EVALUATION OF EFFECTS OF CAPSAICIN IN SUPPRESSION OF PLANT PATHOGENS IN TOMATOES.

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2022

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I **Karanja Charity Wanjiku** declare that this dissertation is my original work and has not been submitted or presented for a degree in any other university

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DEDICATION

I dedicate this project to God who has been my source of inspiration, wisdom, knowledge and understanding. I also dedicate this work to my parents Mr. and Mrs. Karanja who have encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I started. To my siblings Grace and Victor, I dedicate this work to for you became such a strong support system. Finally, I dedicate this work to my supervisors, Dr. Kunyanga, Dr. Njue and Dr Muiru. This work was only possible through your commitment, insight and guidance.

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ABBREVIATIONS

DPPH- Diphenyl-1-picryl-hydrazyl

EU – European Union

KEPHIS – Kenya Plant health Inspectorate Service

MBC- Minimum bactericidal concentration

MIC – Minimum inhibitory concentration

MRLs – Maximum residue levels

PCPB – pest control product board

PHI – pre harvest interval

PTC – plasma total cholesterol

RNS – Reactive nitrogen species

ROS – Reactive oxygen species

SMO – Singlet molecular oxygen

WHO – World Health Organization

ABSTRACT

Chilies contain an active component known as capsaicin. It is hydrophobic and is the main capsaicinoid. Due to this component, chilies have obtained certain bioactive components such as antioxidants, phenolic and anti-microbial properties. Based on the mentioned characteristics, chilies exhibit bio pesticide characteristics which can be used to control pest and diseases in crops.In this study, four varieties of chilies were used, including habanero, bird's eye, cayenne and bullet chilies. This was to assess the potential utilization of capsaicin in control of late blight fungal disease and bacteria speck in tomatoes

To determine the capsaicin content in the four varieties, extraction of capsaicin from both oven-dried and fresh chilies was conducted using three different solvents at different amounts namely 100ml, 150ml and 200ml which included, methanol 99%, a mixture of methanol 99% and acetone and ethanol 99%. Evaluation of the antioxidant and total phenolic composition of capsaicin from the four varieties was analyzed using the Dipheny1-1-picrtyl-hydrazyl (DPPH) method and the absorbance at λmax 760nm measured on a spectrophotometer respectively. Determination of the sensitivity of capsaicin on late blight and bacterial speck in tomatoes was conducted using the agar dilution method. Moreover, determination of the antimicrobial properties of capsaicin from the four varieties of chilies was conducted by examining the minimum inhibitory concentration and the minimum bactericidal concentration.

From extraction using different solvents at different amounts, the ideal solvent was said to be ethanol at 200ml giving an ideal extraction ratio of 1:10. Fresh habanero, bird's eye, cayenne and bullet chilies had the highest amount of capsaicin respectively at an amount of 23.09 mg/g, 12.36mg/g, 7.37mg/g and 3.80mg/g respectively. Oven died habanero, bird's eye, cayenne and bullet chilies had the highest amount of capsaicin respectively at an amount of 16.84mg/g, 9.60mg/g, 6.39mg/g and 3.21mg/g respectively. Therefore, fresh chilies had a higher amount of capsaicin than oven dried chilies.

A positive correlation was observed between the amount of capsaicin present in the chili varieties and the bioactive components available in the chilies. Total phenolic was highest in both fresh and oven- dried habanero, bird's eye, cayenne and bullet chilies respectively, having

fresh chilies with a higher total phenolic than oven-dried chilies. Total phenolic ranged between 2923mg/g- 352mg/g in fresh chilies and 2274.5mg/g – 117mg/g in oven-dried chilies.

Similarly, to total phenolic, there was a correlation between the amount of capsaicin in a variety and antioxidant activity in the same. Antioxidant activity was highest in both fresh and oven-dried habanero, bird's eye, cayenne and bullet chilies respectively, having fresh chilies with a higher antioxidant activity than oven-dried chilies. Antioxidant activity ranged between 50%-86% in fresh chilies and 46%-74% in oven-dried chilies.

Capsaicin from fresh chilies had a higher inhibition than oven-dried chilies. Bacterial speck was more susceptible to capsaicin than late blight across all varieties. Zone of inhibition for bacterial speck ranged between 0.96cm – 2.48cm in fresh chilies and 0.63cm – 1.95cm in oven-dried chili having habanero with highest zone of inhibition and bullet chili the lowest. Zone of inhibition for late blight ranged between 0.68cm – 2.22cm in fresh chilies and 0.56cm – 1.75cm in oven-dried chili having habanero with highest zone of inhibition and bullet chili the lowest as well.

Capsaicin from fresh chilies had a higher MBC than oven-dried chilies. Late blight was more susceptible to the capsaicin than bacterial speck across all varieties. Minimum bactericidal concentration for late blight ranged between 0.01% - 0.18% in the first tube and 0.21% - 0.53% in the second tube incubate, using fresh chilies and 0.02% - 0.20 in the first tube and 0.29% - 0.55% in the second tube incubated, using oven-dried chili having habanero with highest minimum bactericidal concentration and bullet chili the lowest. Minimum bactericidal concentration for bacterial speck ranged between 0.01% - 0.19% in the first tube and 0.26% - 0.55% in the second tube incubate, using fresh chilies and 0.02% - 0.21% in the first tube and 0.30% - 0.61% in the second tube incubated, using oven-dried chili having habanero with highest minimum bactericidal concentration and bullet chili the lowest.as well.

From the study, capsaicin from habanero exhibited the most preferred characteristics of bio pesticides from the four varieties tested. A positive correlation between amount of capsaicin and the efficiency of the bio active components characteristics as a bio pesticide was observed. Thus, the higher the amount of capsaicin, the higher the efficacy of capsaicin as a bio pesticide.

More study of the efficacy of capsaicin in diseases in tomatoes under in vivo environment is recommended to evaluate the influence of environmental conditions in the bio pesticide properties

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Chilies contain an active component known as capsaicin. It is hydrophobic and is the main capsaicinoid (Adiellsson, 2009). It has been shown to contain bioactive compounds that have potential antimicrobial and antifungal properties. Further, it is also very rich in antioxidants and is attributed to the ability to act as a *scavenger* of singlet molecular oxygen (SMO), reactive oxygen species (ROS), peroxyl radicals and reactive nitrogen species (RNS) (Derbalah A, 2019). It has gradually been adapted to control diseases in various crops but the dosage of each of these bioactive compounds required to inhibit the development of a disease and microbial growth is yet to be established (Gervais *et al*., 2008).

Tomato (Solanum lycopersicum) is from the nightshade family and originated from South America in the 16^{th} century. It is known to be rich in various nutrients that play a great role in the functioning of the body systems. Fiber aids in our digestive health and regular bowel movements. Vitamin C which is an essential nutrient acts as an antioxidant, potassium aids in blood pressure control and heart disease prevention, Vitamin K_1 helps in blood clotting and bone health while Folate aids in normal tissue growth and cell functions. It is also rich in carotenoid that is responsible for the red pigmentation in tomatoes and considered an ;antioxidant as well, beta carotene which is an antioxidant and is converted into vitamin A in our bodies. Naringenin is a flavonoid that aids in decreasing inflammation. Chlorogenic acid is a powerful antioxidant and is known to lower blood pressure (Bjarnadottir, 2019) and many other beneficial components. All these beneficial traits in tomatoes are dependent on the physiological and phenological traits in the tomato. If any of the traits is compromised, the levels of the beneficial traits are reduced or depleted (Vliet, 2017)

Tomatoes constitutes 98 % of the vegetables usually consumed at household level and 82% of the most consumed vegetable from high end to low level consumer in Kenya ranking as the highest consumed vegetable (Dijkstra, 2015). This shows the importance of tomatoes in Kenya.

From a report released by Kenya Plant Health Inspectorate Service (KEPHIS) in 2017, the major challenges faced in tomato production is plant pest and diseases and high pesticide residues ranking at 54% and 48% respectively. Many of these diseases occur both in open field and controlled environment and are classified as either fungal, bacterial or viral. Some of the

major diseases include bacterial canker, bacterial speck, bacterial spot, bacterial wilt, buckeye rot, early blight, fusarium wilt, gray mold, late blight, tomato mosaic virus, tomato spotted wilt, tomato yellow leaf curl among others (Jones , et.,al 2014). These diseases have posed to be the largest threat to tomato production in Kenya. Diseases are most prevalent in the tropics (savary, 2014)and damage ranges from reduced plant vigor to plant death by interfering with plant physiological processes therefore the plant losses its ability to synthesize its bio chemical properties and nutritive qualities (Muhammad, 2014). Therefore, the range of diseases attacking tomatoes is large and needs effective disease control methods. The increased demand of tomatoes on account of population explosion and increased consumption frequency of tomatoes has compelled the use pesticides for better crop protection (Tomer, 2013). Continuous use and accumulation of synthetic pesticides have led to pesticide toxicity which has resulted to oxidative stress on plants as a result of the generation of reactive oxygen species (ROS) (Xia *et al*., 2009). Several cases of synthetic pesticide poisoning are recorded every year. Risks of poisoning depend on toxicity, dose and period of exposure. Pesticide toxicity may cause neurological and psychiatric complications, brain tumors, cancers, spontaneous abortions, stillbirths, and birth defects. This has led to use of bio pesticides as an alternative since they are able to breakdown naturally causing no toxicity. Therefore, the extract chili can be used as an alternative to synthetic pesticides to control diseases and increase the level of antioxidants in tomatoes (Sawhney *et al*., 2016).

1.2 Statement of the problem

Chilies are rich in antibacterial and antifungal properties (savary, 2014). Heavy investment on the efficient uses of synthetic products in relation to anti-microbial properties. However, very limited data and information of use of natural antibacterial and antifungal extracts or products in control of microbial growth in tomatoes despite its contribution to nutrition and health (Tsimbiri, 2015).

Bioactive compounds in chili are underutilized and can be used to develop bio products with anti-microbial properties (Mitem S.E, et al., 2018). There are very few products which have been developed from chilies while they have the ability to develop a potential bio product.

Kenya chili production is only 1% of the horticultural production. Production mainly targets export market with local consumption being as low as 6% of the total production (KEPHIS,

2016), this shows that there is low utilization of chili in Kenya as human food and commercial product.

This study will therefore explore ways of utilizing chilies as a functional food and antimicrobial in making bio pesticides in tomato production.

1.3 Justification

According to World's Healthiest Foods, tomatoes stands out in terms of the mix of phytonutrients components such as anti-inflammatory, antioxidants, minerals, vitamins and many more. Besides the various benefits, it is the most consumed vegetable in Kenya proving to be readily available and affordable to most of the people in Kenya (Dijkstra, 2015). According to World Health Organization (WHO), due to the growing population and demand, the need to grow tomatoes has risen but the crop experiences wide range of diseases leading to economic losses, low yields, inability to meet demand and high pricing.

In attempt to control these diseases, high levels of pesticide residues have occurred as pre harvest intervals (PHI) are not observed due to demand. This has led to the loss of the phytonutrients and bio compounds in tomatoes leaving the consumer not benefiting from these properties but experiencing health deterioration caused by pesticide residues. From a study carried out recently, tomatoes ranked highest on having pesticide residues. The tests detected an average of 7.1 pesticides on every conventionally grown tomato sample collected, with a maximum of 19 different pesticides or breakdown products on a single sample. Four pesticides, one insecticide and three fungicides were responsible for the bulk of the residues detected on tomatoes. This came along with lower levels of nutrients and bioactive compounds in the samples (Tomer, 2013).

Several millions of cases of synthetic pesticide poisoning are registered every year. Risks of poisoning depend on toxicity, dose and period of exposure (Alexandra , 2009). Pesticide toxicity may cause neurological and psychiatric complications, brain tumors, cancers, spontaneous abortions, stillbirths, and birth defects. (Adams, 2009) Pesticides are detrimental to the environment and produce considerable damage to ecosystems. They may be harmful to non-target species, pollute air, water and soil. Pesticides considerably affect natural biological equilibrium, diminish biodiversity, reduce nitrogen fixation, contribute to the disappearance of pollinators, threaten fish, and destroy bird and animal habitats (Tomer, 2013). This has led to

recommendation of bio pesticides which naturally breakdown and help in maintaining the beneficial properties of tomatoes and also aid in reducing the levels of pesticide residues and observing MRLs.

Chilies have been recently used as a bio pesticide due to various active compounds which are been known to be antifungal and antibacterial. However, there is limited knowledge on the dosage of the optimum concentration required to inhibit growth of the fungus and reduce microbial activity (Gervais *et al*., 2008). The results of this study will help give a recommended dosage of the optimum concentration of extract chili and the level of antioxidants in the extract chili which will also be beneficial to tomatoes.

1.4 Aim.

To assess the potential utilization of capsaicin in control of major fungal and bacterial plant pathogens in tomatoes.

1.5 Purpose of the study.

To contribute to the reduction of pathogenic contamination in tomatoes with bioactive compounds in chili.

1.6 Objectives

1.6.1 General Objective;

To evaluate the antimicrobial and antioxidant properties of bioactive compounds in capsaicin for use in the suppression of plant pathogens in tomatoes.

1.6.2 Specific Objectives;

i. To determine the capsaicin content of chili.
ii. To evaluate the total phenolic composition of capsaicin and antioxidant activity.
iii. To determine the suppressive effect of capsaicin on late blight and bacterial speck in tomatoes.
iv. To determine the antimicrobial (antibacterial and antifungal) properties of capsaicin.

1.7 Research questions

i. What is the capsaicin content of chili?
ii. What is the total phenolic composition of capsaicin and antioxidant activity?
iii. What concentration of capsaicin inhibits the growth of late blight and bacterial speck in tomatoes?
iv. What is the antimicrobial (antibacterial and antifungal) properties of capsaicin?

CHAPTER 2: LITERATURE REVIEW

2.1 Chili

2.1.1 Nutritional composition

Chilies are rich in vitamins (Chalupowicz, et al., 2019). Some of the vitamins include vitamin C which is a very powerful antioxidant, important for wound healing and immune function. Vitamin B6 which plays a role in energy metabolism. Vitamin K1 which is essential for blood clotting and healthy bones and kidneys (Lukyanenko A, 1991). Vitamin A which is very good for eyesight. It also possesses some minerals which include potassium which reduces the risk of heart disease and copper which is essential for strong bones and healthy neurons (Anarson, 2019).

Chilies are also containing bioactive plant compounds which provide various health benefits. Some of these compounds include, capsaicin, which **is t**he main carotenoid in red chili peppers consisting of up to 50% of the total carotenoid content (Castros, et al., 2008). It is has contributed to the red color and antioxidant properties that help in fighting cancer. Violaxanthin another major carotenoid antioxidant in yellow chili peppers, contributes about 37–68% of the total carotenoid content. Lutein is high in green (immature) chili peppers, which decrease with maturation but its boosts good eyesight if consumed. (Matthews & Hall , 2018). Capsaicin is one of the most studied plant compounds in chili peppers; it is responsible for their pungent (hot) flavor and many of their health effects. Sinapic acid and ferulic acid are also good sources of antioxidants**.** Therefore, chilies' color is provided by carotenoids and its flavor by capsaicinoids, which are considered bioactive compounds due to their beneficial effect on health (Dorantes, *et al* , 2011).

2.1.2 Bioactive components in chili

The bioactive compounds of fruits are the products of secondary metabolism (Mayo, 2018). Chili peppers are a source of vitamin C, which is well known for its antioxidant capacity. Vitamin C not only acts as an antioxidant, but is also an essential bioactive compound that can prevent heart disease, neuro-degenerative diseases, cancer, and hypertension (Matthews & Hall , 2018). One group of bioactive compounds that provide the color of chili peppers is the carotenoids (Anarson, 2019). Among the most important carotenoids in Capsicum are a-

carotene, b-carotene (which is a precursor of vitamin A), violaxanthin, capsanthin, capsorubin, and capsanthin. The main carotenoid that provides an intense red color is the capsanthin family, which is esterified with fatty acids as mono-esters and di-esters, and these represent 50% of the total of carotenoids in ripe fruits. Oxidation is the leading cause of the loss of carotenoids, depending on the amount of available oxygen. Heat, light, metals, enzymes, and peroxides stimulate oxidation, causing the loss of color and reducing the activity of vitamin A and other biological activities (Ware , 2018). Carotenoids, including capsanthin, are considered functional phytochemicals because they act as antioxidants, a function that could prevent colon cancer. Other studies have reported that their properties prevent ulcer development due to their protective effect in the gastric mucosa (savary, 2014). Pungency and part of the biological activity that provides the beneficial health effects of most chilies are due to alkaloids, such as capsaicin (C), dihydrocapsaicin (DHC), nor-dihydrocapsaicin (NHC), homocapsaicin, and homodihydrocapsaicin, which differ from one another in the length of their aliphatic chains (Z & Bode , 2011). This group of compounds is known as the capsaicinoids,of which approximately 20 compounds are known. Capsaicin [(E)-N-(4-hydroxy-3methoxybenzyl)-8-methyl-6-nonenamide)] and DHC [(N-(4hydroxy-3-methoxyphenyl) methyl-8-methylnonanamide)] contribute between 80 and 90% of the total pungency of peppers (Kazuma, *et al*, 2014). Other outstanding beneficial effects to human health provided by capsaicinoids are their stimulation of the cardiovascular system, anti-inflammatory capacity and anticancer effects.

2.1.2.1 Capsaicin in chilli

It is an active component of chili pepper. It is non-polar therefore hydrophobic and mixes freely with fats (dissolves in alcohol or alcoholic beverages), colorless, odorless, and crystalline to waxy compound (Sarwar , 2014). It is the main capsaicinoid (the name given to the class of compounds found in member of the capsicum family and is mainly concentrated in the pith of the pepper (placental tissue where the seeds of the plant grow. Capsaicin, the main bioactive plant compound in chili peppers, has some unique properties. It binds with pain receptors, which are nerve endings that sense pain. This induces a burning sensation but does not cause any real burning injuries (Agoreyo, *et al* , 2011).

2.2 Antimicrobial properties in capsaicin

2.2.1 Antifungal properties

Capsaicin is used as herbal medicine for a variety of ailments of probable microbial origin (Harbant *et al* 2011). It showed that at very low concentrations, it could completely inhibit the growth of Aspergillus niger (Gali *et al*., 2010). It is known to possess antibacterial and antifungal properties by disrupting the membrane integrity of fungal cells (De Lucca *et al*., 2006)

2.2.2 Antibacterial properties

Besides its multiple pharmacological and physiological properties (pain relief, cancer prevention, beneficial cardiovascular, and gastrointestinal effects, capsaicin has recently attracted considerable attention because of its antimicrobial and anti-virulence activity (Nadendla , *et al* , 2018). A bactericidal effect has been described against food-borne pathogens, Helicobacter pylori, and Pseudomonas aeruginos, whereas an anti-virulence activity has been demonstrated against Vibrio cholerae, Staphylococcus aureus, and Porphyromonas gingivalis (Abong'o, 2018).

2.2.3 Insecticidal properties

Capsaicin overwhelms the nerves with a burning sensation and the nerves are unable to report pain for an extended period of time. (I & Oleszek, 2000) With chronic exposure to capsaicin, neurons are depleted of neurotransmitters and it leads to reduction in sensation of pain and blockage of neurogenic inflammation. When capsaicin comes into contact with the skin surface, eyes or mucous membrane it is very painful (Ware , 2018). In large quantities, it causes death by leading to breathing difficulties, blue skin and convulsions. In mites and insects, it damages membranes in the cells and disrupt nervous system.

2.2.4 Antioxidant activity

Capsaicin is an antioxidant especially in its phytochemical profile (chemical compounds produced by plants generally to help them thrive or thwart competitors, predators or

pathogens), it makes it an ideal choice for pesticides. Major antioxidants in capsicum family are carotenoids, tocopherols and capsaicinoids (capsaicin) (Bender & Bender , 2018). This component plays a role as an anti-cancer attributed to its ability to act as scavengers of singlet molecular oxygen, reactive oxygen species (ROS), peroxyl radicals and reactive nitrogen species (RNS). Capsaicinoids intake effectively reduce the triacyclglycerols, plasma total cholesterol (PTC), and non-high-density lipoprotein cholesterol, and thereby helps in the prevention of cardiovascular ailments (Alexandra , 2009). It also exhibit effective and proactive contribution against age-related ailments. Capsaicin exposure expressively repressed the initial adipogenic differentiation, maturation, and lipogenesis of adipocytes. Capsaicin also has ability to target the TRPV1 receptors in the C-fibers lead to their stimulation followed by desensitization that helps to improve the neurogenic bladder. So, it may serve as a potential emerging treatment for patients who are non-respondent to conventional therapy especially those with neurogenic bladder (Alexandra , 2009).

2.3. Tomato

2.3.1 Overview

Tomato (Solanum lycopersicum) is an edible fruit from Kingdom Plantae, Class Tracheophytes, Order Solanales, Family Solanaceae, Genus Solanum and Species S.lycopersicum. It is consumed either raw or cooked and has a significant source of flavor. As much as it is classified as fruit it is mainly consumed as a vegetable ingredient or a side dish. Most varieties are grown in temperate climates across the world and recently greenhouses have allowed production of tomatoes throughout the seasons of the year. There are two main varieties that are grouped as either indeterminate tomatoes which are perennial crops or determinate which are cultivated as annual crops. They originated from South America in the 16^{th} century (Bjarnadottir, 2019).

Tomatoes are a good source of fiber. It provides about 1.5 grams of fiber in an average-sized tomato. Tomatoes have 87% of fiber in an insoluble form of hemicellulose, cellulose and lignin which promote movement of material in the digestive system and increase stool bulk thus preventing constipation and irregular stools (Mayo, 2018). It is also a source of carbs which include simple sugars such as glucose and fructose which are a source of energy to the body and the brain and are fairly easy to digest (Manzella, 2019). They are also a source of vitamins such as Vitamin C which is essential for nutrient and antioxidant activity, Vitamin K which is

important for blood clotting and bone health, Vitamin B9, which is also known as folate, is important for normal tissue growth and cell function. They are also a source of minerals such as potassium which is beneficial for blood pressure control and heart disease prevention (Bjarnadottir, 2019).

According to Food and Agriculture Organization (FAO), tomato consumption increased from 7.5 kg/capita/year in 1963 to 20.5 kg/capita/year in 2009 on world average and has continually increased over the years. In a recent study, Asia ranks highest in tomato consumption at 159 kg/year /capita and America ranking last at 55 kg/year/capita (Garming, 2014). In Kenya, tomatoes are rated at 98 % of the vegetables usually consumed at household level and is at 82% of the most consumed vegetable from high end to low level consumer in Kenya ranking it as the highest usually consumed vegetable and most consumed vegetable (Dijkstra, 2015).

Tomatoes is susceptible to more than 200 diseases and losses to diseases could be as high as 70-95% if they are not managed (Lukyanenko A, 1991). According to a report released by Kenya Plant Health Inspectorate service in 2017, the major challenges faced in tomato production is plant pest and diseases ranking at 54% (Jones , *et al* 2014). Tomato diseases (biotic) could either be fungal, bacterial or viral having soil borne and foliar fungal diseases which are the common cause of infectious plant diseases been the major limiting factor for the vegetable that causes serious yield reduction causing to severe economic losses. These diseases are contagious and can spread from plant to plant in a field especially if the climatic conditions are conducive for the development of the disease. The warm and cool climatic conditions provide an ideal condition for the development of many foliar, stem and soil-borne plant diseases (Kumar , *et al* , 2018). Most of the pathogens are fungal in origin with bacterial and viral borne infections close behinds with symptoms varying with the host. However, there are certain definitive symptoms that help in the positive identification of the target pathogen (Fuentes, 2018).Therefore it is very important to identify and understand the disease agent and determine if it is infectious or not to implement the correct control measures.

2.3.2 Common Diseases in tomatoes

2.3.2.1 Bacteria Spot

Bacterial spot is a bacterial disease which is caused by four species of Xanthomonas (X. euvesicatoria, X. gardneri, X. perforans, and X. vesicatory). X. perforans is the predominant

species which is responsible for bacterial spot in tomatoes. All the four species are strictly aerobic, gram negative and have a flagellum that allows them to move in water thus can easily invade a wet plant tissue easily and cause infection (Amanda , *et al* , 2019). Moist and high humidity climate is very conducive for the multiplication of this bacteria. Primarily, the bacteria cells survive on infected plant debris, seed and solanaceous weeds and then the bacteria cells are transmitted through rain splash, irrigation water, aerosols, and handling wet plants. The bacteria could also invade through the natural openings such as stomata and also wounds (Chalupowicz, et al., 2019). Some of the symptoms include, infected leaves showing small, brown, water soaked, circular spots surrounded with yellowish halo. On older plants the leaflet infection is mostly on older leaves and may cause serious defoliation. The most striking symptoms are on the green fruit where small, water-soaked spots first appear which later become raised and enlarge until they are one-eighth to one-fourth inch in diameter. Centers of these lesions become irregular, light brown and slightly sunken with a rough, scabby surface. Ripe fruits are not susceptible to the disease. Surface of the seed becomes contaminated with the bacteria, remaining on the seed surface for some time (Gilbertson, 2013). However on leaflets, bacterial spot can be easily confused with the early symptoms of bacterial speck, early blight, gray leaf spot, target spot, or Septoria leaf spot. When Xanthomonas is present, bacteria will ooze from infected tissue and can be observed under a light microscope. Oozing will not be observed in lesions caused by fungal pathogens (Amanda , *et al*, 2019).

2.3.2.2. Bacterial Speck

Bacterial speck is caused by the bacteria Pseudomonas syringae pv. The bacteria survives in soil, debris from diseased plant and seeds. Spread of the disease is favored by cool, moist weather and the disease progress is stopped during the hot season. Overhead irrigation and rain splash aids in the spread of the disease. This disease has almost similar symptoms as bacterial spot but is less severe. However, some of the difference is, the leaf lesions are mostly concentrated at the margins of the leaf causing marginal necrosis, they are slightly raised and small and penetrate more than a few cells deep (Gilbertson, 2013).

2.3.2.3 Bacterial Canker

Bacterial canker is caused by the bacteria Clavibacter michiganensis. The bacteria survives in soil, debris from diseased plant, weeds host, volunteer plants and seeds. It can survive in an infected seeds for at least five years and still cause infection, three to four weeks in the soil and

twenty four months in infected debris (Amanda , *et al* , 2019). The bacteria is favored by warm moist conditions. When the plants are tender, the bacteria could be spread through rain splash, irrigation water, aerosols, and handling wet plants and when the plants are mature enough and appears in a field, the pathogen may spread to adjacent plants and infect them through pruning wounds and injury, or through naturally occurring pores along the leaf surface (stomata) or leaf margins (hydathodes). The pathogen can also be moved quite easily by equipment during cultivation. Some of the symptoms include, marginal necrosis in the leaves, discoloration of the vascular tissues, necrotic patches on the leaves and stems, Small dark spots on the fruit surrounded by a white halo and infected leaves die, and light brown streaks or cankers, which may darken with age, develop on infected stems (Ruguo, 2007).

2.3.2.4. Early Blight

Early blight is a fungal disease, Alternaria sp that affects both seedlings and older plants. It thrives in warm moist temperature and is mainly harbored in old plant debris or in the soil. The spores spread by wind, rain and occasionally through pest. The fungal spores enter through wounds in the plant cuticles. Some of the plant symptoms include, irregular dark spots in the lower and mature leaves which enlarge and are surrounded by a yellow area. The entire leaf is killed and can lead to defoliation where the fruit is exposed to sunscald and can lead to yield loss (Tomer, 2013).

2.3.2.5. Late Blight

Late blight is caused by a fungus Phytophthora infestans. It occurs later at the growing season of tomatoes and shows symptoms after blossom. Unlike most diseases, the fungus is not haboured in the soil or plant debris but is introduced to the plant by infected tubers, transplants seeds or by wind from nearby infected areas. Warm and wet conditions promote its rapid spread. This disease appears on the lower mature leaves as water soaked grey spots which later darken and white fungal growth forms on the undersides (Tomer, 2013).

2.3.2.6 Fusarium wilt

Fusarium wilt is caused by fungus Fusarium oxysporum sp. It is a soil borne fungus and develops quickly in soils that are rich n nitrogen and low in potassium. It can survive in the soil or plant debris for about ten years and is favored by warm soil temperature. The fungus enters

the plant through their roots and spreads to the plant through the water conducting vessels. Fusarium causes yellowing of one side of the plant and leaves followed by wilting, browning and defoliation. Growth is stunted and little or no fruits develop and in some cases, the vascular tissues become brown in infected stems (Cilas , *et al*, 2016).

2.3.2.7. Damping off

Damping off is caused by either fungus Pythium, Rhizoctonia or Phytophthorathat. It attacks tomato seeds, tender stems and roots. It thrives well in humid conditions especially in cold wet soils. Although the fungus lives in the soil and water, the spores move fast in air and can easily spread from seed to seed or farm to farm. Healthy young tomato plants infected by damping-off disease look pinched or cut off at the base of the stem. They wilt, droop over, wither away, and die. (Chalupowicz, et al., 2019) A white mold-like growth may appear on the soil surface and on the dead plants. There are two types of damping off: pre-emergent and post-emergent. Pre-emergent damping-off**:** seeds rot in the soil or seedlings decay before they push through the soil. Post-emergent damping-off: seedlings sprout, but then pale, curl, wilt, or collapse at the soil line. The stem is water-soaked and turns gray, brown or black before disintegrating (Brauer, et al., 2019).

2.3.3. Synthetic pesticides in tomatoes

Diseases account for yield losses of tomatoes up to 80 – 100% per growing season. This has therefore led to efforts in getting a solution to controlling diseases. For control of the bacterial and fungal disease, fungicides have been developed to control fungal diseases and bactericides to control bacterial diseases. These pesticides have both active and inert ingredients. The active ingredient controls or inhibit the growth and development of the microorganism while the inert ingredient which often constitutes to over 95% of the pesticide is mixed into the product as a carrier or a sticking agent. Plants uptake these products through the natural openings such as the stomata which then diffuse to the plant or through the root system and is absorbed to the plant through the vascular bundles. Most of these products are copper based and are used throughout the cycle of production from seeds sowing to post harvest (Brauer, et al., 2019). This has therefore seen Kenya import 15,600 tones in 2018 from 6400 tones in 2014 of these pesticides with a growth rate of 144%.This has accounted for 87% increase in terms of volume and 88% in terms of total cost (Abong'o, 2018).

The increase in use of these pesticides requires safe guards to control how they are applied, observation of pre harvest intervals (PHI), maximum residue levels (MRLs) and pesticide residues. This has become very challenging to assess in Kenya since there is no available data concerning use of pesticides, the concentration of pesticides in the soil, water and plants or any related impact (Abong'o, 2018). According to the European Union (EU), the union has funded the Kenya Plant Health Inspectorate Service (KEPHIS) to test food samples but this has been done in an irregular basis and no actual level of pesticides have been made available to the public or any regular monitoring system is in place (EU, 2013). Moreover, there is no published data on the impact of pesticide exposure to human health in Kenya, yet it has raised an alarm on the negative impacts on human health and environment (Adiellsson, 2009).

2.3.4. Negative impacts of use synthetic of pesticide in tomato production.

The heavy use of pesticides in industrial and domestic settings, has resulted in negative health, environmental and economic consequences worldwide (Ochilo , et al., 2019). Pesticides are widely distributed in the environment (like air, soil, water and plants) and as a result, water and soil quality are decreasing and there is an increase in chronic health effects that are suggested to be related to pesticide exposure. Very often not only one pesticide is present, but mixtures of different pesticides at the same time (Anarson, 2019). Many pesticides are either acutely toxic, have long-term toxic effects, are endocrine disrupters (acting on the hormone system), are toxic to different wildlife species or are known to cause a high incidence of severe or irreversible adverse effects. While farmers and rural residents are exposed most frequently and directly to pesticides, residues are found everywhere, in our food, our drinking water, in the rain and in the air (Adiellsson, 2009). No one remains untouched by pesticide exposure. Long-term exposure to pesticides can also result in chronic health effects. Accurately estimating the number of such cases is even more challenging as symptoms may develop only years after exposure, diseases are often multi-causal, and people tend to be exposed to multiple harmful substances throughout their lifetime. A few studies in Kenya established a link between pesticide exposure and acute and chronic health effects (Tsimbiri *et al.*, 2015). Moreover, pesticide manufacturers are only required to list the active ingredients in a pesticide, leaving consumers and applicators unaware of the possible toxics present in the inert ingredients of pesticide products they are using (Fuentes, 2018). Pesticide manufacturers argue they cannot release information on inert ingredients because they are trade secrets, and if released, their products could be duplicated. In addition, pesticides, when subject to various environmental

conditions, break down to other materials known as metabolites, which are sometimes more toxic than the parent material. The Pest Control Products Board (PCPB) is a statutory organization of the Government of Kenya established under the Pest Control Products Act of 1982 to regulate the importation and exportation, manufacture, distribution and use of pest control products in the country. Through the PCPB, 247 active ingredients are registered in 699 products for horticultural use. It is concerning that there are products on the Kenyan market, which are certainly classified as carcinogenic (24 products), mutagenic (24), endocrine disrupter (35), neurotoxic (140) and many which show clear effects on reproduction (Nguetti *et al*, 2018).

2.4 Knowledge Gap

Chilies are rich in antibacterial and antifungal properties (savary, 2014). Heavy investment on the efficient uses of synthetic products in relation to anti-microbial properties. However, very limited data and information of use of natural antibacterial and antifungal extracts or products in control of microbial growth in tomatoes despite its contribution to nutrition and health (Tsimbiri, 2015). Bioactive compounds in chili are underutilized and can be used to develop bio products with anti-microbial properties (Mitem S.E, et al., 2018). There are very few products which have been developed from chilies while they have the ability to develop a potential bio product.

Kenya chili production is only 1% of the horticultural production. Production mainly targets export market with local consumption being as low as 6% of the total production (KEPHIS, 2016), this shows that there is low utilization of chili in Kenya as human food and commercial product. Chilies have been recently used as a bio pesticide due to various active compounds which are been known to be antifungal and antibacterial. However, there is limited knowledge on the dosage of the optimum concentration required to inhibit growth of the fungus and reduce microbial activity (Gervais *et al*., 2008).

CHAPTER THREE: DETERMINATION OF THE CAPAICIN CONTENT OF CHILI VARIETIES GROWN IN KENYA.

ABSTRACT

This study was conducted to determine the capsaicin content in four chili varieties. Thus, the capsaicin content in the various chili varieties was to be used in evaluation and comparison of the various bio active components in capsaicin from the different chili varieties.

The experimental design involved two variables which included the chili variety and the content of capsaicin in the chilies using different amounts of different solvents. This was to demonstrate the relationship between extraction solvents on different varieties. The amount of capsaicin in the different chili varieties was determinant on the amount of the different organic solvents.

Extraction for capsaicin from peppers was done using different organic solvents. The efficiency of extraction varied with different solvents at different amounts, types of peppers and the tissue preparation of these peppers. Evaluation of the effect of different types of solvents (Ethanol, Methanol and Acetone, Methanol and distilled water) at different quantities (100, 150 and 200ml), the tissue preparation (Fresh or oven dried) and different types of peppers (Habanero, Bird's eye, Cayenne and Bullet) in extraction of capsaicin was conducted.

Across all chilies analyzed, only organic solvents managed to extract capsaicin from the peppers making it impossible for distilled water to perform extraction. Ethanol was the solvent that gave the highest yield of capsaicin followed by mixture of methanol and acetone and methanol 99% respectively. Across all solvent's quantities, 200ml of each solvent gave the highest yield of capsaicin giving a ratio of 1:10 the ideal ration while conducting extraction. Higher yield of capsaicin was obtained from fresh peppers than oven-dried peppers across all peppers having habanero, bird's eye, cayenne and bullet produce the highest yield of capsaicin respectively. Overall, higher yields of capsaicin were obtained from fresh habanero peppers using 200ml of ethanol.

This study was only limited to four varieties of chilies and thus does not conclude that habanero is the chili variety with the highest chili content. It is therefore recommended that exploration of other chili varieties is necessary in order to narrow down to the chili variety that has the ability to yield higher capsaicin than habanero.

3.1. INTRODUCTION

Capsaicin, a capsaiciniod, is an active component found in chili peppers (Adams, 2009). Capsaiciniods are flavor compounds found in chilies having capsaicin contribute 90% of its composition. Capsaicin is isolated from chili peppers by extraction using organic solvents (Adiellsson, 2009). Capsaicin is colorless, highly pungent and hydrophobic, thus not soluble in water. It is non-polar therefore hydrophobic and mixes freely with fats (dissolves in alcohol or alcoholic beverages), colorless, odorless, and crystalline to waxy compound (Sarwar , 2014). It is the main capsaicinoid (the name given to the class of compounds found in member of the capsicum family and is mainly concentrated in the pith of the pepper (placental tissue where the seeds of the plant grow. Capsaicin, the main bioactive plant compound in chili peppers, has some unique properties. It binds with pain receptors, which are nerve endings that sense pain. This induces a burning sensation but does not cause any real burning injuries *(Agoreyo, et,al , 2011)*.

Chili peppers varieties have different amounts of capsaicin. Each variety have its unique capsaicin amount which contributes highly on the development of the scoville heat unit (SHU) index (Alvarez, 2012). The higher the concentration of capsaicin in peppers the higher the SHU which measures the level of pungency of the chili pepper. Pure capsaicin rates approximately 16,000,000 SHU on the index, thus, the varieties with closer range to this value have high level of capsaicin (Anand & Bley , 2011).

Extraction of capsaicin is dependent of various factors to produce optimum yields. Some of this factors include type of solvent, amount of solvent used and tissue preparation of the chili peppers (Antonious, 2009). Determination of the suitable solvent and amount necessary for extraction of the optimum yield of capsaicin from different varieties of chili peppers is necessary to evaluate which variety gives the highest yield of capsaici (Antonious G. , 2018) .

Thus, this study aims to evaluate which solvent and its quantity is ideal for capsaicin extraction to determine which variety and under which tissue preparation gives higher yield of capsaicin.

3.2. EXPERIMENTAL DESIGN

The experimental design involved two variables which included the chili variety and the content of capsaicin in the chilies using different amounts of different solvents. This was to demonstrate the relationship between extraction solvents on different varieties. The amount of capsaicin in the different chili varieties was determinant on the amount of the different organic

solvents. The higher the amount of the organic solvent utilized, the higher the yield of capsaicin was expected. The type of the organic solvent that produced the highest yield of capsaicin in its highest amount was the solvent to be recommended for extraction of capsaicin.

3.3. MATERIALS AND METHODS

3.3.1. STUDY SITE

Chili samples were collected from Daroda Farm in Isinya, Kajiado County since the area had a suitable climatic condition for chili growing.Extraction and analysis were carried out at the food chemistry and microbiology laboratories at the Food Science Department in the University of Nairobi Upper Kabete Campus, Uthiru Kiambu County on the Northwest side of the city center, Nairobi. This was located at 247653, 9861440 and 1876 (UTM) longitude, latitude and altitude, respectively. The laboratory had the required equipment and competent staff to help in the analysis of the project. The laboratory had been utilized in many other post graduate projects making it credible for this project's analysis.

3.3.2. MATERIALS

For the process of extraction, the following materials were used; 100 grams of habanero chili, bird's eye chili, cayenne chili and bullet chili each. The reagents included; 99% methanol, Acetone and 99% Ethanol. The equipment required for this process included; a laboratory miller (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland), weighing scale, beakers, vortex mixer, measuring cylinder, test tubes, test tube rack, whatmann filter paper, funnels, rotary evaporator, a fume chamber and a fridge.

3.3.3. METHODOLOGY

3.3.3.1 Sample collection

Habanero (*Capsicum chinense*), bird's eye (*Capsicum annuum*), cayenne (*Capsicum annuum)* and bullet chili peppers were collected at Daroda farm in Isinya, Kajiado county. Harvesting took place at 5pm and samples were collected randomly. The samples were then sorted out to remove any chili with a defect or diseased.

3.3.3.2 Sample preparation

Fifty grams (50 gm) of each variety was divided into two equal portions (25 gm per portion) and stored in labelled boxes. One of the portions of each variety was dried in an air oven at

60^{o}C for 12 hours and the other portions left for milling. In a fume chamber, each variety, both fresh and dried, was milled using a laboratory mil (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland) to 1mm particle size. Using a weighing scale, twenty grams (20gm) powder of each variety, both fresh and dried, was then measured into separate labelled beakers (labelled as per the name of each variety and the tissue preparation used (dried or fresh)). This process was done in duplicate.

3.3.3.3. Methods of chili extraction to obtain capsaicin

3.3.3.3.1. Extraction with methanol 99%

The 20 gm powder in the different labelled beakers was extracted with methanol 99% at different quantities, that is, 100ml, 150ml and 200ml. The mixture was transferred into labelled test tubes and stirred for five minutes using a vortex mixer and left to rest for twenty-four hours (24hrs) in a test tube rack at room temperature. The solution was then filtered using whatmann paper no1 to remove debris and evaporated using the rotary evaporation technique. The extract was then stored in small closed labelled bottles at -20^{O}C for further analysis. This procedure was done in duplicates.

3.3.3.3.2. Extraction with methanol 99% and acetone.

The 20 gm powder in the different labelled beakers was extracted with methanol 99% and acetone at a ratio of 1.1 at different quantities, that is, 100ml, 150ml and 200ml. The mixture was transferred into labelled test tubes and stirred for five minutes using a vortex mixer and left to rest for twenty-four hours (24hrs) in a test tube rack at room temperature. The solution was then filtered using whatmann paper no1 to remove debris and evaporated using the rotary evaporation technique. The extract was then stored in small closed labelled bottles at -20^{O}C for further analysis. This procedure was done in duplicates.

3.3.3.3.3. Extraction with ethanol 99%

The 20 gm powder in the different labelled beakers was extracted with ethanol 99% at different quantities, that is, 100ml, 150ml and 200ml. The mixture was transferred into labelled test tubes and stirred for five minutes using a vortex mixer and left to rest for twenty-four hours (24hrs) in a test tube rack at room temperature. The solution was then filtered using whatmann paper no1 to remove debris and evaporated using the rotary evaporation technique. The extract

was then stored in small closed labelled bottles at -20^{O}C for further analysis. This procedure was done in duplicates.

3.4. RESULTS AND DISCUSSION

3.4.1. Capsaicin content in fresh and dried chili varieties

The use of ethanol, methanol and methanol acetone as solvents are effective in the extraction of capsaicin in chilies. However, the extraction solvents- ethanol, methanol and methanol acetone- presents a significant difference ($p < 0.05$) in the amount of capsaicin for oven-dried and fresh chilies. In addition, in each extraction solvent, there was more capsaicin extracted from fresh than the oven-dried chilies. On a close note, among the extraction solvents, the use of ethanol for both oven-dried and fresh chilies had a slightly highest amount of capsaicin. Even so, there is more capsaicin in habanero that was followed by bird's eye, cayenne and bullet, accordingly (Table 3.1). Ethanol 200ml had the highest extortion in the yield of capsaicin having fresh chilies give the highest yield.

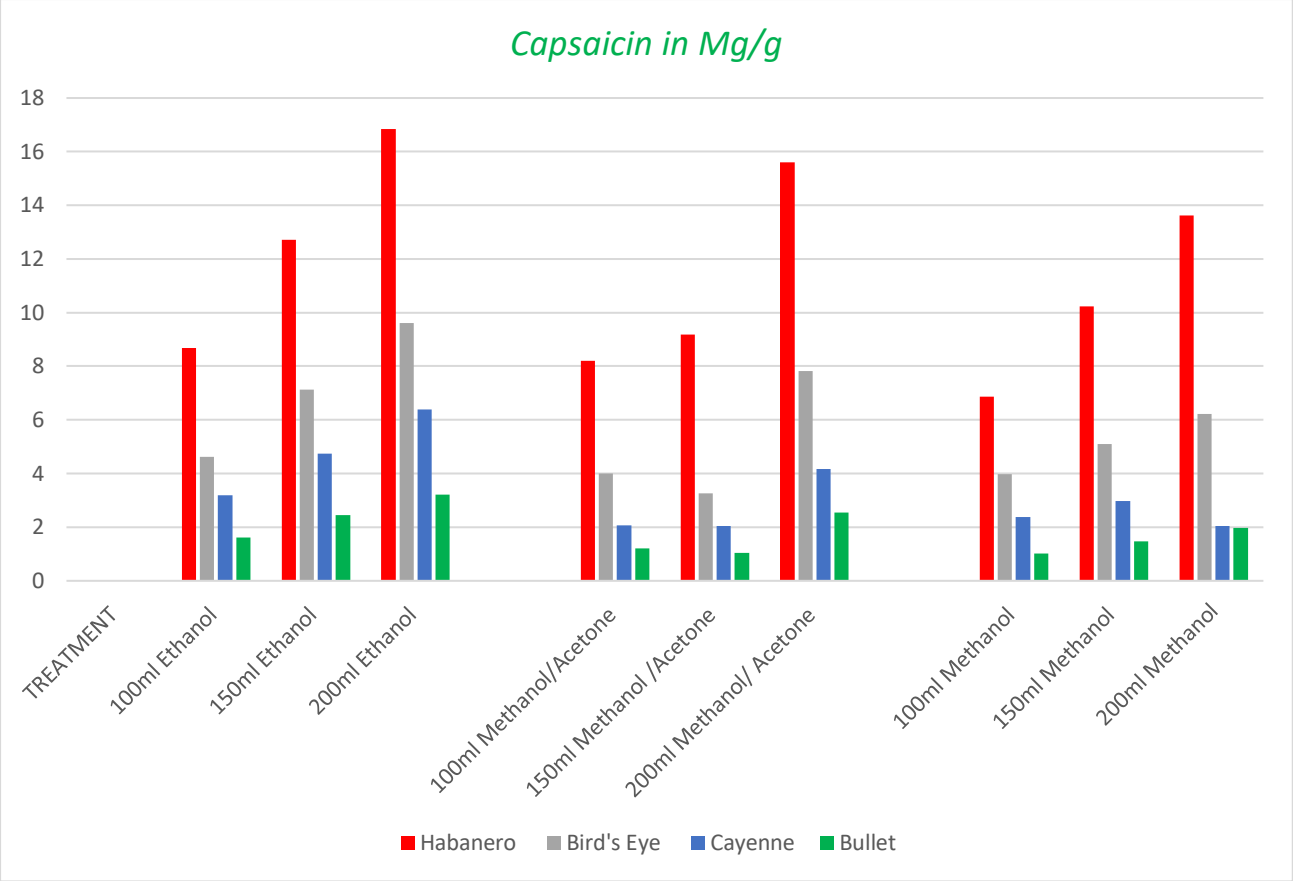

Fig 1; Capsaicin level in different varieties (Dried and Fresh) using various solvents in different quantities.

3.4.1. Effect of extraction solvents on capsaicin of oven-dried and fresh chili varieties

In all the varieties i.e; habanero, bird's eye, cayenne and bullet chilies, the use of ethanol, methanol acetone and methanol showed no significant difference ($p<0.05$) capsaicin from oven- dried chilies. However, ethanol had the slightly highest capsaicin extracted from oven-dried chilies which were followed by methanol acetone and methanol. As for fresh chilies from habanero variety, ethanol had 16.87mg/g that was significantly high than methanol acetone (13.24mg/g) and methanol (10.73 mg/g). In the bird's eye variety, ethanol had significantly highest ($p<0.05$) capsaicin of 9.21 mg/g than methanol with 6.55 mg/g. Furthermore, ethanol had slightly higher capsaicin than methanol acetone but had no significant difference. From the cayenne fresh chili variety, significantly high capsaicin of 5.52 mg/g was observed in ethanol than in methanol which had 3.92mg/g. Ethanol had a slightly higher capsaicin than methanol acetone. Following a probability of 0.072 and 0.307 from capsaicin extracted using methanol and methanol acetone, respectively. Moreover, from the fresh bullet

chilies the highest capsaicin of 2.81 mg/g was obtained using ethanol and was followed by methanol acetone and methanol, accordingly. Unlike ethanol, the amount of capsaicin between oven-dried and fresh chilies for methanol acetone and methanol are likely to be similar with a probability of 0.55 and 0.97, respectively. Therefore, from the results, the use of ethanol led to the highest capsaicin from fresh chilies (Table 3.1).

Table 3.1: Effect of extraction solvents on the level of capsaicin between oven-dried and fresh chili varieties

Treatment	Habanero			Bird's eye			Cayenne			Bullet		
	Oven-dried	Fresh	t-test (p)	Oven-dried	Fresh	t-test (p)	Oven-dried	Fresh	t-test (p)	Oven-dried	Fresh	t-test (p)
Methanol	10.24a	10.73a	0.17 (0.873)	5.11a	6.55a	1.28 (0.270)	2.47a	3.92a	2.44 (0.072)	1.49a	1.86a	0.86 (0.438)
M+A	11.00a	13.24a	0.64 (0.554)	5.03a	6.90ab	0.92 (0.412)	2.76a	4.07ab	1.17 (0.307)	1.60ab	2.34b	1.34 (0.253)
Ethanol	12.74a	16.87b	1.02 (0.367)	7.11a	9.24b	0.93 (0.407)	4.77a	5.52b	0.52 (0.629)	2.42b	2.81b	0.52 (0.633)
CV%	33.2	33.8		33.3	31.7		25	31.6		36.3	29.7	
L.S.D	2.18	2.62		2.55	1.89		2.37	1.23		0.73	0.65	

NOTE: Treatments that were methanol, M+A (Methanol+ Acetone) and Ethanol were a pool of means from 100,150 and 200ml concentrations. Bracket p (p) in the t-test represents the probability. Means in the same column not having a common letter are significantly different (P<0.05).

3.4.2. Effect of extraction solvents concentration on capsaicin of oven-dried and fresh chili varieties

Results in section 3.1 showed that the use of ethanol, methanol acetone and methanol were effective in the extraction of capsaicin among the chili varieties. Given each extraction solvent, there exist significant differences especially in their respective concentrations used and between oven-dried and fresh chili varieties. The highest yield of capsaicin was obtained from extraction solvents at 200ml and in fresh chili varieties. Therefore, an increase in either of the extraction solvent from 100-200ml showed a general increase in the capsaicin in all the chili varieties. Moreover, 200ml of either extraction solvent had almost twice as much capsaicin as that of 100ml for both oven-dried and fresh of all the chili varieties (Table 3.2).

Table 3.2: Effect of solvent concentration in the extraction of capsaicin in oven-dried and fresh habanero and bird's eye chili varieties

Treatment	Concentration (ml)	Habanero			Birds Eye		
		Oven	Fresh	t-test (p)	Oven	Fresh	t-test (p)
Ethanol	100	8.67a	11.75a	18.61 (0.003)	4.61a	6.17a	20.51 (0.002)
	150	12.71b	15.78b	61.4 (< 0.001)	7.12b	9.18b	61.27 (< 0.001)
	200	16.84c	23.09c	109.63 (< 0.001)	9.60c	12.36c	24.21 (0.002)
	CV%	0	0.4		0.8	0.7	
	L.S.D	0.38	0.58		0.12	0.38	
M+A	100	8.2a	9.25a	27.57 (0.001)	4.00b	4.78a	21.63 (0.002)
	150	9.19b	12.39b	67.73 (< 0.001)	3.26a	6.19b	142.37 (< 0.001)
	200	15.61c	18.08c	68.51 (< 0.001)	7.83c	9.74c	50.16 (< 0.001)
	CV%	0.2	0.1		0.5	0.2	
	L.S.D	0.24	0.13		0.12	0.14	
Methanol	100	6.86a	7.11a	13.59 (0.005)	3.98a	4.95a	28.77 (0.001)
	150	10.22b	10.74b	14.42 (0.005)	5.11b	6.56b	102.53 (< 0.001)
	200	13.62c	14.33c	18.05 (0.003)	6.23c	8.13c	53.74 (< 0.001)
	CV%	0.2	0.2		0.1	0.2	
	L.S.D	0.12	0.15		0.17	0.11	

NOTE: Treatments were methanol, M+A (Methanol+ Acetone) and Ethanol. Bracket p (p) in the t-test represents the probability. Means in the same column not having a common letter are significantly different (P<0.05).

In habanero, capsaicin extracted using ethanol at 100, 150 and 200 ml were significantly different ($p<0.05$) from each other in both oven-dried and fresh. Moreover, in oven-dried habanero chili, the use of ethanol at 200ml had 16.84mg/g capsaicin which was significantly highest than that of 150ml (12.71mg/g) and 100ml (8.67mg/g). A similar trend was observed in fresh habanero chili whereby ethanol at 200ml had significantly highest capsaicin compared to 150ml (15.78mg/g) and 100ml (11.75mg/g). (Table 3.2).

In the bird's eye variety, 9.60mg/g of capsaicin in oven-dried bird's eye's chilies was significantly highest ($p<0.05$) compared to 7.12 and 4.61mg/g extracted from 200, 150 and 100ml ethanol, respectively. In fresh bird's eye chilies, capsaicin extracted with 100, 150 and 200ml ethanol were significantly different ($p<0.05$) and were 6.17, 9.18 and 12.36mg/g, respectively. (Table 3.2).

In methanol acetone, each concentration used significantly differed ($p<0.05$) from the other. Of note, in oven-dried bird's eye chili, the least capsaicin of 3.26mg/g was obtained from 150ml methanol acetone whereas the highest of 7.83mg/g was in 200mg/g. This was conversely to previous trends that seen the least capsaicin with 100ml in all the extraction solvents. As of this concentration, 100ml methanol acetone had 4.00mg/g capsaicin as the second-highest in oven-dried birds-eye chili. On the other hand, fresh bird's eye chili was shown to have the highest capsaicin of 9.74, 6.19 and 4.78mg/g from 200, 150 and 100ml methanol acetone, respectively. Fresh chili had more capsaicin than oven-dried bird's eye chilies as denoted by their probability of less than 0.002 of being similar (Table 3.2).

Oven-dried and fresh bird's eye chilies capsaicin to be similar in 100, 150 and 200ml methanol have a low probability, hence differ among each other. However, across the methanol concentrations of 100,150 and 200ml, there is a significant difference ($p<0.05$) in oven-dried and fresh bird's eye chilies. For instance, the highest capsaicin of 6.23mg/g followed by 5.11 and 3.98mg/g was observed in oven-dried whereas 8.13mg/g followed by 6.56 and 4.95mg/g were in fresh bird's eye chilies with 200, 150 and 100ml methanol, respectively (Table 3.2).

Results showed that capsaicin in oven-dried and fresh cayenne chilies was different following a very low likelihood of being similar in each of the ethanol concentrations. Moreover, across the ethanol concentrations which were significantly different ($p<0.05$) from each other, the highest and lowest capsaicin were 6.39 and 3.20mg/g in oven-dried whereas 7.37 and 3.61mg/g were in fresh cayenne chilies. The second highest capsaicin of 4.73 and 5.57mg/g in oven-dried and fresh cayenne chilies, respectively, were observed in 150ml ethanol (Table 3.3).

Use of methanol acetone had 4.18mg/g as the highest capsaicin extracted from 200ml than in both 150ml (2.05mg/g) and 100ml (2.06mg/g) in oven-dried cayenne chilies. The use of methanol acetone at 100 and 150ml had no significant difference in their capsaicin extracted from oven-dried cayenne chilies. Conversely, in fresh cayenne chilies, all the methanol acetone concentrations were significantly different ($p<0.05$) in their capsaicin extracted. From 100 to 200ml methanol acetone, there was an increase of 5.76 to 2.87mg/g in the extracted capsaicin. Given the low probability in oven-dried viz. fresh cayenne chilies, it is evident that their capsaicin differed (Table 3.3).

In methanol, all the concentrations were significantly different ($p<0.05$) for oven-dried and fresh cayenne chilies. The highest methanol concentration of 200ml was observed to have the least capsaicin (2.05mg/g) extracted from oven-dried chilies. However, 150ml methanol (2.97mg/g) had higher capsaicin in oven-dried chilies followed by 100ml(2.38mg/g). As for the fresh cayenne chilies, 200ml methanol (4.85mg/g) was ranked first whereas 150 (3.92mg/g) and 100ml (3.00mg/g) were second and third, respectively, in capsaicin extracted. Oven-dried and fresh cayenne chilies extracted capsaicin was not similar due to an established low probability (Table 3.3).

Bullet chilies had general low capsaicin extracted with the three extraction solvents compared to other chili varieties. Yet, in ethanol, all the three concentrations were significantly different ($p<0.05$) from each on the extracted capsaicin for oven-dried and fresh bullet chilies. In this context, the highest capsaicin of 3.21mg/g followed by 2.44 and 1.62mg/g was observed in oven-dried chili whereas the highest of 3.80mg/g followed by 2.84 and 1.78mg/g were all extracted using 200, 150 and 100ml ethanol, respectively. Differences in capsaicin between oven-dried and fresh were shown by a low probability across the ethanol concentrations. This difference further revealed that there is more capsaicin extracted from fresh than oven-dried bullet chilies within all the ethanol concentrations (Table 3.3).

The use of methanol acetone at 100 and 150ml had no significant difference in capsaicin extracted for both oven-dried and fresh bullet chilies. Only 200ml methanol acetone was significantly different ($p<0.05$) from other concentrations in the extracted capsaicin for both oven-dried and fresh bullet chili. Capsaicin of 2.54 and 2.91mg/g was highest for oven-dried and fresh bullet chilies following the use of 200ml methanol acetone. However, in oven-dried bullet chili, 100ml methanol acetone were ranked second whereas, in fresh bullet chili, it was

150ml. This capsaicin extracted with methanol acetone at the three concentrations differed following a very low probability (Table 3.3).

All the three methanol concentrations were significantly different ($p<0.05$) from each other and still, the more the concentration, the high the capsaicin from both oven-dried and fresh bullet chili. This was evident since 200ml methanol had significantly the highest capsaicin of 1.98 and 2.43mg/g for oven-dried and fresh bullet chili, respectively. Methanol at 150 and 100 were ranked second and third, respectively, with 1.47 and 1.02mg/g for oven-dried bullet chili. However, 1.86 and 1.29mg/g capsaicin were ranked second and third, respectively, as extracted by 150 and 100ml methanol. These two bullet chili conditions, oven-dried and fresh, were different from each other due to a very low probability (Table 3.3).

Table 3.3: Effect of solvent concentration in the extraction of capsaicin in oven-dried and fresh cayenne and bullet chili varieties

Treatment	Concentration (ml)	Cayenne			Bullet		
		Oven	Fresh	t-test	Oven	Fresh	t-test
Ethanol	100	3.20a	3.61a	4.14, 0.054	1.62a	1.78a	1.56, 0.258
	150	4.73b	5.57b	13.90, 0.005	2.44b	2.84b	12.65, 0.006
	200	6.39c	7.37c	39.00, < 0.001	3.21c	3.80c	7.59, 0.017
	CV%	0.6	0		0.5	2.8	
	L.S.D	0.29	0.39		0.35	0.21	
M+A	100	2.06a	2.87a	8.10, 0.015	1.21a	1.91a	24.01, 0.002
	150	2.05a	3.59b	52.82, < 0.001	1.04a	2.21a	40.13, < 0.001
	200	4.18b	5.76c	17.37, 0.003	2.54b	2.91b	12.69, 0.006
	CV%	1.3	2.4		2.2	0.9	
	L.S.D	0.11	0.23		0.4	0.5	
Methankol	100	2.38b	3.00a	24.60, 0.002	1.02a	1.29a	9.26, 0.011
	150	2.97c	3.92b	44.78, < 0.001	1.47b	1.86b	18.68, 0.003
	200	2.05a	4.85c	46.42, < 0.001	1.98c	2.43c	14.96, 0.004
	CV%	0.9	0.5		0.3	0.3	
	L.S.D	0.17	0.18		0.11	0.17	

NOTE: Treatments were methanol, M+A (Methanol+ Acetone) and Ethanol. Bracket p (p) in the t-test represents the probability. Means in the same column not having a common letter are significantly different (P<0.05).

3.4.3. Effect of 200ml of extraction solvents concentration on capsaicin of oven-dried and fresh chili varieties

Section 3.2 revealed that most capsaicin was extracted with ethanol, methanol acetone and methanol at 200ml. Therefore, this section 3.3 further compared only 200ml of ethanol, methanol acetone and methanol in oven-dried and fresh chili varieties to determine the best extraction solvent. Though the three extraction solvents were significantly different ($p<0.05$) from the other, overall, the capsaicin in all the chili varieties were highest when extracted by ethanol followed by methanol acetone and methanol for both oven-dried and fresh. Besides, fresh chili varieties recorded the most capsaicin extracted with the three extraction solvents compared to oven-dried. Across the chili varieties, capsaicin was highest in habanero and was followed by bird's eye, cayenne and bullet (Table 3.4).

Table 3.4: Effect of 200ml solvent concentration in the extraction of capsaicin in oven-dried and fresh cayenne and bullet chili varieties

Treatment	Habanero			Birds Eye			Cayenne			Bullet		
	Oven	Fresh	t-test	Oven	Fresh	t-test	Oven	Fresh	t-test	Oven	Fresh	t-test
Ethanol	16.84c	23.09c	109.63, < 0.001	9.60c	12.36c	24.21, 0.002	6.39c	7.37c	39.00, < 0.001	3.21c	3.80c	7.59, 0.017
M+A	15.61b	18.08b	68.51, < 0.001	7.83b	9.73b	50.16, < 0.001	4.18b	5.76b	17.37, 0.003	2.54b	2.91b	12.69, 0.006
Methanol	13.62a	14.33a	18.05, 0.003	6.23a	8.12a	53.74, < 0.001	2.05a	4.85a	46.42, < 0.001	1.98a	2.43a	14.96, 0.004
	0.3	0.2		0.5	0.3		0.8	0.9		1.6	1.5	
	0.06	0.05		0.03	0.46		0.08	0.32		0.15	0.12	

NOTE: Treatments were methanol, M+A (Methanol+ Acetone) and Ethanol and all had a concentration of 200ml. Bracket p (p) in the t-test represents the probability. Means in the same column not having a common letter are significantly different (P<0.05).

3.5. CONCLUSION

Fresh Habanero yields higher amount of capsaicin when extracted using ethanol at a ratio of 1:10 than bird's eye, cayenne and bullet chili varieties when exposed to the same parameters. Ethanol solution is the most preferred solvent for extraction of capsaicin in comparison to 99% methanol and a mixture of 99% of methanol and acetone. Fresh chilies produce a higher yield of capsaicin than oven dried chilies.

3.6. RECOMMENDATIONS

This study was only limited to four varieties of chilies and thus does not conclude that habanero is the chili variety with the highest chili content. It is therefore recommended that exploration of other chili varieties is necessary in order to narrow down to the chili variety that has the ability to yield higher capsaicin than habanero.

CHAPTER FOUR: EVALUATION OF THE ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC COMPOSITION OF CAPSAICIN.

ABSTRACT

Capsaicin is an antioxidant especially in its phytochemical profile (chemical compounds produced by plants generally to help them thrive or thwart competitors, predators or pathogens), it makes it an ideal choice for pesticides. Capsaicin is also rich in phenolic compounds. These compounds have many ecologic functions such as defense against microbial pathogens. This property is important in the antimicrobial properties of capsaicin. This paper gives a comparison of the content of total phenolic and the antioxidant activity of capsaicin in four varieties that include habanero, bird's eye, cayenne and bullet chili varieties.

The experimental design involved two variables which included capsaicin of the various chili varieties and the antioxidants and phenolic properties. This was to demonstrate the relationship between capsaicin in the various varieties and the number of antioxidants and total phenolics. This was to show the relationship between the amount of capsaicin and the number antioxidants and total phenolics.

Total phenolic absorbance at λmax 760nm was measured on a spectrophotometer while the varieties were also analyzed of their antioxidant activity using the Dipheny1-1-picrtyl-hydrazyl (DPPH) method. The content of phenolic as well as antioxidant activity of capsaicin was higher in fresh chilies than in oven-dried chilies. There was a high correlation between total phenolic content and the amount of capsaicin in the chilies. Similarly, there was a high correlation between the antioxidant activity and the capsaicin amount in the chilies. In the varieties analyzed, i.e. habanero, bird's eye, cayenne and bullet chilies, the total phenolic ranged between 352 – 2923 mg/g in fresh chilies and 117- 2274.5 mg/g in oven-dried chilies with habanero chili having the highest and bullet chili the lowest total phenolic. Antioxidants activity ranged between 50% - 86% in oven-dried chilies and 46% - 74% in fresh chilies with habanero chili having the highest and bullet chili the lowest antioxidant activity. Total phenolic content and antioxidant activity is highest in the chili varieties that have the highest capsaicin content. However, a study on other chili varieties will expound more on the activity of antioxidants and total phenolic in chilies.

4.1. INTRODUCTION

Chilies from the genus capsicum have about 4000 varieties in the world with each variety having a different composition of active phytochemicals (Arora, Gill, & Rana , 2011). These phytochemicals highly contribute to its pungency and its beneficial uses. Chilies have a compound known as capsaicinoids composed of capsaicin and dihydrocapsaicin that constitute for 90% of chili composition with capsaicin accounting for 71% (B, 2008). Capsaicin is one of the major parameters that determine the phytochemicals present in a variety, the commercial value, pharmaceutical properties, pungency and insecticidal properties (Abong'o, 2018). As the content of capsaicin increases, the phenols and antioxidant activity increases, increasing the nutritional, pharmaceutical and insecticidal properties (Barbero, Liazid , Palma, & Barrosso, 2008). Therefore, the quality of a drug, spice or an insecticide from chilies is highly contributed to the amount of capsaicin available in the chili variety.

Capsaicin is an antioxidant especially in its phytochemical profile (chemical compounds produced by plants generally to help them thrive or thwart competitors, predators or pathogens), it makes it an ideal choice for pesticides. Major antioxidants in capsicum family are carotenoids, tocopherols and capsaicinoids (capsaicin) *(Bender & Bender , 2018)*. This component plays a role as an anti-cancer attributed to its ability to act as scavengers of singlet molecular oxygen, reactive oxygen species (ROS), peroxyl radicals and reactive nitrogen species (RNS). Capsaicinoids intake effectively reduce the triacyclglycerols, plasma total cholesterol (PTC), and non-high-density lipoprotein cholesterol, and thereby helps in the prevention of cardiovascular ailments *(Alexandra , 2009)*. It also exhibit effective and proactive contribution against age-related ailments. Capsaicin exposure expressively repressed the initial adipogenic differentiation, maturation, and lipogenesis of adipocytes. Capsaicin also has ability to target the TRPV1 receptors in the C-fibers lead to their stimulation followed by desensitization that helps to improve the neurogenic bladder. So, it may serve as a potential emerging treatment for patients who are non-respondent to conventional therapy especially those with neurogenic bladder (*Alexandra , 2009)*. During drying of chilies, enzymatic and non-enzymatic browning reactions occur (Barceloux, 2008). Enzymatic reactions can be prevented by pretreatment methods. Non-enzymatic reactions i.e. milliard reaction, increase in increase in temperature and intermediate levels of moisture, thus produces color and flavor that changes quality of the chili (Barbero, Palma , & Barroso, Determination of capsaicinoids in pepper by microwave-assisted extraction high-performance liquid chromatography with fluorescence detection, 2006). Moreover, these reactions cause degradation of the pigment,

phenols, ascorbic acid and reduction of the antioxidant activity in chilies (Basith, Cui , Hong, & Choi , 2016). Therefore, fresh chilies have minimal chances of undergoing enzymatic or non-enzymatic reactions if they are not exposed to high temperatures, sun or lose their recommended moisture content. This maintains the photochemical composition, flavor, color and pungency of the chilies.

Therefore, the objective of this study was to give a comparison on the total phenolic content and antioxidant activity of four varieties of chilies, their correlation to capsaicin and their relationship to the tissue preparation of these chilies.

4.2. EXPERIMENTAL DESIGN

The experimental design involved two variables which included capsaicin of the various chili varieties and the antioxidants and phenolic properties. This was to demonstrate the relationship between capsaicin in the various varieties and the number of antioxidants and total phenolics. This was to show the relationship between the amount of capsaicin and the number antioxidants and total phenolics.

4.3. MATERIALS AND METHODS

4.3.1. MATERIALS

In the test for total phenolic of chili varieties, the following materials were used; habanero, bird's eye, cayenne and bullet chilies. The reagents used were; 99% ethanol, 50% methanol, 99% methanol, dpph solution, distilled water, folin's reagent and sodium carbonate (Na_2CO_3). The equipment used was a laboratory miller, weighing scale, beakers, measuring cylinder, test tubes, test tube rack, whatmann filter paper, funnels, rotary evaporator, a fume chamber centrifuge, beakers, cyclomixer and a spectrophotometer.

4.3.2 METHODOLOGY

4.3.2.1. Obtaining chili extract

One Thousand grams (1000 gm) of each variety was divided into two equal portions (500 gm per portion) and stored in labelled boxes. One of the portions of each variety was dried in an air oven at 60^{o}C for 12 hours and the other portions left for milling. In a fume chamber, each variety, both fresh and dried, was milled using a laboratory mil (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland) to 1mm particle size. Using a weighing scale, two

hundred grams (200gm) powder of each variety, both fresh and dried, was then measured into separate labelled beakers (labelled as per the name of each variety and the tissue preparation used (dried or fresh)). The powder was used for extraction to determine total phenolic content and antioxidant activity. This process was done in duplicate.

4.3.2.2. Extraction with ethanol 99%

The 200 gm powder in the different labelled beakers was extracted with two liters (2L) ethanol 99%. The mixture was transferred into labelled test tubes and stirred for five minutes using a vortex mixer and left to rest for twenty-four hours (24hrs) in a test tube rack at room temperature. The solution was then filtered using whatmann paper no1 to remove debris and evaporated using the rotary evaporation technique to obtain the extract. The extract was used to determine total phenolic content and antioxidant activity. This procedure was done in duplicates.

4.3.2.3. Determination of total phenolic

Using a weighing scale, five grams (5 gms) of the extract was weighed into a beaker and 5ml of 50% methanol added into the beaker. The mixture was left to rest for one hour. The solution was then centrifuged at 6000xg for fifteen minutes (15min), filtered using whatmann paper no 1 and maintained at 20ml each using distilled water. Into 1ml of the solution, subsequently 1ml Folin's reagent and 2ml, 20% Na_2CO_3 was added. The test mixture was mixed properly with a cyclomixer and left at room temperature for 30min, maintained at 25ml with distilled water. The absorbance was measured at λmax 760nm on a spectrophotometer. Total phenolic content was expressed as mg/g gallic acid equivalent. This procedure was done in duplicates.

4.3.2.4. Determination of antioxidant activity

The DPPH radical scavenging activity of phenolic extract was analyzed by following the method of Brand-Williams *et al*. (1995) method modified by Sanchez-Moreno *et al*. (1998). A methanol solution (0.1 ml) of the sample extract was added to 3.9 ml (0.025 g/L) of DPPH solution. BHT, (10 mg/10 ml) was used as a positive control. The solutions were incubated at room temperature (25°C) for 30 min and the decrease in absorbance at the end of incubation period was determined at 515 nm with a Spectrometer. From the absorbance value, the free-

radical scavenging activity of phenolic extracts was calculated and expressed as percentage basis. This procedure was done in duplicates.

4.4. RESULTS AND DISCUSSION

4.4.1. TOTAL PHENOLIC CONTENT OF FRESH AND DRIED CHILIES

Total phenolics was present in either oven-dried or fresh chili varieties. Extracted phenolics, however, were significantly different among the chili varieties. There are more phenolics from fresh than from oven-dried of each chili variety. The results too showed more phenolics were in habanero and bullet chili varieties, respectively. Overall, with chili varieties in order of bullet, cayenne, bird's eye and habanero, there is a decrease of phenolics, accordingly, in both oven-dried and fresh (Table 3.5).

Between oven-dried and fresh chili, significant differences in phenolics were observed across the varieties. For example, 2271mg of phenolics that were significantly highest than 1128, 322 and 117mg were recorded from oven-dried habanero, bird's eye, cayenne and bullet chili varieties, respectively. Still, a similar trend though slightly high in phenolics was observed in fresh chili varieties. Habanero (2923mg) had significantly the highest ($p<0.05$) phenolics in fresh chili which was followed by bird's eye (1466mg), cayenne (568mg) and bullet (352mg). Unlike antioxidants, there is more phenolics from fresh than oven-dried chili varieties as demonstrated by a very low probability of being similar.

From correlation analysis, the results show a close to perfect positive correlation between phenolics and the capsaicin extraction solvents that were ethanol, methanol acetone and methanol. Furthermore, the best concentration of 200ml of each extraction solvent in fresh chili obtain from section 3.2 was used in this correlation analysis. In this view, with each of the extraction solvents, an increase in capsaicin also led to an increase in phenolics across all the varieties of chilies (Table 3.6).

Table 3.5: Amounts of Phenolics in oven-dried and fresh chili varieties

Variety	Phenolics		t-test (p)
	Oven-dried	Fresh	
Habanero	2274.5d	2923d	72.85 (< 0.001)
Birds Eye	1128c	1466c	49.77 (<0.001)
Cayene	322b	568b	27.50 (0.001)

Bullet	117a	352a	57.00 (<0.001)
CV%	0.5	0.1	
L.S.D	9.07	33.7	

NOTE: Bracket p (p) in the t-test represents the probability. Means in the same column not having a common letter are significantly different (P<0.05).

Table 3.6: Correlation between phenolics and extraction solvents for capsaicin in chili varieties

Chili variety	Variable		Ethanol=a		Methanol acetone=b		Methanol=c	
			1=a/b/c	2	1=a/b/c	2	1=a/b/c	2
	a/b/c	**1=a/b/c**						
Habanero								
	Phenolics	**2**	0.9997*	0.9948*	0.9996*	0.9948*	0.9964*	0.9948*
	a/b/c	**1=a/b/c**						
Bird's eye								
	Phenolics	**2**	0.9996*	0.9933*	0.9991*	0.9993*	0.9989*	0.9993*
	a/b/c	**1=a/b/c**						
Cayenne								
	Phenolics	**2**	0.9984*	0.9889*	0.9914*	0.9889*	0.9967*	0.9889*
	a/b/c	**1=a/b/c**						
Bullet								
	Phenolics	**2**	0.9867*	0.9667**	0.9955*	0.9667*	0.9976*	0.9667**

NOTE: The three extraction solvents are ethanol (a), methanol acetone (b) and methanol (c). In the ethanol column, denotation a is used in correlation with phenolics whereas b and c are used for methanol acetone and methanol columns, respectively. * represents significance level at 1% and ** at 5%.

4.4.2. ANTIOXIDANT ACTIVITY ON FRESH AND OVEN-DRIED CHILIES

Antioxidants were present in either oven-dried or fresh chili varieties. Extracted antioxidants, however, were significantly different among the chili varieties. There are more antioxidants from fresh than from oven-dried of each chili variety. The results too showed more antioxidants in habanero and bullet chili varieties, respectively. Overall, with chili varieties in order of bullet, cayenne, bird's eye and habanero, there is a decrease of antioxidants, accordingly, in both oven-dried and fresh (Table 3.7).

Each chili varieties were significantly different in antioxidants obtained oven-dried or fresh. Significantly highest antioxidants were observed in habanero (73.50%) followed by bird's eye (58.50%), cayenne (49.65%) and bullet (45.50%) oven-dried chili varieties. On the other hand, habanero (85.50%) had significantly the highest antioxidants ahead of bird's eye (68.50%), cayenne (56.50%) and bullet (49.50%) in fresh chili. Here too, following the very low probability of t-test across all the chili varieties, it showed that there are more antioxidants from fresh than oven-dried chilies (Table 3.7).

From correlation analysis, the results show a close to perfect positive correlation on antioxidants and the capsaicin extraction solvents that were ethanol, methanol acetone and methanol. Furthermore, the best concentration of 200ml of each extraction solvent in fresh chili obtain from section 3.2 was used in this correlation analysis. In this view, with each of the extraction solvents, an increase in capsaicin also led to an increase in antioxidants across all the varieties of chilies (Table 3.8).

Table 3.7: Amounts of antioxidants in oven-dried and fresh chili varieties

Variety	Antioxidants (%)		t-test (p)
	Oven-dried	Fresh	
Habanero	73.50d	85.50d	16.97 (0.003)
Birds Eye	58.50c	68.50c	14.14 (0.005)
Cayene	49.65b	56.50b	13.63 (0.005)
Bullet	45.50a	49.50a	5.66 (0.030)
CV%	0.3	0.0	
L.S.D	2.14	2.60	

NOTE: Antioxidants % =[(Absorbance of control-Absorbance of the sample)/Absorbance of control)]× 100%; where the absorbance of the control was 0.775. Bracket p (p) in the t-test represents the probability. Means in the same column not having a common letter are significantly different (P<0.05).

Table 3.8: Correlation between antioxidants and extraction solvents for capsaicin in chili varieties

Variable		Ethanol=a	Methanol+Acetone=b
		1=a/b/c	1=a/b/c
a/b/c	**1=a/b/c**		
Antioxidants	**2**	0.9963*	0.9960*
a/b/c	**1=a/b/c**		
Antioxidants	**2**	0.9905*	0.9972*
a/b/c	**1=a/b/c**		
Antioxidants	**2**	0.9915*	0.9995*
a/b/c	**1=a/b/c**		
Antioxidants	**2**	0.9537**	0.9707**

NOTE: Antioxidants % =[(Absorbance of control-Absorbance of the sample)/Absorbance of control)]× 100%; where the absorbance of the control was 0.775. The three extraction solvents are ethanol (a), methanol+acetone (b) and methanol (c). In the ethanol column, denotation a is used in correlation with antioxidants and phenolics whereas b and c are used for methanol+acetone and methanol columns, respectively. * represents significance level at 1% and ** at 5%.

4.5. CONCLUSION

Total phenolic content and antioxidant activity is highest in the chili varieties that have the highest capsaicin content. Chili properties such as the insecticidal, pharmaceutical and nutritional properties are enhanced by the increase in the total phenolic content and antioxidant content. Therefore, insecticides, drugs or nutritional products from chilies provide impact based on the amount of capsaicin available in their extract.

4.6. RECOMMENDATION

This study was only limited to four varieties of chilies and thus does not conclude that habanero is the chili variety with the highest chili content. It is therefore recommended that exploration of other chili varieties is necessary in order to narrow down to the chili variety that has the highest antioxidant and total phenolics activity.

CHAPTER FIVE: DETERMINATION OF ANTIMICROBIAL PROPERTIES OF CAPSAICIN ON LATE BLIGHT AND BACTERIAL SPECK IN TOMATOES.

ABSTRACT

Agar dilution is a sensitivity test that measures the susceptibility of a microbe to an antibiotic. Moreover, minimum inhibitory concentration is an antimicrobial activity test that measure the minimum concentration of an antibiotic against a microbe. This paper illustrates incorporating capsaicin from habanero, bird's eye, cayenne and bullet chili varieties as an antimicrobial agent to inhibit the growth of late blight a fungal disease and bacterial speck a bacterial disease that affects tomatoes. These techniques are suitable for antibacterial and antifungal susceptibility and minimum inhibitory/bactericidal concentration testing.

The experimental design involved two variables which included capsaicin of the various chili varieties and the susceptibility of the capsaicin to bacterial speck and late blight diseases in tomatoes. This was to demonstrate the relationship between capsaicin in the various varieties and the susceptibility of the capsaicin to bacterial speck and late blight diseases in tomatoes. The higher the level of capsaicin was to reflect higher susceptibility of bacterial speck and late blight diseases in tomatoes to capsaicin.

Chili varieties that were used were either oven dried or freshly prepared and extracted to obtain capsaicin. These chilies were used as an antimicrobial agent using the agar well dilution method for sensitivity test and minimum inhibitory concentration method to analyze the level of inhibition of bacterial speck and late blight in tomatoes.

In both techniques, bacterial speck and late blight were most susceptible to habanero, bird's eye, cayenne and bullet capsaicin respectively. Capsaicin from fresh chilies from all varieties, inhibited the growth of bacterial speck and late blight more than oven-dried chilies. Therefore, there was a high correlation between the level of inhibition of the diseases and the level of capsaicin present in the chili.

From the sensitivity test, bacterial speck was more susceptible to the capsaicin than late blight across all varieties. Zone of inhibition for bacterial speck ranged between 0.96cm – 2.48cm in fresh chilies and 0.63cm – 1.95cm in oven-dried chili having habanero with highest zone of

inhibition and bullet chili the lowest. Zone of inhibition for late blight ranged between 0.68cm – 2.22cm in fresh chilies and 0.56cm – 1.75cm in oven-dried chili having habanero with highest zone of inhibition and bullet chili the lowest as well.

From the minimum inhibitory/bactericidal concentration test, both late blight and bacterial speck had no growth at the 10^{-1} dilution, 10^{-2} dilution and 10^{-3} dilution while using capsaicin from both fresh and oven-dried bird's eye and habanero chilies. Growth was observed from 10^{-4} dilution. Therefore, minimum inhibitory concentration was at dilution $10^{-3.}$ Moreover, both late blight and bacterial speck had no growth at the 10^{-1} dilution and 10^{-2} dilution while using capsaicin from both fresh and oven-dried cayenne and bullet peppers. Growth was observed from 10^{-3} dilution. Therefore, the minimum inhibitory concertation was at 10^{-2}.

Late blight was more susceptible to the capsaicin than bacterial speck across all varieties. Minimum bactericidal concentration for late blight ranged between 0.01% - 0.18% in the first tube and 0.21% - 0.53% in the second tube incubate, using fresh chilies and 0.02% - 0.20% in the first tube and 0.29% - 0.55% in the second tube incubated, using oven-dried chili having habanero with highest minimum bactericidal concentration and bullet chili the lowest. Minimum bactericidal concentration for bacterial speck ranged between 0.01% - 0.19% in the first tube and 0.26% - 0.55% in the second tube incubate, using fresh chilies and 0.02% - 0.21% in the first tube and 0.30% - 0.61% in the second tube incubated, using oven-dried chili having habanero with highest minimum bactericidal concentration and bullet chili the lowest.as well. Capsaicin from fresh habanero chilies gave 0.01% minimum bactericidal concentration in both late blight and bacterial speck diseases in tomatoes.

Therefore, habanero chili is the most preferred variety as an antimicrobial agent against bacterial speck and late blight diseases in tomatoes.

5.1. INTRODUCTION

Chilies are grown for uses such as flavor, preservative, aroma, pharmaceutical purposes and in cuisines (Baskaran, Markert , Bennis , & Zimmerman , 2019). Chilies have many varieties and are classified by their level of pungency. Pungency is due to accumulation of capsaicinoids which are naturally produced compounds in the capsicum genus (Bley, Boorman , Mohammad , McKenzie , & Babbar , 2012). The higher the capsaicinoids in the chilies, the higher the pungency which ranges from zero (0) to millions of Scoville heat units (SHU). The highest value of the capsaicinoids is 16,000,000 SHU in the purest form. Capsaicinoids include capsaicin (69%), dihydrocapsaicin (22%), nordihydrocapsaicin (7%) and homocapsaicin (1%). Capsaicinoids have beneficial effects in treatment of various diseases such as obesity, diabetes, gastro protective and anticancerogenic activity (F, Tundis , Bonesi, Conforti, & Satti , 2009). It is also used in pepper sprays and in security firms in dispersing crowds. In the recent past, they have been explored for their potential in antimicrobial and antifungal properties (FS, Conceicao, Bandeira , Costa , & Silva, 2014). They have gradually been adapted to control diseases in various crops but the dosage of each of these bioactive compounds required to inhibit the development of a disease and microbial growth is yet to be established (*Gervais et al., 2008*).

Tomatoes constitutes 98 % of the vegetables usually consumed at household level and 82% of the most consumed vegetable from high end to low level consumer in Kenya ranking as the highest consumed vegetable (Dijkstra, 2015). This shows the importance of tomatoes in Kenya. From a report released by Kenya Plant Health Inspectorate Service (KEPHIS) in 2017, the major challenges faced in tomato production is plant pest and diseases and high pesticide residues ranking at 54% and 48% respectively. Some of the major diseases include bacterial speck and late blight. Diseases are most prevalent in the tropics (savary, 2014)and damage ranges from reduced plant vigor to plant death by interfering with plant physiological processes therefore the plant losses its ability to synthesize its bio chemical properties and nutritive qualities (*Muhammad, 2014*). Therefore, these diseases need effective disease control methods.

The increased demand of tomatoes on account of population explosion and increased consumption frequency of tomatoes has compelled the use pesticides for better crop protection (Tomer, 2013). Continuous use and accumulation of synthetic pesticides have led to pesticide toxicity that has

resulted to oxidative stress on plants because of the generation of reactive oxygen species (ROS) (*Xia et al., 2009*). Several cases of synthetic pesticide poisoning are recorded every year. Risks of poisoning depend on toxicity, dose and period of exposure. Pesticide toxicity may cause neurological and psychiatric complications, brain tumors, cancers, spontaneous abortions, stillbirths, and birth defects. This has led to use of bio pesticides as an alternative since they are able to breakdown naturally causing no toxicity.

There has also been a strong consumer demand on production of safe and quality food. Concerns on food safety are due to increased occurrences of food borne diseases caused by pathogenic microorganisms during farm production, manufacturing, processing and handling (FS, Borges , Paula , & Conceicao , 2012). This raises more concerns due to the increased use of chemical anti-microbial in the attempt to inhibit the growth of the microorganisms which have also become a threat to human health if used inappropriately. Thus, this has administered the need to come up with alternatives that ate safe, cost effective and simple to administer.

Bacterial speck in tomatoes is caused by the bacterium Pseudomonas syringae pv. tomato (Gonzalez, Nunez Palenius , & Ochoa , 2011). This disease has no cure once tomatoes are infected. The disease is introduced to the farm by contaminated seeds or transplants and multiplies in conducive conditions such as cool and humid conditions (I & Oleszek, 2000). This can be influenced by the environmental conditions or irrigation systems used. The disease spreads by splashing water (rain or overhead irrigation), contaminated farm tools or worker's garments, thus farm hygiene is important in preventing the spread of this disease (Linanfu & Zelong , 2008). Therefore, seed treatment is prudent before planting using antimicrobial agents. This study looks at using chili extract that has high capsaicinoids level as an antimicrobial agent against bacterial speck.

Late blight in tomatoes is caused by the oomycete Phytophthora infestans (HF, Yang , Zhao , & Wang , 2009). It also thrives in cool humid weather. The disease is introduced in a farm by infected transplants. The disease can be severe since P.infestans can infect and produce thousands of sporangia per lesion in less than five days, destroying the entire fields in a short period if not well managed (Martins & Cardoso , 2015). This disease can be managed in several ways including cultural control or chemical control. Cultural control can only prevent the disease on the onset of planting by destroying any diseased part of the plant, removal of infected plants, transplant

inspection or farm hygiene. Chemical control is by use of fungicides where farmers are encouraged to make applications prior to infections when the environment conditions favor the disease (MS, Sharma , & Cotter , 2011). The fungicides are to be applied in ratios advised by the manufacturer. However, most farmers do not adhere to the ratios and the pre harvest intervals (PHI) instructed leading to exceeding of the maximum residue levels (MRI) (Riera, Gallego , & Mason , 2006). This become hazardous to human and animals. This study looks at using capsaicin as an antifungal agent against late blight.

Over the recent past, efforts to develop antimicrobial agents from natural products due to the belief that natural products are safe and dependable compared to synthetic antimicrobial agents that have adverse effects if used incorrectly and have high cost, have risen. Moreover, besides its multiple pharmacological and physiological properties (pain relief, cancer prevention, beneficial cardiovascular, and gastrointestinal effects, capsaicin has recently attracted considerable attention because of its antimicrobial and anti-virulence activity (*Nadendla , et,al , 2018*). Therefore, this study measures the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of capsaicin om the various varieties and its efficacy against bacterial speck and late blight in tomatoes.

5.2. EXPERIMENTAL DESIGN

The experimental design involved two variables which included capsaicin of the various chili varieties and the susceptibility of the capsaicin to bacterial speck and late blight diseases in tomatoes. This was to demonstrate the relationship between capsaicin in the various varieties and the susceptibility of the capsaicin to bacterial speck and late blight diseases in tomatoes. The higher the level of capsaicin was to reflect higher susceptibility of bacterial speck and late blight diseases in tomatoes to capsaicin.

5.3. MATERIALS AND METHODS

5.3.1. MATERIALS

5.3.1.1. Variety of chilies used

In both tests for MIC/MBC and sensitivity of bacterial speck and late blight against capsaicin, the following materials were used; habanero, bird's eye, cayenne and bullet chili extract as well as one-day old bacterial speck and late blight cultures.

5.3.1.2. Reagents and Equipment used

The reagents used were; 99% ethanol, distilled water, Mueller Hinton agar, 1.5% Acetric acid and media. The equipment used was a laboratory miller, weighing scale, beakers, measuring cylinder, test tubes, test tube rack, what Mann filter paper, funnels, rotary evaporator, a fume chamber, beakers, vortex mixer, rotary evaporator, petri dish, sterile loop, 11mm cork borer, micropipette tips, culture tubes and incubator.

5.3.1.3. Test Microorganisms

The microorganisms used were bacterial speck (*Pseudomonas syringae pv.tomato*) and late blight (*Phytophthora infestans*) in tomatoes.

5.3.2. PREPARATION OF CHILI EXTRACT

Fifty grams (50 gm) of each variety, habanero, bird's eye, cayenne and bullet chili was divided into two equal portions (25 gm per portion) and stored in labelled boxes in a well-ventilated room. One of the portions of each variety was dried in an air oven at 60^{o}C for 12 hours and the other portions left for milling. In a fume chamber, each variety, both fresh and dried, was milled using a laboratory mil (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland) to 1mm particle size. Using a weighing scale, twenty grams (20gm) powder of each variety, both fresh and dried, were then measured into separate labelled beakers (labelled as per the name of each variety and the tissue preparation used (dried or fresh)). This process was done in duplicate.

5.3.2.2. Extraction with ethanol 99%

The 20 gm powder in the different labelled beakers was extracted with 200ml ethanol 99% at a temperature of 40^{o}C using the rotary evaporation technique for 20 minutes. The mixture was transferred into labelled test tubes and stirred for five minutes using a vortex mixer and left to rest for twenty-four hours (24hrs) in a test tube rack at room temperature. The solution was then filtered using what Mann paper no1 to remove debris and evaporated using the rotary evaporation technique. The extract was then stored in small closed labelled bottles at -20^{O}C for sensitivity analysis. This procedure was done in duplicates.

5.3.2.3. Sensitivity test on bacterial speck in tomatoes.

One-day old bacterial speck culture was prepared from isolates obtained from Plant Pathology laboratory. One petri dish with colonies of the bacteria was constituted in 4ml of sterile distilled water and 0.5ml of the constituted colony was evenly spread on the pre prepared muller Hinton agar. Using a standard cork borer (11mm) two duplicate wells were made at equidistant points on the inoculated on the Muller Hinton agar (9 cm), and the agar plug aseptically removed. The chili extract from the various varieties at 99.9% concentration used at 100µl of the extract prepared at a concentration ratio of extract to water of 1:1 mg/ml, and transferred into the well using sterile micropipette tips. The same volume of 1.5% Acetic acid, 10µ which acted as a positive control and sterile distilled water (negative control) were used. Plates were incubated at 37^{o} C for 18 h and inhibition zones diameters (mm) measured and recorded. This method was used to measure the concentration of the chili extract from the various varieties that inhibits the growth of bacterial speck pathogen in tomatoes.

5.3.2.4. Sensitivity test on late blight in tomatoes.

One-day old late blight culture was prepared from isolates obtained from Plant Pathology laboratory. One petri dish with colonies of the bacteria was constituted in 4ml of sterile distilled water and 0.5ml of the constituted colony was evenly spread on the pre prepared muller Hinton agar. Using a standard cork borer (11mm) two duplicate wells were made at equidistant points on the inoculated on the Muller Hinton agar (9 cm), and the agar plug aseptically removed. The chili extract from the various varieties at 99.9% concentration used at 100µl of the extract prepared at a concentration ratio of extract to water of 1:1 mg/ml, and transferred into the well using sterile micropipette tips.The same volume of 1.5% Acetic acid, 10µ which acted as a positive control and

sterile distilled water (negative control) were used. Plates were incubated at 37º C for 18 h and inhibition zones diameters (mm) measured and recorded. This method was used to measure the concentration of the chili extract from the various varieties that inhibits the growth of late blight pathogen in tomatoes.

5.3.2.5. Minimum Inhibitory concentration (MIC)/Minimum bactericidal concentration (MBC) of bacterial speck in tomatoes.

Standard bacterial strain of *bacterial speck* was used to determine minimum Inhibitory concentration (MIC) of extract, One day old microbial cultures prepared from the corresponding media, was constituted in 4mls of normal saline in culture tubes and adjusted to that of 0.5 McFarland standards which will be equivalent to 1-2 × 10^8 CFU/ml.

Four hundred milligrams of the extract was dissolved in 2ml of sterile Muellar Hinton Broth in culture tubes. Serial dilution of 1ml was carried out up to the 5^{th} serial dilution. Preparations was carried out in 2 sets. One set had broth and extract alone, the other set broth, extract and microorganism (100µl of *late blight*). The other set had broth and microorganism (100µl of *late blight*) alone. The tubes were then incubated at 37°C for 18h and turbidity observed. Tubes without turbidity were considered to have minimum Inhibitory concentration (MIC). Sub- culturing was done from the tubes without turbidity in duplicates on Potato Dextrose agar. They were incubated at 37°C for 18h and microbial count done. The concentration of the tubes with no growth or tubes where 99.9% of the microorganisms were killed, were considered to be the one having minimum bactericidal concentration (MBC) (*Njue et al., 2017*).

5.3.2.6. Minimum Inhibitory concentration (MIC)/Minimum bactericidal concentration (MBC) of late blight in tomatoes.

Standard fungal strain of *late blight* was used to determine minimum Inhibitory concentration (MIC) of extract, one day old microbial cultures prepared from the corresponding media, was constituted in 4mls of normal saline in culture tubes and adjusted to that of 0.5 McFarland standards which will be equivalent to 1-2 × 10^8 CFU/ml.

Four hundred milligrams of the extract were dissolved in 2ml of sterile Muellar Hinton Broth in culture tubes. Serial dilution of 1ml was carried out up to the 5^{th} serial dilution. Preparations was carried out in 2 sets. One set had broth and extract alone, the other set broth, extract and

microorganism (100µl of *late blight*). The other set had broth and microorganism (100µl of *late blight*) alone. The tubes were then incubated at 37°C for 18h and turbidity observed. Tubes without turbidity were considered to have minimum Inhibitory concentration (MIC). Sub- culturing was done from the tubes without turbidity in duplicates on Potato Dextrose agar. They were incubated at 37°C for 18h and microbial count done. The concentration of the tubes with no growth or tubes where 99.9% of the microorganisms were killed, were considered to be the one having minimum bactericidal concentration (MBC) (*Njue et al., 2017*).

5.4. RESULTS

Table 3.7: Effect of capsaicin extracted from chili varieties on the in vivo inhibition zones against bacterial speck and late blight of tomato

Variety	The bacterial speck of tomato inhibition zone (mm)		Late blight of tomato inhibition zone (mm)	
	Oven-dried	Fresh	Oven-dried	Fresh
Habanero	19.5d*	24.8c*	17.5d	22.2c*
Birds Eye	16.5c	18.8b*	12.3c	17.0b
Cayene	9.8b	10.3a	8.2b	9.7a
Bullet	6.3a	9.6a	5.6a	6.8a
CV%	4	0	4	0
L.S.D	0.3	0.7	0.3	0.7

NOTE: A concentration of 200ml was used in the extraction of capsaicin from each chili variety. Bracket p (p) in the t-test represents the probability. The control diameter is 1.80cm whereby values above it represents susceptibility while below it represents the resistance of either bacterial speck or late blight of tomato. The asterisk (*) denotes the cut-off value from 1.80cm. Means in the same column not having a common letter are significantly different (P<0.05).

Table 3.8: Effect of chili varieties on the minimum inhibitory concentration against bacterial speck and late blight of tomato

VARIETY	Bacterial speck of tomato (%)		Late blight of tomato (%)	
	Tube A	Tube B	Tube A	Tube B
Habanero	0.01a	0.275a	0.01a	0.235a
Bird's eye	0.05a	0.388b	0.235a	0.285b
Cayenne	0.125b	0.41b	0.555a	0.355c
Bullet	0.18b	0.53c	0.165a	0.51d
CV%	1.9	4.4	28.6	10.7
L.S.D	0.038	0.029	1.201	0.028

Means in the same column not having a common letter are significantly different (P<0.05

5.5. DISCUSSIONS

5.5.1. Effect of capsaicin extracted from chili varieties on the in vivo inhibition zones against bacterial speck and late blight of tomato

Inhibition zone of bacterial speck of tomato significantly differs ($p<0.05$) in each chili variety. In this regard, habanero oven-dried chilies (19.5mm) showed a significantly widest inhibition zone against bacterial speck of tomato and was followed by that of bird's eye (16.5mm), cayenne (9.8mm) and bullet (6.3mm). In fresh habanero chilies (24.8mm), too had the widest inhibition zone against bacterial speck of tomato whereby bird's eye (18.8mm), cayenne (10.3mm) and bullet (9.6mm) were ranked second, third and fourth, respectively. However, cayenne and bullet fresh chili varieties were not significantly different in their inhibition zone against bacterial speck of tomato (Table 3.7).

In terms of the inhibition zone, between oven-dried and fresh chilies, habanero had the widest inhibition zone that was followed by bird's eye, cayenne and bullet. A much wider inhibition zone was observed with capsaicin extracted from fresh rather than oven-dried chilies of all the varieties. Moreover, both habanero and bird's eye developed about twice as wide inhibition zone as cayenne and bullet (Table 3.7).

Though on a close note, there is a slight narrow inhibition zone in late blight of tomato as compared to that of bacterial speck of tomato across the chili varieties, still, a similar trend was observed. For example, in oven-dried, 17.5mm inhibition zone which was observed in habanero against late blight of tomato and was followed by 12.3, 8.2 and 5.6mm as were observed in bird's eye, cayenne and bullet, respectively. Habanero (22.2mm) had the widest inhibition zone which was significantly different from that of bird's eye (17mm) against late blight of tomato. Both cayenne (9.7mm) and bullet (6.8mm) were not significantly different in their inhibition zone against late blight of tomato (Table 3.7).

Moreover, the control diameter of 18mm was used in determining resistance as well as the susceptibility of 200ml capsaicin against either bacterial speck or late blight of tomato. Regarding the 19.5mm* inhibition zone obtained by oven-dried habanero chili; it further showed the susceptibility of bacterial speck to be inhibited. Other varieties which are bird's eye, cayenne and bullet (all with 1.8.mm inhibition zone), revealed that bacterial speck was resistant to capsaicin extracted when oven-dried. Whereas capsaicin extracted from fresh habanero (24.8mm*) and bird's eye (18.8mm*) chili varieties showed that bacterial speck was susceptible to inhibition, that from cayenne and bullet showed that it was resistant. Late blight of tomato was susceptible to inhibition only by capsaicin extracted from fresh habanero chili (22.2mm*). Overall, either bacterial speck or late blight of tomato was susceptible to inhibition by capsaicin extracted from fresh habanero chili (Table 3.7).

5.5.2. Effect of chili varieties on the minimum inhibitory concentration against bacterial speck and late blight of tomato

After incubation, the positive control had growth of both bacterial speck and late blight microorganisms. This was because there was no control (antibiotic) thus the microorganisms grew. The negative control was determined by visual comparison. After capsaicin was mixed with the broth, a whitish substance was observed. Hence, after serial dilution, the solution lost its color at the 3rd serial dilution and turned colorless. Serial dilution of the tubes that had microorganisms present denoted that, both bacterial speck and late blight microorganisms grew at the 3rd serial dilution under the control of capsaicin from bullet and cayenne and the 4th serial dilution from bird's eye and habanero chilies. These was observed in both fresh and oven dried chilies. Therefore, the minimum inhibitory concentration of bacterial speck and late blight in tomatoes

inhibited by capsaicin from bullet and cayenne chilies was the 2nd serial dilution and 3rd dilution from inhibition caused by capsaicin from bird's eye and habanero chilies. By this, we conclude that both fresh and oven-dried bird's eye and habanero chilies had the ability to have the minimum inhibitory concentration against both bacterial speck and late blight microorganisms in tomatoes.

The four chili varieties, habanero, bird's eye, cayenne and bullet, exert different minimum inhibitory concentrations against bacterial speck and late blight of tomato. Among the tubes, there were slightly more of the minimum inhibitory concentration in tube B than in A in bacterial speck and late blight of tomato. In addition, there is slightly more minimum inhibitory concentration in late blight than in bacterial speck of tomato (Table 3.8).

In bacterial speck of tomato, habanero (0.01%) and bird's eye (0.05%) were not significantly different in terms of their minimum inhibitory concentration in tube A. Additionally, cayenne (0.125% and bullet (0.18%) were not significantly different in tube A. However, both bullet and cayenne in tube A had significantly ($p<0.05$) higher minimum inhibitory concentration than both habanero and bird's eye. In tube B, bullet (0.53%) had significantly ($p<0.05$) highest minimum inhibitory concentration whereas habanero had the least (0.275%). After bullet, both cayenne (0.41%) and bird's eye (0.388%) were ranked second since they were not significantly different in their minimum inhibitory concentration for bacterial speck of tomato (Table 3.8).

In late blight of tomato, all the chili varieties were not significantly different in their minimum inhibitory concentration in tube A. Rather, cayenne (0.555%) had a slightly higher minimum inhibitory concentration which was followed by bird's eye (0.235%), bullet (0.165%) and habanero (0.01%). However, tube B revealed a significant difference ($p<0.05$) in minimum inhibitory concentration in each variety whereby bullet (0.51%) was the highest and was followed by cayenne (0.355%), bird's eye (0.285%) and habanero (0.235%) (Table 3.8).

5.6. CONCLUSION

Bacterial speck and late blight diseases in tomatoes are both susceptible to fresh habanero chilies extracted from 200ml 99% ethanol. Resistance of these diseases to capsaicin extract from bird's eye, cayenne and bullet, could be experienced thus no recommendation of the use of the latter chilies.

From the recommended MBC of 0.01% of any antibiotic against microorganisms, capsaicin from fresh habanero chilies is the most preferred to inhibit the growth of bacterial speck and late blight in tomatoes. The other chilies have minimal ability to inhibit the growth of bacterial speck and late blight in tomatoes.

5.7. RECOMMENDATIONS

A study on other chili varieties will expound more on the activity of antioxidants and total phenolic in chilies. Chilies with higher capsaicin levels could be tested for susceptibility of bacterial speck and late blight diseases in tomatoes.

CHAPTER SIX; GENERAL CONCLUSIONS AND RECOMMENDATION

6.1: GENERAL CONCLUSIONS

From habanero, bird's eye. Cayenne and bullet chilies, habanero had the highest amount of capsaicin followed by bird's eye. Cayenne and bullet chilies respectively. This was also evident in the amounts of total phenolics and antioxidants activity being highest from the varieties with the highest capsaicin to the lowest. Moreover, habanero showed the highest anti-microbial activity followed by bird's eye. Cayenne and bullet chilies respectively. Therefore, there is a significant correlation between the amount of capsaicin and total phenolics, antioxidant activity and anti-microbial properties.

However, to achieve a high yield of capsaicin, use of fresh habanero chilies extracted using 200ml ethanol is recommended. Exploration of other chili varieties is necessary in order to narrow down to the chili variety that has the ability to yield higher capsaicin than habanero. Moreover, a study on other chili varieties will expound more on the activity of antioxidants and total phenolic in chilies. Chilies with higher capsaicin levels could be tested for susceptibility of bacterial speck and late blight diseases in tomatoes.

References

Abong'o. (2018). Occurence and distribution of orgaochlorine pesticide residue levels in water, sediment and aquatic weeds in Nyando river catchement, Lake victoria, Kenya. . *Afr. J of aquatic science*, 255- 270.

Adams, M. (2009). Effect of capsaicin and dihydrocapsaicin on in vitro blood coagulation and platelet aggregation. *Thrombosis*, 721-723.

Adiellsson, j. r. (2009). Monitoring of pesticides in swedish rivers. *ISSN O347-9307*.

Alexandra , B. (2009). Chemistry, antioxidant properties and bioavailability . *A review od quercetin*.

Alvarez, P., Rosa, d. L., & Shahidi , F. (2012). Food Chemistry . *Protective effect of fresh and processed jalaperio and serrano peppers against food lipi and human LDL cholesterol oxidation* .

Anand, P., & Bley , K. (2011). Topical capsaicin for pain management: Therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8% patch. *British journal of anesthesia* , 107.

Anarson, A. (2019). Chillies nutritional facts and benefits . *Chillies 101*.

Antonious, G. (2018). Capsaicinoids and vitamins in hot pepper and their role in disease therapy. *Capsaicin and its human theraputic development* .

Antonious, G., Berke , T., & Jarret , R. (2009). Pungency in Capsicum chinense: Variation among countries of origin. *Environmental science and health* , 179-184.

Arora, R., Gill, G., & Rana , A. (2011). An overview about versatile molecule capsaicin. *Pharmaceutical sciences and drug research* , 280-286.

B, P. (2008). Genetic resources and breeding of capsicum spp. *Genetic resources and breeding of capsicum spp*, 96-129.

Barbero, G., Liazid , A., Palma, M., & Barrosso, C. (2008). Fast determination of capsaicinoids from peppers by high-performance liquid chromatography using a reversed phase monolithic column. *Food chemistry* , 107.

Barbero, G., Palma , M., & Barroso, C. (2006). Determination of capsaicinoids in pepper by microwave-assisted extraction high-performance liquid chromatography with fluorescence detection. *Analytica Chimica Acta* , 227- 233.

Barceloux, G. D. (2008). Pepper and capsaicin . *Disease a month* , 380-390.

Basith, S., Cui , M., Hong, S., & Choi , S. (2016). Harnessing the therapeutic potential of capsaicin and its analogues in pain and other diseases. . *Molecules* , 966.

Baskaran, P., Markert , L., Bennis , J., & Zimmerman , L. (2019). Assessment of pharmacology, safety, and metabolic activity of capsaicin feeding in mice. *Scientific reports* , 9.

Bender , D. A., & Bender , A. E. (2018). A dictionary of food and nutrition. . *Newyork oxford university press* .

Bjarnadottir, A. (2019). Nutritional facts and health benefits. *Tomatoes 101*.

Bley, K., Boorman , B., Mohammad , B., McKenzie , D., & Babbar , S. (2012). A comprehensive review of the carcinogenic and anticarcinogenic potential of capsaicin. *Toxicologic Pathology* , 847-873.

Brauer, V. S., Rezende, C. P., Pessoni , A. M., De Paula , g. R., Rangappa, S. K., Nayaka , C. S., . . . Fausto, A. (2019). Antifungal agents in Agriculture . *Friends and foes of public health* .

Castros, S. M., Saraiva , J. A., Lopes da Silva, Delgadillo, I., VAn Loey , A., & Scout, C. (2008). Effects of thermal blanching and of high pressure treatments on sweet green and red bell pepper fruits. . *Food Chemistry* .

Chalupowicz, L., Dombrovsky, A., Gaba, V., Luria, N., Rueven, M., Beerman, A., . . . Manulis-Sasson, S. (2019). Diagnosis of plant diseases usisng Nanopore sequencing platform. *Plant pathol 68*, 229-238.

Derbalah A, T. K. (2019). Monitoring sources, discharges and fluxes of and assessing risks from pesticides in the kurose and asida rivers, Japan. . *International journal of enviromental science and technology.* , 1035- 1050.

Dijkstra, T. (2015). Trading fruits of the land. *Horticultural marketing channel*.

F, M., Tundis , R., Bonesi, M., Conforti, F., & Satti , G. (2009). The influence of fruit ripening on the phytochemical content and biological activity of capsicum . *Food chemistry* , 114-553.

FS, M., Borges , L., Paula , J., & Conceicao , E. (2012). Impact of different extraction methods on the quality of extraxts . 23-52.

FS, M., Conceicao, E., Bandeira , E., Costa , R., & Silva, J. (2014). The effects of extraction method on recovery rutin from calendula. *Pharmacognosy magazine* , 10-73.

Fuentes, A. F. (2018). High performance deep neural based tomato plant diseases and pest diagnosis system with refinement filter bank. *frontiers in plant science*.

Garming, H. (2014). Global Consumption . *Tomatoes are the superlative vegetable* .

Gilbertson, R. (2013). Tomato yellow leaf curl. *Plant Pathology*.

Gonzalez, A., Nunez Palenius , H., & Ochoa , A. N. (2011). Molecular biology of capsaicinoid biosynthesis in chili pepper (Capsicum spp.). . *Plant Cell*, 695-706.

HF, Z., Yang , X., Zhao , L., & Wang , Y. (2009). Ultrasonic assisted extraction of epimedin C from fresh eaves of Epimedium and extraction mechanism . *Innovative food science & emerging tecnologies* , 54-60.

I, P., & Oleszek, w. (2000). Food Chemistry . *Extrcation and determination of capsaicinoids in fruit of hot pepper capsicum annum* , 91.

Linanfu, Z., & Zelong , L. (2008). Optimization and comparison of ultrasound/microwave assisted extraction and ultrasonic assisted extractio of lycopene from tomatoes. *Ultrasonics sonochemistry* , 7.

Lukyanenko A, N. (1991). Genetic improvement ot tomato. *Disease Resistance in Tomato*.

Manzella, D. (2019). How simple carbs and cmplex carbs affect bloos sugar . *The differnt tyoes of carbohydrates* .

Martins, F., & Cardoso , E. (2015). Evaluation of extraction method on the chemical composition in extracts. 11-73.

Matthews , R. F., & Hall , J. W. (2018). Ascorbic acid, dehydroascorbic acid and diketogulonic acid in frozen greeb peppers. . *Journal of Food Science* .

Mayo, c. (2018). *Nutrtional and healthy eating.*

Mitem S.E, J., H. N., Imungi , J. K., Okoth , M. W., Wang'ombe , J., Mbacham, W. F., & Mitem , S. E. (2018). Assessment knowledge and use of pesticide by the tomato farmers in Mwea Region, Kenya. .

MS, C., Sharma , S. R., & Cotter , J. (2011). Solvent extraction and quantification of capsaicnoids from capsicum chinese food and bio products processing . 89-97.

Ochilo , W. N., Nyamasyo, G. N., C, D. K., A, W. O., D, M. O., A, F. C., . . . Lingeera, E. K. (2019). Characteristics and production constraints of smallholder tomato production in Kenya . *CABI*.

Riera, E., Gallego , J. J., & Mason , T. (2006). Airborne ultrasound for the precipitation of smokes and powders and the destruction of foams . 13-107.

Sarwar , M. (2014). Frequency of insetcts and mite fauna in chillies, Onion and garlic culiticated areas and their intergrated management. . *Int J Argron plant production* , 173-178.

savary, S. (2014). cropping ractices and disease management. *The root of crop health*.

Schlafer, O., Onyeche , T., Bormann , H., Schroder , C., & Sievers , M. (2012). Ultrasound stimulation of micro organisms for enhanced biodegradation . *Ultrasonics* , 9-25.

SL, A., Pearson , D., Okun , D., Lane , C., Williamson, I., & Pinkert, D. (2008). Imports of peppers. *International Trade commission*, 4-33.

Tomer, N. (2013). Determination of chlrinated pesticide in vegetables, cereals and pulses by gas chromatographyin east national capital region, Dehli, India. . 27-80.

Tsimbiri. (2015). Health impact of pesticides on residents and hrorticultural workers in the lake naivasha region, Kenya. . *Enviromental Medicine* .

U, S., Carle, R., & Schieber, A. (2019). Characterization of major and minor capsaicinoids and related comounds in chili pods. 112-123.

Wang, L., Yang , B., & Du, X. (2008). Optimisation of supercritical fluid extraction of flavanoids from pueraria lobota . *Food Chemistry* , 108-373.

Ware , M. (2018). How antioxidants benefit our health .

Y, W., Cui, S., Tang, J., & GU, X. (2007). Optimization of extraction process of crude polysaccharides fron boat- fruited sterculia seeds by response surface methodology . *Food Chemistry* , 105.

Z, D., & Bode , A. (2011). The two faces of capsaicin. *Cancer Research* , 2809-2814.

ZA, A. O., Ahmed , Y., Habila, M., & Ghafar , A. (2011). Determination of capsaicin and dihydrocapsaicin in capsicum fruit samples using high performance liquid chromatography . 16-29.