ANTIBACTERIAL EFFECT OF *Zingiber officinale* RHIZOME EXTRACT

A

Project work submitted to

Department of Microbiology

Central Campus of Technology, Tribhuvan University

In Partial Fulfillment for the Award of the Degree of

Bachelor of Science in Microbiology

Submitted by

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August 2017

RECOMMENDATION

This is to certify that **Mr. Himal Kafle** has completed this project work entitled **"ANTIBACTERIAL EFFECT OF *Zingiber officinale* RHIZOME EXTRACT"** as a part of partial fulfillment of the requirements of Bachelor's degree in Microbiology under my supervision. To my knowledge, this work has not been submitted for any other degree.

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ACKNOWLEDGEMENTS

It is a great privilege to be able to acknowledge the people without whom this project work wouldn't have been possible. I would take this opportunity to express my deepest appreciation to all those who provided me the possibility to complete this research and helped me all the way by providing their precious time, knowledge and guidance. First, I owe my deepest gratitude of thankfulness to my supervisor Mr. Hemanta Khanal. His supportive guidance was the base for my work. I would also like to thank my co-supervisor Mr. Suman Rai and Lecturer Prince Subba for their help and support. I am also thankful to my respected teacher and HOD of Department of microbiology, Mr. Shiv Nandan Sah for providing me idea for work and helping me on literature. I am also thankful to my entire teaching and non-teaching staffs for their constant support.

I would also like to thank Mr. Aindra Bahadur Karki**,** Mrs. Kamana Bantawa Rai and Mrs. Saru Budhathoki, laboratory persons of microbiology department for providing necessary materials and their help on my works.

My loving and respective parents, Mr. Loknath Kafle and Mrs. Pabitra Kafle for their love, support and encouragement to make me stand here today.

Lastly, I want to thank my very dear friends: Chetan Maskey, Mahesh Karki, Pawan Gurung, Rojina Subedi, Santoshi Ghimire, Yasoda Rai and Chunsha Limbu for their great support and encouragement during the work and study.

Above, I have gratefully acknowledged people who have been invaluable to me in completing this project work and I owe my sincere gratitude to everyone who directly or indirectly supported me during the research activities. However, all the opinions expressed in this work, and all errors, are mine.

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ABSTRACT

Ginger (*Zingiber officinale*) is an herbaceous, perennial flowering plant of *Zingiberaceae* family whose rhizome is widely used as a spice or a folk medicine. This study was conducted to determine the antibacterial effect of ethanol extract of dried ginger powder using agar well diffusion method against both, gram-positive bacteria i.e. *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus* spp. And gram-negative bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella* spp. The result showed the potent antibacterial activities of ginger extract against all test bacterial pathogens. Ginger purchased from local market of Itahari were washed, peeled, sliced and air dried for 15 days and then grounded to fine powder using mechanical blender. By soxhlet extraction technique using ethanol as the solvent, the ginger extract was prepared. After different phytochemical tests of the extract, the antibacterial activities of ginger extract was tested using agar well diffusion technique. Similarly, to compare the zone of inhibition of ginger extract with that of antibiotics, antibiotics susceptibility tests were also done. Minimum inhibitory concentration (MIC) of the extract was also determined by adding the different concentrations of extracts on bacterial swab at MHA agar plates. Ginger extract showed distinct antibacterial activity toward all six isolates on Agar well diffusion method. Ethanol extract of ginger showed highest zone of inhibition (22.0±1.1mm) against *Streptococus* spp. and lowest zone of inhibition (11.1±1.7mm) against *Pseudomonas aeruginosa.* Ginger extract also showed lower zone of inhibition (15.2±1.10mm) against *Staphylococcus aureus*, compared to gram-negative bacteria. Lastly, comparison between the effect of antibiotics and ginger extract on the bacterial sample were made and interpreted. Thus, from the result it is concluded that ginger have medicinal value and can be potential source for production of antibacterial drugs.

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LISTS OF ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
FDA	Food and Drugs Administration
GRAS	Generally Recognized As Safe
MDR	Multi Drug Resistant
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
mL	Milliliter
NB	Nutrient Broth
spp	Species
WHO	World Health Organization
XDR	Extremely Drug Resistant
µL	Microliter
µg	Microgram
µm	Micrometer
Z. officinale	*Zingiber officinale*
B. subtilis	*Bacillus subtilis*
E. coli	*Escherichia coli*
P. aeruginosa	*Pseudomonas aeruginosa*
S. aureus	*Staphylococcus aureus*

CHAPTER I

INTRODUCTION

1.1 Background

It is believed that the history of medicine is as old as human civilization. This mean to say that the use of plants and herbs had commenced from the starting of human civilization. Humans of ancient past time used to have plant parts, such as flowers, stems, barks, seeds and roots as the medicines. This mean to sum up that, many infectious diseases have been known to be treated with herbal remedies throughout the history of humankind. Most of the ancient study and documents revealed that plants were used medicinally in China, India, Egypt, and Greece long before the beginning of Christian era. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drugs leads because of the unmatched availabilities of chemical diversities. At that same period, particular efforts had been progressing to examine and classify the medicinal herbs. Plants were used as antimicrobial agent before the microbiological study had ever been started. (Gyawali, 2013)

The increased usage of antibiotics has induced microorganisms to acquire resistance factors, which have become a burning predicament. (Abimbola et al 1993) As a result, there is an urgent need to find the alternative of chemotherapeutic drugs in disease treatment particularly those of plants origin, which are easily available and have considerably less side effects. (Khulbe & Sati, 2009) The use of higher plants and there extracts for treating the infectious diseases has long been practiced in many parts of the world. (Sofowora, 1984) The plant-derived medicines may be used in many different forms including; powder, liquid or mixtures, which could be raw or boiled, such as, Liniments, Ointments and Incisions. (Apata, 1979)

An antimicrobial (i.e. antibacterial, antifungal and antiviral) substance is anything that destroy the microorganism (i.e. bacteria, fungi, and virus) or suppresses their growth or their ability to reproduce. In this context, we shall discuss about the antibacterial effects. In the 1940s, antibacterial (also called antibiotics) was defined as the substances produced by one microorganism, which

in low concentration inhibits the growth of other microorganisms. The meaning of term has changed over the years, both because of increasing number of synthetic analogues as well as their production from other biological sources like plants and animals. (Hugo & Russel, 2013)

Plants are rich sources of antibacterial components. Different plant extracts are being used in daily life to combat bacterial and fungal infections. (Duhan, 2013) In this study, the ginger extract that was extracted from ginger by using ethanol was explored for their antibacterial activity against various bacteria (*Bacillus subtilis, Staphylococcus aureus, Streptococcus* spp, *Escherichia Coli, Salmonella* spp, and *Pseudomonas aeruginosa*).

Ginger (*Zingiber officinale*) is a flowering plant, whose rhizome, ginger root or simply ginger is widely used as a spice or a folk medicine. It is a small plant of the family *Zingiberaceae*. It is called '*Aduwa*" in Nepali. Ginger originated in tropical rain forest in Southern Asia. Although ginger no longer grows wild. It is thought to have originated on the Indian subcontinent because of ginger plants grown in India show the largest amount of genetic variation. Ginger is widely cultivated in countries like India, China, Nepal, Indonesia and Nigeria. They are cultivated for medicinal and culinary purposes. (Roscoe, 1807)

Ginger is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases. (Ali BH, 2008) Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections. (Tan & Vanitha, 2004) The Zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes. (Chen IN, 2008) Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also "Generally Recognized as Safe" (GRAS) by the US FDA.

Bacteria now a day, due to improper uses of drugs are getting antibiotics resistant day by day. Emerging of new diseases as a new threat has become common. In order to solve these types of problems, new drugs are to be examined

and prepared. The target of present study is to unravel the effect of Ginger on some common gram positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus* spp) and gram negative (*Escherichia Coli*, *Salmonella* spp, *Pseudomonas aeruginosa*)

Escherichia coli are usually a commensals bacterium of humans and animals. Pathogenic variants cause intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia. (Baum & M, 2005 & Sodha et al) *Pseudomonas aeruginosa* is a non-sporing, non-capsulated, gram-negative bacillus. It can affect almost any external site or organ. Infection in hospital includes urinary tract infection, infected ulcers, burns and eye infections. (Greenwood, Slack & Peutherer, 2007) *Salmonella* is a genus of rod shaped, gram-negative bacteria of *Enterobacteriaceae* family. Different strains of *Salmonella* causes illness such as typhoid fever, paratyphoid fever and food poisoning (Salmonellosis). *Salmonella* infection mostly affects the gastrointestinal tracts causing bloody diarrhea, abdominal cramp and fever. It also causes bacteremia, meningitis etc. (Greenwood, Slack & Peutherer, 2007)

Staphylococci are gram-positive cocci about 1µm in diameter that are commonly found on the skin of healthy individuals. *Staphylococcus aureus* is present in nose of 30% healthy individuals but can cause infection at site of lowered host resistance, such as damaged skin or mucous membranes. Methicillin resistant Staphylococcus aureus (MRSA) in many cases has been a major public health issue. *Streptococci* are typically gram-positive cocci that are typically grown on chain or pairs. They cause wide range of suppurate infection (pus formation) in respiratory tract and skin, life threatening soft tissue infection and certain types toxin-associated reactions. Similarly, *Bacillus subtilis* is a gram-positive, rod shaped, spore forming and non-capsulated bacteria, which are abundantly found in soil and gastrointestinal tract of ruminants and humans. The spore of *Bacillus* germinates in the tissue at the site of entry and growth of organism results in the formation of gelatinous edema and congestion. It also reaches to blood stream and multiply freely in blood causing bacteremia and causes death of animals and humans. (Greenwood, Slack & Peutherer, 2007)

Essential oil derived from many aromatic plants are well known to possess cytotoxic, antioxidant, antifungal, insecticidal and antimicrobial activities (Shah & Qadir, 2013) A wide range of technologies is available for the extraction of active components and essential oils from medicinal and aromatic plants. The choice depends on the economic feasibility and suitability of the process to the particular situation. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicines. (Duhan J. S., 2013) The main aim of this research was to study the antibacterial effect of rhizome extract of *Zingiber officinale* among potential bacterial pathogen.

1.2 Rationale of the study

People have been using plant as medicine since a very long time and the knowledge of plant as medicine have been transferred from generation to generation. However, in past time, much of the knowledge has been based only on the experience of the people and in present time, much of the study had been done in Nepal. Now, approach that is much more scientific is to be made to determine the effect of plants, reasoning is to be done and applications are to be determined. Nowadays, due to the emergence of new drug resistant diseases, there is a necessity for the discovery of new drugs in scientific way. As ginger is used in most of the traditional system, the finding will support for scientific validation for the advance use of this plant for antibacterial activity against the most common gram-positive (*Bacillus subtilis, Staphylococcus aureus*, *Streptococcus* spp) and gram negative (*Escherichia Coli, Salmonella* spp, *Pseudomonas aeruginosa*). It can also be used as proof for the implication of this plant in the field of medicine and pharmaceuticals.

1.3 Objectives

1.3.1 General objective

- To determine the antibacterial effect of *Zingiber officinale* rhizome extract on some common gram-positive and gram-negative test bacteria.

1.3.2 Specific objectives

- To extract essential oil from ginger (*Zingiber officinale)* using soxhlet extraction.
- To determine the antibacterial effect of ginger extract on *(Bacillus subtilis, Staphylococcus aureus, Streptococcus* spp, *Escherichia Coli, Salmonella* spp *and Pseudomonas aeruginosa*).
- To determine minimum inhibitory concentration of the extract on each test bacterium.

1.4 Limitations of the study

In this study, antibacterial effect of ginger extract was determined on some common pathogenic bacteria on culture media. Thus, this study does not guarantee for its direct usage on treatment of diseases for human. This study is also not concerned with the inhibitory effect of ginger on fungi, actinomycetes, plant pathogens and other untested bacteria. Similarly, presence of some of the preliminary phytochemical tests of the extract were done, but this study does not show exact phytochemical components responsible for the antibacterial activity of ginger.

CHAPTER II
LITERATURE REVIEW

2.1 Ethnobotany

Ethnobotany is the study of the relationship between plants and people: From "ethno- study of people and "botany"-study of plants. Ethnobotany is considered a branch of ethnobiology. Ethnobotany studies the relationships between (uses of) plants and cultures. The complex focus of ethnobotany is on how plants have been or are used, managed and perceived in human societies and includes plants used for food, medicine, divination cosmetics, dyeing, textiles, for building, tools, currency,clothing, rituals, social life and music. Ethnobotany is a multidisciplinary science defined as the interaction between plants and people. The relationship between plants and human cultures is not limited to the use of plants for food, clothing and shelter but also includes their use for ornamentation and health care. (Choudhary, 2008)

2.2 Medicinal plants

Medicinal plants, medicinal herbs or simply herbs have been identified and used from prehistoric times. Plants make many chemical compounds for biological functioning, including defense against insects, fungi, and herbivores mammals. Over 12,000 compounds are known to science. These chemicals work on the human body is exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effect just like conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant as medicine can be complex. Medicinal plants are widely used to treat disease in non-industrialized societies, not least because they are far cheaper than modern medicines. The annual global export value of pharmaceutical plants in 2012 was over US $2.2 billion. (Gyawali, 2013)

Medicinal plants are one of the major sources of drugs all over the world. They are used, mostly in the form of extracts, as traditional drugs or as source of semi-synthetic bioactive drugs. The WHO estimates that medicines, derived

directly or indirectly from plants constitute about 25% of the pharmaceutical arsenal. The Nepalese biosphere is a very rich one due to different climate that have high biodiversity with about 1900 medicinal and aromatic plants. There is undeniably a growing need for pharmacists to understand the role of medicinal plants to develop new drugs especially with the emphasis on Himalayan medicinal plants. (Gyawali, 2013)

Natural products have long been investigated for their potential benefits. By continuous process of trails and selection, primitive man has learnt to use certain plant juice and crude extracts as antidotes for human disorder. In the 1900s, most medicines were obtained from the cooking, infusion, or maceration of roots, barks, leaves, or flowers. Today natural products still have a huge importance as a source of new drugs and leads. (Reddy & Grac, 2016)

Among nearly 300,000 species of higher plants available, only a small proportion has been investigated for medicinal properties, and still smaller number yield well-defined drugs. The same is the case with lower plants and with plants of sea. Approximately, only 10% of the organic constituents of plants are reported to be known and the remaining 90% are yet to be explored. (Farooqi & Sreeramu, 2014b)

In ancient time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plant's usage gradually abandoned the empiric framework and became founded on explicatory facts. People have tried to find medications to alleviate pain and cure different illnesses. In every period, every successive century from the development of humankind and advanced civilizations, the healing properties of certain medicinal plants were identified, noted, and conveyed to the successive generations. An estimated 400,000 tons of medicinal and aromatic plants are traded every year, with around 80 percent of the species harvested from the wild. About 15000 species of medicinal and aromatic plant species are used in traditional and modern medicine in the world. (Gyawali, 2013)

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal product today symbolize safety in contrast to the synthetics that are regarded as unsafe to the human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic product of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Over three quarters of the world population relies mainly on plants and plants extracts for health care. (Agrawal & Paridhavi, 2012)

Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious disease have led to increased emphasis is on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion populations cannot afford the products of western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant materials. (Apata, 1979)

In spite of the overwhelming influences and our dependence on modern medicines and tremendous advances in synthetic drugs, a large segment of the world population still like drugs from plants. In many of the developing countries, the use of plant drugs is increasing because modern life saving drugs are beyond the reach of the three quarter of third world's population although many of such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future. (Sofowora, 1984)

2.3 Medicinal plants of Nepal

Nepal has always been the center of herbal richness with more than Ten thousand species of the herbs in its alpine belts. The medical herbs database listing for Nepal showing 1,624 species of medical and aromatic species of medical

and aromatic species. These herbs have been integral part of traditional medicines practices of indigenous community in Nepal, seeing the same impact and long history of herbal use, these herbs of Nepal are exported to many countries and companies for medical purposes. Many big companies import such herbs to make medical extracts and derivatives that are used in treating various diseases and health deficiency. (Jha & Shah, 2013)

Either its Eastern medical homeopathic treatment or modern days' medicines, the herbs are used directly or indirectly, ultimately making a huge dependency in the natural herbs. Nepal being benefited with such geographical setting, these herbs grow wild without human interference, which make it even more effective. Medical herbs plants of Nepal are mainly used in making extract for cancer cure, liver and many chronic diseases. Local herbs are also used for antifungal, antioxidant, antibacterial purpose.

Various medicinal plants are common in use in Nepal for the treatment of different diseases. Different parts of medicinal plants serve for its medicinal property. For e.g. leaves of *Ocimum santum (Tulsi)* are used in daily life to treat cough, cold, fever and other viral infections. Juice of *Aloe Vera* leaves is used for burns and skin treatment. Dried stem barks of *Saraca indica (Asoka)* are used in uterine disorder and dysentery. Stem and flower of *Swertia chirata (Chiraito)* have its significant use on stomachic, febrifuge, and bitter tonic (Agrawal & Paridhavi, 2012)

Medicinally, leaves of *Azadriacita indica (Neem)* are used to cure many diseases of bladder, kidney, eyes and skin. Oil extract of flower are used for skin diseases. Trunk bark is useful in fever, thirst, nausea, vomiting, skin diseases and snakebite (Farooqi & Sreeramu, 2014a) . Similarly, *Allium sativum* (Garlic) has traditional dietary and medicinal application as an anti-infective agent. It is mainly used to cure the abnormal digestive problems such as Gastric and in curing common cold and sore throat. Similarly, *Thuja (Dhupi)* has also its use as Antiseptic, expectorant and in tuberculosis and diabetes. Ginger is another major medicinal plant of Nepal, which is commonly used by the Nepalese family in order to cure different health problems such as; sprains, rheumatism,

sore throat, muscular ache, vomiting, constipation, fever etc. (Agrawal & Paridhavi, 2012)

Beside the above-mentioned names, some of the other prevalent medicinal plants in are *Acorus calamus (Bojho), Mimosa pudica (Lajjawati), Aegle marmelos (Bel)* ¸ *Syzygium cumins (Jamun), Bhumiraj* etc. (Jha & Shah, 2013)

2.4 Use of ginger as medicinal plant

Ginger is widely used in the preparation of boiled food and beside that, it mostly used as folk medicine. Many scientific research and study showed that ginger contains foremost important phytochemicals that are beneficial for fighting against different diseases. It has been a popular spice and herbal medicine for thousands of years. It has a long history of use in Asian, Indian and Arabic herbal traditions. In China, for example, ginger has been used to help digestion and treat stomach upset, diarrhea, and nausea for more than 2,000 years. Ginger has also been used to help treat arthritis, colic, diarrhea, and heart conditions. (Felter, 1983)

Fig: Fresh rhizome of ginger

Ginger is native to Asia where it has been used as cooking spice at least 4,400 years. The medicinal part of this plant is composed of oil extracted from the rhizome. Ginger is a medicinal plant that has been widely used all over the

world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throat, muscular ache, pains, constipation, vomiting, hypertension, indigestion, dementia (chronic or persistent disorder of the mental process caused by brain disease or injury and marked by memory disorders, personality changes, and impaired reasoning), fever and infectious diseases. (Ali BH, 2008)

Today, health care professionals may recommend ginger to help prevent or treat nausea and vomiting from motion sickness, pregnancy, and cancer chemotherapy. It is also used to treat mild stomach upset to reduce pain and osteoarthritis and may even be used in heart disease.

i) Motion sickness

Several studies, but not all, suggest that ginger may work better than placebo in reducing in reducing some symptoms of motion sickness. In one trial of 80 new sailors who were prone to motion sickness, those who took powdered ginger had less vomiting and cold sweats compared to those who took placebo. Ginger did not reduce their nausea, however. A study with healthy volunteers found the same things.

However, other studies found that ginger does not work as well as medication for motion sickness. In one small study, people were given either fresh root or powered ginger, scopolamine, a medication commonly prescribed for motion sickness, or a placebo. Those who took scopolamine had fewer symptoms than those who took ginger. Conventional prescription and over-the-counter medicines for nausea may also have side effects that ginger does not, such as dry mouth and drowsiness. (Felter, 1983)

ii) Pregnancy-related nausea and vomiting

Human studies suggest that 1g daily of ginger may reduce nausea and vomiting in pregnant women when used for short periods (no longer than 4 days). Several studies have found that ginger is better than placebo in relieving motion sickness.

In a small study of 30 pregnant women with severe vomiting, those who took 1 gram of ginger every day for 4 days reported more relief from vomiting than those who took placebo. In larger study of 70 pregnant women with nausea and

vomiting, those who got a similar dose of ginger felt less nauseous and did not vomit as much as those who got placebo. Pregnant women should ask their doctors before having ginger and not taking more than 1 gram a day. (Felter, 1983)

iii) Chemotherapy nausea

A few study suggest that ginger reduces the severity and duration of nausea, but no vomiting, during chemotherapy. However, one of the studies used ginger combined with another anti-nausea drug. Therefore, it is hard to say whether ginger had any effect. More studies are needed. (Felter, 1983)

iv) Osteoarthritis

Traditional medicine has used ginger for centuries to reduce inflammation. In addition, there is some evidence that ginger may help reduce pain from osteoarthritis (OA). In a study of 261 people with OA of the knee, those who took a ginger extract twice daily had less pain and needed fewer painkiller medications than those who received placebo. Another study found that ginger was no better than Ibuprofen (Motrin, Advil) or placebo in reducing symptoms of OA. It may take several weeks for ginger to work.

Similarly, preliminary studies suggest that ginger may lower cholesterol and help prevent blood from clotting. That can help treat heart disease where blood vessel can become blocked and lead to heart attack or strokes. Other studies suggest that ginger may help to improve blood sugar control among people with type 2 diabetes. More research is needed to determine whether ginger is safe or effective for heart disease and diabetes.

Ginger's therapeutic properties effectively stimulates circulation of the blood, removing toxins from the body, cleansing the bowels and kidneys, and nourishing the skin. Other uses for ginger root include treatment of asthma, bronchitis, and other respiratory problems by loosening and expelling phlegm from the lungs. Ginger root may also be used to help break fevers by warming the body and increasing perspiration. (Felter, 1983)

2.5 Chemistry of Ginger

The primary known constituent of ginger root include gingerols, zingibian, bisabolene, oleoresin, starch, essential oils (zingibrene, zingiberole, camphene, cineole, borneol), mucilage and protein. Mainly the volatile oils present in ginger are bisabolene, cineole, phellandrene, citral borneol, citronellol, geranial, linalool, limonene, zingiberol, zingibrene, and camphene. Similarly, oleoresin such as gingerol and shagoal are as present in ginger. Zingibian is a proteolytic enzyme present in ginger. Besides that, Vitamin B6, Vitamin C, Calcium, Magnesium, Phosphorus, Linoleic acid, Gum, Starch, Lignin, Vegeto matter, Asmazone, Acetic acid, Acetate of potassa, and Sulphur are other abundant constituent of Ginger. (Connell & Jordan, 1971)

The pungency of ginger is due to gingerol which is the alcohol group of the oleoresin (when resins are associated with volatile oils, they are called Oleoresins). Ginger owes its aroma to about 1 to 3% of volatile oils, which are bisabolene, zingibrene, and zingiberol. Ginger contains several chemical components such as Starch (50%), Protein (9%), Lipids (including glycerides, phosphatidic acid, lecithins, and fatty acids; 6-8%), Protease (2.26%), volatile oils (including gingerols, shagaol, zingiberene, and zingiberol; 1-3%). The pungent principles (including the volatile oils i.e. gingerols) are the most medicinally potent because they inhibit prostaglandin and leukotriene formations (products in the body that influence blood flow and inflammation). They also give ginger its pungent aroma. (Denniff, Macleod, & Whiting, 1981)

The lemony character of fresh ginger is due to citral. The major components of the essential oil are the sesquiterpenes, beta-sesquiphellandrene and zingibrene. The ‘sharp’ constituent, causing the burning sensation of the mucous membrane, are substituted phenols (gingerols /shogaols). The secondary metabolites found in the rhizome of ginger that are of primary interest can broadly be divided into volatile compounds (extractable by steam distillation) and non-volatile phenolic compounds, the major ones of which have pungent properties. It is generally considered that the pharmacological activity of ginger rhizome

resides with compounds from these classes, in particular the non-volatile pungent phenolic compounds. (Connell, 1969)

The term oleoresin, when applied to ginger, refers to the volatile oil, the pungent compounds and other compounds extracted by means of solvents (ethanol or acetone) (Connell, 1969; Govindarajan, 1982a).

2.5.1 Non-volatile compounds

Ginger owes its pungency to phenolic compounds. In the fresh rhizome, the major type comprises a series of homologous phenolic alkanones known as gingerols and derivatives thereof such as gingerdiols. The principal of these compounds is [6]-gingerol with 8- and 10- gingerol occurring in lower concentrations (Connell & Sutherland, 1969; Denniff et al 1981). When subjected to heat or alkali treatment, however, gingerols are converted to a corresponding series of homologous shogaols by dehydration and/or to the compound zingerone (Connell, 1969) (Connell & Sutherland, 1969). The shogaols possess greater pungency than the corresponding gingerols (Denniff, Macleod, & Whiting, 1981).

2.5.1.1 Historical background

In 1879, Tresh isolated an oily pungent concentrate from ginger oleoresin and called it gingerol (Tresh, 1879). In 1917, English and Japanese researchers independently isolated two pungent ginger compounds, gingerol (Fig. 2-1) and zingerone (Fig. 2-2). (Lapworth, Pearson, & Royale, 1917)

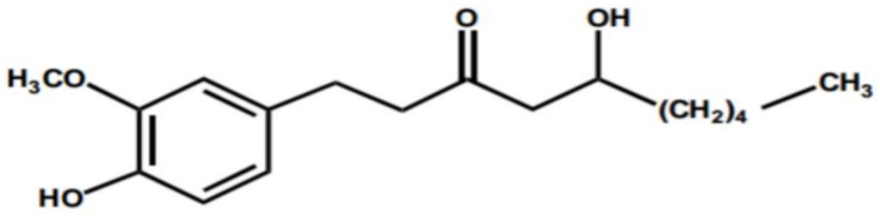

Fig. 2-1. Structure of [6]-gingerol, the most abundant gingerol in ginger rhizome.

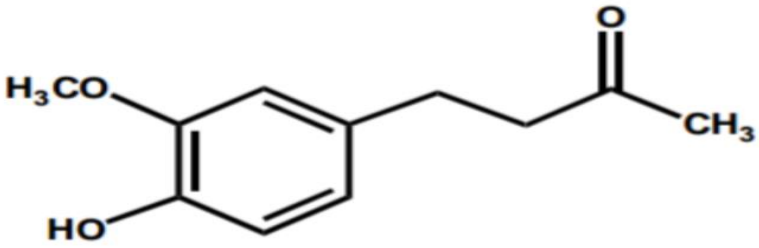

Fig. 2-2. Structure of zingerone.

In 1927, the Japanese group published the structural characterization of another pungent ginger compound, shogaol (Fig. 2-3) (after shoga, the Japanese word for ginger) (Connell, The pungent principles of ginger and their importance in cartain ginger products, 1969) (Govindarajan, 1982 a)

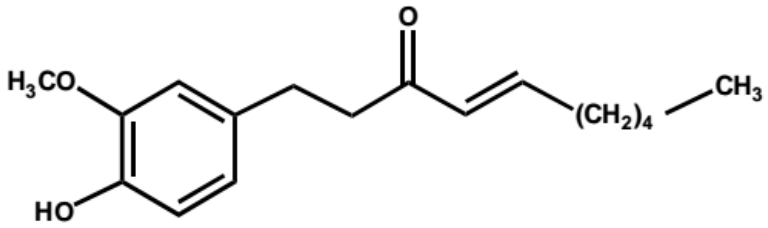

Fig.2-3. Structure of [6]-shogaol.

2.5.1.2 Gingerols

Comparing the chemical composition of commercially prepared ginger oleoresin and an oleoresin extracted with cold solvent, Connell's group found surprising differences. The major pungent compound identified in the commercial oleoresin was shogaol and despite repeated attempts, gingerol could not be isolated from this sample. The freshly prepared oleoresin extracted with cold solvent, however, had a different major constituent, which was isolated and identified as gingerol (Connell, 1969) ; Connell & Sutherland, 1969).

Extensive work on ginger oleoresin led Connell and Sutherland to suggest that although both shogaol and zingerone had been isolated from oleoresins; they were in fact either artefacts, or at the most minor constituents of ginger rhizomes. The presence of shogaol and zingerone in easily detectable quantities, according to Connell and Sutherland, were indicative of the oleoresin having

been exposed to excessive heat in the course of extraction (Connell & Sutherland, 1969)

Although gingerol is normally an oily substance, Connell and Sutherland were able to obtain a crystalline solid when storing the gingerol in hexane at -30^{o}C. This solid was shown to consist of a mixture of homologous phenolic ketones, identified as [6]-, [8] - and [10] gingerol (Fig. 2-4). [4]- And [12]-gingerols were not identified, but their presence in trace amounts could not be excluded.

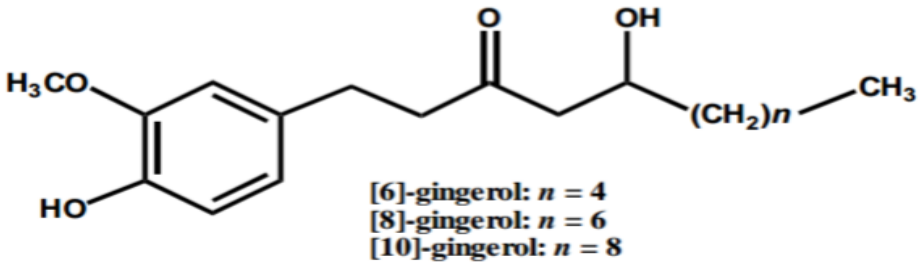

Fig. 2-4. Structures of the major gingerols in ginger

2.5.2 Volatile oil

Commercial ginger oil is obtained by steam distillation of coarsely ground dried ginger rhizome, and most published compositional analyses refer to oil prepared from dried raw material. Ginger oil distilled from dry material is characterized by a high proportion of sesquiterpene hydrocarbons and relatively small amounts of monoterpene hydrocarbons and oxygenated compounds (Govindarajan, 1982a). The major sesquiterpene hydrocarbons are zingiberene, ar-curcumene, β -bisabolene, (-) β -sesquiphellandrene and (E, E) α –farnesene (Lawrence, 1995b),although published data indicate the relative abundance of these compounds varies greatly. Fig. 2-5

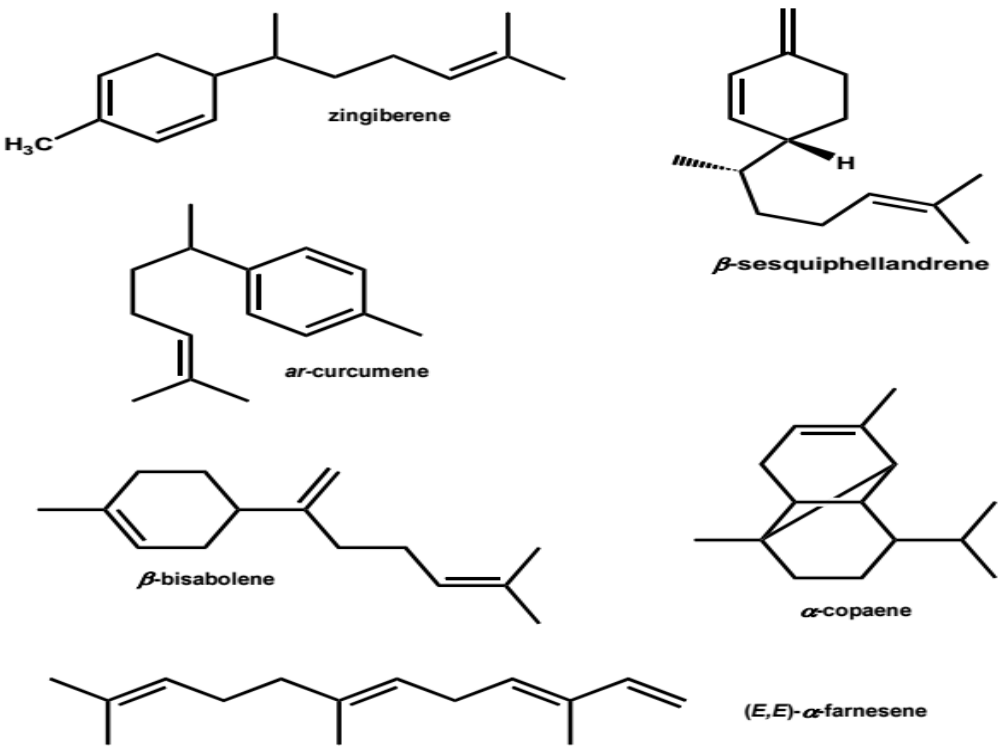

Fig. 2-5. Volatile sesquiterpenes from *Zingiber officinale*

Both zingiberene and (-) β-sesquiphellandrene can be oxidized to ar-curcumene in oil stored under unfavorable conditions (Connell & Jordan, 1971; Govindarajan, 1982b). High levels of ar-curcumene may therefore be an indicator of degraded oil, but could also possibly reflect distillation conditions (Govindarajan, 1982 a) Other constituents of ginger essential oil widely reported include α -pinene, camphene, 6-methyl-5-hepten-2-one, myrcene, α - and β -phellandrene, limonene, 1,8-cineole, linalool, borneol, α -terpineol, citronellol, neral, geraniol, geranial, bornyl acetate, 2-undecanone, citronellyl acetate, α -copaene and geranyl acetate. (Lawrence, 1995b) (Lawerence, 1997) (Lawrence, 2000) Some of the monoterpenoids in the oil are shown in Fig. 2-6.

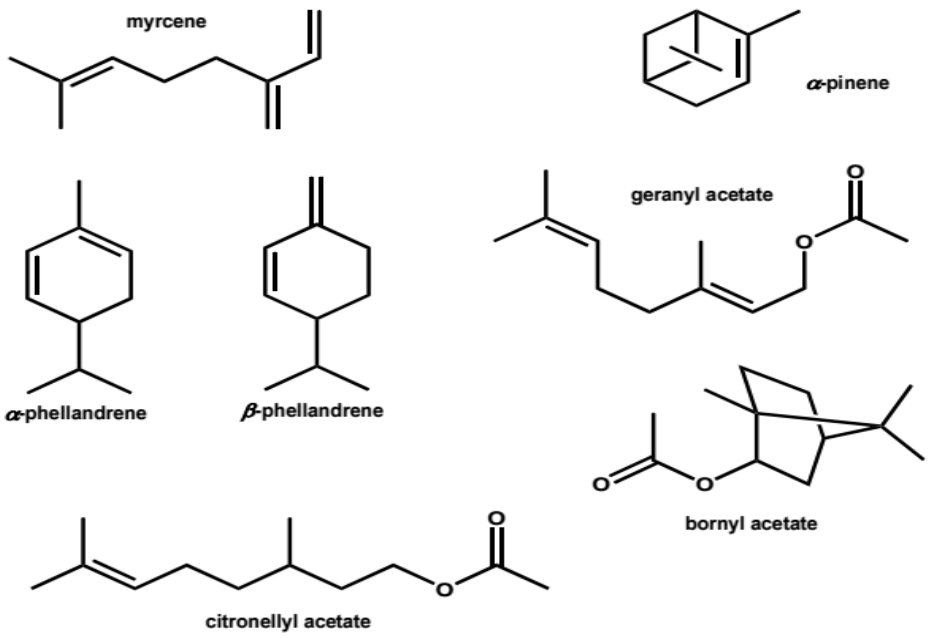

Fig. 2-6. Volatile monoterpenoids from *Zingiber officinale.*

2.6 Antibiotic resistance of diseases

The emergence of new infectious diseases, the resurgence of several infections that appeared to have been controlled and the increase in bacterial resistance have created the necessity for studies directed towards the development of new antimicrobials. Considering the failure to acquire new molecules with antimicrobial properties from microorganisms, the optimization for screening methods used for the identification of antimicrobials from other natural sources is of great importance. (Valgas, SMD, & Smania, 2007). There are high proportions of antibiotic resistance in bacteria that cause common infections (e.g. urinary tract infections, pneumonia, bloodstream infections) in all regions of the world. Increased usage of antibiotics has induced bacteria to acquire resistance factors which have become a burning predicament (Difficult or unpleasant situation). For e.g. MRSA, MDR *Pseudomonas*, XDR *tuberculosis* and other multi drug resistance gram negative bacteria which cause a high percentage of hospital-acquired infections. (WHO)

In a study among *E. coli* isolates, resistance to sulfonamide was one of the most common resistance profiles identified among our study isolates and

showed a monotone increasing resistance trend over time. Tetracycline resistance was the most common type of resistance observed and the most prevalent resistance phenotype in animal isolates (71.1%). A small percentage of *E. coli* showed resistance to chloramphenicol. One human *E. coli* isolate recovered in 1997-showed resistance to ceftiofur and ceftriaxone. This isolate was also resistant to nine other antimicrobial drugs. Gentamicin was approved for use in 1963. Although gentamicin resistance was rare in human *E. coli* isolates, we found resistance rates <40% among animal *E. coli* in 2002. Since 1980, resistance to gentamicin has increased among animal E. coli isolates. (Tadesse et al 2012).

In Europe, significant decline in susceptibility rates to β-lactams, aminoglycosides, and quinolones was recently observed in this pathogen, and nosocomial outbreaks of MDR *P. aeruginosa* have been described in various European hospitals. (Moniri et al 2005) Some strains of *Pseudomonas aeruginosa* have been found to be resistant to nearly all or all antibiotics including aminoglycosides, cephalosporin, fluoroquinolones, and carbapenems. Approximately 8% of all healthcare-associated infections reported to CDC's National Healthcare Safety Network are caused by *Pseudomonas aeruginosa*. About 13% of severe healthcare-associated infections caused by *Pseudomonas aeruginosa* are multidrug resistant, meaning several classes of antibiotics no longer cure these infections. An estimated 51,000 healthcare-associated Pseudomonas aeruginosa infections occur in the United States each year. More than 6,000 (or 13%) of these are multidrug-resistant, with roughly 400 deaths per year attributed to these infections. (CDC, 2013)

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes a range of illnesses, from skin and wound infections to pneumonia and bloodstream infections that can cause sepsis and death. MRSA in many hospitals has become a major public health issue, with concern expressed by patients and members of the public about the clinical implications. (Greenwood, Slack, & Peutherer, 2007)

Staphylococcus has also been showing resistance to vancomycin. These are also resistant towards methicillin and related antibiotics (e.g. nafcillin, oxacillin) and resistance to cephalosporin are of concern. CDC estimates 80,461 invasive MRSA infections and 11,285 related deaths occurred in 2011. An unknown but much higher number of less severe infections occurred in both the community and in healthcare settings. (CDC, 2013)

Multidrug-resistant (MDR) Pseudomonas aeruginosa also causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments and resistance to many antibiotics and antiseptics. A main problem is the emergence of multidrug-resistant P. aeruginosa strains resistant to different antimicrobial agent classes. Perhaps, this high degree of multidrug resistance related to the presence of antibiotic efflux systems which provide resistance to multiple antimicrobial agents (Adwan et al., 2009).

Streptococcus pneumoniae (*S. pneumoniae*, or *pneumococcus*) is the leading cause of bacterial pneumonia and meningitis. It also is a major cause of bloodstream infections and ear and sinus infections. *S. pneumoniae* has nowadays developed resistance to drugs in the penicillin and erythromycin groups. Examples of these drugs include amoxicillin and azithromycin (Zithromax, Z-Pak). *S. pneumoniae* has also developed resistance to less commonly used drugs. In 30% of severe *S. pneumoniae* cases, the bacteria are fully resistant to one or more clinically relevant antibiotics. Resistant infections complicate treatment and can result in almost 1,200,000 illnesses and 7,000 deaths per year. (CDC, 2013)

2.7 Antibiotic Susceptibility Test (AST)

Antibiotics are microbially produced anti-microbial agents. In other word, antibiotics are the substances produced by a microorganism, which in lower concentration can inhibit the growth of other microorganisms. Antibiotics have wide spectrum of action and is widely used in curing different diseases caused by microorganism. Less than 1% of the thousands of known antibiotics are clinically useful, other are not much used because of their toxicity and or lock

of uptake cells. Antibiotic susceptibility test is done to check whether the prescribed antibiotic is effective against the targeted microorganism or not. It is done to check how wide is its mode of action and how effective it is to cure the disease caused by any particular disease. This test is performed mainly in Muller Hinton agar. Selected antibiotics disc, when kept on the bacterial swab and incubating it at 37^{o}C for 24 hours, shows the distinct zone of inhibition. Based on the size of zone of inhibition, the effectiveness of antibiotics can be analyzed.

2.8 Minimum Inhibitory Concentration

The minimum inhibitory concentration was defined as the lowest concentration that completely inhibited the growth of microorganisms for 24 hours. MIC of the extracts can be carried out using agar well diffusion technique and tube dilution method. (Duhan et al, 2013).

The method allows comparisons between the microorganisms exposed to the same antimicrobial agents, but does not allow analog comparisons between the activities of different agents. This method is also utilized to select the commercial chemical agent presenting the better performance, as compared with other. (Mazzola et al, 2009).

The hole-plate method is the only suitable diffusion technique for testing aqueous suspensions of plant ethanol extract. (Hugo and Russel, 2013). In this method, the presence of suspended particulate matter in the sample being tested is much less likely to interfere with the diffusion of the antimicrobial substance into the agar than in the filter paper disc. (Valgas, SMD, & Smania, 2007)

CHAPTER III

MATERIALS AND METHODS

3.1 Materials Used

The materials used in this research is mentioned in Appendix-I.

3.2 Site of the study

Ginger rhizomes were bought from local market of Itahari and the intended work on its antibacterial effect was carried out in the microbiology lab of Central Campus of Technology, Hattisar, Dharan. The laboratory was provided with all the necessary materials and equipments that were required for this study.

3.3 Research method

The method for this study was qualitative as well as quantitative. This study was based on the culture method.

3.4 Type of study

The study was of descriptive type.

3.5 Bacterial Species

Standard culture of *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus* spp, *Escherichia coli*, *Salmonella* spp and *Pseudomonas aeruginosa*.

3.6 Work flow-chart

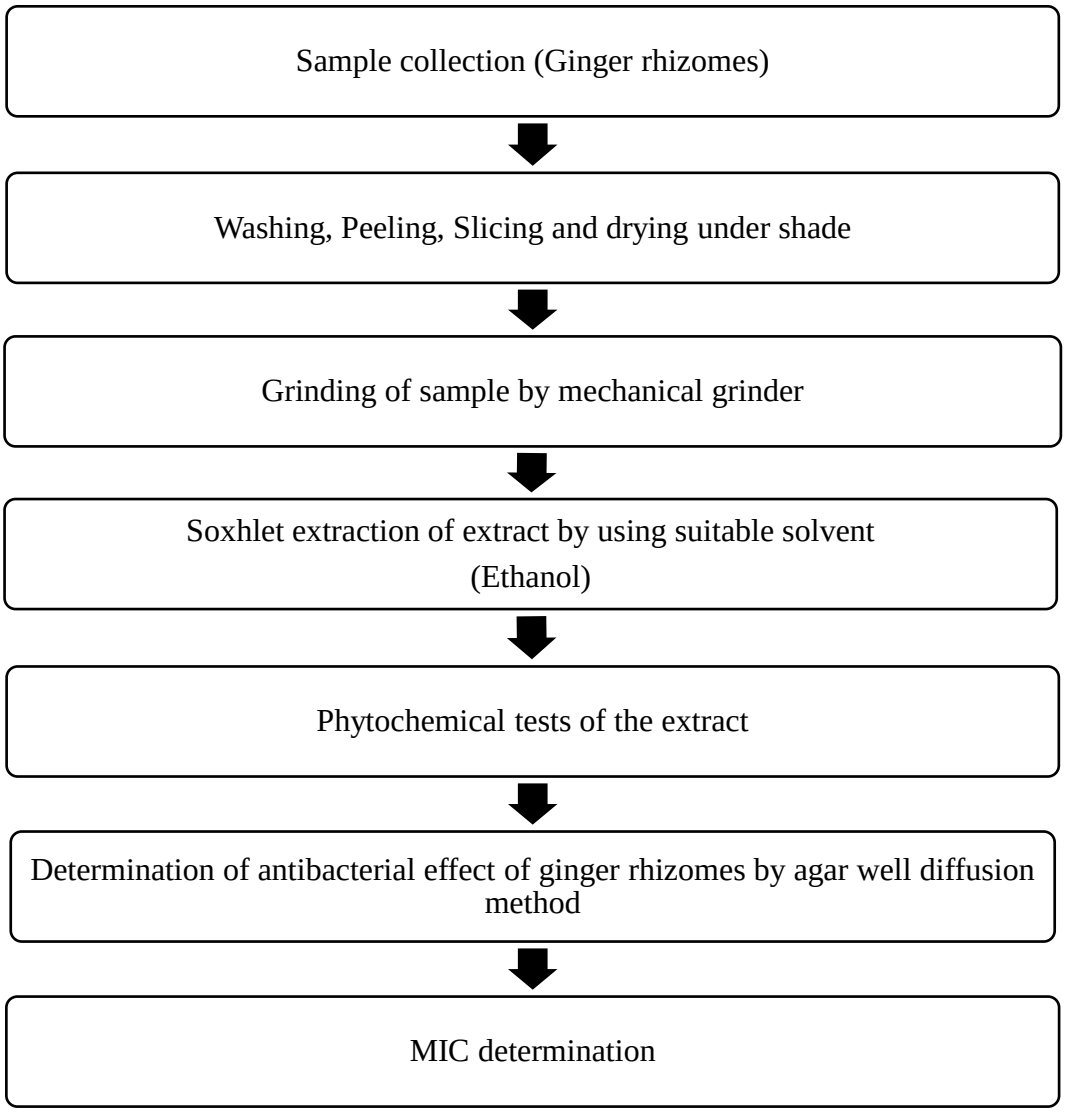

Fig: Work flow-chart of the study

3.7 Preparation of Ginger powder

The first step for extraction involved the preparation of dry powder from ginger rhizomes. For this, fresh gingers were brought from the local market of Itahari. The gingers were then thoroughly washed. After washing, they were peeled, sliced into pieces and then air dried at room temperature for 15 days to remove the moisture. Ginger pieces might get discolored during the sun drying so best dried under shade. Removing of sufficient moisture content of crude drugs was necessary to improve its quality and make it resistant to the growth of microorganisms. The dried ginger samples were then powered by mechanical grinder and sieved to give powdery form. The powder was stored in polythene bag at room temperature before extraction.

3.8 Preparation of extract

Firstly, 10g sample (powder+ fiber) was weighed using a digital balance. The sample was then placed in a thimble and enclosed in it. After enclosing the sample, suitable solvent was taken. For this study, Ethanol was chosen as the solvent. The thimble was placed on the soxhlet apparatus for solvent extraction. The solvent was then placed on the soxhlet apparatus. The solvent was left to siphoned single time. Then again, the solvent was added, so as to just cover the thimble. The temperature was adjusted at around 55-60^{o} C, where the boiling point of ethanol is 78.4^{o} C. The temperature at a once was not set to the boiling point, but the subsequent and mild heating was done. Then the process of siphoning was started. Siphoning was done until the extraction becomes transparent. The extracts were poured on a beaker and concentrated to dryness using rotary evaporator. This process was repeated when more ginger extract was required. The extracts were then ready for testing antibacterial activity.

3.9 Phytochemical analysis of ginger extract

Tests for the presence of the following plant secondary metabolites: alkaloids, saponins, tannins, flavonoids, steroids, phlobutannins, and cardiac glycosides were carried out on the extracts of the rhizome of ginger as described by Sofowora, 2008.

3.9.1 Alkaloids test: 2ml of the ginger extracts were taken and transferred into a flask and stirred with 3ml of 1% aqueous hydrochloric acid on a steam bath. Then 1ml of that filtrate was treated with few drops of Dragendorff's reagent (solution of potassium bismuth iodide). A color to blue black was the evidence of presence of alkaloids.

3.9.2 Saponins test: 2ml of the ginger extracts and 3ml distilled water in a test tube were taken in a test tube. Appearance of frothing on shaken with water showed the presence of saponins.

3.9.3 Tannins test: 2ml of the ginger extracts along with 50ml distilled water and filtered, then ferric chloride reagents was added, blue black or blue green precipitate appeared which showed the presence of Tannins.

3.9.4 Phlobotannins test: when an aqueous extract of the test sample (ginger) was boiled with 1% hydrochloric acid, deposition of red precipitate had confirmed the presence of phlobotannins.

3.9.5 Flavonoids test: When 5ml of diluted ammonia solution was added to aqueous filtrate of the test sample (ginger extract) followed by the addition of concentrated H_2SO_4, a yellow coloration was observed which determined the presence of Flavonoids.

3.9.6 Cardiac glycosides (keller-killiani test): When 3 ml of the ginger extracts dissolve in 1 ml of glacial acetic acid solution containing a drop of ferric chloride solution was underplayed with 1ml of concentrated H_2SO_4. A brown ring appeared at interface indicated adeoxy-sugar characteristics of cardenolides. A violet ring may appear below the brown ring, hile in the acetic acid layer, a green ring may form just gradually spread throughout this layer.

3.9.7 Steroid tests: When 2ml of acetic anhydride was added to 0.5g of the ginger extract and 2ml of sulphuric acid was added by the side of the test tube a color change was observed to violet or blue-green which showed the presence of steroids.

3.9.8 Terpenoids test: When 2ml of chloroform was added to 1ml of the extract, and Conc. H_2SO_4 (3ml) was added to form a layer, a reddish brown coloration at the interface indicated the presence of terpenoid.

3.10 Preparation of standard inoculum of test organism.

The antibacterial activity of Ginger rhizomes was tested against six bacterial species: *Bacillus subtilis, Staphylococcus aureus*, *Streptococcus* spp, *Escherichia coli*, *Salmonella* spp and *Pseudomonas aeruginosa*. Nutrient broth was inoculated with freshly sub cultured bacteria and incubated at 37^{o}C for 24 hours to match the turbidity to that of 0.5 McFarland standard. Such prepared inoculum was used to spread onto Mueller Hinton Agar using sterile cotton swab to make a lawn of bacteria.

3.11 Antibiotic Susceptibility Test

For this test, the fresh inoculums of test microorganism were taken. Six sterile petri plates were taken and Mueller Hinton agar was poured to it and allowed to solidify at room temperature. Sterile Mueller Hinton agar plate was uniformly swabbed with a specific bacterial culture. Then, three quadrants were divided and into which three different antibiotics discs namely ciprofloxacin, azithromycin and gentamycin having respective concentrations of 5µg, 15µg and 30µg were placed. The plates were incubated at 37^{o}C for 24 hours.

3.12 Screening of antibacterial activity (Evaluation method)

In order to access the antimicrobial activity, one method was performed, i.e. agar well diffusion method. Besides, we use well diffusion method for the determination of minimum inhibitory concentration.

3.13 Agar well diffusion method

Agar well diffusion method was used to determine the antibacterial activity. In this assay, Mueller-Hinton agar (MHA) plates were used for the growth of each bacterial species. MHA media was prepared and autoclaved at 121^{o}C for 20-30 minutes. It was then allowed to cool and plated at about 50^{o}C. Each plate was uniformly swabbed with bacteria by dipping in the standardized suspension with sterile swab and streaking it on the surface of the agar plate. (Basri and Nor, 2014). Into the wells of 5mm diameter created in the inoculated agar media with sterile cork borer, extract was loaded into each well and incubated at 37^{o}C for 24 hours and the plates were checked to determine the effect of the extract on desired bacteria by appearance of zone of inhibition by around the well.

3.14 Minimum inhibitory concentration

MIC was determined using well diffusion method as given by (Mazzola et al, 2009). The prepared MHA plates were inoculated with respective test organisms, i.e. *Bacillus subtilis, Staphylococcus aureus, Streptococcus* spp*, Escherichia Coli, Salmonella* spp and *Pseudomonas aeruginosa*. Four wells of 5mm diameter were made at least 1.5cm from edge of the plate. Each well was labeled for the amount of extract to be kept on. Various ginger extracts of 50µl, 25µl, 12.5µl and 6.25 µl of extract were respectively poured in the wells and were allowed to dry for few minutes. The plates were incubated at 37^{o}C for 24 hours for the determination of minimum inhibitory concentration.

3.15 Data collection

Data collection was based in experimental outcomes. The zone of inhibition was measured in millimeter (mm) using scale.

3.16 Data analysis

The data was analyzed, inferred and presented in tables and figures as required. Data were analyzed to determine the MIC values. Comparision of extract's effect on bacteria was done by comparing zone of inhibition on different bacteria and amount of extracts used.

CHAPTER IV

RESULTS

After collection of sample, they were successfully washed, peeled, cut into small pieces and dried under shade. Then they were subjected to mechanical grinder and small sieved particle i.e. powder was obtained. Ginger powder was packed in thimble and soxhlet apparatus was run which at the end gave extract with some residue that was pale yellow in color. The extract was then used to determine the antibacterial activity.

4.1 Percentage Yield

Percentage yield of the extract from ginger powder was calculated by using this formula:

% Yield of extract = (Weight of extract / Weight of ginger powder) ×100%

During extraction, 0.4g extract was obtained from a thimble containing 10 gram of ginger powder. Therefore, percentage yield of extract from each thimble containing 10g of ginger powder was

% Yield of extract = (0.4/10) ×100%

= 4%

Hence, the percentage yield of extract was found to be 4%.

4.2 Phytochemical tests

After performing different phytochemical tests of the ginger extract, the following results were obtained.

Bioactive principles (Phytochemicals)	Results
1. Alkaloids	Positive
2. Flavonoids	Positive
3. Glycosides	Positive
4. Phlobotannins	Negative
5. Tannins	Positive
6. Terpenoids	Positive
7. Saponins	Positive
8. Steroids	Negative

4.3 Effect of extract on test bacteria

Test of extract against the test bacteria were performed by agar well diffusion method. It was observed that ginger extract has inhibitory effect on both gram positive as well as on gram negative bacteria. All six test bacteria were inhibited by the extract whereas none were resistant to it. Extract was poured in the well with 5mm diameter and after overnight incubation zone of inhibition was observed.

The zone of inhibition by ginger extract and selected antibiotics upon all the six test bacteria were compared as follow:

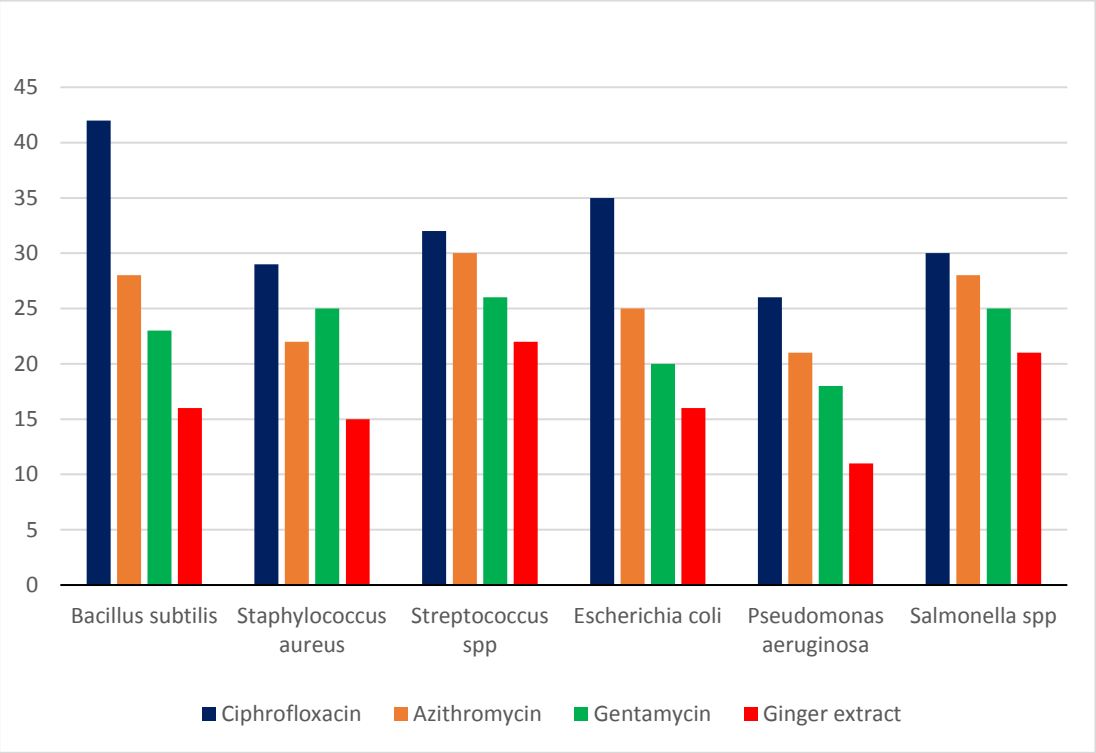

Fig: Bar diagram for zone of inhibition (mm) shown by selected antibiotics and ginger extract

Table I: Effect of extract on test bacteria on well diffusion

S.N	Materials used	Organisms used	Zone of inhibition (in mm)
1	Ginger extract	*Bacillus subtilis*	16 mm
2	Ginger extract	*Staphylococcus aureus*	15 mm
3	Ginger extract	*Streptococcus* spp	**22** mm
4	Ginger extract	*E. coli*	16 mm
5	Ginger extract	*Pseudomonas aeruginosa*	11 mm
6	Ginger extract	*Salmonella* spp	21 mm

Note: Most effective towards *Streptococcus* spp and *Salmonella* spp on well diffusion

Bacillus subtilis showed 16mm zone of inhibition, *Staphylococcus aureus* showed 15mm of zone of inhibition, *Streptococcus* spp showed 22mm zone of inhibition, *E. coli* showed 16 mm zone of inhibition, *Pseudomonas aeruginosa* showed a zone of inhibition of 11mm and *Salmonella* spp showed a zone of inhibition of 21mm. It revealed that ginger extract was effective against all six test bacteria.

4.4 Minimum inhibitory concentration (MIC) assay

MIC was determined for all six test bacteria. *Pseudomonas aeruginosa*, *Salmonella* spp and *Streptococcus* spp had MIC of 12.5µl whereas *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* had MIC of 25µl.

Table II: Zone of inhibition of test bacteria for MIC determination

Organisms	**Zone of inhibition (in cm)**			
	50µl	25µl	12.5mµl	6.25µl
E. coli	1.6	0.7	-	-
P. aeruginosa	1.1	0.9	0.7	-
Salmonella spp	2.1	1.9	1.2	
Streptococcus	2.2	1.8	1.1	-
S. aureus	1.5	0.8	-	-
Bacillus subtilis	1.6	1.1	-	-

4.5 Antibiotics susceptibility test

On testing three differents antibiotics i.e. Azithromycin, Ciprofloxacin and Gentamycin against six test bacteria, these results were obtained.

Table III: Zone of inhibition of standard antibiotics disc against different test bacteria

Organisms	**Zone of inhibition by using antibiotics**		
	Azithromycin	Ciprofloxacin	Gentamycin
E. coli	25 mm	35 mm	20 mm
Pseudomonas aeruginosa	21 mm	26 mm	18 mm
Salmonella spp	28 mm	30 mm	25 mm
Streptococcus spp	30 mm	32 mm	26 mm
Staphylococcus aureus	22 mm	29 mm	25 mm
Bacillus subtilis	28 mm	42 mm	23 mm

CHAPTER V

DISCUSSION

Our ancients recognized the healing power of plant many years ago and the practice of using plants as the source of medicine is practiced until up to date. Peoples on all continents have long applied thousands of indigenous plants, dating back to prehistory. Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new target is a matter of urgency. Despite the access to large chemical drugs for the treatment of different diseases, use of herbs as the natural drugs used to remain the alternative to treat deformities made in the normal physiological system by foreign organism or by any malfunctioning of the body. Our present study design to obtain preliminary information on the in vitro antimicrobial activity of ginger on six common pathogenic gram positive and gram negative bacteria, the agar well diffusion method was preferred to be used in this study. (Agrawal & Paridhavi, 2012) (Gyawali, 2013)

Fresh ginger rhizomes were brought from local market of Itahari. Then they subsequently washed, peeled, sliced to small pieces and dried under shade. According to (Gyawali, 2013) immediate drying prevents microbial fermentation and degradation of metabolites. In addition, protection from direct sunlight is essential to minimize chemical reactions induced by ultra violet rays. The sample was grinded to powdery form using mechanical blender. Grinding of ginger to its powdery form is necessary because it reduces the surface area of ginger to greater extent, which facilitates the easy extraction. Smaller surface area allows easy penetration of solvents into cells. For the extraction, different types of solvents can be used as per our choice. Ethanol, ethyl acetate, chloroform, acetone, n-hexane, toluene, petroleum ether etc. are commonly used extraction solvents. Among them, ethanol was used as the extraction solvent because it could easily enhance the extraction of bioactive compounds of different polarities without showing any adverse change on the required product. Soxhlet extraction was performed for the extraction of essential oil, as fresh solvent could

continually and easily extract the herbal materials efficiently with minimum solvent being used. (Duhan et al, 2013)

After extracting the extract from ginger by using ethanol as the extraction solvent, different tests of the extract were done. First test was for phytochemical tests for tannins, saponins, flavonoids, terpenoids, alkaloids, steroids, glycosides and phlobotannins. Similarly, ginger extract was also used to perform the agar well diffusion assay and MIC in order to know about its antibacterial effect. Ginger oleoresin was found to be effective against all six isolates. On well diffusion assay technique, oleoresin showed a distinctive zone of inhibition on tested bacteria, having zone of inhibition between 14mm and 25 mm in diameter. MIC of *Pseudomonas aeruginosa, Salmonella* spp and *Streptococcus* spp was found to be 12.5µl and that of *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* was found to be 25µl, which supports the validity of work. (Duhan et al, 2013) (Chen IN, 2008)

The present work reveals that the ginger rhizome is found to have therapeutics uses in treating various diseases. A detailed research work in the characterization and standardization is strongly required for this potential plant in developing its various formulations, which can ultimately be beneficial for human as well as animals. Antibacterial activity of ginger rhizome was observed against both gram positive and gram negative bacteria. Ginger rhizome shows a good antibacterial activity against these selected species i.e. *Bacillus subtilis, Staphylococcus aureus, Streptococcus* spp, *Escherichia Coli, Salmonella* spp, and *Pseudomonas aeruginosa.* Thus this study confirms its use as medicinal plant.

Ginger can inhibit an antibiotic resistant strain of *E. coli,* a bacterium that cause intestinal and food-borne illness. In recent years, several reports have been published concerning the composition and/or the biological properties (antimicrobial, antioxidants, anticancer, and a stimulated effect on the immune system) of *Zingiberaceae* extracts oils extracted from different species or varieties. These variations are likely to influence the antimicrobial activity of the oil and are generally a function of three factors: genetically determined properties, the age of the plant and the environment. (Abimbola, 1993)

In addition, the results for all extracts were more effective against the Gram-positive bacteria compared to the results for the Gram-negative ones. The higher resistance of the Gram-negative bacteria could be due to the complexity of the cell wall of this group of microorganisms. Indeed, the external membrane of Gram-negative bacteria renders highly hydrophilic surfaces whereas the negative charge of the surface of the gram-positive wall may reduce their resistance to antibacterial compounds. (Abimbola, 1993)

Ginger compounds are active against specific type of diarrhea, which is leading to cause death in infant in developing countries. Moreover, it has been found that ginger is effective in treating nausea caused by seasickness, morning sickness and chemotherapy, though it was found superior over a place for post-operative nausea. In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, shogaol and diarylheptanoids work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic and anti-tumor. (Felter, 1983)

The gingerols have analgesic, sedative, antipyretic, antibacterial and gastrointestinal tract motility effects. Ginger has the capacity to eliminate harmful bacteria, such as *Escherichia coli*, responsible for most of the diarrhea, especially in children. Ginger eases both diarrhea and constipation; hence, it should have impact on the growth of *Bacillus subtilis*, which mainly causes diarrhea and nausea. It has been shown to reduce the stickiness of blood platelets, hence may help reduce risk of arthrosclerosis. (Felter, 1983)

It has been reported that ginger extract and its pungent compounds demonstrated greater antibacterial activity against a variety of bacterial species including *Salmonella* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, although mixed result is attributed to different ginger preparations and varying strength. (Connell, 1969)

From these results, it is concluded that there might be some secondary metabolites that are present in the Ginger rhizome that had more potent antibacterial activity. These metabolites can be subjected to isolation and purification for the production of new antibacterial agent.

A critical factor for which ginger is used as a medicinal herb is its content of essential oils. The ginger volatile oil is consisted of monoterpenes, camphene, cineole, linalool, limonene, citral, geraniol, citronellol, and borneol and sesquiterpenes viz. α-zingiberene, ar-curcumene, β-bisabolene, β-sesquiphellandrene, zingiberol and zingiberenol along with some aliphatic aldehyde and alcohols. The volatile oil composition is highly variable depending upon a variety of factors including their geographical origin, distillation procedures, postharvest treatment, processing and drying conditions and temperature. The major pungent compounds in ginger are active gingerols and their derivatives, viz. shogaols, zingerone. (Govindarajan, 1982 a)

Ginger owes its unique flavoring properties to a combination of pungency and its aroma. Nonvolatile phenolic compounds provide the pungency, whereas the essential oil gives ginger its characteristic aroma. the ginger possess analgesic, anti-emetic, antiseptic, antispasmodic, bactericidal, carminative, cephalic, expectorant, febrifuge, laxative, rubefacient, stimulant, stomachic and tonic properties. Ginger is used to treat fractures, rheumatism, arthritis, bruising, carbuncles, colds, nausea, vomiting, hangovers, travel and seasickness, flu, cough, sinusitis, sores on skin, sore throat, diarrhea, colic, cramps and fever. That is why ginger is the widely used herbs for medical purposes and the further study about curing the fatal diseases by using ginger as the medicine seems to be the urgency to be done. (Connell & Sutherland, 1969) (Felter, 1983)

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

It is concluded that extract from rhizome of ginger (*Zingiber officinale*) is inhibitory to *Bacillus subtilis, Staphylococcus aureus, Streptococcus* spp, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* spp. Therefore, the rhizomes of ginger are ethno-botanically used and has great significant role in the treatment of many diseases. This study also revealed that the ginger rhizome might be useful as an antibacterial agent following extensive investigation.

The results obtained from our investigation confirm the use of Ginger as medicinal plant. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation. The result of the present study supports the medical usage of the studied plant and suggested that plant extract possess certain constituents with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious disease caused by pathogens. The most active extract can be subjected to isolation and purification of the therapeutic antimicrobials and carry out further pharmacological evaluation.

The results of our experiments showed that different bacterial species exhibited different sensitivities towards the extract of ginger. Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the spices extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus spices, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens.

6.2 Recommendations

Based on the above study, following recommendations are suggested

6.2.1 Recommendation

- Awareness programme should be performed for people to understand about conservation of biodiversity and medicinal uses of ginger.
- Pharmaceuticals should give emphasis on use of ginger for treatment of disease and production of pharmaceuticals.
- Isolation of active ingredients responsible for antibacterial activity.

6.2.2 Recommendation for further study

- Research can be performed to determine its phytochemical constituents, their use and effect on different microorganisms (including drug resistant species).
- Research can be carried out to determine the use of ginger and its impact in human.
- Research can be performed to determine the effect of ginger on plant pathogens for the control of plant diseases.

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APPENDICES

APPENDIX I: MATERIALS USED

Glasswares

Pipettes

Test tubes

Petri plates

Conical flask

Round bottom flask

Beaker

Glass rod

Glass tubes

Equipments:

Autoclave

Soxhlet apparatus

Hot air oven

Microscope

Incubator

Refrigerator

Chemicals:

Ethanol

Lysol

Materials:

Test tube racks

Wash bottle

Burner

Markers

Price tag

Thimble

Others:

Cotton swabs

Sample:

Ginger (*Zingiber officinale*) rhizomes

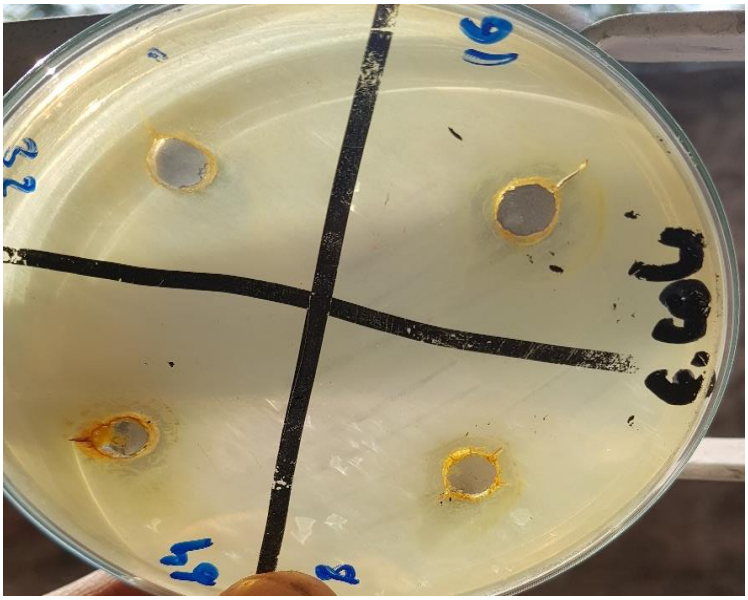
APPENDIX II: COMPOSITION OF MEDIA USED

Nutrient broth

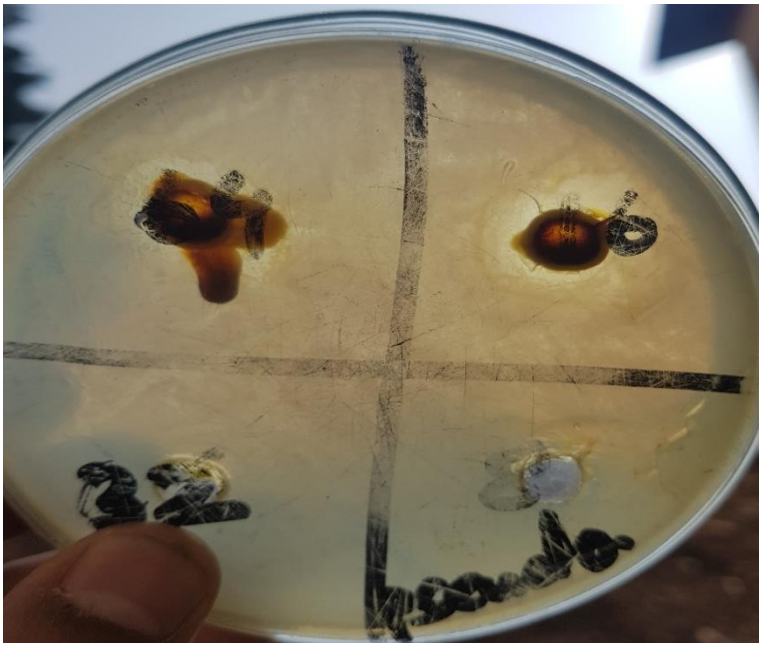
Ingredients	gm/l
Peptone	5.0 g
Sodium chloride	5.0 g
Beef extract	1.5 g
Yeast extract	1.5 g
Agar	15 g
Final pH	7.2

Mueller Hinton Agar Medium

Ingredients	gm/l
Beef extract	2.0 g
Acid hydrolysis of casein	17.5 g
Starch	1.5 g
Agar	17 g
Final pH	7.3

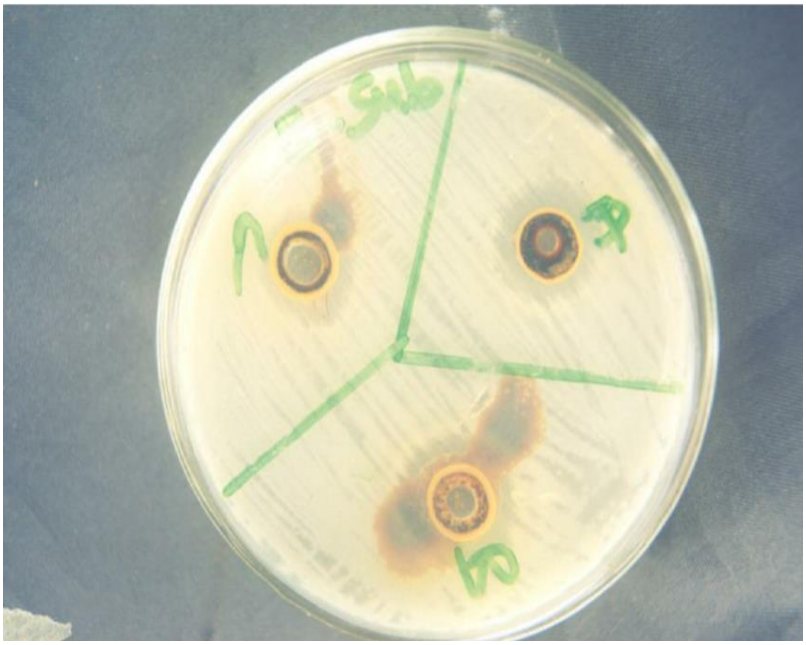


Photograph 1: Effect of extract on *Escherichia coli* on well diffusion

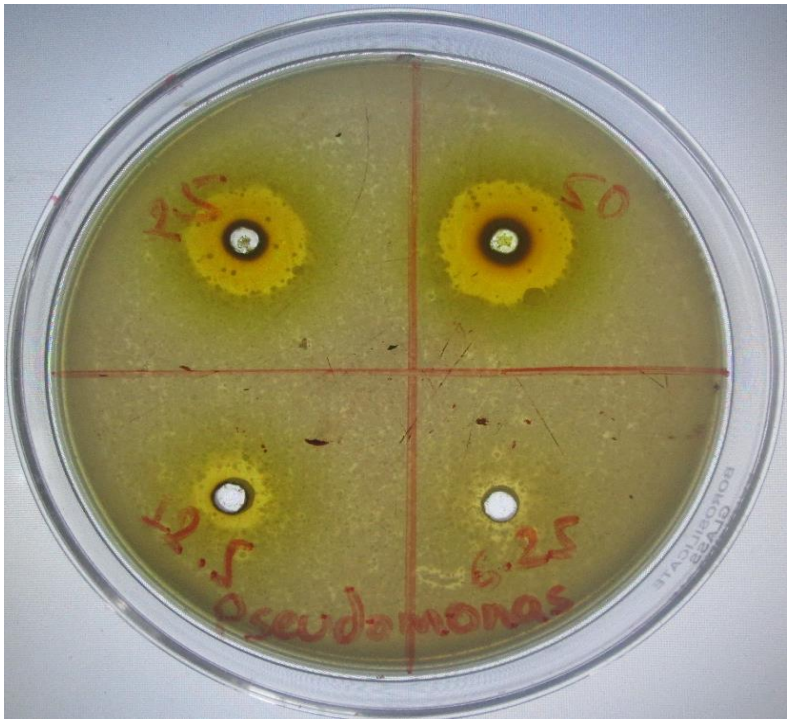


Photograph 2: Effect of extract on *Pseudomonas aeruginosa* on well diffusion

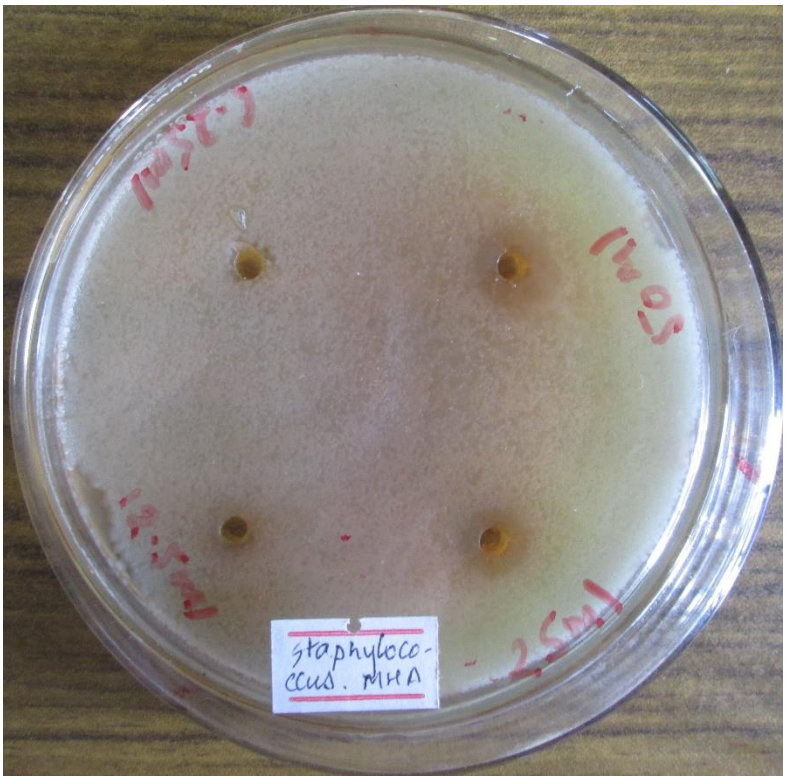
Photograph 3: Effect of extract on *Staphylococcus aureus* on well diffusion



Photograph 4: Effect of extract on *Bacillus subtilis* on well diffusion



Photograph 5: Determination of MIC for *Pseudomonas aeruginosa* [MIC value- 12.5µl (0.7cm)]



Photograph 6: Determination of MIC for *Staphylococcus aureus* [MIC value- 25 µl (0.8cm)]