UNIVERSITY OF NAIROBI

COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES

RESEARCH PROJECT REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF VETERINARY MEDICINE

PROJECT TITLE: SENSITIVITY OF STREPTOCCOCCUS AGALACTIAE, STAPHYLOCCOCCUS AUREUS AND ESCHERICHIA COLI TO ZINGIBER OFFICINALE(GINGER) AND ALLIUM SATIVUM(GARLIC)

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J30/2031/2010

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DECLARATION

This research project is my original work and has not been presented for a degree in any other university to the best of my knowledge.

ChemutaiEmily (J30/2031/2010)…… Date……..

This research project report has been submitted with my approval as the supervisor

Dr.D.KKagunya (Department of Veterinary Pathology, Microbiology and Parasitology)

signature …… Date……

ACKNOWLEDGMENT

I acknowledge God almighty for giving me good health throughout my studies, The chairman department of Veterinary pathology, microbiology and parasitology and The chief technologist Mr.Weda for allowing me to use the microbiology laboratory for my project, my supervisor Dr. D.K Kagunya for his continuous support, guidance and suggestions throughout the course of this research study I wish you God's blessings.

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I thank my classmates for the moral support and suggestions throughout my studies. Last but not least gratitude goes to my parents Mr. and Mrs. Mosonik for their continual financial and moral support; this far it has been the hand of God.

ABSTRACT

There are a number of *Streptococcus* serotypes, *Staphylococcal* serotypes and *Escherichia coli* all of which have varying degrees of pathogenicity and as such there is need to use traditional herbs and spices to control and treat certain conditions caused by the same micro-orgarnisms such as controlling mastitis caused by *Streptococcus agalactiae*, abscesses on human skin(boils) caused by *Staphylococcus aureus,* enteric collibacillosis caused by *Escherichia coli*among other common conditionscaused by the same micro organisms using extracts of *Allium sativum*(garlic) and *Zingiber officinale*(ginger).Culture and sensitivity tests therefore are of key importance to test the efficacy of the traditional spices in controlling the micro-organisms.

CHAPTER ONE

1.0 INTRODUCTION

The traditional herbs have been in use for a long time especially among the Indian communities even though the use of them in the modern day has spread to more communities due to increased social interactions and education system that has brought the human races together.

On the other hand micro-organisms are present everywhere in the environment. Some bacteria are pathogenic while others are non pathogenic. The pathogenic ones cause disease only when certain conditions prevail such as:

1. When the host immune system is compromised

2. When they multiply to very large numbers

3. Virulence of the strain involved

4. When there are favorable conditions of water, oxygen and substrate

Also antibiotic resistances have been on the rise making treatment of bacterial disease conditions difficult both in animals and human. For the last 30 years no new antibiotics have been synthesized or discovered making resistance to antibiotics by bacteria a major problem. Therefore this necessitates search for antibacterial treatment substances from other alternative sources like traditional herbs and spices.

Due to the above factors there is need to use locally available and affordable traditional spices(ginger and garlic) in controlling *Streptococcus agalactiae*, *Staphylococcus* aureus and *Escherichia coli*.

1.1 JUSTIFICATION

Streptococcus agalactiae, Staphylococcus aureus and Escherichia coli are common in the environment; they can be pathogenic or non-pathogenic.The pathogenic strains cause diseases in both animals and humans. The diseases caused by these organisms are a threat to production by the animals for example streptococcal mastitis results in reduced milk production, slow growth in young calves(0-3 months old not weaned) as milk is the main source of nutrients for them. Also reduced labor output where humans are involvedfor example in cases of enteric colibacillosis resulting in diarrhea caused by enteropathogenic *Escherichia coli*. Therefore it is important to study the sensitivity of these strains to traditional spices and herbs in an attempt to counter the rising cases of antibiotic resistance. From the past studies *Zingiber officinale* and *Allium sativum* were widely used as spices in food especially by the Indian communities even though the use has spread across the world due to education and races interacting; hence, the purpose of this study was to ascertain the efficacy of *Zingiber officinale* and *Allium sativum*on the above mentioned micro-orgarnisms.

1.2 OBJECTIVES

GENERAL OBJECTIVE

To determine the sensitivity of *Streptococcus agalactiae,Staphylococcus aureus* and *Escherichia coli* to *Zingiber officinale and Allium sativum.*

SPECIFIC OBJECTIVES

1. To determine the sensitivity of *Streptococcus agalactiae, Staphylococcus aureus* and *Escherichia coli* to *Zingiber offinnale* and *Allium sativum* when used individually
2. To determine the combined effect of *Zingiber officinale* and *Allium sativum* on *Streptococcus agalactiae ,Staphylococcus aureus* and *Escherichia coli*
3. Recommend how the effects of *Zingiber officinale*and *Allium sativum* can be put into clinical use

CHAPTER TWO

2.0 LITERATURE REVIEW

- *Zingiber officinale* and *Allium sativum* extracts have been documented to have antibacterial activity against gram negative bacteria such as enterobacter coli and gram positive bacteria such as Streptococcus and Staphylococcus(Azu N, Oneagba R : Internatinal journal of Tropical Medicine ; Brazillian journal of microbiology volume 37 no.2 sao Paulo april/june 2006)
- *Allium sativum* has shown activity against *Salmonella typhimurium, Escherichia coli, Vibrio cholerae, Pseudomonas aeruginosa* and *Helicobacter pylori*(newsletter archives by Linda B White M.D; infection fighting herbs 2013.
- *Allium sativum* proved to have a higher antibacterial power than three conventional antibiotics used against two strains of staphylococcus infections common in hospitals(Journal of microbiology and antimicrobials 2010- www.pharmanewsonline.com)
- Allicin an active ingredient of *Allium sativum* has a potentially effective treatment for chronic inflammatory disease caused by *Streptococcus, Staphylococcus* and *Enterobacter coli* among other bacterias (food and chemical toxicology 2013)
- Participants who took one capsule of allicin containing supplement(active ingredient of *Allium sativum*) were less likely to contract cold virus(advances in therapy 2001- www.pharmanewsonline.com)
- In a study of fifty medicinal plants belonging to 26 families 72% showed antibacteial activity. 15 families exhibited activity against gram-postive and gram-negative bacteria. *Allium cepa* and *Allium sativum* exhibited activity against both filamentous and non-filamentous fungus.(journal of ethanopharmacology march 2001, vol.74(3) ; authors: D.Srinivasan, Sangeetha Nathan, P.Lakshmana)

- 1. Ginger and its constituents show antioxidant activity and prevent the damage of macromolecules, caused by the free radicals/oxidative stress.

  2. Ginger and its constituents also show a vital role as anti-inflammatory agents. Earlier studies on *in vitro* investigations of ginger preparations and some isolated gingerol-related compounds showed that anti-inflammatory effects of ginger such as inhibition of COX and inhibition of nuclear factor.

  3. Ginger also acts as antitumor via modulation of genetic pathways such as activation tumour suppressor gene, modulation of apoptosis and inhibition of VEGF. Earlier study has shown that terpenoids, constituents of ginger induce apoptosis in endometrial cancer cells through the activation of p53 (Shukla Y, Singh M ; Cancer preventive properties of ginger: a brief review; Food Chenical toxicology 2007, 45; 683-690.)

  4. Ginger also shows antimicrobial and other biological activities due togingerol and paradol, shogaols and zingerone. An important finding showed that 10% ethanolic ginger extract was found to possess antimicrobial potential against pathogens].(International journal of physiology ,patho-physiology and pharmacology vol. 6(2) 2004)

- Drug resistance is increasing worldwide and it is considered as a main culprit in the failure of treatment. The use of antibiotics against bacteria is an effective mode of treatment but also causes adverse complications. Earlier investigators have shown that, ginger and its constituents play a vital role in the prevention of microbial growth. An important study in the flavors of ginger as anti-microbial activity showed that ginger has antimicrobial activity against *Escherichia coli, Salmonella typhimurium* and *Bacillus subtilis*. Ethanolic extract of ginger showed widest zone of inhibition against *Salmonella*

typhimurium. Ginger rhizome contains several constituents which have antibacterial and anti fungal effects. The gingerol and shagelol are identified as more active agents. Earlier studies have shown that, ginger has broad antibacterial activity and the ethanolic extract of ginger powder has pronounced inhibitory activities against *Candida albicans* and other report also showed that antifungal properties of ginger extract, Gingerol. Chief constituents such as gingerol and gingerol, isolated from ginger rhizome, showed antibacterial activity against periodontal bacteria] and]-gingerol has been reported as active inhibitor of *M. avium* and *M. tuberculosisin vitro*. (International journal of physiology,pathophysiology and pharmacology vol 6(2) 2004)

2.1 ACTIVE INGREDIENTS OF ZINGIBER OFFICINALE AND ALLIUM SATIVUM

- *Zingiber officinale* : The active ingredients are terpene and oleoresin(ginger oil). Ginger oil is made up of volatile oils(1%-3%) and non- volatile pungent components. Terpene is composed of sesquiterpene hydrocarbons which is a major component and phenolic compounds(gingerol and shagoal). The lipophilic rhizome extracts yielded potentially active gingerols which can be converted to shagoals, zingerone and paradol.(Hassan HA, RasheedRaaufAM.AbdRazik BM , Rasool Hassan BA. Pharmaceut Chemical AnatActa 2012 : 3 : 184)
- *Allium sativum*: Allicin is the active ingredient and is responsible for many of its therapeutic benefits(Advances in therapy 2001, Food and chemical toxicology 2013)

Their mechanism of action is not clearly understood but it has anti-bacterial, anti-fungal and anti viral properties.

CHAPTER THREE

3.0:MATERIALS AND METHODS

3.1 MATERIALS

Rhizomes of *Zingiber officinale*

Bulbs of *Allium sativum*

Pure cultures of *Staphylococcus aureus, Streptococcus agalactiae* and *Escherichia coli*

Agar plates (Blood agar/ nutrient agar : 12 plates)

Mortar and pestle

Wire loops

Bunsen burner and lighter on working bench

Scalpel blade

Incubator

3.2 METHODS

PURE CULTURES

Sub-cultures from previous clinical cases of mastitis in a cow with Streptococcal mastitis, Staphylococcal mastitis and enteric coli-bacillosis from a rabbit made as follows:

Using a sterile wire loop a colony of bacteria was picked from the samples, then streaked on blood agar plates. This was done for *Streptococcus agalactiae, Staphylococcus aureus* and *Escherichia coli* .Also streaking on MacConkeydone for *Escherichia coli*. Working was around Bunsen burner flame. Once the streaking was over I placed them in the incubator for 24 hours.

FRESH PLANT EXTRACTION

With hands gloved bulbs of garlic and rhizomes of ginger were washed using distilled then peeled and final rinsing using distilled water.

It was then cut into smaller pieces put into the mortar then ground using a pestle

Picture 1.
GARLIC

PICTURE 2. GINGER

After grinding it was then weighed

TRIAL 1

2,5grams of both ginger and garlic weighed then put inseparate 100ml beaker, 15ml of distilled water added to each beaker and shaken to mix uniformly after which a drop of each extract was taken using a Pasteur pipette and put in the pre-made holes in nutrient agar already streaked with the respective bacteria(*Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli).* Also a 1:1 ratio of both ginger and garlic and ginger by taking 2ml of ginger and mixed with 2 ml of garlic and a drop taken and placed in a pre-made holes too. I left it for 15minutes to diffuse before incubating the plates for 24 hours.

TRIAL 2

5 grams of both ginger and garlic weighed,put in 2 separate a100 ml beaker, 15 ml of distilled water added to eachthen shaken to mix uniformly. A drop using a Pasteur pipette was taken and put in pre-made holes in nutrient agar already streaked with the respective

bacteria(*Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli*)for each extract. 2ml of ginger taken and added to 2ml of garlic them mixed well and a drop taken and put in pre-made holes then left for 15minutes to diffuse before incubating the plates for 24 hours.

TRIAL 3

A neat extract made by taking 20 grams of ginger and garlic crushed using mortar and pestle then its juice squeezed out using a gauze and the juice collected in the mortars used to crush them. A drop of each extract taken using a Pasteur pipette then placed in pre-made holes in blood agar already streaked with the respective bacteria(*Staphylococcus aureus,Streptococcus.agalactiae, Escherichia coli*). A mixture of the extracts then made by taking a drop from each extract and mixed in a glass slide then a drop was placedin a hole in the streaked plate.

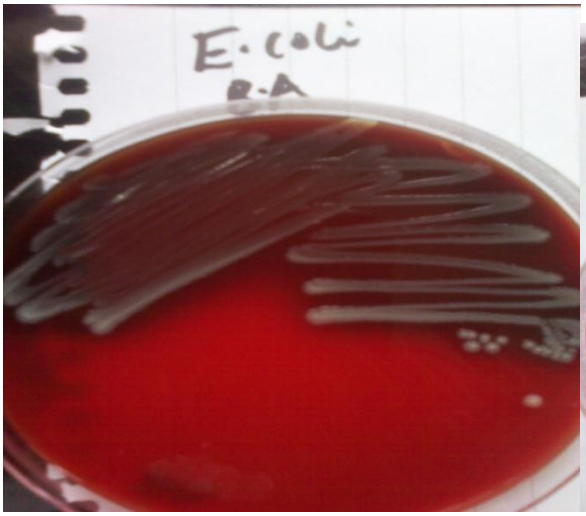
The number of holes made in each plate was four: ginger and garlic mixture and one for control

CHAPTER FOUR

PICTURE 3 ; HOLES MADE FOR EXTRACT DROPS

4.0 RESULTS4.1 :PURE CULTURE RESULTS

Colonial morphology



Picture 4

Picture 5

Picture 6

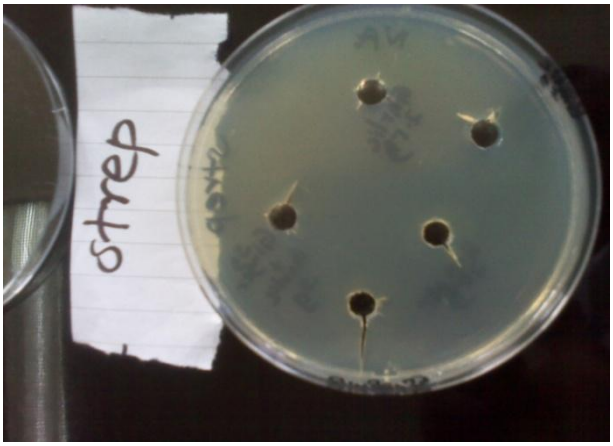
Picture 7

The above pictures show the gross appearances of the bacteria in the agar plates.

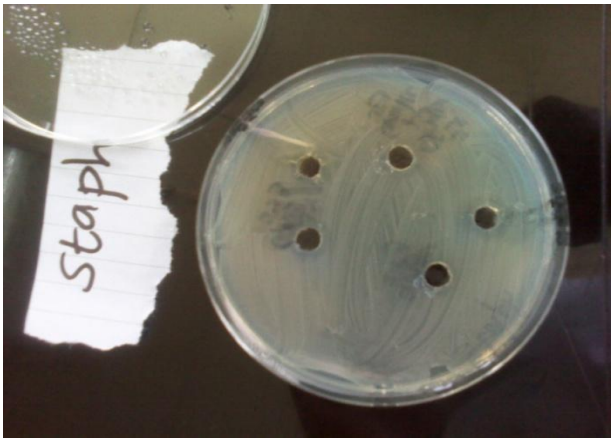
4.2 EFFECT OF 2.5 GRAMSAND 5 GRAMS GINGER AND GARLIC IN 15ML OF DISTILLED WATER ON THE BACTERIA

The dilute extracts did not meet the minimum inhibition concentration required to wipe out the bacteria cultured in the plates(the concentration was toolow).

Picture 8



Picture 9



Picture 10

4.3 EFFECT OF THE NEAT EXTRACT ON THE BACTERIA

Picture 11

Escherichia coli

Picture 12

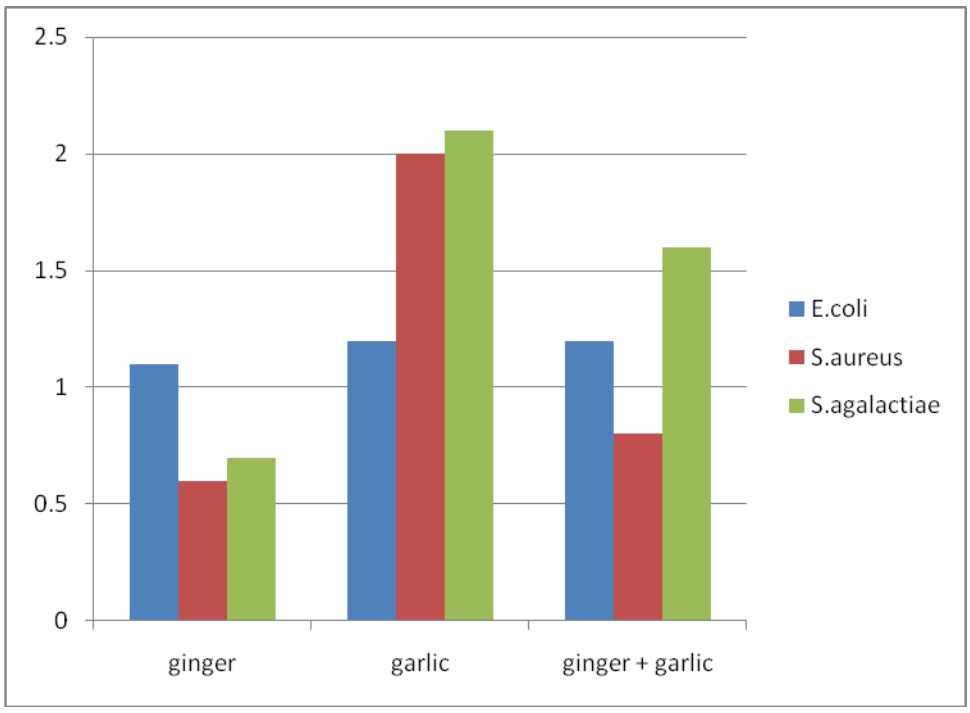
Staphylococcus aureus

Picture 13

Steptococcusagalactiae

The bacteria were sensitive to the neat extract as follows:

BACTERIA	DISTANCE CLEARED IN CENTIMETERS		
	GINGER	GARLIC	GINGER + GARLIC
Escherichia coli	1.1	1.2	1.2
Staphylococcus aureus	0.6	2	1.4
Streptococcus agalactiae	0.7	2.1	1.6



Picture 14

Key: vertical axis; measurements in centimeters

From the above chart the following observations could be made for the specific micro-orgarnisms whose sensitivity to Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) was tested for.

- *Escherichia coli*: Garlic extract was more effective then the (ginger+garlic)mixture and finally ginger.
- *Staphylococcus aureus*: Garlic extract was very effective, then the ginger+garlic mixture andfinallyginger
- *Streptococcus agalactiae*: Garlic extract very effective, then ginger + garlic mixture and finallyginger.

Of the three sensitivity agents used garlic was themost effective followed by the ginger + garlic mixture and last ginger. *Escherichia coli* was the most susceptible to ginger while *Streptococcus agalactiae* was the most susceptible to pure garlic extract and the ginger +garlic mixture.

Comparing the effectiveness of the dilute extracts and the neat extracts, It is clear that the more concentrated the extract, the more effective it is.

CHAPTER FIVE

5.0 DISCUSSION

The micro-orgarnisms(*Escherichia coli, Staphylococcus aureus* and *Streptococcus agalactiae*) tested for their sensitivity to *Allium sativum* and *Zingiber officinale* are classified into the domain bacteria and kingdom Monera. They are single celled prokaryotes, multiply by binary fission and are broadly divided into two groups: gram positive(*Staphylococcus aureus* and *Streptococcus agalactiae)* and gram negative(*Escherichia coli*). Bacteria are found everywhere; on air, water soil, plants, animals, inanimate objects and surfaces.

Bacteria cause diseases normally when they multiply to very large numbers, when the host immune mechanism is compromised and when the environmental conditions are favorable (air, water, temperature and nutrients availability).

Disease conditions caused by these bacteria are usually treated using conventional drugs and with continuous use, resistance hasprogressively developed hence the need to seek for alternative medicine such as the use of herbal drugs (Kaul PN, Joshi BS. Alternative Medicine : Herbal drugs and their critical appraisal Part II .Prog. Drug Res. 2001:57:1-75).

In this research study the micro-orgarnisms above are common mastitides (causes mastitis), also *Staphylococcus aureus* causes abscesses(boils) in humans while *Escherichia coli* causes enteritis resulting in diarrhea. When subjected to *Zingiber officinale* and *Allium sativum* at various concentrations they responded differently. When dilute extracts were used the organismsgrew on the entire plates as the extract did not meet the minimum inhibition concentration required to wipe out the bacteria. The neat extract was very effective and zones of clearance could be observed.

From the tests conducted garlic was the most effective followed by the ginger + garlic extract and finally ginger extracts. Therefore use should be limited to individual extract so that it exploits its full potential especially for garlic while for the use of ginger incorporating garlic extract into it would greatly improve its efficiency.

Garlic showed good results on *Staphylococcus aureus* compared to ginger. *Escherichia coli* were almost equally sensitive to both thus use of either of the extracts would still work for it. Streptococcus on the other hand was very susceptible to garlic. This therefore indicates that gram negative bacteria (*Escherichia coli* being an example) could be susceptible to both ingredients with an almost equal strength. Gram positive bacteria (*Staphylococcus aureus, Streptococcus agalactiae*) are more susceptible to garlic than ginger.

CONCLUSION

This study therefore shows that ginger and garlic are potential anti-biotics, and therefore quicker means of extraction and good storage qualities should be identified so that challenges due to resistance resulting from the current use of conventional drugs can be countered.

RECOMMENDATIONS

- Farmers should be enlightened on the potency of herbal drugs and their effectiveness as they are available locally,affordable and easy to use in an attempt to control mastitis in dairy farms.
- With the rising cases of anti-biotic resistance, global awareness on the use of alternative medicines (herbs and spices i.e*Allium sativum* and *Zingiber officinale* should be used to counter it.
- Occassional addition to feed to counter infections and boost immunity for example skin abscesses (boils) in humans caused by *Staphylococcus aureus*.

- Use on bruises and minor wounds should be encouraged as it counter any infections on the wounds and promote quick healing.

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