is culturally entrenched, accessible, and affordable, up to 80% of the Ethiopian population relies on traditional remedies as a primary source of health care (Kassaye et al., 2006).

Moreover, Western medicine has become more focused on preventative measures and people seeking curative practices still rely on indigenous medicine as the primary source for health care (Pankhurst, 1990). The influence of traditional medicine is also seen in Ethiopian migrant populations. In countries with substantial Ethiopian immigrant populations, traditional herbs, medical devices, and practitioners are readily available (Papadopoulos, 2002).

Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including food borne pathogens (Hara-Kudo *et al.*, 2004). It has been reported that spices owe their antimicrobial properties mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins (Ebana, 1991). As reviewed by López-Malo *et al*., (2006) some of antimicrobial components that have been identified in spices and herbs are: eugenol from cloves, thymol from thyme and oregano, carvacrol from oregano, vanillin from vanilla, allicin from garlic, cinnamic aldehyde from cinnamon, allyl isothiocyanate from mustard, etc.

2.2. The use of spices

The use of plant extract (phytochemicals) believed to constitute the major parts of therapy apart from their use in the Ethiopian traditional systems of medical care at the local level (Desta, 1993). Ethiopia have long tradition of using spices and condiments for curinary as well as medicinal purpose. Here spices are important ingredients of food and beverages of many ethnic cultures that are usually consumed in relatively large quantities. There fore can have significant health effects on a large sector of the Ethiopian population (Worku Abebe, 2006). In a study published in *bioscience,* it was indicated that Ethiopia was one of the ten countries in the world where spice are used the most, in particular in meat-based recipe(Sharman and Billing, 1999).

The typical Indian spices and herbs like cumin, garlic, ginger, mustard, fenugreek, ajowain, curry-leaf, nutmeg etc. are usually used in curries, pickles, sauces etc. Mostly spices are known to have some ethno-medicinal or anti- microbial properties (Singh *et al.*, 2002). Active compounds of spices have been included in class of naturally occurring food preservatives (Brull and Coote, 1999). Inhibitory effect of spices on a variety of microorganisms has been reported, although considerable variation for resistance of different microorganisms to a given spice and of the same microorganisms to different spices has also been observed (Akgul and Kivanç, 1988).

Allium sativum, known to most as garlic is known for having an array of antiviral, anti-fungal, and antibacterial properties. Along with its protective abilities, allicin is believed to be the natural chemical component responsible for the antimicrobial effects of garlic. Various studies have shown that garlic is known to be effective against gram-negative as well as gram-positive bacteria, such as *Escherichia coli, Salmonella, Staphylococcus,* and *Streptococcus* species (Sivam, 2001; Joe *et al.*, 2009; Iram *et al*., 2012; Ismail *et al.*, 2012).

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 (Joe *et al*., 2009; Iram *et al*., 2012; Ismail *et al*., 2012; Revati *et al*., 2013 and Shabaan *et al*., 2013) Ginger has been used as a spice and as natural additives for more than 2000 years (Bartley and Jacobs, 2000). Ginger has been identified as an herbal medicinal product with pharmacological effect. Ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase-1andcyclooxygenase- 2.

Allium cepa is used in treatment of common ailments like cold, allergies, toothaches, laryngitis and cough. It is used for healing both internally and externally. A tint of onion is used in homeopathy to treat a variety of conditions such as diarrhea, facial paralysis, hay fever, hernia, laryngitis, pneumonia and trauma (Patil and Patil, 2007). It has been recommended to treat bronchitis, whooping, asthma and other respiratory problems. A blend of rue and onion rids the digestive system of parasites. It stimulates the appetite; reduce arteriosclerosis by lowering blood cholesterol levels and prevent the formation of blood clots. Fresh onion juice is used to prevent microbial infections, removes warts, reduce superfluous skin blemishes when applied externally (Kashyapa, 1997). Dropping warm onion juice in the ear can comfort earaches. The Roman Gladiator used onions for snakebites; prevent hair loss and firming muscles. (http://www.herballegacy.com/peret_History.html).

Out of 29 spices and herbs studied, clove (genus *Caryophyllus*), star anise (*Illicium anisatum*) and all spice (genus *Pimenta*) completely inhibited the growth of 3 different *Aspergillus* species (*Aspergillus ochraceus*, *Aspergillus versicolor* and *Aspergillus flavus*) and also inhibited the toxin production. Eugenol extracted from clove and anethol extracted from star anise were incorporated in PDA (Potato Dextrose Agar) medium to test its growth inhibition ability.

2.3. Indigenous Knowledge and People

Over centuries indigenous people of different localities have developed their own specific knowledge on plant resource use, management and conservation (Cotton, 1996).Systematic application of indigenous knowledge is important for sustainable use of resources and sustainable development (Thomas, 1995).

Kim (2005) added that in ancient cultures, people methodologically and scientifically collect information on herbs and developed well defined herbal pharmacopoeias. Leonti *et al*., (2003) noted that the relevance of the historical depth of medicinal plant use from a variety of perspectives. Spices and herbs have been added to food since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (Bauchat and Nakatani, 1994; Cutler, 1995). They are essentially flavoring agents used in small amounts and are reported to have both beneficial effect and antimicrobial properties (Oluwafemi, 2000). Nowadays, plenty of spices and herbs are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities (Shan *et al.*, 2007).

2.4. Microbiological Quality and safety of spices.

Spices are important group of agricultural commodities, because of their taste and aroma. They are widely used to flavor the food preparations. Spices do not have much nutritive value but the importance of Spice in daily diet is due to the fact that they enhance the aroma and flavor of food preparations. There is lot of heterogeneity found with respect to the plant parts used. India is one of the largest producers of Spices and condiments.

The classification of the Spices samples is mainly based on morphology and the plant part which is used for the purpose. Based on a review of the published literature, a number of studies were identified that examined the microbial quality of different spices and dried herbs at point-of-sale around the globe. *Cronobacter* species, an opportunistic pathogen, has been isolated from a number of LMFs and food ingredients including spices and dry herbs.

Bedada *et al*., (2018), assessed a total of 162 samples of 25 spices for *Heterotrophic bacteria*, *Mould* and *Yeast* enumerations, total *Coliform* and *Thermo tolerant Coliforms*, *E. coli, S. aureus*, *and Salmonella* species. *Moulds, yeasts, total Coliforms* and *thermo tolerant Coliforms* larger than 10^4 CFU/g, HPC larger than 10^6 CFU/g. Out of 162 spices samples, 139 (85.8%) samples or 11 (44%) various spices had acceptable limits of *Yeast, Mould*, HPC, total and *Thermo tolerant coliforms*, *E. coli, S. aureus* and *Salmonella* spp. However, the rest 40 (24.7%) samples or 14 (56%) of the spices had one or more of the microbes.

(Baumgartner *et al*., 2009), Similarly, Vero toxigenic *E.coli* (VTEC), *Salmonella, Staphylococcus, Bacillus cereus* (spores), *Clostridium botulinum and Clostridium perfringens* (spores), and *Listeria monocytogenes* have also been isolated from a variety of LMFs.

As Baunchat *et al.,* (2013) reported, Spices and spice products that were sampled within these studies had varying degrees of contamination from pathogenic microorganisms. In a study conducted in the United Kingdom, 750 samples of spices and spice ingredients as well as 1,946 samples of ready-to-eat foods where spice had been incorporated were collected to test for a number of pathogens including *Salmonella* spp. *B. cereus* and *Bacillus* spp. (Little *et al*., 2003).

Based on their results, ready-to-eat food samples tested positive for *B. cereus* (17%) and *Bacillus* spp. (17%), *Enterobacteriaceae* (11%), *E. coli* (4%).
In addition, spices and spice ingredients tested positive for *B. cereus* (19%), other *Bacillus* spp. (53%) and *Salmonella* (<1%). Little *et al.,* (2003) examined 42 un opened & opened spices sample of Red pepper, turmeric & Corriander in Bangladish and reported the prevalence of Total Coliform, *E. coli, Staphylococcus*, yeast and moulds. The study reported higher levels of contamination in opened spices; although, microbial counts varied by region, year of production and the harvest and storage conditions prior to drying(Parveens *et al.,* 2014). In a 2007 study conducted in Iran, 351 samples of black pepper, caraway, cinnamon, cow parsnip, curry powder, garlic powder, red pepper, sumac, and turmeric were tested for the presence of a number of *aerobic mesophilic bacteria, E. coli*, and *Molds*.

Based on their results, 63.2% of samples exceeded the standard limits for *Mesophilic bacteria* (>$5x10^5$ CFU/g), 23.4% for *E. coli* (>0.3MPN/g), and 21.9% for molds (>$5x10^3$ CFU/g) (Koohy-Kamaly-Dehkordy *et al*., 2013). In addition, two studies conducted in Turkey had identified various pathogens (aerobic bacteria, *S. aureus, B. cereus, E. coli, sulphite reducing bacteria*, *molds/yeast*, *Salmonella* spp. and *E. coli* 0157 H:7) in sampled spices and dried herbs.(Kahraman and Ozmen 2009; Hampikyan *et al*., 2009).

Donia *et al*., (2008) performed microbial and aflatoxin analysis on 303 samples of different spices and medicinal dried herbs in Egypt. From their analysis, *aerobic bacterial count, spore-forming bacteria, coli form*, *E. coli, S. aureus*, *yeast* and *mould* were detected.

Total viable counts of microorganisms were found in different spices at various level, For example, *E. coli* was detected in all samples except for tea, black pepper, karakade and saffron, while *S. aureus* was only detected in basil, peppermint and spearmint. The highest and lowest mean counts were found in peppermint and black pepper respectively. All samples tested were free of aflatoxins (B1, B2, G1 and G2). (Donia, 2008).

Ahene *et al*., (2011) performed microbial analysis of aniseed, rosemary and several spice products in Ghana. Microorganisms isolated from the spices varied depending on the product tested. For example, aniseed had the highest count of bacterial load, and Royco shrimp cube and Royco beef cube had the least.

Aeromonas salmonicida, Enterobacter cloacae, Enterobacter amnigenus, Enterobacter agglomerans, Enterobacter Sakazakii, Flavobacterium spp, Chromobacterium violaceum, Pseudomonas putida, Pseudomonas aeruginosa, Acinetobacter spp, Pseudomonas cepacia and *Serratia plymuthica* were detected in the tested samples. (Ahene *et al*., 2011).

In Brazil, Moreira *et al*., (2009) analyzed different spices and dried herbs for the presence of *mesophilic bacteria*, *thermo tolerant coli forms*, *B. cereus, S. aureus*, and *Salmonella.* Twenty one percent of all samples tested positive for *thermo tolerant coli forms*, while 5.6% were positive for *Salmonella.* Black pepper had the highest level of contamination; 18.2% of black pepper samples were positive for *Salmonella* spp. and 8.3% of dehydrated green onion samples tested positive for *B. cereus*.

Turcovsky *et al*., (2011) tested 602 food items for the presence of *Cronobacter* spp. The highest contamination was observed in foods of plant origin (spices, teas, chocolate, nuts, pastries and vegetables). Sixty two per cent (13/21) of spice samples tested were positive for *Cronobacter* spp. (Turcovsky, 2011).

The 2014 systematic review by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) identified 77 studies investigating the prevalence and/or concentration of microbial hazards in spices. The report concluded that many spices can be contaminated with various microbial hazards (FAO/WHO, 2014).

The evidence identified by this review suggests that spices and dried herbs available at point-of-sale can be contaminated with pathogens. A number of studies also concluded that spices and dried herbs may be high risk products, and when contaminated, may pose a potential risk to consumers.FAO/WHO, 13–16. As with many other agricultural products, herbs and spices may be exposed to a wide range of microbial contaminants before, during and post-harvest (Koci-Tanackov *et al*., 2007).

Although used in small quantities, herbs and spices are recognized as significant carriers of microbial contamination primarily xerophilic storage molds and some bacteria (Dimic *et al*., 2000; Romagnoli *et al*., 2007). On a global scale, contamination of spices by mycotoxins has been reported as significant in Ethiopia (Fufa and Urga, 1996), Egypt (Selim *et al*., 1996; Aziz *et al*., 1998), Turkey (Gurbuz *et al*., 2000), Portugal (Martins *et al*., 2001), Italy (Romagnoli *et al*., 2007) and Morocco (Zinedine *et al*., 2006). Control of microbial contamination relies on the application of good hygiene practice in production and harvesting and post-harvest processing including storage.

During the last decade of the 20th century, food-borne infections and intoxication due to spices increased in several European countries (Buckenhüskes *et al*., 2004). The European Spice Association (ESA) and the European Commission (EC) Recommendation 2004/24/EC also specify that *Salmonella* spp. should be absent in 25g of spice (ESA, 2007), *Escherichia coli* must be less than 10^2 cfu g-1, and other bacteria requirements should be agreed between the buyer and the seller(Muggeridge *et al*., 2001).

Studies on the microbiology of these commodities have shown the presence of high microbial total counts (up to 8 log CFUg-1in black pepper, paprika, chilli powder and cumin seeds (McKee, 1995).Thus, herbs and spices may provide a conduit to introduce food spoilage organisms to a range of meals (Garcia *et al*., 2001). When added to high moisture foods, low levels of microbial contamination in herbs and spices may develop quickly causing the food to deteriorate. Rani and Singh (1990) found that 89% of their fennel, coriander and cumin samples were contaminated with aflatoxin B1 at 3000 ppb, 1640 ppb and 1580 ppb, respectively.

Similarly, Roy and Chourasia (1990) determined that the seeds of *Piper nigrum* and *Mucuna prurians*, and the barks of *Acacia catechu*, *Coriandrum sativum* and *Elettaria cardamomun* were contaminated with aflatoxin B1 at or below 20 µg kg-1. Results similar to those described in this study have also been reported by El-Kady *et al*., (1995). Spices like other food substances, may carry some bacteria, yeasts, molds spores and even some insects. The predominant flora is generally composed of aerobic spore and non spore forming bacteria, indicator organisms and some pathogens may also be found International Commission on Microbiological Specifications for Foods (ICMSF, 1986).

Spice ingredients are thought to have some antimicrobial activities, and yet meat treated with spices have high microbial load (Shamsuddeen and Ameh, 2008). However, spices are raw agricultural materials and if the moisture content is too high, toxigenic molds, like *Aspergillus spp., Penicillium spp.* and *Fusarium spp. (*Halt, 1998; Martins *et al*., 1999) may grow offering the opportunity for aflatoxins production (Reddy *et al*., 2001).

2.5. Food Borne Pathogens.

While a wide range of pathogens can cause food borne diseases, viruses, bacteria, and parasites pose the greatest share of preventable food borne threats (Fischer Walker *et al*., 2010). Food is only a vehicle for virus and parasite transmission to a new host. However, for many bacteria, food offers an opportunity to grow exponentially to infectious levels. Some bacteria, such as *Staphylococcus aureus* and *Bacillus cereus*, will produce toxins while growing in food, resulting in food borne intoxications (often called food poisoning).

This basic difference in etiology is reflected in the variability in the time to onset of disease symptoms, which range from a few hours for food borne intoxications to possibly weeks for food borne infections.

2.5.1. Major food borne diseases

Staphylococcus aureus

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastro-enteritis with rapid onset of symptoms. It is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans. *S. aureus* is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus.

The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces staphylococcal-enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning (FDA, 2012). *S. intermedius*, a *Staphylococcus* species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning (Le Loir *et al.,* 2003).

The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (aw), PH, the presence of oxygen and composition of the food. These physical growth parameters vary for different *S. aureus* strains .And the temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C. *S. aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. *S. aureus* is readily killed during pasteurization or cooking. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of 6–7 (Stewart, 2003).

S. aureus is uniquely resistant to adverse conditions such as low aw, high salt content and osmotic stress. In response to low aw, several compounds accumulate in the bacterial cell, which lowers the intracellular aw to match the external aw (Montville and Matthews, 2008). As such, most *S. aureus* strains can grow over a aw range of 0.83 to >0.99 (FDA, 2012). *S. aureus* is a poor competitor, but its ability to grow under osmotic and pH stress means that it is capable of thriving in a wide variety of foods, including cured meats that do not support the growth of other food borne pathogens (Montville and Matthews, 2008). *S. aureus* is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart, 2003).

Escherichia coli

Escherichia coli are a versatile microorganism within the family *Enterobacteriaceae*, genus *Escherichia* (Nataro and Kaper, 1998). The German paediatrician and bacteriologist, Theodore *Escherichia* first described the bacterium in 1885 and named it bacterium coli commune. Later it was named *Escherichia coli* (Geyid, 1995). These organisms are gram negative, can be non motile or motile with peritrichous flagella. *E. coli* is non-spore forming oxidase negative bacilli and predominantly facultative anaerobe (Boyed, 1995).

It forms gas from glucose, ferments lactose, gives positive methyl red, a negative Voges-Proskauer reaction and does not utilize citrate. It is a consistent inhabitant of the human intestinal tract, which often remains harmlessly confined. However, in immune compromised host, or when gastrointestinal barriers are violated, even normal 'nonpathogenic' strains of *E. coli* can cause infection (Nataro and Kaper, 1998). Most *E. coli* strains are harmless commensals but others are pathogenic.

Differentiation of the pathogenic strains from the commensal ones was accomplished on the basis of virulence properties, mechanisms of pathogenicity, clinical syndromes and serotyping of distinct "O" (somatic), "H" (flagella) and "K" (capsule) antigens (Wilshaw *et al*., 2000).The pathogenic strains may further be classified into virotypes which include entero pathogenic *E. coli* (EPEC), entero invasive *E. coli* (EIEC), entero toxigenic *E. coli* (ETEC), entero aggregative *E. coli* (EAggEC), diffuse adhering *E. coli* (DAEC) and verocytotoxin producing *E. coli* (VTEC) also referred to as Shiga toxin-producing *E. coli* (STEC)(Bell, 2002).

Entero pathogenic *E. coli* cause a watery diarrhoea accompanied by vomiting and fever in children under the age of three (Wilshaw *et al.,* 2000). Entero invasive *E. coli* cause *Shigella* like dysentery bacillary diarrhoea (Harris, 2001) which is acute and watery at first accompanied by fever and abdominal cramps.

The diarrhoea can worsen leading to bloody and mucoid stools (Wilshaw *et al*., 2000). Enteroaggregative *E. coli* cause persistent watery diarrhoea accompanied by vomiting, dehydration and abdominal pain in children (Harris, 2001).

Diffuse adhering *E. coli* cause childhood diarrhoea and they have been associated with diarrhoea in children in Mexico (Doyle *et al*., 1997). Verocytotoxin producing *E. coli* were first described in 1977 by Konowalchuk and his co-workers (Wilshaw *et al*., 2000). They were recognized as significant causative agents of Haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP).

Salmonella typhiymurium

Salmonella typhimurium is a pathogenic Gram-negative bacteria predominately found in the human intestinal lumen. Not only in human but also found in the wild birds such as house sparrow and fiches (Une *et al.*, 2008). In most cases *Salmonella typhimurium* has the ability to undergo acetylation of the O-antigen, which changes its conformation, and makes it difficult for antibodies to recognize (Slauch *et al*., 1995).*Salmonella typhimurium* is rod shaped bacteria that contain peritrichous flagella and produce hydrogen sulfide.

Surette *et al., (*1998) describe the cell structure and metabolism of *Salmonella typhimurium.* They stated that *Salmonella typhimurium* able to secrete small signaling molecules called auto inducers. The LuxS gene is responsible for initiating a series reaction that produce this molecule and allow for cell to cell communication. Sugar compounds, preferably glucose, activate LuxS and the resulting auto inducer concentration increases with the bacterial concentration until the substrate is depleted. At this point the auto inducer is degraded and can be recycled by the bacterial cell. This quorum sensing allows cells to determine the metabolic potential of the environment. *Salmonella typhimurium* is an important pathogenic organism in both humans and animals (Guerin *et al.,* 2005).

Pseudomonas aeruginosa

P. aeruginosa is a common bacterium that can cause disease in animals, including humans. It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. It is a Gram-negative, aerobic, cocco bacillus bacterium with unipolar motility. *P. aeruginosa* is the type species of the genus *Pseudomonas* (Anzai *et al.,* 2000). Although classified as an aerobic organism. *P. aeruginosa* is considered by many as a facultative anaerobe, as it is well adapted to proliferate in conditions of partial or total oxygen depletion.

Adaptation to micro-aerobic or anaerobic environments is essential for certain lifestyles of *P. aeruginosa*, for example, during lung infection in cystic fibrosis patients, where thick layers of lung mucus and alginate surrounding mucoid bacterial cells can limit the diffusion of oxygen. *Aeruginosa* secretes a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown).

Pathogenic fungi

Yeasts are a subset of a large group of organisms called fungi. *Yeasts* can grow with or without oxygen (facultative) and are well known for their beneficial fermentations that produce bread and alcoholic drinks. They often colonize foods with a high sugar or salt content and contribute to spoilage of maple syrup, pickles, and sauerkraut. They usually grow slowly, producing off-odors and flavors and carbon dioxide that may cause food containers to swell and burst.

Candida and related genera are a heterogeneous group of yeasts, some of which also cause human infections. They are involved in spoilage of fruits, some vegetables and dairy products. (Cosey and Dobson, 2003).

Molds are filamentous fungi that do not produce large fruiting bodies like *mushrooms*. *Molds* are very important for recycling dead plant and animal remains in nature but also attack a wide variety of foods and other materials useful to humans. They are well adapted for growth on and through solid substrates, generally produce airborne spores, and require oxygen for their metabolic processes. Most *molds* grow at a pH range of 3 to 8 and some can grow at very low water activity levels (0.7–0.8) on dried foods. Spores can tolerate harsh environmental conditions but most are sensitive to heat treatment.

Spoilage molds can be categorized into: *Penicillium* and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions. They are distinguished by their reproductive structures that produce chains of conidia. *Penicillium* spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in these crops. They also spoil other fruits and vegetables, including cereals.

Some species can attack refrigerated and processed foods such as jams and margarine. *Aspergillus* and related molds generally grow faster and are more resistant to high temperatures and low water activity than *Penicillium* spp. and tend to dominate spoilage in warmer climates.
Many aspergilla produce mycotoxins: aflatoxins, ochratoxin, territrems, and cyclopiazonic acid. *Aspergilli* spoil a wide variety of food and nonfood items (paper, leather, etc.) but are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices.

2.6. Anti-microbial activities of spices

2.6.1. Anti-bacterial activity

The antimicrobial activity exhibited by plant extracts against food poisoning bacteria has been demonstrated by several researchers (Verma *et al*., 2012; Akinpelu *et al*., 2015). Gupta *et al*. (2010) investigated antibacterial activity of five ethanolic and aqueous plant extracts against S. aureus, Pseudomonas aeruginosa and Bacillus subtilis and their results showed that the ethanolic extracts of four plants (Achyranthes aspera, Cynodon dacynodon dactylon, Lantana camara and Tagetes patula) were effective against all tested microorganisms with MIC's ranged from 25 to 125 mg/ml.

Sapkota *et al*. (2012) studied antibacterial effect of guava leaves, garlic and ginger against some human microbial pathogens and they ascertained that ginger was only effective against S. aureus while guava and garlic were effective against all tested microorganisms. Akinpelu *et al*. (2015) investigated antibacterial potential of crude and butanolic extracts of Persea americana against Bacillus cereus implicated in food poisoning. The extracts exhibited antibacterial activity at concentrations of 25 and 10 mg/ml with MBC of both extracts ranged between 3.12 and 12.5 mg/ml respectively.

Moreover, antimicrobial activity of different natural substances such as medicinal plant extract have been investigated against food borne bacteria. For example; Ateb and Erdo_Urul, (2003), and Rios and Recio (2005) tested the suppression of food borne bacteria and their diseases by medical plant extracts. The extract of three medicinal plants used in Nigerian folk medicine showed a highly antibacterial activity against some food borne pathogens. All extracts exhibited a strong antimicrobial activity against Salmonella enteritidis, E. coli and S. aureus but in variable degree and with different MIC's depending upon the plant extract and pathogenic organism.

Akinyemi *et al*., 2006. In addition, Sher (2009), Pirbalouti *et al*. (2010) investigated antimicrobial activity of eight medicinal plants against E. coli, Bacillus cereus and Listeria monocytogenes. The most effective extracts were those obtained from Myrtus communis and Thymus daenensis with MIC values ranged between 0.039 and 10 mg/ml. Antimicrobial activity of Punica granatum against food poisoning bacteria was proved by several investigatorsm (Voravuthikunchai *et al*., 2005; Nuamsetti *et al*., 2012). Antibacterial activity of Punica, Citrus and Allium extracts against food borne spoilage bacteria was investigated by Verma *et al*. (2012). All plant extracts was potentially effective against S. typhi, E. coli, B. cereus and S. aureus implicated in food spoilage but the extract of Punica granatum was the most effective extract with concentration of 500 mg/ml. Ethanolic P. granatum peels extracts was found to be potentially effective against Micrococcus luteus, S. aureus, Bacillus megaterium and Gram negative bacteria like E. coli and P. aeruginos in concentration ranged between 30 and 50 mg/ml. (Dey *et al*., 2012).

Antimicrobial activity of ethanolic Punica granatum extract and its fractions showed a highly antibacterial activity against Gram positive (S. aureus and B. cereus) and Gram negative bacteria (E. coli and S. typhi) causing food poisoning and these extracts can be used for prevention of food borne diseases or as preservative in food industry (Alzoreky, 2009; Mahboubi *et al*., 2015). Spices extracts used as food additives were potentially effective against some food poisoning bacteria and their antibacterial activity was investigated by several researchers (Parekh and Sumitra, 2007; Abdulrahman *et al*., 2010). Cinnamon extract was found to be the most effective spice against all tested strain while the weakest antimicrobial activity was displayed by cumin, ginger and clove respectively. Antimicrobial activity of clove (S. aromaticum) against Gram negative bacteria and food borne pathogens was investigated

2.6.2. Anti-fungal activity

Antifungal activity of spices and derivatives has been studied regarding viable cells count, mycelia growth and mycotoxins synthesis. Juglal *et al*., (2002) studied the effectiveness of nine essential oils to control the growth of mycotoxins producing moulds and noted that clove, cinnamon and oregano were able to prevent the growth of *Aspergillus parasiticus* and *Fusarium moniliforme*, while clove (ground and essential oil) markedly reduced the aflatoxin synthesis in infected grains. These findings could be useful for rural communities to prevent the synthesis of fungal toxins in contaminated grains by simple measures.

Basílico and Basílico (1999) studied the inhibitory effect of oregano, mint, basil, sage and coriander on the mycelial growth of *Aspergillus ochraceus* NRRL 3174 and its ochratoxin synthesis and the results showed that oregano (750ppm) completely inhibited the fungal growth and ochratoxin A synthesis up to 14 days at 25°C.Basil (750 ppm) was effective to inhibit the mycelial growth up to 7 days.

Thyagaraja and Hosono (1996) assayed the ability of chilli, coriander, pepper, cumin and asafetidato inhibit food spoilage moulds (*Rhizopus azygosporus, Mucor dimorphosphorous, Penicillium commune*, *Fusarium solani*) and asafoetida showed promising results in inhibiting the fungal growth. Among the ethanolic and aqueous asafoetida extract, only ethanolic fraction showed antifungal property.

Abel-Hafez and El-Said (1997) analyzed the effect of garlic and onion extract on the mycoflora of pepper, cinnamon and rosemary and reported effectiveness of garlic extract up to 0.25% (v/v) to inhibit the growth of *Aspergillus flavus, A.fumigatus, A. niger, A. ochraceus, A. terreus, Penicillium chrysogenum, P. puberulum, P.citrinum, P. corylophilum, Rhizopus stolonifer, Stachybotrys chartarum, Eurotium chevalieri* and *Emericella nidulans*. Elgayyar *et al*., (2001) analyzing the antimicrobial effect of selected plant essential oils found that anise essential oil was highly inhibitory on *Aspergillus niger, Geotrichum* and *Rhodotorula*, although it was not active on bacteria.

Arora and Kaur (1999) assayed the sensitivity of yeasts to spices aqueous extracts and found that garlic and clove extract were able to inhibit *Candida acutus, C. albicans, C. apicola, C.catenulata. C. inconspicua, C. tropicalis, Rhodotorula rubra, Sacharomyces cerevisae* and *Trignopsis variabilis* and in some cases strong cidal effect was observed. Grohs and Kunz (2000) examined the mixtures of ground spices (2 and 5%w/v) were effective to inhibit the growth of *Candida lipolytica*.

Adam *et al*., (1998) analyzed the antifungal activity of essential oils from oregano, sage, lavender and mint on human pathogen fungi and found prominent inhibition on *Malassezia furfur, Trichophyton rubrum* and *Trichosporum beigelii* with minimum inhibitory concentration and lethal minimum inhibitory of, respectively, 0.25% and 1% (v/v).

Minimum inhibitory concentration was understood as the lower essential oil concentration that caused total inhibition of fungal growth noted by formation of growth inhibition halos, while the minimum lethal inhibitory was understood as the lower essential oil concentration that killed the fungi inoculums detected by viable cells count Little information on spices and derivatives action on/in the fungal cell in order to promote fungi static or fungicide effect.

These biological events could take place separately or concomitantly culminating with mycelium germination inhibition (Cowan, 1999).Also, it is reported that plant lytic enzymes act in the fungal cell wall cousing breakage of B-1 3 glycan, B-1 6 glycan and chitin polymers (Brull and Coote, 1999).

2.6.3. Chemical compounds of spices

In addition to the studies on antimicrobial activity of spices and their extracts and essential oils, the antimicrobial effectiveness of their chemical compounds have also been investigated in order to improve the understanding about the cell targets of the molecules found in spices (Karatzas *et al*.,2000; Vasquez *et al*., 2001). Phyto-chemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activity (Madhuri and Pandey, 2009).

Alkaloids

Alkaloids constitute a chemical group that includes many molecules of vegetable origin that are very well known, such as caffeine or cocaine. In terms of antimicrobial properties, molecules like berberine and piperine seem have the ability to intercalate into cell wall or DNA (deoxyribonucleic acid). Some activity against protozoa is also referred, mainly anti-*Plasmodium* and anti-trypanosome, although *Giardia* and *Ent amoeba* infections, common in HIV patients, can also be eliminated through the consumption of some alkaloids. Solamargine, a glycol alkaloid extracted from *Solanum khaniasum* is helpful in HIV infections (Cowan, 1999).

Flavonoids:

Flavonoids are derived from 2-phenylchromen-4-one (2-phenyl-1-4-benzopyrone) and are commonly known for their antioxidant activities. Flavonoids, which are widely distributed in plants, fulfill many functions including producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects. Compared to other active plant compounds, they are low in toxicity. Flavonoids are referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Ayoola *et al*., 2008).

Saponins:

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom triterpenes in plants. They are found in various plant parts; leaves, stems, roots, bulbs, flowers and fruits. They are characterized by their bitter taste and their ability to haemolyzed red blood cells. They are used medically as expectorant, emetic and for the treatment of excessive salivation, epilepsy, chlorosis and migraines. They are used in Ayurvedic medicine as a treatment for eczema, psoriasis and for removing freckles.

Saponins are believed to be useful in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to jump more efficiently Saponins also inhibit cancer tumor growth in animals, particularly, lung and blood cancers, without killing normal cells. Saponins are the plant's immune system acting as anti-biotic to protect the plants against microbes and fungus.(Ayoola *et al*., 2008)

Anthraquinone:

Anthraquinones are aromatic organic compounds and is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is fairly stable under normal conditions. Anthraquinones naturally occur in some plants, fungi, lichen and insects, wherein they serve as a basic skeleton for their pigments. Anthraquinones are used in the production of dyes and are also used as a laxative.

Cardiac glycosides:

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as 7secondary metabolites in several plants and in some animals. Some of these compounds are used as arrowhead poisons in hunting (Filippos *et al*., 2007).

3. Materials and method

3.1. Description of the sample site

The study was conducted in Oromia Regional State, Kelem Wollega Zone, Hawa gelan Woreda, Gaba Robi Town. Hawa Gelan is located at 634 km west of Addis Ababa, the capital of Ethiopia. Currently it has 32 administration kebeles, including 29 rural kebeles and two urban centers (namely Geba Robi and Mechara). The topography of the district is rigged with elevation varying between 1200-2200 m.a.s.l. (HGWANRO, 2014).

The administrative center of the district is Geba Robi, located at 28 km from the zonal capital, Dembi Dollo, towards the North West. The population size of the district is estimated to be 129486 of which 65662 are males and 63854 are females. The district is boarded by Illu-Ababor zone in the South and South West, Dale Wabera district in the Northeast, Yemalogi Walal district in the North and Sayo district in West direction.

The community in the study area cultivates maize (*Zea mays*), sorgum and finger millet (*Eleusine coracana*) in all areas of the district. However, some crops likes teff (*Eragrostis tef*), wheat (*Triticum* spp.) and barley (*Hordeum vulgaris*) are produced in parts of Hawa Gelan. Among the major source of income of population of the study area are coffea (*Coffea arabica*), khat (*Catha edulis*), and red pepper (*Capsicum* spp.). Spices like *Allium sativumL, Zingiber officinale Roscoe, Trigonella faenum graecumL, Allium cepaL* and *Aframomum corrorima* are also among the product of the study area.

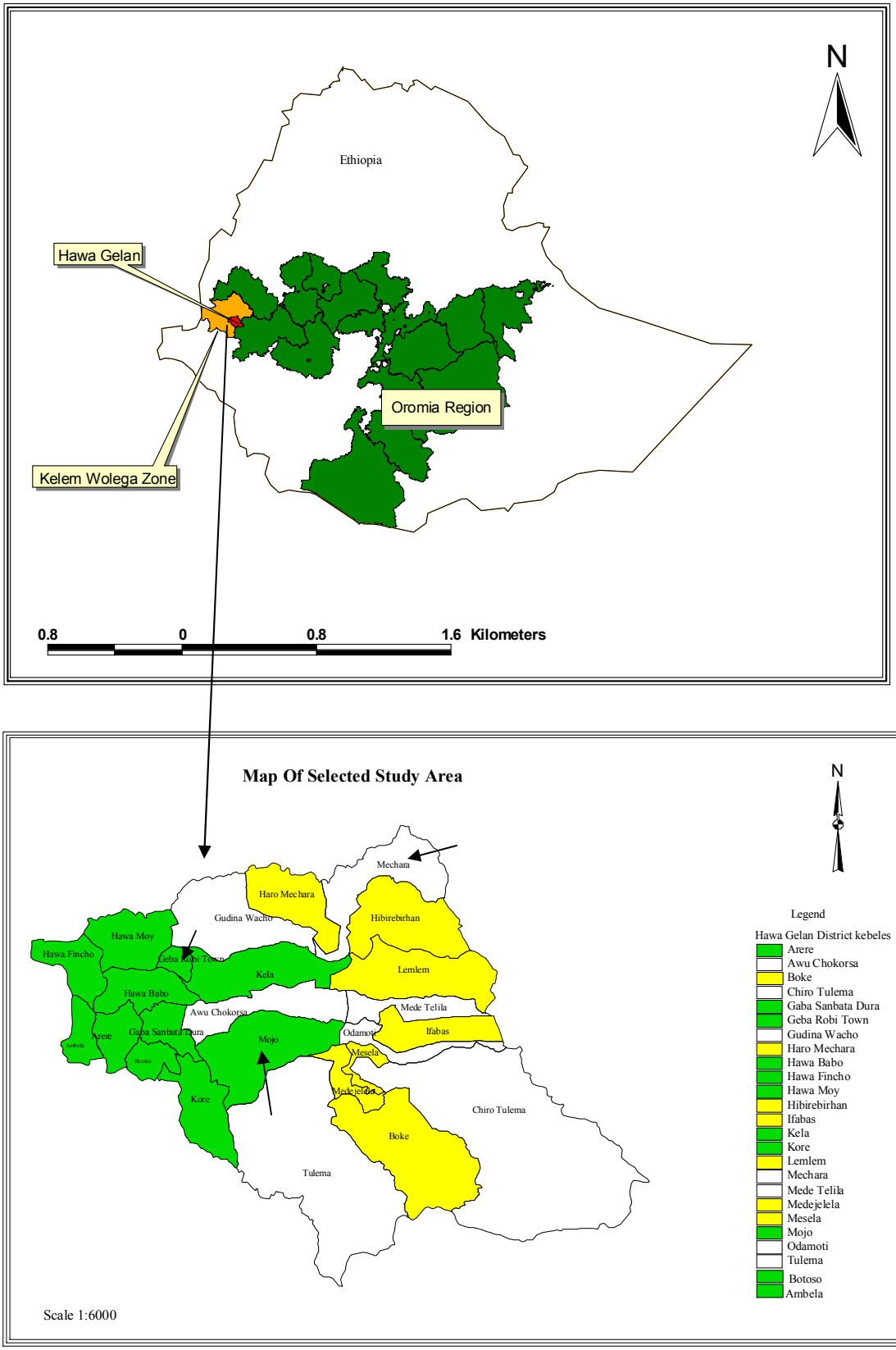

Fig.1 Map of the study area (Hawa Galan) (Ayana, 2017). Sample collected area.

3.2 Study Design and population

Cross sectional study design for venders and experimental were used to collect frequently used spices in the study area.

3.3. Sampling techniques and sample collection

Purposive sampling techniques were used to collect spices (medicinally) frequently used in the study area having identified the dominant spices in use. Questionnaire was used to assess the common spices (medicinal plants) used in the study area, their medicinal values and frequency of uses. Depending on frequency of use and ethno-medicinal information of the spices, decision was made to select the spices most frequently used in the study areas.

About 60 samples of 3 different spices were collected randomly from different localities of Hawa Galan. Accordingly, *Curcuma (L.) domestica Valeton*, *Zingiber officiale Roscoe* and red pepper *(Capsicum annuum spp.)* were selected for the study. Therefore, 20 rhizome of *Curcuma (L.) domestica Valeton,* 20 rhizome *of Zingiber officiale Roscoe* and 20 fruits of red pepper *(Capsicum annuum spp.)* spices were purchased from different local market of Geba Robi Town, machara town and modjo. The purchased spices were collected in to sterilized polyethylene bag and transported to Jimma university and stored in a laboratory blender at Research Laboratory of Staff and Postgraduate Studies until used up.

The collected spices samples were processed for microbiological quality and safety followed by evaluation of antimicrobial activities of their extracts against pathogenic microorganisms. Investigation of the microbiological quality and safety of the spices and the antimicrobial activity of their extract was tested at Research Laboratory of Staff and Postgraduate Studies, Department of Biology, Jimma University.

3.4. Microbiological analysis

3.4.1. Sample preparation

A 25g of spices samples were separately suspended in 225ml of buffered peptone water and homogenized in erylenmer flask for 5min using shaker at 160rpm. A 1ml of homogenized sample was transferred into 9ml of sterilized 0.85/100ml of saline solution and mixed thoroughly using vortex mixer.

The homogenized spices sample was further diluted from 10^{-1}-10^{-8} and 0.1ml aliquot of the dilutions was spread plated on pre-solidified agar plate and incubated at appropriate temperature (32^0c for 18--48h).

3.4.2 Microbiological Enumeration

Determination of total aerobic mesophilic counts (TAMC), counts of *Staphylococci*, *Enterobacteriaceae*, *Coliforms*, *Yeast* and *Molds* were done according to the procedure described by Dabassa and Bacha (2012). Accordingly, standard plate count method using PCA (Plate Count Agar) (Oxoid) were implemented for counts of TAMC after incubation at 30-32^{0}C for 72h; Mannitol Salt agar (Oxoid) for *Staphylococci* count, potato dextrose agar for *Yeast* and *Molds* counts (after incubation at 25^{0}C for 2-5 days), VRBGA (violet red bile glucose agar) (Oxoid) for counts of *Enterobacteriacea* and VRBA (violet red bile agar) (Oxoid) for total *Coli forms* count (TCC) after incubation for 24h at 32^{0}C.

Then Colonies were counted from plates containing microbial colonies between30-300 and were expressed in colony forming units per gram (CFU g^{-1}⁄). The detailed procedures for specific microbial enumeration are as given below.

Aerobic mesophilic bacterial **count:** From the appropriate dilution, 0.1ml of the aliquot was spread plated on Plat Agar Count (PAC) (Oxoid) and the plates were incubated at 32^{0}c for 48h. (Weil. *et al*., 2006).

Enterobacteriaceae **Count:** From appropriate dilution, 0.1ml of the aliquot was spread plated on VRBGA (violet red bile glucose agar) (Oxoid) and incubated at 32^{0}c for 18-24h, after which pink to red purple colonies are counted as member of the family *Enterobacteriaceae* (Spencer *et al*., 2007).

Staphylococcus **Count:** From appropriate dilution 0.1ml of aliquot was spread plated onto Mannitol Salt Agar (MSA) (Oxoid) and incubated at 32^{0}c for 48h (Acco *et al*., 2003).

Coliform **Count:** From appropriate dilution 0.1ml of aliquot was spread plated on pre-solidify surface of Violet Red Bile Agar (VRBA) (Oxoid) plates. Then the plates were incubated at 32^{0}c for 18—24h. Then after, purplish red colonies surrounded by reddish zone of precipitated bile were counted as *coli forms* (Weil *et al*., 2006)

***Yeast* and *Moulds* Count**

From appropriate dilution, 0.1ml of aliquot was spread plated on pre-solidify surface of potato dextrose agar (Oxoid) supplement with 0.1g Chloromphenicol to control the growth of bacteria and incubated at 25^0c for 5-7 days (Spencer *et al*., 2007). The colonies of each isolates were identified using taxonomic and morphological keys of Cheesbrough (2006) and the pure culture of each isolate was maintained separately. Then, smooth (non-hairy) colonies without extension at periphery were counted as yeast; whereas hairy colonies with extension at periphery were counted as *moulds*. About 10 distinct colonies were randomly picked from countable plates of moulds and was further purified by repeated streaking on appropriate agar plate and identified morphologically at least to genus level.

3.5. Preparation of spices extracts

The fresh spice samples collected from different local markets were processed for antimicrobial activities following standard procedure. Accordingly, husk was removed from ginger and *Curcumae longae* (Fig. 1A) and washed with distilled water and dried (Fig.1B) before grounded finely in a laboratory blender. To obtain the spices' extracts, about 100g each of washed and dried spices were crushed with mortar and pestle and extracted with different solvents of varying polarity (Fig. 1C), to get as many possible active compounds as possible (Fig.1D). Accordingly, spice extracts were extracted using Methanol, chloroform, and petroleum ether (Appendix 2).

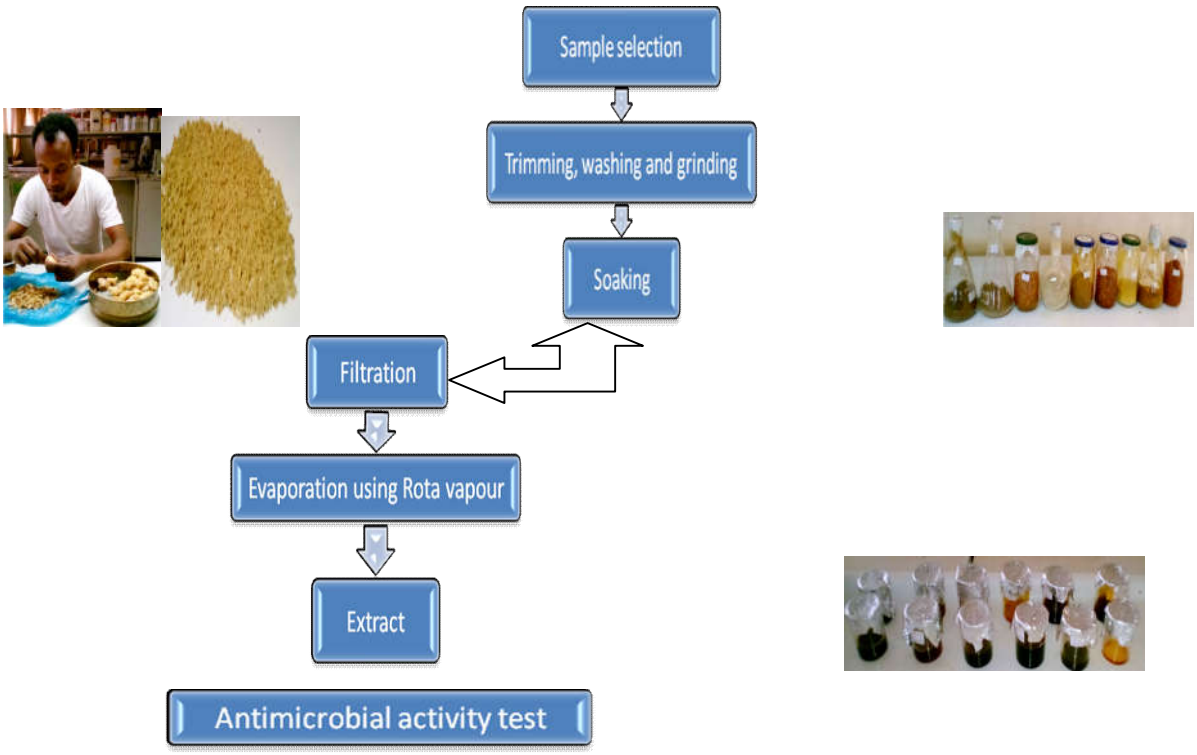

Fig.2. Flow chart for extraction of spices extract.

The spice soaked with extraction solvent was filtered by filter paper (whatman №1) to remove debris of the spice. Then, the extract added to Rota flask. The flask then applied on the Rota vapor machine, the power supply turned on, and the solvent evaporated from the extract. After few minutes (3 to 7min.) based on the types of solvents, only spices extract were left in the flask. Then the extract was transferred to pre-prepared and sterilized equipment (Fig. 2). Basically, the extracts were sieved through a fine mesh cloth and sterilized using a membrane filter (0.45-micron sterilized filter).

This extract was considered as 100% concentration extract. Other levels of concentrations (75%, 50%, 25% and 10%) were made by diluting the concentrated extract with appropriate volumes of DMSO

3.5.1. Antibacterial activity testing using agar disc diffusion method.

In vitro screening of the spice extracts for antimicrobial activities was tested using selected pathogens of reference strain took from Microbiology laboratory of Addis Ababa University: including *Salmonella typhiymurium* (ATCC-13311), *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25925) and Yeast (*Candida albicans)*.

Experimental design for bioassays involved varying treatments. These are test group, positive control and negative controls. Test group consists of the standard reference microorganisms' [*Salmonella typhimurium* (ATCC-13311), *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC25925), and Yeast (*Candida albicans*)] and the extracts of different spices applied on pre-sterilized Muller Hinton Agar plate.

The 2nd treatment contain negative controls where Dimethylsafoxide (DMSO) was applied on reference strains of bacteria pre inoculated on sterilized Muller Hinton Agar plate. The standard antibiotic disc, namely Chloramphenicol (1mg\ml) was used as positive control. Before activity evaluation, pure cultures of the test organisms were activated overnight. The 24 hours cultures of cell density equivalent to 0.5 McFarland standards (1-2 x 10^8 cfu /ml) were uniformly spread onto the entire surface of a Mueller – Hinton Agar (MHA) plate with sterile cotton swab. Test solutions were prepared by dissolving appropriate weight of plant extracts to achieve final stock concentrations of 30 and 25 mg/ml in duplicate.

About 25 mg of the various spices extracts (i.e. methanol, chloroform and petroleum ether extract) were aseptically transferred to each disc at all dilutions that were made in duplicate. Standard disc of chloramphenicol (30 μg/disc) was also included as controls for bacteria. Steriled filter paper discs (6 mm) that contain different spice extract and controls were evenly placed on the agar plate surface previously inoculated with the 0.1 ml standard suspensions of each test microorganisms. The plates were placed inverted and incubated for 24 hours at 37^{o}C.

After 24hrs the diameter of zone of inhibition were measured (in mm) and results were recorded. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active (Junior and Zanil, 2000). The mean and standard deviation of the diameter of inhibition zones were calculated.

3.5.2. Antifungal activity test

In vitro antifungal activity of crude extracts was tested by disc diffusion method. The spoilage fungi/yeast (Candida albicans) was grown on PDA (Hi-Media, Pvt. Ltd. Mumbai,India) medium and the spore suspension (1×10^4 spore/ml) was prepared. Sterilized filter paper discs (What man No.1, 6 mm in diameter) was aseptically placed on each Petri plates containing PDA, pre-inoculated with respective spoilage fungi.

A 30 µl of each crude extracts was impregnated onto the disc by sterilized micropipette tips and the same amount of DMSO and kanazole were added as negative and positive control, respectively. The Petri plates were left for 30 min at room temperature to allow the extract diffusion, and were incubated at 25 °C for 5 days. At the end of the incubation period, anti-fungal activity was evaluated by measuring zone of inhibition against the tested spoilage fungi using a ruler. All treatments were replicated twice.

3.6. Determination of minimal inhibitory concentration (MIC)

Agar dilution method was used to determine the MICs of spice extracts. Equal volumes of each bacterial strain culture containing approximately 10^5CFU/ml was applied onto nutrient broth supplemented with different concentration of methanol extract, Chloroform extract, and petroleum ether extract with concentrations ranging from 1 to 100 mg/L in a test tubes. Cultures were then incubated at 37 °C for 24 hr, subsequently; 100µl of each culture was inoculated onto nutrient agar and further incubated at 37 °C for 24 h. The lowest concentration at which no growth occurred was taken as the minimum inhibitory concentration value of extract (Manila *et al.*, 2014). MIC is defined as the lowest concentration of spice extract that completely suppresses colony growth.

3.7. Data analysis

The data collected from laboratory experiment were analyzed both quantitatively and qualitatively. The data of different microorganisms that are counted from different spices sample collected from different places of Hawa Gelan district were entered in to computer, processed, edited and analyzed by using Microsoft excel and the results were then displayed by using table and graphs. The same is true of data on antimicrobial activity of the spices against food borne pathogens.

4. RESULT AND DISCUSSION.

4. Result

Description of spices in the study area

Out of many different kinds of spices being produced and used, *Capsicum annuum, Curcuma longa, and Zingiber officinale* are among the frequently used spices in the study area (Table 1). The medicinal values associated with the spices are their use for the treatment of different bacterial disease, fungal disease, and cancer. The names, frequency of use and the medicinal values of the spices are as presented in Table 1.

Table 1. List of spices, their frequency of uses and proposed medicinal values, Hawa Galan district, Oromia region, Western Ethiopia, 2018

Types of spice/medicinal plant	Local name	Scientific name	Frequency of use (%)*	Proposed medicinal value
Red pepper	*barbare diimaa*	*Capsicum annuum*	98.3	Capsicum exerts a variety of desirable actions on the entire cardiovascular system.
Turmeric	*Irdii*	*Curcuma longa*	85.57	It is a natural antiseptic and antibacterial agent, useful in stopping and curing colds, and disinfecting cuts and burns
Ginger	*Jinjibila*	*Zingiber officinale*	82.77	Anti-clotting agent, anti-inflammatory, circulatory stimulant
Garlic	Qullubbii adii	Allium sativumL	95.2%**	Reduces cholesterol, an anti-cancer strengthens the immune system
Shallot/Onion	Qullubbii diimaa	Allium sepaL	99.3%**	Onion prevents Atherosclerotic plaques**.** Onions reduce blood coagulation, prevents atherosclerosis and other cardiovascular diseases**.**
fenugreek	*Sunqoo*	*Trigonella foenum graecumL*	52.33	To prevent stomach dryness from children
Cororima	Eegihoo	Aframomum corrorima(A.Braun)P.C.M.Jansen	33%	Used medicinally as a tonic, carminative and purgative.

* Proportion of respondent that mentioned its use value in the study area

** Well investigated for their antimicrobial activity and were not considered for this study

Microbial count from spices

In the present study, mean microbial load were varied from spice to spice. Among the locally collected spices samples, the highest mean count of AMB (6.32±0.56 Log cfu/g) was recorded in red pepper while the lowest mean count (6.03±0.014 Log cfu/g) was observed in ginger. Mean *Yeast* and *Mould* count was higher in turmeric (6.36±0.01Logcfu/g) and lower in Ginger (6.02±0.03Logcfu/g). The highest coliform count was found in Red pepper (4.07±0.15 Logcfu/g) and lowest count was in Turmeric (4.01±0.06 Log cfu/g). Highest mean *Enterobacteriaceae* count was detected in Turmeric (6.29±0.021Logcfu/g). The highest mean count of *Staphylococci was recorded in* ginger sample (4.03±0.011Log cfu/g. *Staphylococci* were not detected in red pepper sample and turmeric (Table 2).

Table 2: Mean microbial counts (Log cfu/ml) of different spices collected from, Hawa Galan district, Oromia region, Western Ethiopia, 2018.

Sample source	Mean ±SD (Log cfu/ml)				
	TAMB	TCF	Entero.	Staph.	Yeast and molds
Red pepper (*Capsicum* spp.)	6.32±0.56	4.07±0.15	6.23±0.021	<2	6.15±0.22
Turmeric (*Curcuma Longa*	6.26±0.11	4.01±0.06	6.29±0.021	<2	6.36±0.01
Ginger (*Zingib officinale*)	6.03±0.014	<2	<2	4.03±0.011	6.02±0.03
P-value	.043	.386	.751		.355

Where: TAMB, total *aerobic mesophilic bacteria*; TCF, total *coli form*; Entero., *Enterobactericeae*; Staph, *Staphylococci*; <2, below detectable level.

According to one way ANOVA analysis at (CI = 95%) only TAMB had significantly associated between groups (P= 0.043). Other than this, almost all of the microorganisms (TCF, Entero, and Yeast and Mould) have no significant association based on the P-values; that means there p- value was larger ($P > 0.05$).

The highest mean count of *Enterobacteriaceae* was recorded in Red pepper from Modjo town (6.37 Log cfu/g) (Fig.2). The same is true of total aerobic mesophilic bacteria and Yeasts and Molds. The mean counts of total coliforms were relatively low in all samples from the three sampling sites with count ≤ 4 log cfu/g. Accordingly, the mean microbial count (Log cfu/g) of AMB, TCF, *Enterobcteriaceae* and Yeast and Moulds in Red pepper collected from Modjo site were 6.36, 3.99, 6.37, and 6.4, respectively. The mean microbial counts of AMB, Coliform, *Enterobacteriaceae* and Yeast & Moulds in Red pepper from machara site were 6.28, 4.24, 6.10, and 6.0, respectively. The mean counts of *Staphylococci* were below detectable level (Fig. 2).

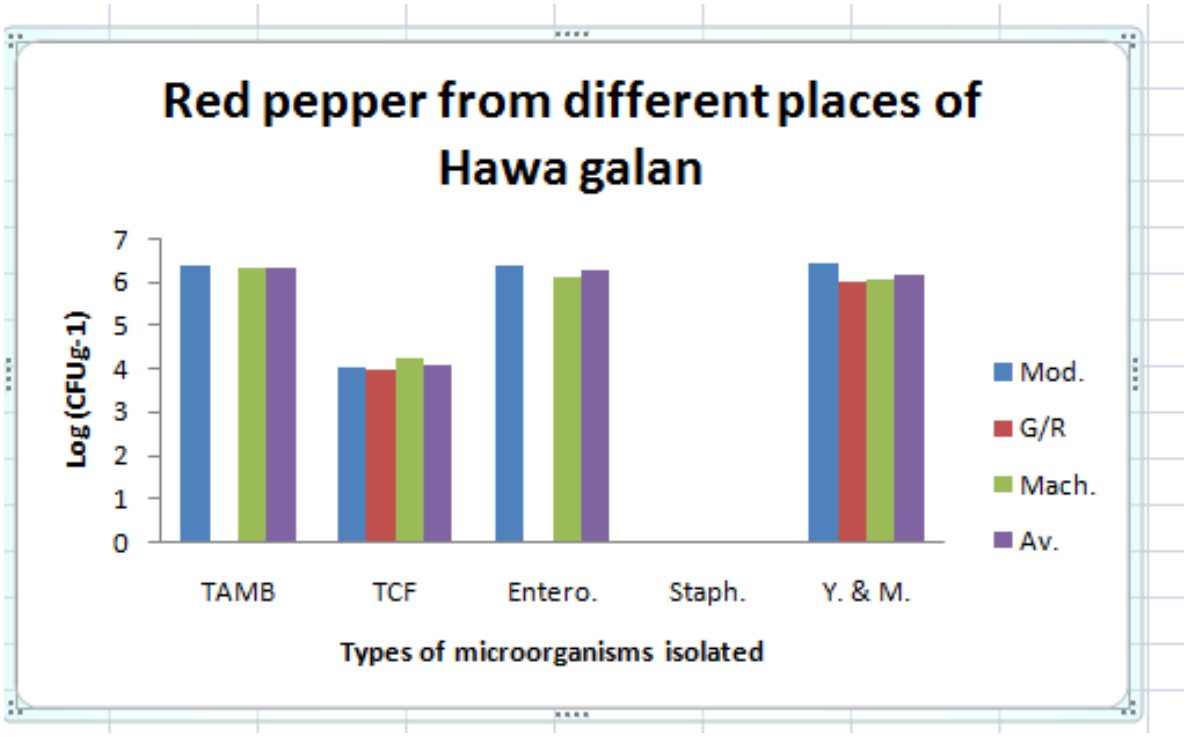

Fig. 2 Mean microbial count (log cfu/g) of Red pepper spices collected from different places of Hawa Galan Western Ethiopia.

Where: TAMB Total aerobic mesophilic bacteria, TCF Total Coliform, Entero Enterobacteriaceae, Staph Staphylococcus, Y. & M. Yeast and Moulds, Mod.= Modjo, G/R= Gaba Robi, Mach= machara, Av.=Average.

Unlike the case of red pepper, the highest mean count of *Staphylococci* was recorded in sample of Ginger collected from Gaba Robi town (4.04Logcfu/g) and Machara town (4.04Log cfu/ g^{-}(Fig. 3). Furthermore the highest mean count of *Yeast* and *Moulds* were detected in Turmeric from Machara town (6.43Logcfu/g) (Fig.3).

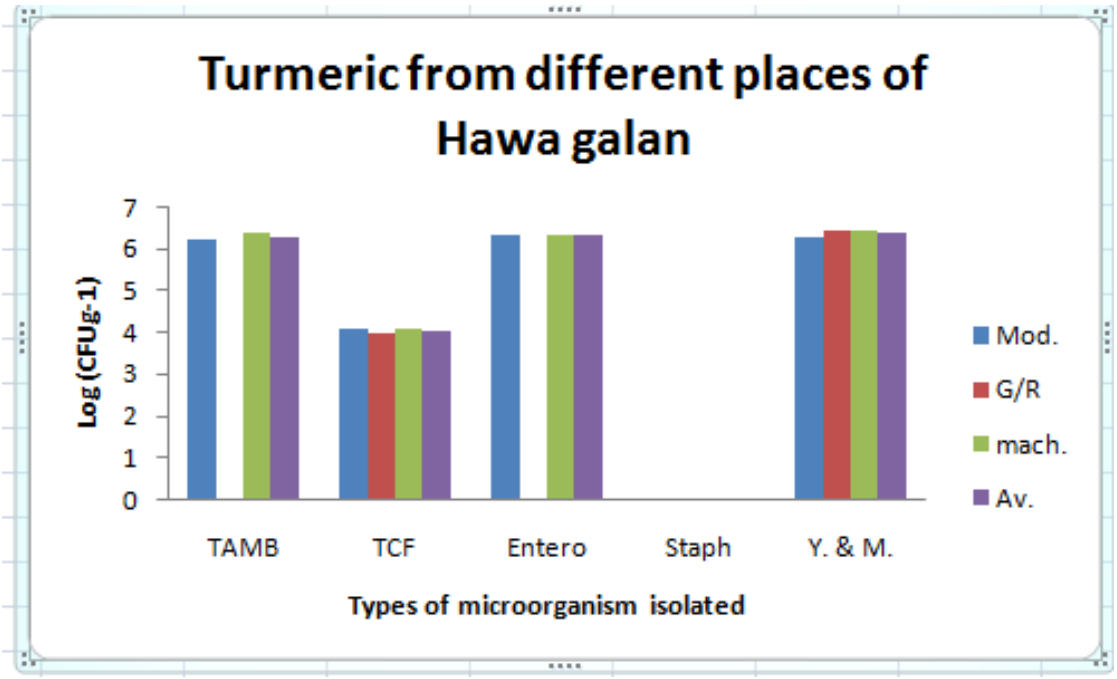

Fig. 3 Mean microbial count of Turmeric spices sample collected from different places of Hawa Galan Western Ethiopia. The mean is taken from duplicates of petridishes.

Where: TAMB Total aerobic mesophilic bacteria, TCF Total Coliform, Entero Enterobacteriaceae, Staph Staphylococcus, Y. & M. Yeast and Moulds, Mod.= Modjo, G/R= Gaba Robi, Mach= machara, Av.=Average.

Similarly, the mean microbial count (Logcfu/g) of AMB, coli form, *Enterobacteriaceae*, and *Yeast & Moulds* in Termeric from Modjo were 6.19, 4.05, 6.28 and 6.25 and in turmeric from machara are 6.34, 4.04, 6.31 and 6.43, respectively (Fig 3).

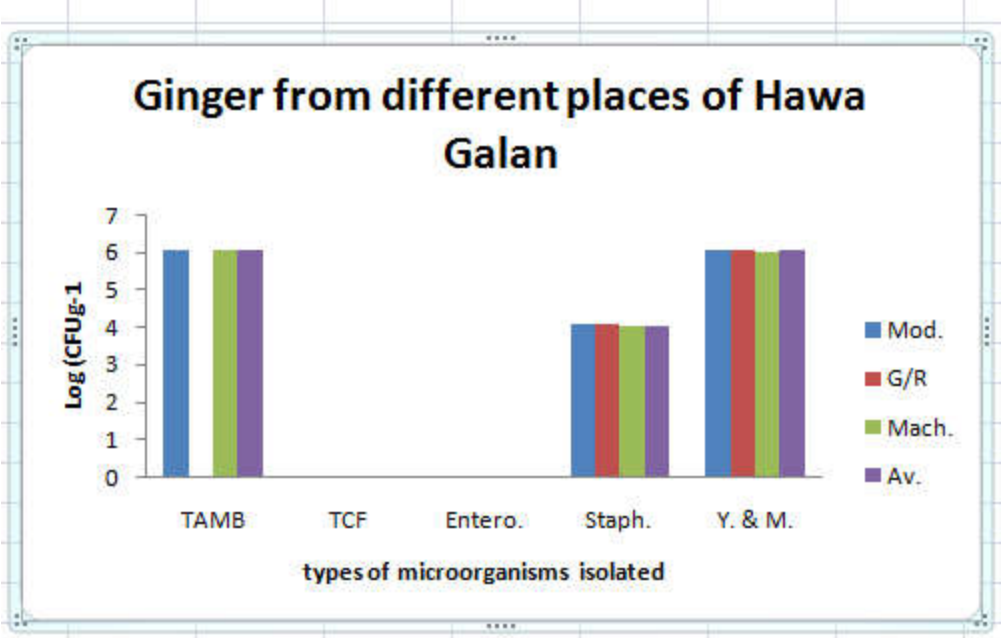

Fig. 4 Mean microbial count of Ginger spices sample collected from different places of Hawa Galan, Western Ethiopia.The mean is taken from duplicate of petridishes.

Where: TAMB Total aerobic mesophilic bacteria, TCF Total Coliform, Entero Enterobacteriaceae, Staph Staphylococcus, Y. & M. Yeast and Moulds, Mod.= Modjo, G/R= Gaba Robi, Mach= machara, Av.=Average.

Furthermore, the mean microbial count (log cfu/g) of AMB, *Staphylococci* and *Yeasts* & *Mould* in Ginger from modjo were 6.02, 4.04 and 6.05, respectively, the same mean counts in ginger from machara were 6.04, 4.02 and 5.99, respectively (Fig 4). Although much of the data narrated above are on mean microbial counts of spices collected from mojo and machara sites of Hawa Galan district, the patterns were the same for samples collected from Gaba Robi sites.

Likewise, the mean microbial count of Coliform in Red pepper was 3.97 Logcfu/g and that of Yeast and Moulds were 6.0 Log cfu/g. The mean microbial counts of Coliform and Yeast & Moulds in turmeric were 3.94 and 6.41, respectively. Similarly the mean microbial count of Staphylococci and Yeast and Moulds in Ginger from Gaba Robi were 4.04log cfu/g and 6.02Logcfu/g, respectively. In the current study, yeast and moulds were detected in all samples tested.

4.1.2. Morphological Identification of detected fungi.

Table 4 Typical colony morphology of dominant fungal genus isolated from spices collected from the study area, 2018

Code of fungal isolates	Characteristics of colony	Structural morphology	Tentatively identified fungi
JUR00	Cottony, brown, pink colony	Extensive septate mycelium, conidiophores simple or branched. conidia are septate fusiform.	*Fusarium*
JUR01-03 JUG03-05	White to dark grey colonies, dense cottony mycelium producing mass of sporongia	Non-septate mycelium with root like rhizoids sporongiophores in clusters dark sporangia.	*Rhizopus*
JUR04-06 JUT02-04	white/ cream Colonies	No hyphae, smooth appearance	Yeast
JUR07-09 JUG06-08	Colonies with loose White to yellow Mycelium. Become Dark brown to black On the development Of conidian	Black, brownish black or purple brown conidiophores. conidia are yellow to green, conidiophores arising from a foot cell.	*Aspergillus* Spp.
JUT05-07 JUG09-11	Greenish /blue green colonies	Conidia in long chain on branched conidiophores brush like heads (penicillus) smooth conidiophores, irregular and asymmetrical pattern of branched penicilia.	*Penicillium* spp.
JUR10-12 JUT08-10	Very fast growing, cottony to fluffy, white to yellow color	Non-septate hyphae, has stolon, Zygospores formation.	*Mucor* spp.
JUG12-14	Downy to powdery colonies, yellow or dull green to bluish green	Green conidia, separate septate.	*Eurotium* spp.
JUT11-13	Fast growing, flat, white to cream(yeast like or slimy) color	Colorless conidia, separate septate.	*Geotrichum* spp.

Where: R-red pepper, T-turmeric and G-ginger.

4.1.3 Prevalence of spoilage fungi isolated from tested spices.

A total of 25 isolates of fungi were recovered from the three types of spices, out of which 16 (64%) isolates were identified to genus level while 6 (36%) isolates remained unidentified. The highest numbers of spoilage fungi were isolated from red pepper (26.31%) followed by turmeric and ginger (21.05%). The relatively dominant spoilage fungi were *Aspergillus* sp. (23.08%), *Penicillium* sp. (23.08%), Yeast sp. (23.08%), and *Rhizopus* sp. (23.08%) followed by *mucor* sp. (15.38) *Fusarium* sp. (15.38) (Table 4). Table: 3 Dominant fungal genera isolated from the tested spices.

Spices sample	Isolated fungal genus	Frequency
Red pepper	*Fusarium*	(2)(67.7)
	Aspergillus	(2)(67.7)
	Mucor	(1)(33.3)
	Rhizopus	(1)(33.3)
Turmeric	*Penicillium*	(2)(67.7)
	Mucor	(1)(33.3)
Ginger	*Aspergillus*	(1)(33.3)
	Penicillium	(1)(33.3)
	Rhizopus	(2)(67.7)

Yield from different spices extracted with three solvents (methanol, petroleum ether and chloroform).

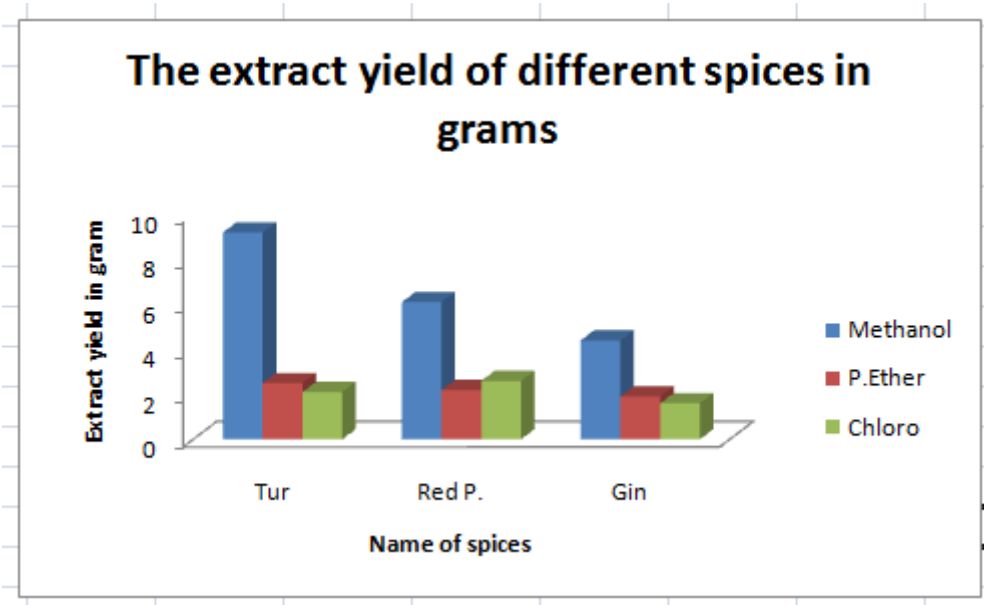

Fig.5 The extract yield of different spices in grams with different solvent.

Where: Tur = Turmeric, red p.= Red pepper, Gin= Ginger, p. Ether= Petroleum ether.

Percent yields of red pepper, ginger and turmeric extracts from 100g sample ranged from 1.6 to 9.2 on dry weight basis. The highest yield of spices extract was obtained from methanolic extract of turmeric (9.2 g) followed by methanolic extract of red pepper (6.1 g). The methanolic extract yield of ginger was 4.4g while that of petroleum extracts of Turmeric, Red pepper and ginger were 2.5g, 2.2g and 1.9g, respectively. Similarly, the yields of chloroform extract of Turmeric, Red pepper and Ginger were 2.1, 2.6 and 1.6 respectively (Fig. 5).

The entire individual extracts showed broader antimicrobial activity as they revealed inhibitory activities against bacteria as well as fungus (*C. albicans)*. The diameter of inhibition zones of extracts of different spices ranged from 7 to 23 mm against bacteria and from 7.2 to 15 mm against *C. albicans.* Accordingly, antimicrobial activity or inhibitory zone diameters raged between 7 to 23 mm for Red pepper, 8 to 19 mm for turmeric and 9.25 to 15 mm for Ginger extract, indicating that Red pepper was the most effective spice in inhibiting the microbial growth. As evident from the inhibition zones diameter values given above, the individual extracts of all the three spices were most inhibitory against the growth of *C. albicans* except for petroleum ether extract of red pepper which was resisted by the tested pathogens (Table 5)

4.1.3. **Antimicrobial Activity of the Tested Spices Extract against Food Borne Pathogens.**

Table 5 Antimicrobial activity of spices against food borne bacteria and fungi (*C. albicans*).

No	Spices	Extraction method	Tested microorganisms Zone of inhibition							
			E. coli (ATCC-25922)	Chloramphenicol	*S.typhi-murium* ATCC-13311	Chloramphenicol	*Staphylococcus aureus* (ATCC-25925)	Chloramphenicol	*Candida albicans*	Kanazole
`1	*Capsicum annuum* (Red pepper)	Petroleum ether	17.8	14	7	15	NA	30	NA	11.5
		Chloroform	19.5	20.5	NA	13.4	14.5	20	7.2	13
		methanol	22	18	11	18	23	25	9	12
2	*Curcuma longa domestia Valeton* (Turmeric)	Petroleum ether	NA	15.3	9	15	12	14	12	14
		Chloroform	11	15	NA	14.6	NA	18.3	13	12.8
		methanol	9	15.8	8	14.3	19	24	15	10
3	*Zingiber officiale* (Ginger)	Petroleum ether	NA	14.9	9.25	15	11.5	21	15	13
		Chloroform	18.5	20	10	19.5	15	21	11	12.25
		methanol	NA	12.9	10	21	NA	15.6	8	9

NA: no activity

The minimal inhibitory concentrations for spice extracts against examined bacterial strains are as presented in Table 6. The lowest MIC (20 mg/ml) was recorded for methanol and chloroform extracts of red pepper against *Escherichia coli*. The MIC values recorded for other extracts were above or equals to 25mg/ml.

Table 6 Minimum inhibitory concentrations MIC (mg/ml) of Methanol, Petroleum ether and Chloroform extract of spices against food borne pathogens

Nameof spices	solvent used	Types of microorganisms and MIC in mg/ml			
		E. coli	*S. thyphimurium*	*S. aureus*	*C. albicans*
Red pepper	Methanol	20	25	25	25
	Petroleum ether	25	25	30	30
	Chloroform	20	30	25	25
Turmeric	Methanol	25	25	25	25
	Petroleum ether	30	25	25	25
	Chloroform	30	30	25	25
Ginger	Methanol	30	25	30	25
	Petroleum ether	30	25	30	25
	Chloroform	25	30	25	25

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5. Discussion

In the current study, mycological and bacteriological quality and safety of spices collected from different localities in Hawa Galan district were assessed. Accordingly, it was observed that the counts of *Enterobacteriaceae and Staphylococcus* exceeded the standard set by International Commission on Microbiological Specifications for Foods (ICMSF) while the total aerobic mesophilic bacteria and total coli forms were within acceptable range of limit of the specifications (ICMSF, 2005). The observed levels of bacterial and fungal contaminations of spices could be accounted to poor harvesting and processing, exposure to dust, wastewater, animal and human excreta during production or in retail markets (Freire and Offord, 2003). The levels of contamination depend on variations in spices processing of users. (Schweiggert*et al*., 2007).

The detection rate of yeasts in spices was one in 154 samples as reported in the study done in India (Banerjee and Sarkar, 2003) while in the study done in Turkey, yeasts and moulds were detected in 45.5% of the samples (Hampikyan*et al*., 2009). In the current study yeast and moulds were detected in 99% of the tested spices sample. Similarly, Banejee and Sarkar found 97% contamination of Indian spices by moulds in the spice samples investigated (Banerjee and Sarkar, 2003).

Also in study done by Abu Donia (2008) Fungi were found in all of the collected spices samples which exactly similar with our current study. Following cooking, the presence of fungal toxins might cause food poisoning or valuable food products' deterioration. Contamination of mycotoxins is a serious problem if spices are stored for long periods of time, without temperature and moisture controls (Bugno *et al*., 2006).

In this study, two of the three spices samples exceeded the ICMSF criteria for total coli forms, suggesting a faecal contamination. These results indicated that ginger was the least contaminated product, followed by turmeric, whereas the highest microbial counts were associated with red pepper. The studied spices were not subjected to any washing or selection procedures before the analyses, which if properly applied are important to remove soil particles, contaminants and damaged or altered spices and spices part.

The highest levels of yeast & mould, total coliform, and *Enterobactericeae* contaminations were observed in turmeric and red pepper, respectively, while maximum counts of staphylococci were observed in ginger. Enterobacteriaceae counts are used more generally as an indicator of hygienic quality rather than of faecal contamination and therefore say more about general microbiological quality than possible health risks posed by the product (Adams & Moss, 1995).

The members of Enterobacteriaceae occurred in 2 out of 3 kinds and 66.67% of the samples of spices. When the microbial counts in spices collected from different shops were compared, the most unhygienic shops showed the highest counts of total aerobic mesophilic bacteria (TAMB) and Enterobacteriaceae. Mesophilic bacteria were also reported from ginger, black pepper, and red pepper in India (10^6 CFU/g in 50% of the samples) *(*Seenappa *et al*., 1981*)*. As per ICMSF specifications, TAMB count of $<10^4 g^{-1}$ is of acceptable quality and $10^4–10^6$ $g^{-1}$is of marginal quality.

Our results indicate a high level of contamination (66.67%) of the samples falling in the unacceptable range ($>10^6$ cfu g^{-1}), showing marginal quality. Dried spices and herbs are commonly used in a variety of ways and food preparations. Recently, the microbiological concerns of these products are recognized by center for Food Safety and Applied Nutrition (CFSAN) of the US Food and Drug Administration (FDA) (Vibha *et al*., 2006).

The results of hygienic quality parameter analysis demonstrated that the population size of aerobic Mesophilic bacteria had a varied count depending on sample type. Similar reports of varied aerobic mesophilic bacteria in food related spices and herbs have been documented(Hampikyan *et al*., 2009; Sago *et al*., 2009; Sospedra *et al*., 2010; Vitullo *et al*., 2011; Witkowska *et al*.,2011; Dababneh, 2013). Generally, plate count of aerobic bacteria in spices is considered as a sign of general sanitation and quality parameter (Kneifel *et al*., 2002).

In the current study, 33.33% (20 of 60) of retail spices samples did not comply with the ICMSF standards for total aerobic mesophilic bacterial counts (TAMB >6 log10 CFU g-1). These findings are similar to data reported by several researchers (Moreira *et al*., 2009; Sago *et al*., 2009; Vitullo *et al*.,2011; Witkowsk *et al*., 2011). Contamination with coli forms denotes unhygienic conditions.

The bacterial populations of coliforms were detected at a low limit among samples (< 4 log10 CFU g-1). Similar population sizes have been reported in other studies (Abou-Donia, 2008; Vitullo *et al*., 2011; Khanzadi *et al*., 2012). However, higher coli form counts (> 4 log10 CFU g-1) have been reported by other workers (Czech *et al.*, 2001; Phianphak *et al.*, 2007; Idu *et al.*, 2008). The occurrence of coli forms show the possibility of fecal contamination and inadequate sanitation conditions while the plants were being grown. Although herbs and spices are not major contributors to food borne disease they occasionally contain Pathogenic microorganisms which may pose a risk to public health, especially when added to meals without further treatments (Banerjee and Sarkar, 2003; ICMSF, 2005).

In a 2007 study conducted in Iran, 351 samples of black pepper, caraway, cinnamon, cow parsnip, Curry powder, garlic powder, red pepper, sumac, and turmeric were tested for the presence of a number of aerobic mesophilic bacteria, *E. coli*, and yeast and moulds. Based on their results, 63.2% of samples exceeded the standard limits for mesophilic bacteria (>$5x10^5$ CFU/g), 23.4% for *E. coli* (>0.3MPN/g), and 21.9% for moulds (>$5x10^3$ CFU/g) (Koohy-Kamaly-Dehkordy *et al*., 2013). In addition, two studies conducted in Turkey had identified various pathogens (aerobic bacteria, *S. aureus, B. cereus, E. coli,* sulphite reducing bacteria, moulds/yeast, *Salmonella* spp. and *E. coli* 0157 H: 7) in sampled spices and dried herbs. (Kahraman and Ozmen, 2009; Hampikyan *et al*., 2009).

The same results with the current research also observed from Donia (2008) report, who performed microbial and aflatoxin analysis on 303 samples of different spices and medicinal dried herbs in Egypt. From their analysis, aerobic bacterial count, spore-forming bacteria, coliform, *E. coli, S. aureus*, yeast and mould were detected.

Even though spices are not the most important contributors to food borne illness, if they are added without further cooking to ready to eat foods, the spices can cause high risk (Little*et al*., 2003).While spices have been implicated in large scale outbreaks of food borne illness if they are not further cooked, the impact of contaminated spices on the incidence of food borne illness in Hawa Gelan is not documented. Microbial counts vary according to the region, the time of production, the harvest and Storage conditions prior to drying. So, the observed counts were thus a reflection of the original bio-load, of growth, as well as of die-off which were probably enhanced by oxidation and the presence of active compounds in herbs and spices (Donia, 2008).

The variation in the microbial count depends on the different origin of spices, conditions of Processing, storage and type of spices. The highest count of bacteria, Coliform and yeast and mould may be due to poor hygienic standard of preparation and or handling.

The International recommended Microbiological Standard limits for bacteria contaminant in spices are in the range of 10^1 to 10^3 cfu/g for Coliform, 10^1 to 10^5 cfu/g for total microbial plate count, 10^1 to 10^3 cfu/g for yeast and mould, 0/20 g for *S. aureus* and 0/20 g for *E.coli* (Awe *et al.*, 2009). Although some of the results of our study are comparable to the International Microbiological Standards and most of the mean counts were above the recommended limits.

The highest mean count of aerobic mesophilic bacteria were recorded in red pepper samples from Modjo (6.36Logcfu/g) Filiz, (2001) reported total aerobic mesophilic bacteria count of $8x10^6$cfu/g. Elmali *et al*.,(2005) examined 15 powdered red pepper and found $2.7x10^6$cfu/g of mesophilic bacteria,. which is exactly the same with our current result obtained. As many other agricultural commodities, spices are exposed to a wide range of environmental microbial contamination during harvest, processing, and in retail markets by dust, waste water, and animal and even human excreta (Freire and Offord, 2003).

The International Commission on Microbiological Specifications for Foods (1974) has set up maximum limit of 10^6, 10^4 and 10^3 CFU of total aerobic mesophilic bacteria (TAMB), fungi, coliforms and *E. coli*, respectively, per gram of spice (Zamboni *et al*., 1991). The current result of contamination of spices with total aerobic mesophilic bacteria and total coliform was 10^6 and 10^4 respectively; which was equal with this specification. While the contamination of spices with yeast and moulds, Enterobacteriaceae and staphylococcus were 10^6, 10^6 and 10^4respectively which exceeds this specification.

Brazilian Microbiological Standard for Foods (ANVISA, 2001) has set maximum limit of 5 x 10^2 e 10^2 CFU/g for faecal coli forms and positive coagulase *Staphhylococcus*, respectively, and absence in 25g of spices for *Salmonella*. In German legislation, standard limit value for TAMB, *Bacillus cereus* and *S. aureus* is 10^5, 10^4 and 10^2 CFU per gram of spices, respectively (Mousuymi and Sarkat, 2003). Our current result was exceeds these German legislation, standard limit value which is 10^6, for Total aerobic mesophilic bacteria and 10^4 for coagulase *Staphhylococcus.*

Moreover, spices are collected in tropical areas by simple methods and are commonly exposed to many contaminants before, being dry enough to prevent microbial growth. The most frequent fungal contaminants of spices are species from the genera *Aspergillus* and *Penicillium* (Silliker *et al*., 1992; Dimic´ and S˘krinjar, 1995). Fungal contamination of spices usually occurs when spices are not properly dried or when stored in a highly humid environment (Dimic´ *et al*., 2008).

From total 16 fungal population isolated *Aspergillus* and *Rhizopus* spp. were the predominant fungus in both red pepper and ginger. Followed by *yeast* and *mucor* spp. in red pepper and turmeric. In the same way penicillin were predominant in turmeric and ginger. On the contrary, *Fusarium* were the less dominant fungal strain isolated from red pepper. The emergence of *Aspergilli, Penicillin* and *Rhizopus* on the three different media greatly indicates the presence of these fungi as the dominant mycoflora of different spices. This observation was greatly in agreement with other investigators who dealt with mycoflora of spices and medicinal plants (Dimić *et al*., 2008; Bugno *et al*., 2006).

Spices have been used for not only flavor and aroma of the foods but also to provide antimicrobial properties (nanasombat *et al.,2011*). Some of the natural compounds found in various spices posses antimicrobials (Haltha *et al.,2006*). Recent studies indicate that spices in low doses are beneficial to human beings (Banerjee & Sarkar, 2003a; De, 2004).Wide range of plant spices are used as active ingredients for the food preparations by the people Living in the Hawa Galan district. In the traditional ayurvedic treatments, a number of plant extracts and plant products are used to prepare drugs to treat different human ailments (Bonjar *et al*., 2004).

Different studies have been carried out to understand the role of such plant extracts in human body and their anti-microbial properties (Vaishnavi *et al*., 2007). Three spices were tested for their antimicrobial property against three standard bacterial pathogens and one fungal pathogen in the present study. All the spices tested have been confirmed having antimicrobial activities. A large number of plants are used to combat different types of diseases and possess antimicrobial activity.

In an era characterized by increasing consumer choice, self-medication and quest for natural therapy, herbal products are used increasingly as an alternative to drugs and supplements (Mansaray, 2000). In particular, extracts from many kinds of oriental spice plants are known to possess antimicrobial effect besides being used for the purpose of food preservation, appetizer

promotion and medicinal purposes (Tassou *et al.*, 2000). Arora & Kaur (1999) tested various spices for antimicrobial activity.

The entire individual extracts showed broader antimicrobial activity as these were more or less inhibitory against bacteria as well as fungus *C. albicans*. The diameter of inhibition zones of individual extracts of different spices ranged from 7 to 23 mm against bacteria and from 7.2 to 15 mm against fungus *C. albicans.* The agar disc diffusion assay for antimicrobial activity yielded the inhibitory zones of 7 to 23 mm diameter for Red pepper, 8 to 19 mm diameter for turmeric and 9.25 to 15 mm diameter for Ginger extract indicating that Red pepper was the most effective spice in inhibiting the microbial growth. Gram positive bacteria and fungus were more prone to the inhibitory activity in comparison to gram negative bacteria.

Generally, gram negative bacteria are supposed to be more resistant to antibiotics than gram positive bacteria (Zaika *et al.*, 1983), the present study revealed the reality of these observations that gram positive bacteria were more susceptible to inhibition by the crude spice extracts. As evident from the inhibition zones diameter values, given in table 5, the individual extracts of all the three spices were most inhibitory against the growth of *C. albicans* except petroleum ether extract of red pepper which is resisted by the pathogen.

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 (Hoult and Paya, 1996; Rios and Recio, 2005). Antimicrobial activity of alcoholic extracts of ginger has been reported against *B. subtilis* and *C. albicans*. and agar well diffusion method has shown inhibition zones from 15 to 35 mm diameter for ginger (Mishra and Behl, 2010).

Out of the three types of spices extracts tested, petroleum ether, chloroform and methanolic extracts had demonstrated better activity against pathogenic bacteria. Alcoholic solvents might have exhibited better solubility of active ingredients of spices than water. Most of the examined spices extracts showed varied inhibitory activity against the tested food borne strains (table 5). The methanolic solutions of the extracts were found to have potent antimicrobial activity against all the Gram positive, Gram negative and fungus organisms tested.

Red pepper extract proved to be the most inhibitor against all of tested bacteria. The extracts of turmeric exhibited notable antibacterial activities toward most of examined bacteria with different potentialities. On the other hand, the spices extracts of ginger showed weak anti- bacterial activities against most of the tested strains. The efficacy of spices was compared between gram negative, gram positive bacteria and fungi C. Albicans.

Extract of spices like ginger had shown highest antimicrobial zones against gram negative bacteria while extract of Turmeric exhibited maximum zones against gram positive bacteria, The extract of Red pepper had exhibited maximum zones in both. The gram positive bacteria were more sensitive to the extract of spices than gram negative bacteria because of the differences in the organization and components of the cell wall structure (Shihabudeen *et al*., 2010; Ceylan and Fung, 2004). Generally, gram negative bacteria have been reported to be more resistant than Gram positive to essential oils antimicrobial effect because of their cell wall lipopolyssaccharide (Russel, 1991).

Cell wall lipopolyssacaride may prevent that essential oils active compounds reach the cytoplasmic membrane of Gram negative bacteria (Chanegriha *et al*., 1994). Anyway, the present work shows that the spices have been very effective against gram positive, gram negative bacteria and pathogenic fungi C. albicans chosen for this study.

The antibacterial activity of the plant extract of spices was compared with the standard antibiotic Chloramphenicol (10 U/disc) and antifungal kanazole. This indicates that the spices specified exhibited similar antimicrobial activity with the antibiotic Chloramphenicol which is the most common used for the infections caused by bacterial pathogens. This study concludes that the extracts of the spices reduce and inhibited the growth of the selected bacterial pathogens. This could also be applied to food borne and food poisoning organisms as diverse spices are used in various food preparations. Thus the present study confirms the therapeutic potential of the generally used spices.

The minimal inhibitory concentrations for spices extracts against examined bacterial strains are presented in table (8); the lowest MIC which could inhibited microbial growth was recorded for methanol extracted red pepper and chloroform extracted red pepper. Methanol extracts of other spices exhibited marked antimicrobial potentialities by determining their MICs against tested bacteria and fungus *C albicans*.

Minimum inhibitory concentration values ranging from 3.125 to 12.5 mg/ ml has been reported by Mishra and Behl (2010) for ginger extract against *B. subtilis, S. aureus, E. coli, P. aeruginosa* and *P. chrysogenum, C. albicans.* Antimicrobial activity of alcoholic extracts of ginger has been reported against *B. subtilis* and *C. albican.*

5. CONCLUSION and RECOMMANDATION

5 Conclusion

- The results of this study have shown that majority of the spices samples tested contained unacceptable limits of microorganism as compared to ICMFS guidelines of fungi, Enterobacteriaceae and *S. aureus*. However, few spices samples had microbiological indicators, spoilages or pathogens that were acceptable limits of ICMFS guidelines of TAMB and Total coli forms. From the research red pepper spice sample was found to present the largest contaminated sample and ginger was the lowest contaminated spices sample. The use of these contaminated spices may cause high risk to human health. The results of this study have shown that spices sold in the Hawa Galan markets harbored high bacterial count and fungi. This indicates poor handling, storage and a general lack of hygiene, possibly during cultivation and transportation of these Spices.
- Considering the importance of medicinal values in the human health and the large practice of the spices in various forms for disease prevention and cure; the results of this study clearly indicated that the antibacterial and anticandidal activity vary with the species of the spices, the solvent type, and the microorganisms tested. Further, the active crude extract of these plants against bacteria and *C. albicans* should be characterized and their toxicity should be evaluated *in vitro*.
- The data supports the hypothesis that some common Indian spices have an inhibitory effect on the growth of certain food borne pathogens. In the antimicrobial study of spices, antimicrobial activity against *E.coli,* maximum activity was shown in methanol extract of red pepper, and minimum activity was shown in methanol extract of turmeric. The antimicrobial activity against *S.tyhimurium* maximum was recorded in methanol extract of Red pepper and minimum activity was shown in petroleum ether extract of red pepper.
- Antimicrobial activity against *Staphylococcus aureus* maximum activity was shown in methanol extract of Red pepper, and minimum activity was shown in both petroleum ether extract of turmeric and Zinger. Moreover, the antimicrobial activity of the spices against fungi *Candida albicans* maximum activity was shown in petroleum ether extract of turmeric and minimum activity was shown in methanol extract of zinger. Based on our results this study established a good base for developing future drugs for treatment of infectious diseases caused by *C. albicans* and some pathogenic bacteria such as *Staphylococcus aureus, Salmonella typhimurium*, and *E. coli*.

- In summary, the present study indicates bacterial contamination and occurrence of antimicrobial resistance in bacterial populations in spices at retails that can affect the products quality and shelf life and may constitute threats to consumers,.
- It can be also concluded that these spices can be used as effective antimicrobial agents against both gram positive and gram negative bacteria as well as pathogenic fungi.

7. RECOMMENDATION

- The microbial loads of spices is high, there was also in the spice indicator organisms and organisms that are potentially pathogenic. It could therefore be recommended that; Spices should be produced under strict hygienic measures.
- The spice should be subjected to treatment that would reduce their microbial load. This is to avoid the introduction of undesirable kinds of organisms that might bring about spoilage and contamination.
- Culturing, harvesting, conveying and processing of these spices should be done in sanitary conditions. Thus, it is important to control environmental conditions and improvements in hygiene procedures during production and processing of spices.
- A suitable sanitization method for disinfection before and when packaging spices would be recommended.
- Further study of these microbiological quality and safety of spices would be recommended for the presence of spore former microbes and different fungal species.
- In addition, a further investigation is necessary to assess antimicrobial activity by performing various solvent (ethanol, hot and cold-water) extraction method. The clear zone depends on the solubility and rate of diffusion in agar medium and its volatilization.
- Thus, there is a need for detailed scientific study of traditional spices to ensure their action with diverse bacterial species and this would provide scientific evidence for their efficacies.
- This study recommends further investigations on the possibilities of using different spices around the world as supplementary or alternative medicines for bacterial infections.

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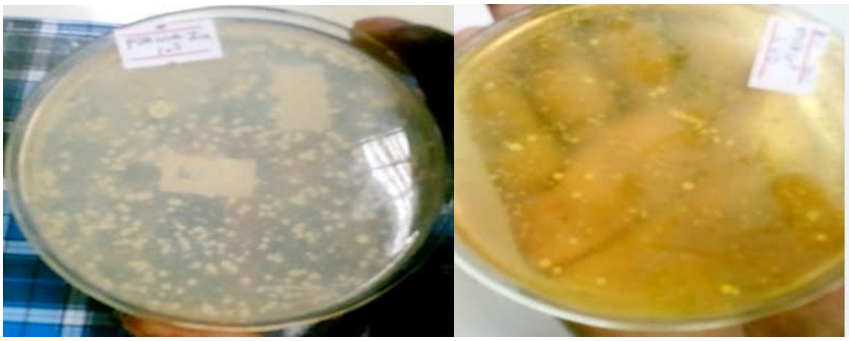
Appendix 1

Sample of spices contamination level.

Highly contaminated

Moderately contaminated



No contamination

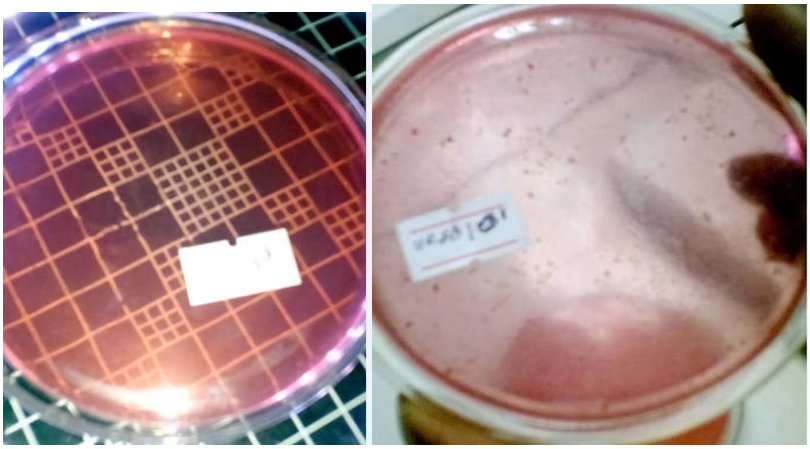


Fig. pictorial representation of spices contamination level

Appendix 2

Fig. 1 Pictorial presentation of processing of spices (medicinal plants) for extraction:

Removing the husk of zinger and Curcuma L. (A), drying zinger and Curcuma L (B), and Solvents used for extraction (C), Spices soaked in different Solvents (D), crude extracts of spices extracted with different solvents (E)

Where: Z, Zinger; C, Chloroform; M, Methanol; R, Red pepper; P.E Petroleum ether, and Cu is Curcuma longa

Appendix 3

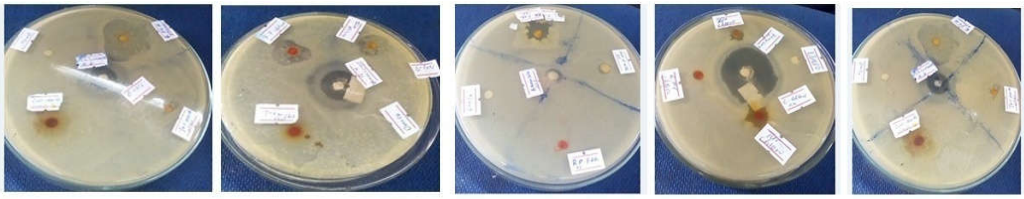
Fungal genus isolated from spices sample tested.

Yeast spp. Asprgillus spp. Fusarium spp.

Fig. pictorial representation of fungal genus isolated from tested spices sample.

Appendix 4

Fig. Inhibition zone of antimicrobial activity of spices sample tested against some food borne pathogens



Staph with red pepper staph witgh turmeric and Staph with zinger *C.albicans* with Red pepper *C.albicans* with Zinger C. albicans with turmeric *E.coli* with Red pepper

Fig. pictorial representation of Antimicrobial activity of the spices extract against food borne pathogens.

Appeendiksii 5

YUNVERSIITII JIMMAATTI

KOLLEEJJII SAAYINSII UUMAMAA

MUUMMEE BAYOLOOJII

Gaaffilee

Faayidaan Gaaffilee kanaa odeeffannoo waa'ee mi'eessituu Aanaa kanatti oomishamanii, gosoota mi'eessituu hawwaasni aanaa kanaa baayinaan itti gargaaramuu fi faayidaa mi'eessituun ni kenna jedhee hawwaasni aanaa kanaa yaadu argachuuf waan ta'eef qorannoo kanaaf ga'een keessan ol'aanaa dha. Kanaafuu, Gaaffilee armaan gadiitiif hubannoo qabdan akkaa tarreesitan kabajaan isin gaafadha. Galatoomaa!

I. Haala gaafatamtootaa

Saala: dhiira □ dhalaa □ Umurii: 20__ 30 □ 30__ 40 □ 40 ol □

Sadarkaa: barnootaa; M/barumsa Kan hin galiin □ dubbisuu fi barreessuu □ sadarka1^{ffaa} (1-4) □ Sadarkaa 1ffaa marsaa lammaffaa(5-8) □ Sadarkaa 2ffaa(9-10) □ Qophaa'ina(11-12) □ dippiloomaa kolleejjii fi isaa ol □

Miindeffamaa mootummaa □ Hojjetaa Guyyaa □ Hojii dhuunfaa kan hojjetu □

Kan biraa_____

Iddoo jireenya: magaalaa □ Baadiyyaa □

II. Haala gaafii: gaaffileen marti gaaffii banaadha.

waa'ee gaaffichaa waanta beektan hunda barreessuun ykn dubbachuun deebisaa!

1. Gosoonni mi'eessituu aanaa kana keessatti oomishaman maal fa'aadha?

2. gosoota mi'eessituu mana keessan keessatti itti gargaaramtan tarreessaa!

3. mi'eessituu maalif nyaatatti dabaltu?

4. faayidaa mi'eessituun qaama namaaf kennu ibsaa!

5. mi'eessituun dhukkuboota attamii fa'a qaama namaa irraa ittisuu danda'a jettu?

JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

DEPARTIMENT OF BIOLOGY

1: Questionnaire

The purpose of this questionnaire is to obtain information about the perception of spices. Your genuine response contributes a lot to the research to be undertaken. Hence you are requested to kindly give what you know based on the questionnaire. Thank you

About respondents

Sex of respondent, 1. Male □ 2.Female □; Age: 20__30, □ 30__40 □ 40 ol □

All questionnaire are open ended

1. What types of spices are produced in Hawa galan district?

2. List the types of spices used at your home!

3. Why you add spices to food?

4. Explain the purpose of spices for human body!

What types of disease do you think that spices fight from our body?

Appendix 6 List of informants interviewed during data collection.

Name	Age	Sex	Kebele		Name	Age	Sex	Kebele
Hasan Gemechu	57	M	Kella		Wakjira Dano	82	M	Hawababo
She Mahamad Said	74	M	Hawa Moyi		Birane megarsa	38	F	Harere
Mokonin Deresa	37	M	Hawa Fincho		Askala Mamade	75	F	Harere
Mohamad Gomol	61	M	Hawa Moyi		Shashitu Ayana	62	F	Harere
Shibiru Bulcha	57	M	Hawa Moyi		Tafasa Adaba	45	M	Harere
Dasale Kajela	38	M	>>		Dasalegn Dinka	50	M	Harere
Galane Gabisa	67	F	>>		Marga Wayesa	70	M	Harere
SheAbrihamGalmo	68	M	>>		Solomon Bakala	36	M	Harere
Herega Tumsa	86	M	>>		Dasalegn Deresa	29	M	Harere
Fakade Nagasa	58	M	Hawa Babo		Dula Abdisa	45	M	Lemlem
Abdisa Wage	83	M	Hawa Fincho		Nazifa Dalju	40	M	Botoso
Gamme Gute	67	M	Hawa Babo		Getacho Biranu	24	M	Botoso
Alami Sori	63	F	Hawa Bano		Rashida Aliyi	25	F	Botoso
Tujube Daka	71	F	Hawa Babo		Idiris Alamu	70	M	Botoso
Tasama Biri	64	M	Hambela		GamachuMagarsa	22	M	Botoso
Tilahun Sori	47	M	Hambela		Tayech Tesema	21	F	Botoso
Tariku Regasa	52	M	Hambela		Ashanafi Tasema	27	M	Botoso
Bayisa degefa	41	M	Gaba Robi		Kabade Bula	55	M	Machara
Sintayo Tesfa	31	F	GabaSanbatdura		Kababu Tariku	47	F	Machara
Diribe Gemechu	40	F	Kela		Dasatu Abera	28	F	Machara
Getacho Mokonin	32	M	kela		Gutu Tamiru	22	M	G/S/Dura
Muzamil Hasan	23	M	kela		Tariku Tarefa	54	M	G/S/Dura
Buseri Duki	67	M	Kela		Nabiyu Shabe	26	M	G/S/Durar
Fatuma Hordofa	63	F	Kela		Diriba Suki	75	M	H/ fincho
Marsha Zarihun	41	M	Gaba Robi		Yosef Jaleta	39	M	G/Robi
Ahimad Ayale	40	M	GabaSanbatdura		Kancher Diriba	35	M	H/fincho
Olika Wayesa	96	M	Modjo		Motu Warkina	54	F	G/Robi
Kenea Denki	52	M	Modjo		Ligidi Musa	87	M	H/Babo
Diriba Hambisa	47	M	Modjo		Kenatu Sima	50	F	Modjo