ANTIBACTERIAL PROPERTIES OF CERTAIN ESSENTIAL OILS AGAINST BACTERIA ISOLATED FROM ROTTED CARROT (*Daucus carota*)

BY

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UNIVERSITY OF BENIN

BENIN CITY

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFESCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) HONOURS IN MICROBIOLOGY.

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFESCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

MARCH, 2024.

CERTIFICATION

This is to certify that this project was carried out by Destiny Osemeke OCHEI in the Department of Microbiology, Faculty of Life Sciences, University of Benin, under the supervision of Mr. G. O. Oribhabor, submitted to the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, in partial fulfillment for the requirement of the award of Bachelor of Science (B.Sc.) degree in Microbiology.

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MR. G. O. ORIBHABOR **DATE**

Project Supervisor

APPROVAL

This is to certify that this project work was accepted in partial fulfillment of the requirement for the award of Bachelor of Science B.Sc.(Hons) Degree in Microbiology of the University of Benin, Benin City.

_____ _____

PROF. (MRS) F.I. AKINNIBOSUN **DATE**
Head of Department

DEDICATION

I dedicate this project to God Almighty for his guidance, provision, wisdom, understanding, strength and protection during the course of this project.

ACKNOWLEDGMENT

It is my earnest intention to express my profound gratitude to Almighty God for His wisdom and understanding on the successful completion of my project.

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ABSTRACT

Carrot (*Daucus carota*) is one of the most important economical root vegetable crops worldwide and the largest source of provitamin A and carotenoids in the human diet. Storage is a prerequisite for a year-round supply of Carrot, but longer duration can affect its quality. Essential oils also has diverse and relevant biological activities. This study was aimed towards isolating the bacteria associated with rotted carrot, characterizing and isolating three bacteria isolate and determining the antibacterial properties of selected essential oils against the isolates using Agar well diffusion method. Suspected bacteria pathogens were isolated from a diseased carrot tuber, the isolates were identified using biochemical and cultural characterization. Essential oils were used in the antimicrobial sensitivity treatment using agar well diffusion method against the isolated bacteria to determine the antibacterial property of the essential oils. The results showed that *Enterobacter* sp., *Pseudomonas* sp. and *Agrobacterium tumenfacien* were the isolated pathogenic bacteria from the carrot tubers. The essential oils which are (Neem oil, Tea tree oil and Bergamot oil), showed no antibacterial activity on the isolated bacteria pathogens. Further investigations can be done using essential oils against bacterial isolates but it should be extracted from the plant source to avoid destroying its potential antimicrobial properties.

CHAPTER ONE

INTRODUCTION

Carrot (*Daucus carota*) is one of the most important economical root vegetable crops worldwide and the largest source of provitamin A and carotenoids in the human diet (Constance, 1971). Mostly, it was cultivated for its nutritional status of α and β carotene, vitamins (A, B6, and K), and minerals (Ca and potassium) with edible fiber (Heinonen, 1990). Naturally, it is grown in temperate regions with high-humidity agroecosystems. *Daucus carota* is one of the most crucial root vegetables in the family *Apiaceae*, which is cultivated worldwide. Current studies are mainly focused on nutrient content, breeding, cultivation, increasing yield, tissue culture, and regulating carotenoid synthesis.

The cultivated carrot can be mainly classified into the anthocyanin or eastern-type carrot (e.g., yellow or purple) and the carotene or western-type carrot (e.g., yellow, orange, or red) based on the pigmentation in the roots (Banga, 1957). Western carrots are always cylindrical or tapered cylindrical in shape and has less pubescent leaves, higher provitamin A carotenoid content, and higher sugar content when compared to eastern carrots (Baranski *et al.,* 2012). Eastern carrot always have thicker, shorter conical roots, pubescent leaves and are poor in provitamin A carotenoid content (Rubatzky *et al.,* 1999).

Storage is a prerequisite for a year-round supply of Carrot, but longer duration can affect its quality. After harvest, carrots are susceptible to a wide variety of spoilage organisms including phytopathogenic fungi (Suojala, 2000) and many species of bacteria and yeasts (Snowdon, 1991). Bacterial spoilage occurs when there is poor storage conditions or when the soil conditions are wet (Farrar *et al.,* 2000). Bacterial degradation of carrots begins at the crown or root tip and then progresses rapidly through the core region (Towner and Beraha, 1976). Such spoilage is characterized by a brownish flesh and smelly decay which could be extremely soft and slimy to touch (Godfrey and Marshall, 2002).

Essential oil also known as “essence” or “volatile oil” is a mixture of complex volatile constituent biosynthesized by many living organisms (Bauer *et al.,* 2001). They carry a

distinctive scent of the source plant and are generally extracted by steam distillation and other processes like maceration, cold pressing, or solvent extraction. Essential oils (EOs), their fractions and isolates are used in a large variety such as, in flavour and fragrance, food, perfumery, cosmetics and toiletries, fine chemicals, pharmaceutical industries and therapy (Baser, 1995). EOs also has diverse and relevant biological activities. They are used in the medical field due to their biocidal activities (bactericidal, virucidal and fungicidal) and medicinal properties. Numerous studies have highlighted EOs antimicrobial effects even against multi-resistant bacteria (Mayaud *et al*., 2008, Burt, 2004). Furthermore, EOs have been used as a cleaning liquid for disinfecting medical equipment and surfaces (Warnke *et al*., 2009) or as an aerosol in operating blocks and waiting rooms for air cleaning to limit contaminations (Billerbeck, 2007). They could also provide a pleasant feeling of psychic comfort for patients as a result of their pleasant odor. Use of EOs as food preservatives has also been described (Burt, 2004, Tiwari *et al*., 2009).

1.1 AIM AND OBJECTIVES

The aim of this study was to investigate the antibacterial properties of essential oils against bacteria isolated from rotted carrots. The specific objectives were to;

I. Isolate the bacteria associated with rotted carrot.
II. Characterize and identify three bacteria isolate.
III. Determine the antibacterial properties of selected essential oils against the isolate using the Agar well diffusion method.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Research

Carrot (*Daucus carota* L.) is a biennial herbaceous species that is a member of the *Apiaceae* family (Mandrich *et al.,* 2023). It is among the most widely cultivated root vegetables in the world. The root, the stem, and the umbel make up a carrot. The stalk beneath the white flower canopy is approximately one meter tall. The portion of the carrot that is most frequently consumed is the root; it is tasty, big, and has a good shelf life. The pulpy outer cortex, or phloem, the inner core, or xylem, and the peel, or periderm, combine to produce the root. (Mandrich *et al.,* 2023).

Carrots are divided into eastern and western varieties according to the color of their roots (Heywood, 1983). Only a small percentage of eastern carrots have yellow roots; the majority have purple roots. Conversely, the roots of western carrots can be orange, red, or white. Based on the first molecular investigation about carrot domestication, it was believed that the western-type cultivated carrots directly came from the eastern-type carrots (Lorizzo *et al.,* 2013). Heywood, on the other hand, believed that western-type cultivated carrots did not directly come from eastern-type carrots (Heywood, 1983). He summarized the theory that the domestication of western-type cultivated carrots involved a subsequent domestication event. A recent study suggests that genetic improvement from wild carrots may also be the source of western-type orange carrots from eastern carrots (Rong *et al.,* 2014).

The most well-researched and well-known *D. carota* L. subspecies are *D. carota* subsp. *Sativus*, also known as the domestic carrot, and D. carota subsp. carota, well known as the wild carrot. The primary differences between these subspecies are in the color and thickness of the roots as well as in flavor. Domestic carrot roots come in a variety of color, ranging from purple to white. They taste sweet and are thick. Queen Anne's lace, another name for wild carrots, are bitter-tasting plants with thin, white roots (Heywood, 1983).

There is a common misconception that domestic carrots are directly descended from wild carrots. Moreover, this belief is supported by their shared characteristics, which include the way they grow, the scent, and the design on the leaves (Carrot, 2011). Wild and domestic carrots intercross

freely, and they are predominantly cross-pollinated by a large diversity of insects, so the gene-flow frequency may be very high when both the spatial distribution and flowering overlap.
Recently, a review reported several studies performed on the biology and origins of carrots, their cultivation, their chemical composition, and "omic" analyses (Que, 2019), supporting their abundant contents, including beneficial nutrients for human health. For instance, as with other fruits and vegetables, the consumption of carrots is recommended by global dietary guidelines advocating five portions of fruits and vegetables per day (Sadler, 2019).

2.2 HISTORY OF CARROT

A prevalent issue during the early days of carrot cultivation was the mix-up of parsnip and carrot. When Linnaeus published Species *Plantarum* in 1753, establishing scientific nomenclature, the difference between the two was finally made clear. He dubbed parsnips *Pastinaca sativa* and carrots *Daucus carota*, a combination of Greek and Latin names.
It was Galen who first distinguished between carrot and parsnip (Pastinaca) in the second century CE by using the terms *Daucus* and *Carota*. Galen's statement that the domestic carrot is a better food source than the wild one indicates that *Carota* was domesticated.
Carrots were initially grown in the Persian Empire, then in the Iranian Plateau (Afghanistan, Pakistan, and Iran). The main criterion for domestication selection was color and flavor. Over the course of domestication, the color of the roots changed dramatically. While the first domesticated carrots were either purple or yellow, wild carrots are white or pale yellow. The domesticated types were divided into two subgroups: Eastern/Asiatic Group (var. *altorubens*) and Western Group (var. *sativus*) as described by (Harris 1990).
The Eastern/Asiatic group, the original domesticates, have anthocyanin-pigmented roots, purple, pink, or orangey-yellow, that are often branched, with pubescent slightly dissected leaves that give the plant leaves a grey green appearance. Plants are prone to early flowering. The center of diversity was the Himalayan-Hindu Kush region (Kashmir-Afghanistan) and around Turkestan (Mackevic, 1929; Heywood, 1983).

The purple varieties grow erratically and have poor quality storage. Cooking causes the anthocyanin-based purple/red pigment to turn brown, staining hands and kitchenware. With unbranched, carotenoid-pigmented roots that are yellow, orange, or red, and sometimes white, the Western group evolved later. The leaves are bright yellowish green, slightly hairy, and

heavily dissected. Before bolting, plants need a prolonged period of exposure to cold conditions. The Anatolian region of Asia Minor (Turkey) and Iran is the center of diversification for the western carrot. Discovering orange carrots in wild germplasm may indicate Turkish ancestry (Simon, 2000). Due to human desire and selection, these orange varieties replaced the purple varieties throughout Europe and the Mediterranean by the 17th century. Their better taste, adaptability, and nutritional content also served as the foundation for contemporary commercial cultivars worldwide. Because of this, the bulk of contemporary commercial cultivars of carrot are thought to have originated in temperate Europe and Asia Minor/Mediterranean basin (Turkey). Carotenoids, carotene, and xanthophyll are pigments that are attached to plastids that give western carrots their characteristic yellow/orange color. Carotene and xanthophyll are the primary pigments found in minor amounts in white carrots. It was stated that mutation was the source of the yellow and white varieties.

Purple carrots contain anthocyanins, a powerful antioxidant, whilst red contain lycopene, good for eye health, and also found in tomato (Rubatzky et al., 1999). A diverse gene pool appears to have contributed to both selection and mutation in the development of the contemporary carrot. These include cultivated white-rooted wild carrot derivatives (cultivated as therapeutic plants since classical times), yellow-rooted eastern carrots, and wild, unselected populations from Europe and the Mediterranean (Banga, 1957, 1963; Heywood, 1983). Orange carrots probably arrived from mutations of yellow forms, and then from human selection, commonly thought to be originated in the Netherlands.

2.3 NUTRITIONAL CONTENT OF CARROT

It has been established that the moisture content varies between 86 and 89%. Carrots have been shown to contain minerals such as iron (Fe), manganese (Mn), calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), and sodium (Na). There have also been reports of trace amounts of zinc (Zn, 0.2 mg/100 g) and copper (Cu, 0.02 mg/100 g). Among them, varying amounts of iron, sodium, and magnesium have been noted based on the type of carrot. Furthermore, one of the key factors that determine this vegetable's quality is its mineral content value (Sharma, 2012).

Carrots also include tiny amounts of starch and fiber along with simple sugars like fructose, glucose, and sucrose (Yusuf, 2021). The contents of carrot cultivars may vary, and

environmental and storage circumstances might have an impact (Sistrunk, 1967). Thus, the carrot, as with other vegetables, is considered a source of prebiotics by CODEX Alimentarius (Yusuf, 2021). Carrots have a high dietary fiber content as well. Dietary fiber is beneficial to human health since it lowers cholesterol, promotes intestinal health, and lowers the risk of heart disease. Carrot roots include cellulose as the primary source of crude fiber, with smaller amounts of hemicellulose and lignin (Sharma, 2012). Furthermore, it has been discovered that the cellulose concentration varies based on the variety of carrot, ranging from 35 to 48%. Additionally, very minute concentrations of lactic acid, glycolic acid, succinic acid, and α-ketoglutaric acid have been found. According to reports, fresh carrots typically contain 40 and 0.41 mg/100 g of nitrate and nitrite, respectively. However, significant levels of thiamin, riboflavin, niacin, folic acid, and vitamin C have also been identified (Miedzobrodzka, 1992). Furthermore, carrot roots are a good source of anthocyanins; the major ones have been identified as cyanidin 3-(2-xylosylgalactoside), cyanidin 3-xylosylglucosylgalactoside, and cyanidin 3-ferulylxyloglucosyl galactoside. Their contents can vary from trace amounts in pink cultivars to 1750 mg/kg in black carrots. On the other hand, the flavor of carrots is mostly caused by the glutamic acid concentration and the buffering effect of free amino acids.

Carrot roots are also a good source of vitamins, whose levels are another crucial factor used to determine the vegetable's quality along with its mineral content (Szczepanek, 2015). The two main vitamin types found in carrot varieties are vitamin E (191–703 μg/100 g), derived from α-tocopherol and essential for cell signaling, gene expression, and cell membrane stability in the human body, and vitamin A (derived from β-carotene), which can boost immune system functions and play a significant role in the prevention of night blindness (Luby, 2014). Additionally, carrot cultivars are high in vitamin B derivatives (pyridoxine, thiamine, riboflavin, and cobalamin), which are critical for brain and digestive system functions as well as cell growth (Naidu, 2003).

Carrots, however, are a great source of organic acids. Of them, ascorbic acid, or vitamin C, is the most well-known. Because of its strong antioxidant properties, it helps to regulate blood pressure, avoid iron shortage, and strengthen the immune system. Gallic acid operates as an anti-mutagenic agent, whereas benzoic acid and hydroxycinnamic acid exhibit antibacterial and anti-inflammatory properties, respectively. Many additional compounds, however, assist normal

bodily processes. Additionally, iron absorption is stimulated by acetic, succinic, citric, lactic, and malic acids, as well as their salts (Yusuf, 2021).

Carrots are also a good source of C17-polyacetylenes (PAs), which are oxylipins generated from dehydrocrepenynic and crepenynic acids. These are also referred to as "unusual" polyunsaturated fatty acids. The PAs falcarinol, falcarindol, and falcarindol-3-acetate have been shown to be the most prevalent in carrots. Depending on the source (wild or farmed orange carrot), storage duration, age, physiological stage, and root size, the total PA contents and their relative distribution can change (Dawid, 2015).

2.4 CAROTENOIDS

Beyond their use as natural hues in food, carotenoids are important because they are increasingly linked to biological processes and activities. Carotenoids are found inside cells, where they regulate gene expression and impact biological processes such as platelet activation and monocyte adhesion inhibition (Rock, 1997). These biological effects have been linked to carotenoids' antioxidant properties, which deactivate free radicals and quench singlet oxygen, and are independent of pro-vitamin A activity (Krinsky, 1989; Palozza and Krinsky, 1992). Carotenoids are often divided into two categories in food: carotenes and xanthophylls. Carotenoids give food an appealing red or yellow color and improve its quality. The carotenoids' structures can either be acyclic or have a ring with five or six carbons at one or both of the molecule's ends (Carle and Schiber, 2001).

Carotenoids are important micronutrients for human health (Castermiller and West, 1998). The total carotenoids content in the edible portion of carrot roots range from 6,000 to 54,800 µg/100 g (Simon and Wolff, 1987). The main physiological function of carotenoids is a precursor of vitamin A (Nicolle *et al.,* 2003). Over the last ten years, carotenoids, like β-carotene, have garnered significant interest due to their potential to provide protection against specific types of cancer (Bast *et al*., 1996; Santo *et al*., 1996; Van, 1996). In human system, the physiological activity of α- and β-carotene has been 50 and 100% of the pro-vitamin A activity, respectively (Panalaks and Murray, 1970; Simpson, 1983) and one molecule of β-carotene yields two molecules of retinol in human system. Increased immunity and a lower risk of degenerative diseases like cancer, heart disease, age-related macular degeneration, and cataract development have been associated with carotenoids (Mathews-Roth, 1985; Bendich and Olson, 1989;

Krinsky, 1990; Byers and Perry, 1992; Bendich, 1994; Krinsky, 1994; Faulks and Southon, 2001). Carotenoids have been identified as a potential inhibitor of Alzheimer's disease (Zaman *et al*., 1992).

Carrots' biological and therapeutic effects may be attributed to their high concentration of antioxidant carotenoids, particularly β-carotene. It has been observed that carrots are good in removing uric acid and have diuretic and N-balancing qualities (Anon, 1952). Carotenoids are shown to suppress carcinogenesis in mice and rats and may have anticarcinogenic properties in humans based on a multitude of animal trials and epidemiological studies. Within biological systems, β-carotene has antimutagenic, chemopreventive, photoprotective, and immunoenhancing qualities in addition to acting as a single oxygen quencher and free radical trapper (Deshpande *et al*., 1995). Carrot intake may also enhance the immune system, protect against stroke, high blood pressure, osteoporosis, cataracts arthritis, heart diseases, bronchial asthma and urinary tract infection (Beom *et al*., 1998; Sun *et al*., 2001; Seo and Yu, 2003). Carotenoids also act as free-radical scavengers and are very important for health (Bast *et al*., 1998; Bramley, 2000). (D'Odorico *et al.,* 2000) have shown that the presence of α- and β-carotene in blood has a protective effect against atherosclerosis. (Nicolle *et al.,* 2003) has demonstrated that high carotenoid diets are associated with a reduced risk of heart disease.

2.5 PHENOLICS

The physiological properties of phenols, also known as polyphenols, such as their anticancer, antioxidant, and antimutagenic properties, have drawn a lot of attention. According to reports, they could be a viable option to fight free radicals, which are bad for our bodies and food systems (Nagai *et al*., 2003). Although, phenolic compounds do not have any known nutritional function, they may be important to human health because of their antioxidant potency (Hollman *et al*., 1996). Phenolics are ubiquitous plant components that are primarily derived from phenylalanine via the phenylpropanoid metabolism (Dixon and Paiva, 1995). Phenolics in carrots are present throughout the roots but are highly concentrated in the periderm tissue (Mercier *et al*., 1994). Two major classes of phenolics are hydroxycinnamic acids and para-hydroxybenzoic acids (Babic *et al*., 1993). Furthermore, Zhang and Hamauzee (2004) investigated the antioxidant characteristics and distribution of phenolic compounds in carrots and discovered that the

majority of them were hydroxycinnamic acids and their derivatives. One of the most important hydroxycinnamic acids among them was chlorogenic acid, which accounted for 42.2–61.8% of all phenolic compounds found in the various carrot tissues. The tissues with decreasing phenolic contents were peel, phloem, and xylem in that order. Carrot peel could supply 54.1% of total phenolics, compared to 39.5% from phloem tissue and just 6.4% from xylem tissue, even though it only made up 11% of the fresh weight of the carrot. The degree of antioxidant and radical scavenging activity declined in relation to phenolic concentration in various tissues. These results showed that carrots and other hydroxycinnamic derivatives, such dicaffeoylquinic acids and chlorogenic acid, may have significant antioxidant qualities due to the presence of phenolics. Carrot peel, which is handled as waste in the processing sector, has a greater amount of phenolics and antioxidant characteristics, therefore it may be evaluated for value-added usage. Oviasogie *et al*., (2009) have reported that the total phenolic content in carrot is 26.6 ± 1.70 µg/g. Total phenols in violet carrot juice have been reported to be 772 ± 119 mg/l (Karakaya *et al*., 2001).

2.6 DIETARY FIBER

Dietary fiber is a complex carbohydrate that is indigestible and present non plant structural elements. They have no calorific value since they are not absorbed by the body, but eating a diet high in fiber has numerous health benefits, such as preventing constipation, controlling blood sugar, preventing heart disease, lowering high blood pressure, and preventing some types of cancer. Based on their solubility, fibers are divided into soluble and insoluble categories. Soluble fibers include non-cellulosic polysaccharides including pectin, gums, and mucilage, while insoluble fibers are mostly made up of cell wall components like cellulose, hemi-cellulose, and lignin (Yoon *et al*., 2005). Carrots are high in dietary fibers (Bao and Chang, 1994) and these fibers play an important role in human health (Anderson *et al*., 1994) and diets rich in dietary fibers are associated with the prevention, reduction and treatment of some diseases such as diverticular and coronary heart diseases (Anderson *et al*., 1994; Gorinstein *et al*., 2001; Villanueva-Suarez *et al*., 2003). Nawirska and Kwasniewska (2005) have reported the composition of dietary fiber constituents in the fresh carrot on dry weight basis as pectin (7.41%), hemi-cellulose (9.14%), cellulose (80.94%) and lignin (2.48%). Dietary fibers are advantageous for their functional and technological qualities in addition to their nutritional value, which makes them suitable as food ingredients (Thebaudin *et al*., 1997; Schieber *et al*., 2001).

2.7 HEALTH BENEFITS OF CARROT

2.7.1 ANTIOXIDANT, ANTICARCINOGEN, AND IMMUNOENHANCER BENEFITS

Carotenoids, polyphenols, and vitamins, among other ingredients, have anti-oxidant, anti-carcinogenic, and immune-boosting properties. Orange carrots contain abundant amounts of carotenoids, which are strong antioxidants that can counteract the effects of free radicals. It has been demonstrated that they reduce mutagenesis activity, which lowers the likelihood of developing some malignancies (Dias, 2012). Carrot roots contain flavonoids and phenolic derivatives, which have been shown by Zhang and Hamauzuet (2004) to have significant antioxidant properties. Moreover, they lessen inflammatory insult, have anticarcinogenic properties, and alter immunological response (Dias, 2012).

Zaini *et al*., (2011) reported the anti-carcinogenic effect of carrot juice extracts on myeloid and lymphoid leukemia cell lines. After extracting carrot juice for 72 hours, leukemia cell lines and non-tumor control cells were subjected to in vitro study. In leukemia cell lines, it was found that carrot juice extract might induce apoptosis and cause cell cycle arrest. The impact on myeloid and hematopoietic stem cells was less pronounced. The researchers hypothesised that the presence of β-carotene and falcarinol in the carrot juice extract could be the cause of the advantageous effect of "killing" leukemia cells and preventing their spread.

Carrots have anti-clastogenic properties against human lymphocytes and Chinese hamster ovary (CHO) cells, according to research by Darroudi *et al*., (1988).

The effect of carrot and its component falcarinol against the development of azoxymethane (AOM)-induced colon preneoplastic lesions in rat colon was investigated by Kobaek-Larsen. The rats were given a variety of treatments, including AOM treatment, carrots, and extracted falcarinol from carrots. The tumors and aberrant crypt foci (ACF) in the rats fed carrot and falcarinol were significantly reduced, according to the data. According to the researchers' findings, a diet rich in carrot and falcarinol may be able to prevent or slow the growth of colon cancer and big ACF tumors (Kobæk-Larsen *et al.*, 2005).

According to Purup *et al*., (2009), carrot extracts with varying concentrations of falcarinol, falcarindiol, and falcarindiol 3-acetate significantly slowed the growth of cancer and normal cells. According to the study, carrots may contain aliphatic C17-polyacetylenes that have anti-cancer properties. Additionally, the bioactivity of these polyacetylenes may be influenced by their synergistic interactions. According to other studies, falcarinol destroys precancerous cells in tumors by exhibiting cytotoxic action against a number of human tumor cell lines in vitro (Matsunaga *et al*., 1990).

Using 24 albino rats, Ekam *et al*., (2006) evaluated the immunomodulatory effect of carotenoid derived from carrots. It was determined what percentage difference there was in the platelet count, eosinophils, monocytes, and lymphocytes. It's interesting to note that rats given carotenoids had far higher concentrations of platelets, eosinoplils, lymphocytes, and monocytes. The α- and β-carotenoids in carrots were responsible for the positive outcome. Vision issues might arise from the deterioration of the photoreceptors in the eyes caused by a vitamin A deficiency. The carotenoid in carrots with the highest provitamin A activity, β-carotene, protects against macular degeneration and the development of senile cataracts, the primary cause of blindness in the elderly. It also helps to preserve eyesight, particularly at night (Dias, 2012). Carrots are known to be beneficial for the eyes, and eating carrots high in β-carotene may help recover vision. One of the best foods for provitamin A is carrots, and eating a lot of them has been associated with a marked reduction in the risk of post-menopausal breast cancer (Swamy *et al.*, 2014).

Additionally, studies have shown that eating carrots more than thrice a week lowers the incidence of lung cancer in smokers (Pisani *et al*., 1986), and a diet high in beta-carotene may protect against prostate cancer as well (Wu *et al*., 2004). Hung *et al*., (2006) have also reported on the protective properties of carotenoids, antioxidant polyphenols, and dietary fibers against carcinomas, including bladder cancer.

Carrots have β-carotene and other carotenoids in addition to vitamins C and K, thiamin (B1), riboflavin (B2), pyridoxine (B6), and folates (B9), which are important for the metabolism of proteins, carbohydrates, and healthy growth (Dias, 2012).

While vitamin K aids in the process of blood clotting, vitamin C encourages the absorption of non-heme iron and is necessary for battling infections. Our nervous system and mental attitude benefit greatly from thiamin (B1); riboflavin is required for red blood cell formation and respiration; pyridoxine prevents homocysteine from being formed and lowers the risk of heart disease; and folates may lower homocysteine levels, which may lower the risk of a heart attack. Elevated homocysteine levels have been linked to a higher risk of artery hardening brought on by the buildup of fatty plaques. Additionally, it guards against birth abnormalities in infants (Dias, 2012).

2.7.2 WOUND HEALING BENEFITS

In an excision wound model, Patil *et al*., (2012) observed that mice treated with topical cream prepared at different doses of ethanolic extract from carrot root showed substantial decreases in wound area, epithelization duration, and scar breadth, in contrast to the animals in the control group.

Concurrently, it was observed that animals administered a topical cream formulation containing an ethanolic extract of carrot seeds showed notable improvements in wound tensile strength, hydroxyproline content, and protein content (Patil *et al.*, 2012). The antibacterial and antioxidant properties of the carrot root's ethanolic extract, which mostly consist of flavonoids and phenolic derivatives, may also be responsible for the healing effect.

2.7.3 HEPATOPROTECTIVE AND RENOPROTECTIVE BENEFITS

Bishayee *et al*., (1995) observed that carrot extract shields the liver from acute damage caused by the harmful effects of environmental pollutants. In this investigation, the impact of carrot extract on acute liver injury in mice produced by carbon tetrachloride (CC14) was assessed. The pre-treatment with the carrot extract considerably reduced the elevated serum enzyme levels by CC14-induction. The administration of CC14 resulted in higher serum bilirubin and urea concentration, which were reduced by the carrot extract. Carrot extract reversed the dose-responsive reduction in CCl4-produced succinic dehydrogenase, glucose-6-phosphatase, and cytochrome P-450, as well as the increased activities of hepatic 5'-nucleotidase, acid phosphatase, and acid ribonuclease. The researchers came to the conclusion that the outcome showed that

carrot might provide a notable protective activity in the reduction of hepatocellular acute damage generated by CCl4 (Bishayee *et al*., 1995).

In four biofortified flesh carrot cultivars (purple/orange, purple/orange/red, orange/red, and orange), Mills *et al*., (2008) assessed the potential effects of bioactive substances on the provitamin A bioefficacy and antioxidant capacity in the liver of Mongolian gerbils. When Mongolian gerbils were fed colorful flesh carrots instead of white flesh carrots and vitamin A supplemented groups, the authors found that the liver's antioxidant potential and vitamin A reserves were higher in the former group (Mills *et al.*, 2008).

Carrot root extract's renoprotective effects on acute renal ischemia reperfusion injury in rats were investigated by Mital *et al*., (2011). Malondialdehyde levels were dramatically elevated in rats with renal reperfusion injury, while superoxide dismutase, catalase, and glutathione activity were significantly reduced. According to the study, one of the processes underlying the kidney damage caused by ischemia reperfusion is the reduction of free radical scavenging activity, which is why carrot extract exhibits renoprotective efficacy against ischemia reperfusion-induced kidney acute injury (Mital *et al.*, 2011).

2.8 DISEASES OF CARROT

2.8.1 ALTENARIA BLACK ROT

Carrot black rot was initially documented in 1888 and was widespread in Denmark and other northern European nations (Rostrup, 1888). The fungus *Sporidesmium exitiosum* (Kühn) v. dauci (Kühn) 1855 (syn. *Alternaria dauci*), which causes *Alternaria* leaf blight, was wrongly blamed for the illness at the time. The illness was initially discovered in 1918 in Washington, DC, and Long Island, New York, where it resulted in large losses in carrots kept in cold storage and during transportation. Subsequent research properly identified a novel species of *Alternaria* as the causative culprit (Simmons, 1995). Since these first reports, black rot has been documented in every major global location that grows carrots. While there are no accessible statistics regarding the yearly losses attributed to black rot, field studies reveal significant damage.

Up to 88% of disease incidence on mature plants was observed by Coles and Wicks (Coles and Wicks, 2003) in a few South Australian areas. (Pryor et al., 1998) conducted a survey of eight

fields in the Cuyama Valley of California and found that the disease incidence on mature plants ranged from 63 to 99%. Due to severe petiole deterioration, whole fields were abandoned in some of the California cases before harvest. Estimates of disease incidence from marketplaces and storage facilities also show how common black rot is. For instance, research done on carrot shipments from New York farms between 1918 and 1926 showed infection rates as high as 62% (Meier *et al.*, 1922). Black rot on stored carrots ranged from 1 to 50% on average, according to surveys done in Europe and Asia (Geeson *et al.*, 1988).

Causal agent:

The fungus Alternaria *radicina* is the cause of black rot in carrots (Ellis and Holiday, 1972). Asexual conidia, which are abundantly formed on upright conidiophores with one to three geniculate conidiogenous sites each, are used for reproduction. Conidia are generally dark olive-brown to natal brown, broadly ellipsoid to ovoid, 20–50 × 10–25 µm, with one–three longisepta and two–five transepta in any or all segments, with the exception of the basal and apical segments, which are typically septa-free. Conidia are borne singly or occasionally in chains of two. Conidia rarely grow into a long, narrow shape that is broadly ellipsoid to obclavate, measuring 50–65 × 15-20 µm, with one or two longisepta and seven–eight transepta in most segments (Groves and Skolko, 1944). Sexual reproduction by *A. radicina* is not known to occur. *A. radicina* is the representative species for the radicina species-group of *Alternaria* fungi (Pryor *et al.*, 2002).

Carrot roots with black rot are characterized by depressed, dry lesions that are black in color. Carrots stored in cold storage are particularly vulnerable to the disease, as lesions can grow rapidly, decompose the entire root, and spread to neighboring carrots (Rostrup, 1888). *A. radicina*, however, can infect the majority of carrot tissues. Since the fungus is spread by seeds, planting carrot seeds contaminated with *A. radicina* frequently starts the disease cycle (Meier, 1922). *A. radicina* infects the hypocotyl during seed germination, causing preemergence damping-off and a black necrosis of the growing seedling (Mounce and Bosher, 1943).

As newly emerged seedlings become infected at or close to the soil line, post-emergence damping-off takes place. After that, the fungus sporulates, and the spores infect other seedlings through wind, irrigation water, or rain, lowering stands and yields. *A. radicina* can infect carrot plants at any stage of growth in the right circumstances. Vigorously growing carrot plants are generally more resistant to infection once they have passed the seedling stage. Plants are

vulnerable to *A. radicina* infection once more after they grow to a marketable size. The carrot crown can become infected by older, senescing petioles, which are especially prone to *A. radicina* infection and colonization. This causes a black ring of deterioration to form at the petiole attachment points; this distinctive symptom gave rise to the term "black crown" for carrots.

A. radicina infections in the field frequently stay limited to the petioles and carrot crown. While the quality of the carrot root is not always affected by these illnesses, deterioration of the petioles and crown can make mechanical harvesting difficult. Petiole infections can get so bad that they force farmers to leave entire fields before they can be harvested.

A. radicina can produce foliar blight when the disease conditions are ideal, since it spreads from the crown to the canopy. Small necrotic areas that may have a chlorotic edge surrounding them initially manifest as symptoms. Entire leaflets or leaves may have a black necrosis when lesions grow and fuse together. Because of their decreased ability to photosynthesize, these blighted carrots have smaller roots and a lower yield as a result. Carrot root infection can also result from crown infection. In these situations, the top section of the root develops necrosis, which has the potential to spread to the lower root sections.

Although *A. radicina* can also cause carrot root infections below ground, storage roots seem to be rather resistant to this kind of infection (Maude, 1996). Abrasions like insect or mechanical damage can lead to belowground diseases. Carrots with visible huge black lesions during sorting will be removed if root infections emerge early in the growing season.

Potential sources of inoculum during storage could arise from later-season carrots that did not exhibit apparent lesions at harvest. One of the main ways that *A. radicina* damages crops economically are when they are being stored (Maude, 1966). Carrots are grown in warmer areas and preserved for up to 200 days, depending on the climate in which they are grown. As low as -0.6°C can cause *A. radicina* to infect carrot tissue, as long as the humidity level stays high. The development of black rot and the growth of *A. radicina* are thus made possible by the cold, damp conditions that come with storing carrots for an extended period of time. If the infection spreads widely, it might eventually infect every carrot in a storage bin.

A. radicina can coexist with crop detritus in the interim between carrot crops. The fungus sporulates on infected waste when conditions are right, and the spores infect newly planted carrot crops. Long after the infected plant material has decomposed, *A. radicina* can continue to exist in

the soil as conidia (Pryor *et al*., 1998). For instance, in soils infected with *A. radicina* that had not been planted with carrots in up to 8 years, carrots developed black rot (Maude and Bambridge, 1991).

2.8.2 SCLEROTINIA ROT

Sclerotinia rot of carrot is a destructive disease, both in the field and during storage, throughout carrot production worldwide (Rubatzky *et al*., 1999; Kora *et al*., 2003). The disease causes severe losses by 50–100% during long-term storage (Finlayson *et al*., 1989). The disease-causing necrotrophic homotallic fungus, *Sclerotinia sclerotiorum*, affects 408 distinct plant species from 278 genera and 75 plant families (Boland and Hall, 1994). Depending on the environmental conditions, which include several biological and physical elements, the fungus can live in soil as dormant sclerotia for up to 10 years (Harvey *et al*., 1995; Liu *et al*., 2017).

According to Huang and Kozub (1993) and Liang *et al*., (2010), mature sclerotia can germinate carpogenically to produce apothecia or myceliogenically to produce mycelia. Out of all the cultivated carrot varieties, there is currently no cultivar that is resistant to *S. sclerotiorum* (Kora *et al*., 2005). The most widely used fungicide to prevent sclerotinia rot in carrots is synthetic chemical fungicide; unfortunately, there isn't a commercial fungicide that can stop the disease during its storage stage (Hildebrand *et al*., 2008).

On the other hand, persistent use of chemical fungicides promotes the emergence of resistant *S. sclerotiorum* strains (Chen *et al*., 2011). Many of these artificial fungicides also have lengthy degradation times, toxicity, and carcinogenic qualities. Additionally, growing worries about pesticide residues on storage goods have prompted researchers to look at novel, alternative methods for managing postharvest infections by lowering disease incidence (Bautista-Banos *et al*., 2006).

2.8.3 BACTERIAL SOFT ROT

Pectobacterium species are plant pathogenic bacteria that are Gram-negative and are members of the *Pectobacteriaceae* family. Along with *Dickeya* species, these bacteria are the primary cause of diseases including blackleg, soft rot, and aerial stem rot in potatoes, as well as a variety of vegetables like celery, carrots, and tomatoes, as well as several crops that are stored and grown in fields across the globe (Perombelon and Kelman, 1980). These bacteria's capacity

to produce and secrete large amounts of a variety of extracellular plant cell wall-degrading enzymes (PCWDE, exoenzymes), such as pectate, lyases (Pel), polygalacturonases (Peh), proteases (Prt), and cellulases (Cel), is what gives them their virulence and pathogenicity. These enzymes cause extensive tissue maceration, rotting, and ultimately the death of the entire plant (Barras *et al.*, 1994).

The symptoms of bacterial soft rot include pitting along the taproot, soft decay of sections or the entire taproot, and decay that frequently advances from the taproot tip to the crown. When pitting happens, the lesions are sunken, becomes dull orange, and the affected tissues' epidermis either rots or is left intact. Pectin-degrading enzymes dissolve the middle lamella between the cells in affected tissues, causing the tissue to collapse into a soft mass. In certain cases, a soft rot develops that preserves the epidermal tissue while the entire core rots (Towner and Beraha, 1976). The odor of infected tissue is generally not foul unless secondary organisms invade.

Pathogenicity of soft rot and the generation of exoenzymes Comprehensive networks of positive and negative regulatory proteins are involved in the transcriptional and posttranscriptional processes that govern *Enterobacteriaceae* in a coordinated manner. Both the pathogen's and the host's chemical signals, among other physiological and environmental variables, have an impact on this control (Collmer and Keen, 1986).

Bacterial soft rot, caused by *Pectobacterium carotovorum* was reported as the most common causal organism for the soft rot disease (Mansfield *et al*., 2012). The bacterial soft rot pathogen can infect a wide variety of vegetables, including lettuce, potatoes, carrots, and cabbage (Walker, 1998). Previously, bacterial soft rot of vegetables was managed through a variety of techniques, such as formaldehyde fumigation of a storage building (Walker, 1998), cleaning of the storage house with formaldehyde solution, calcium hypochlorite, or sodium hypochlorite (Bhat *et al*., 2010), and treatment with certain salts such as aluminum acetate, copper sulphate pent hydrates, and alum (Mills *et al*., 2006).

This disease limits the pre and post-cultivation of carrots and results in significant losses to the crop. The disease spreads by cuts and contaminated seed inoculum from harvest to storage settings due to incorrect crop handling during transportation combined with an unbalanced air environment (Phillips, 1991). Preventing infections in the field and losses during storage and transportation are essential for achieving a rise in production.

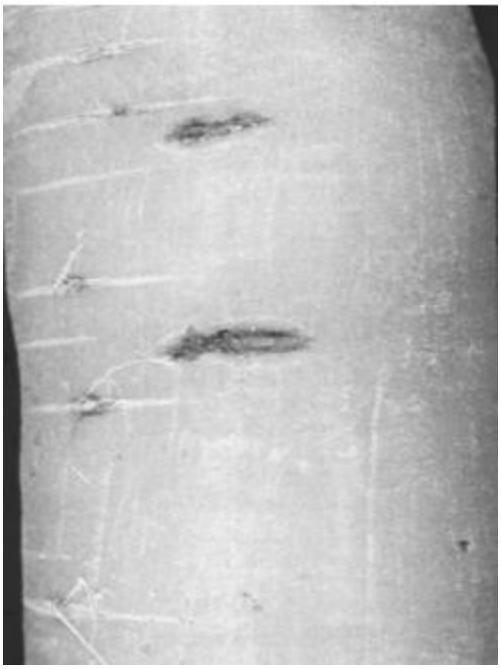

Figure 1: Pitting of carrot caused by the bacterium *Erwinia carotovora* subsp. *Carotovora* (Source: Davis, 2004).

2.9 ESSENTIAL OILS

Researchers are looking for new sources of broad-spectrum biocides since microbes are becoming more resistant to traditional chemicals and medications (Abad *et al*., 2007). Plants and their byproducts, such as essential oils (EOs), have been utilized in traditional medicine since ancient times. EOs is crucial for protecting plants in the natural world. Additionally, they might draw in some insects to aid in the spread of seeds and pollen or repel other unwanted insects. Consequently, EOs may operate as a mediator in how plants interact with their surroundings (Bakkali *et al.,* 2008).

As secondary metabolites, aromatic plants release concentrated natural compounds with potent scents known as essential oils. Although diterpenes (C20) may also be present, these oils are mainly composed of terpenoids, particularly monoterpenes (C10) and sesquiterpenes (C15). Numerous additional molecules can also be found, including homologues of phenylpropanoids, uncommon compounds containing nitrogen and sulfur, acids, alcohols, aldehydes, aliphatic hydrocarbons, acyclic esters, and lactones. EOs are soluble in lipids and organic solvents with a density less than water. They are liquid, volatile, limpid, and colored. All plant parts, such as buds, blossoms, leaves, seeds, twigs, stems, blooms, fruits, roots, wood, or bark, may contain

them, although secretory cells, cavities, canals, glandular trichomes, or epidermic cells are typically where the plant stores them. Essential oils (EOs) are derived from a variety of fragrant plants that are typically found in warm, temperate regions of the world, where they are frequently a significant component of customary pharmacopoeias. These plants are frequently used in food preservation and as analgesics, sedatives, anti-inflammatories, spasmolytics, and local anesthetics. They may be well-known for their antioxidant benefits as well as their antiseptic and therapeutic qualities and smell (Chorianopoulos *et al.,* 2008).

Numerous secondary metabolites found in EOs have the ability to prevent or reduce the growth of mold, yeast, and bacteria. The targets of the EOs and their constituents are diverse, especially the cytoplasm and membrane; in rare cases, they even entirely change the shape of the cells (Burt and Reinders, 2003).

2.9.1 ACTIVITY OF ESSENTIAL OILS AGAINST BACTERIA

In general, Gram-negative bacteria have greater resistance to EOs in comparison to their Gram-positive counterparts. The differences between the architecture of the cell walls of Gram-positive and Gram-negative bacteria contributes to their resistance or suceptibility. Peptidoglycan, to which proteins and teicoic acid are attached, makes up around 90%–95% of the cell wall of Gram-positive bacteria (Trombetta *et al*., 2005).

Hydrophobic compounds can readily enter Gram-positive bacteria's cell walls and act on them as well as the cytoplasm due to the nature of the bacterial cell wall. Additionally found in EOs, phenolic chemicals often have antibacterial action against Gram-positive bacteria. The compound's action varies with concentration; at low levels, it can disrupt energy-producing enzymes, while at greater levels, it can cause proteins to become denaturized (Tiwari *et al*., 2009).

Gram-negative bacteria have a more complex cell wall. Its peptidoglycan layer, which makes up around 20% of the dry weight of the cell, is 2-3 nm thick and thinner than that of the cell walls of Gram-positive bacteria. On the exterior of the thin layer of peptidoglycan is an outer membrane (OM). Braun's lipoprotein, which is embedded in the OM and chemically bonded to the peptidoglycan, is what holds the two substances together tightly. One characteristic that sets Gram-negative bacteria apart from Gram-positive bacteria is the existence of an OM. It consists of two phospholipid layers connected to the inner membrane by lipopolysaccharides (LPS). LPS

and a variety of proteins make up the OM that covers the peptidoglycan layer. The O-side chain, which supplies the "quid" that makes Gram-negative bacteria more resistant to EOs and other natural extracts with antibacterial activity, and lipid A, the core polysaccharide, make up LPS. Gram-negative bacteria are relatively resistant to hydrophobic antibiotics and harmful medicines because small hydrophilic solutes can flow through the OM via numerous porin proteins that operate as hydrophilic transmembrane channels (Nikaido, 1994).

The hydrophobic molecules some of which can slowly pass through porins can pass through the OM nearly but not entirely (Plesiat and Nikaido, 1992). The chemical makeup of EOs and/or their constituent parts determines how they work. For example, whereas thymol and carvacrol both have antibacterial properties, their modes of action against Gram-positive and Gram-negative bacteria differ. The antibacterial activity of these compounds may vary depending on where one or more functional groups are located. Although thymol and carvacrol share structural similarities, the two compounds' hydroxyl groups are located differently.

Nevertheless, neither antimicrobial agent's activity is impacted by these variations. The alkyl group determines the antibacterial action of various compounds, including limonene and p-cymene. As a result, according to Dorman and Deans (2000), limonene may occasionally be more effective than p-cymene. EOs and/or their constituents may choose to focus their efforts on one or more specific goals. (De Martino et al., 2009) found that when exposed to the same EOs and their single components, two strains of Bacillus cereus exhibited distinct behaviors. Finding the EOs' mode of action necessitates a thorough examination of the raw material until the unique components are found. The mode of action should also be investigated in a variety of microorganism strains and species. Expanding our basic knowledge of the molecules present in the EOs will support future studies into the comprehensive modes of antimicrobial action of EOs.

2.9.2 TERPENES

Hydrocarbons known as terpenes are created when multiple isoprene units combine (C5H8). The cytoplasm of the vegetal cell is where they are synthesized; the mevalonic acid pathway initiates with acetyl CoA. Cyclases have the ability to rearrange the hydrocarbon backbone of terpenes into a cyclic form. Longer chains, such as diterpenes (C20H32), triterpenes (C30H40), and so on, are also present in the plant cell. Monoterpenes (C10H16) and sesquiterpenes (C15H24) are the most prevalent types of terpenes. The most well-known terpenes include pinene, limonene,

terpinene, sabinene, and p-cymene. Majority of terpenes don't naturally have strong antibacterial properties. One of the key ingredients in thyme essential oil, p-cymene, is ineffective against a variety of Gram-negative bacteria (Bagamboula *et al*., 2004). According to Dorman and Deans (2000), terpenes including limonene, α-pinene, β-pinene, γ-terpinene, δ-3-carene, (+)-sabinene, and α-terpinene exhibited negligible or no antibacterial action against 25 different genera of bacteria. Based on these in vitro experiments, terpenes alone do not exhibit significant antibacterial action.

2.9.3 TERPENOIDS

Terpenoids are terpenes that have had oxygen molecules added to them or that have had certain enzymes shift or completely eliminated their methyl groups (Caballero *et al*., 2003). The most prevalent and well-known terpenoids are thymol, carvacrol, linalool, menthol, geraniol, linalyl acetate, citronellal, and piperitone. The hydroxyl group of phenolic terpenoids and the presence of delocalized electrons are critical components for their antibacterial action. The antimicrobial activity of most terpenoids is linked to their functional groups. Carvacrol, for instance, works better than other essential oils, including *p-cymene* (Ultee *et al*., 2002). Carvacrol's hydrophobicity and antibacterial activity may be impacted by the replacement of hydroxyl group with a methyl ether. The hydroxyl group's location within the phenolic molecule has no bearing on the antibacterial action's general direction. Though its hydroxyl group is positioned differently from carvacrol's, thymol exhibits comparable antibacterial action against *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Lambert *et al*., 2001).

Carvacrol and thymol are known to have strong OM dissolving qualities. (Helander *et al*., 1998) showed that increased LPS release and made cells more sensitive to detergents. Nevertheless, unlike EDTA or polyethylenimine, which break down OM at sub-lethal quantities, thymol and carvacrol do not directly function as OM permeabilizing agents (Vaara, 1992). These substances also have the ability to make the cytoplasmic membrane more permeable to ATP. The monoterpene p-Cymene, which has a benzene ring without any functional groups on its side chains, is the precursor of carvacrol. According to Mann *et al*., (2000), p-cymene has been shown to exhibit antibacterial action when taken alone. Additionally, research has shown that p-cymene can boost the antimicrobial activity of other substances, such as its derivative carvacrol

(Rattanachaikunsopon *et al*., 2010). With its strong affinity for microbial membranes, p-Cymene has the ability to disrupt them, causing them to swell and altering the membrane potential of whole cells. P-cymene has no effect on the permeability of the membrane, but it may lower its enthalpy and melting temperature. These characteristics support the theory that p-cymene functions as a substitutional impurity in the membrane (Cristallani *et al*., 2007). P-cymene does not only function at the membrane level, though. According to (Burt *et al*., 2007), the substance altered the membrane potential in *E. coli* but had no effect on protein production because the proton motive force is necessary for flagellar movement, treatment with p-cymene reduced cellular motility.

Thyme oil (EO) contains thymol, a phenolic monoterpenoid. It shares a structure with carvacrol and has hydroxyl groups that are arranged differently on the phenolic ring. Thymol's antibacterial action, like that of carvacrol, causes structural and functional changes in the cytoplasmic membrane, which can harm both the inner and outer membranes. It can also interact with intracellular targets and membrane proteins (Sikkema *et al*., 1995). K+ ions and ATP are released when thymol interacts with the membrane, influencing membrane permeability (Walsh *et al*., 2003). Thymol has the ability to cause lipopolysaccharide release under certain conditions, but it has no effect on chelating cations (Helander *et al*., 1998).

Thymol integrates within the lipid bilayer's polar head-groups, causing changes to the cell membrane. The membrane can modify its lipid profile to preserve membrane integrity and function at low thymol levels (Turina *et al*., 2006). As shown by Juven *et al*., (1994) using a model system containing bovine serum albumin, thymol also interacts with proteins. Thymol interacts with proteins at many locations inside the cell, which can have an impact on a range of physiological processes.

The main source of carvacrol, a phenolic monoterpenoid, is oregano oil extract (EO). Carvacrol is among the EO ingredient that has been studied the most, along with substances like thymol. Carvacrol works on microbial cells similarly to thymol, causing structural and functional damage to their membranes that leads to increased permeability (Sikkema *et al*., 1995). One of the rare elements of an EO with a disintegrating impact on the outer membrane of Gram-negative bacteria is carvacrol (La storia *et al*., 2011). In addition to releasing LPS, it modifies ion

transport by acting on the cytoplasmic membrane (Helander *et al*., 1998). Carvacrol's ability to operate as a transmembrane carrier of monovalent cations by bringing H+ into the cell cytoplasm and removing K+ appears to be related to its hydroxyl group (Ben arfa *et al*., 2006). This theory contradicts other results that suggest the presence of non-hydroxyl groups rather than hydroxyl groups is what gives carvacrol its antibacterial activity (Veldhuizen *et al*., 2006).

Carvacrol appears to work via increasing the permeability and fluidity of membranes. Microbial cells may exhibit alterations in the makeup of their membrane fatty acids upon exposure to carvacrol. This is a widely recognized process that keeps cells' membrane shape and function at their ideal levels. The content of fatty acids changes in response to carvacrol, which may have an impact on membrane fluidity as well as permeability (Di Pasqua *et al*., 2006).

The impact of carbvacrol on membrane permeability was verified by observing the entry of nucleic acid stains and the efflux of ATP, H+, K+, and carboxyfluorescein (Xu *et al*., 2008). Carvacrol may potentially have intracellular targets, and there are evidence that it influences membrane proteins and periplasmic enzymes (Horvath *et al*., 2009). Carvacrol may have an impact on how OM proteins fold or insert. (Burt *et al*., 2007) demonstrated that carvacrol impacted protein folding by producing considerably more GroEL in *E. coli* cells cultured in the presence of a sub-lethal dosage of the drug. Additionally, carvacrol prevented the synthesis of flagellin, another microbial protein, and caused cells to develop without flagella, which resulted in a reduction in motility. But even flagellar cells showed reduced motility, which was dependent on the concentration of carvacrol; this suggests that the chemical also reduced the proton motive force required to propel flagellar movement (Gabel and Berg, 2003).

2.9.4 PHENYLPROPENES

Cinnamic acid, which is created during the first stage of phenylpropanoid biosynthesis, contains a three-carbon propene tail and a six-carbon aromatic phenol group. Only a minor fraction of EOs is made up of these substances. These five phenylpropenes have been investigated the most: eugenol, isoeugenol, vanillin, safrole, and cinnamondehyde. Its free hydroxyl groups are responsible for most of these compounds' antibacterial action (Laekeman *et al*., 1990).

The presence of a methyl group in the γ position and a double bond in the α β locations of the side chain are responsible for eugenol's antibacterial activity (Jung and Fahey, 1983). Like most other EOs, the antibacterial activity of phenylpropenes is also dependent on the kind and quantity of substitutions made to the aromatic ring, as well as the microbial strain and testing conditions (Pauli and Kubeczka, 2010). The phenylpropenes have a variety of antibacterial action in general. According to Zemek *et al*., (1979), isoeugenol is more potent than eugenol against bacteria and also works well against yeasts and molds. It's interesting to note that eugenol and isoeugenol work best against Gram-negative bacteria than they do against Gram-positive ones (Hyldgaard *et al*., 2012).

While eugenol is often more effective than cinnamonaldehyde (Gill and Holley, 2004), cinnamonaldehyde exhibits activity comparable to thymol and carvacrol, the strongest essential oils, when used against *E. coli* and *S. typhimurium* (Helander *et al*., 1997). Eugenol modifies the fatty acid composition of many bacteria, modifies the membrane, and influences the movement of ions and ATP. Additionally, it inhibits a variety of bacterial enzymes, including as ATPase, amylase, protease, and histidine carboxylase (Thoroski, 1989). There are three main ways that cinnamonaldehyde works against germs. It serves as an ATPase inhibitor at higher concentrations while inhibiting enzymes involved in cytokine interactions or other less significant cell processes at lower quantities. Cinnamaldheyde disturbs the membrane at a deadly dose. The information on cinnamonaldheyde's ability to disrupt membranes has been inconsistently published in certain studies. For instance, in *E. coli*, a sub-lethal concentration of the molecule does not compromise membrane integrity, but it can suppress the microbe *Photobacterium leiognathi's* growth and bioluminescence; this indicates that cinnamaldheyde enters the periplasm and possibly the cytoplasm. According to Wendakoon and Sakaguchi (1995), cinnamonaldehyde can change the lipid profile of microbial cell membranes.

Vanillin is a phenolic aldehyde derived from phenylpropene, and its exact mode of action is unknown. On the other hand, other research indicates that it acts against some lactic acid bacteria, including *L. plantarum*, by upsetting pH and K+ homeostasis and inhibiting the respiration pathway in *E. coli* and *L. innocua* (Fitzgerald *et al*., 2004). Fitzgerald *et al*., (2005) noted that although the membrane is vanillin's principal target, there may be additional targets inside the microbial cell. Carvone has the ability to interfere with a cell's membrane potential and pH gradient. The growth rates of *E. coli*, *Streptococcus thermophilus*, and *Lactobacillus lactis*

decreased with increasing concentrations of carvone. These findings led to the notion that the substance may function by altering the metabolic energy status of cells (Osterhaven *et al*., 1995).

2.9.5 MECHANISM OF ACTION OF ESSENTIAL OILS

The action of an EO on bacterial cells has been explained by a number of different methods. Both the cytoplasm and the cell's outer membrane can be impacted by an EO's action. Due to the difficulty to detach the EOs from the bacterial cell membrane, the hydrophobicity characteristic of EOs causes breakdown of bacterial structures and thereby increases permeability. The cell membrane's permeability barrier is essential for a number of cellular processes, such as solute transport, metabolic control, membrane-coupled energy-transduction pathways, and preserving the cell's energy status.

Controlling the turgor pressure is also dependent on the cell membrane (Poolman *et al*., 1987). The antibacterial action of EOs is typically explained by toxic effects on membrane structure and function (Andrews *et al*., 1980). In actuality, the mechanisms of action of EOs include the following: cytoplasmic membrane damage, cytoplasm coagulation, cell wall degradation (Gill and Holley, 2006); membrane protein damage; increased permeability resulting in cell contents leakage (Juven *et al*., 1994); reduction of the proton motive force; decrease in intracellular ATP pool through decreased ATP synthesis and enhanced hydrolysis, which is distinct from the increased membrane permeability; and membrane potential reduction through increased membrane permeability.

Helander *et al*., (1998) provided an account of how various EO components affected the permeability of the OM. All three of the microorganisms in their investigation showed reduced viability in response to tea tree oil-induced damage to their cell membrane structures, and it was determined that this damage was most likely what led to the cell deaths. Because EOs are hydrophobic, they can therefore enter microbial cells and change their structure and function.

This could explain why EOs are generally most effective, with some exceptions (Kim *et al.,* 1995), against Gram-positive microorganisms. Some Gram-negative bacteria have an outer capsule that restricts or eliminates the ability of EOs to enter the microbial cell. Additionally, the chemicals found in EOs have the ability to disrupt wall proteins, which are frequently implicated in the entry of vital substances into the cell. Some writers have suggested that the various components of the EO cause the microbial viability to be lost through distinct mechanisms. The

effects of EOs typically result in the loss of essential intracellular components, the deactivation of enzymatic processes, the destabilization of the phospholipid bilayer, and the destruction of the plasma membrane's composition and function.

Certain components of essential oils, such as carvon, thymol, and carvacrol, raise intracellular ATP concentrations, which is connected to the breakdown of the microbial membrane. In certain situations, essential oils also change membrane permeability by disrupting the electron transport system. Physiological alterations that can cause cell lysis and death include blocking electron transport for the creation of energy, interfering with the proton motive force, protein translocation, and the synthesis of cellular components (Turina *et al*., 2006).

Since the cell membrane is a crucial component of the basic biological processes occurring inside the cells, its integrity is vital to the survival of bacteria. The cell membrane serves as an efficient barrier that separates the cytoplasm from the outside world. It is via this membrane that ions and metabolites that are necessary for all microbial cell processes are imported and exported. Microorganisms may respond to antimicrobial substances in their environment by changing the synthesis of membrane proteins and fatty acids, which changes the fluidity of the membrane (Mrozik *et al*., 2004).

The double lipid layer of the membrane is permeable to the EOs and their constituents due to their hydrophobicity. The permeability and functionality of membrane proteins can be changed by the EOs. Certain essential oils, especially those high in phenolics, have the ability to penetrate the phospholipid bilayer of bacterial cell walls, attach to proteins, and stop them from doing their intended jobs. This event suggests that an EO's initial target is the membrane. As was previously mentioned, the EO's mode of action is not solitary; rather, it entails a number of processes that take place in the cytoplasm and on the surface of the cell. A "disbalance" develops within the microbial cell as a result of changes in membrane permeability and flaws in the movement of molecules and ions. As a result, there is a loss of ions and metabolites, cytoplasm coagulation, denaturation of various enzymes, and cellular proteins (Burt and Reinders, 2003). Microorganisms respond to various situations by upregulating the expression of stress-responsive proteins, which helps to repair damaged proteins. Examples of these conditions include the presence of sub-lethal quantities of EOs or other antimicrobial substances (Burt *et al*., 2007). However, this response is not able to stop cell death when the concentration of EOs or other natural antimicrobials is higher. The impact on Gram-positive bacteria is more pronouncedGram-

negative bacteria's cell walls are more resilient to the effects of EOs and their constituent parts. Hydrophobic compounds cannot enter the Gram-negative cell wall as easily as they can in Gram-positive bacteria, which mean that EOs has less effect on the growth of Gram-negative bacteria. The antibacterial action of the natural extracts (EOs) cannot be explained by a single mechanism due to the large range of chemicals present. Rather, many biochemical and structural processes are engaged at various locations within the cell and on the cell surface (Carson *et al*., 2002). These methods, which have the ability to entirely alter the shape of the microbial cell, involve chemical alterations of the cytoplasm, enzymes, proteins, and cell membrane. Moreover, exposure to an EO might cause a persistent loss of ions or metabolites, which can impair microbial metabolism and cause cell death. Tea tree oil, for instance, can result in E. Coli to perish without the cell being lysed (Burt, 2004).

2.10 TYPES OF ESSENTIAL OILS

EOs are composed of combinations of around fifty distinct scent components, making them incredibly complex in nature. Tea tree, lemon, clove, cinnamon, thyme, mustard, oregano, lavender, eucalyptus, and peppermint oils are a few examples of essential oils (EOs). They are essential for food preservation and the suppression of the growth of harmful microorganisms. For instance, lemon essential oil's terpenes and oxygenated terpenes demonstrated strong antifungal ability against *Candida* spp. like *C. albicans*, *C. tropicalis*, together with *C. glabrata* (Ooi *et al*., 2006). Cinnamon oil is a volatile compound extracted from the bark, leaf, and root of *Cinnamomum zeylanicum*. It contains three important components such as *trans*-cinnamaldehyde, eugenol, and linalool (obtained from the bark extract), which represent 82.5% of the total composition. Cinnamaldehyde is the most active compound of cinnamon EOs, which showed inhibitory effects on the growth of various pathogens including both gram negative and positive bacteria and possesses potential growth inhibitory effects on fungi (Ramage *et al.,* 2012). Several studies revealed that the cinnamon EOs also had antioxidant, antiparasitic and free radical scavenging properties (Terzi *et al.,* 2007). Moreover, the tea tree oil (TTO) from *Melaleuca alternifolia* (*Myrtaceae*) majorly contained terpinen-4-ol, *γ*-terpinene, *p*-cymene, *α*-terpinene, 1,8-cineole, *α*-terpineol, and *α*-pinene (Chaieb *et al.,* 2007). It showed a strong inhibitory activity on fungal strains (Benabdelkader *et al.,* 2011).

Clove oil is mostly extracted from clove buds and it contains the phenylpropanoids viz. eugenol, eugenyl acetate, carvacrol, thymol, cinnamaldehyde, *β*-caryophyllene, and 2-heptanone, as major

compounds. Among these, eugenol is frequently employed as an antibacterial agent, has the ability to decrease the synthesis of ergosterol, a particular component of cell walls, and may prevent the formation of the germ tube in C. *albicans*. In tests against tert-butylated hydroxytoluene, it demonstrated potent radical scavenging activity and shown inhibitory effects on multiresistant *Staphylococcus* species. (Vegh *et al*., 2012).

Lavender oil is majorly extracted from the family *Lamiaceae*, especially, *Lavandula angustifolia* by steam distillation and it was found to contain linalool, linalyl acetate (3,7-dimethyl-1,6-octadien-3yl acetate), linalool (3,7-dimethylocta-1,6-dien-3-ol), lavandulol, 1, 8-cineole, lavandulyl acetate, *B*-ocimene, terpinen-4-ol, l-fenchone, viridiflorol and camphor as major components (Prashar and Locke, 2004). However, the concentration levels of these compounds were varying from species to species.

Linalool is one of the active compounds in lavender oil (Zuzarte *et al.,* 2012). Lavender oil showed antibacterial activity against antibiotic resistant bacteria, yeasts, dermatophytes*, Cryptococcus neoformans, Aspergillus* strains and *C.* species (posadzki *et al.,* 2012).

Eucalyptus oil comprises of 1,8-cineole as the major compound and have other compounds such as cryptone, *α*-pinene, *p*-cymene, *α*-terpineol, *trans*-pinocarveol, phellandral, cuminal, globulol, limonene, aromadendrene, spathulenol, and terpinene-4-ol (Tyagi and Malik, 2011). EOs extracted from eucalyptus are mainly used as flavoring agents and showed significant activities in controlling the growth of pathogenic and food spoilage microorganisms (Lambert *et al.,* 2001).

The bioactive components of EOs, particularly the antibacterial and antifungal ones, can target different cell structures or chemical pathways, including the breakdown of cell walls, damage to membranes, and disruption of the proton motive force, among other things. On the other hand, not much is known about the mechanism underlying eucalyptus oil's antifungal and antibacterial properties. Rather than being attributed to a single molecule, it was observed that the antibacterial action of eucalyptus oil was strongly correlated with the synergistic effects of its major and minor constituents. Among the eight different species of eucalyptus, EOs extracted from *Eucalyptus odorata* showed strong cytotoxic effects and inhibitory activities against *S. pneumonia, S. aureus*, *H. influenza* and *S. pyogenes* (Tyagi and Malik, 2011).

Peppermint oil showed momentous growth inhibitory activity on *Staphylococci.* Several studies reported that it exhibited antifungal activity against both standard and clinical pathogenic fungal strains of *Candida* species at IC_{50} concentrations ranging from 0.5 to 8 μg/mL, and showed good antifungal effects on azole-resistant and azole-susceptible strains (Cox *et al.,* 2008).

2.10.1 EUCALYPTUS OIL

Eucalyptus is a large genus of the Myrtaceae family that includes some 900 species and subspecies (Brooker and Kleinig, 2004). While eucalyptus is planted extensively throughout the world, Australia is arguably the only country where a single plant species predominates over the majority of the terrain. The Aboriginal people, who are thought to have arrived in Australia more than 60,000 years ago, acquired a highly developed empirical knowledge of local flora like eucalyptus. Its leaves were traditionally used to treat fungal infections and wounds. There's currently a resurgence of interest in indigenous herbal traditions, even if most of this information has disappeared along with its caretakers (Chevallier, 2001).

Leaf extracts of *Eucalyptus* have been approved as food additives, and the extracts are also currently used in cosmetic formulations. Recently, attention has been focused on the functional properties of these extracts. Research has shown that the extracts exhibit various biological effects, such as antibacterial, antihyperglycemic and antioxidant activities, with essential oils playing a central role in these biological functions (Takahashi *et al.*, 2004).

Essential oils are the odorous, volatile products of the secondary metabolism of an aromatic plant, which are often concentrated in a particular organ of the plant such as leaves, stems, bark or fruit (Conner, 1993). Of the approximately 3000 essential oils that are recognized, roughly 300 are significant from a business standpoint and are mostly used in the flavor and fragrance industries (Burt, 2004). The inclusion of certain terpenoid and phenolic chemicals, which have been demonstrated to have antibacterial activity in pure form, is responsible for the antimicrobial activity of most essential oils. These characteristics are somewhat linked to their lipophilic nature, which causes accumulation in membranes and ensuing membrane-related phenomena such as energy depletion (Conner, 1993).

Phenolic components of essential oils sensitize the phospholipids bi-layer of the cell membrane, causing an increase of permeability and leakage of vital intracellular constituents or impairment

of microbial enzyme systems (Moreira *et al.*, 2005). The chemical composition and biological effects of essential oils, including antimicrobial properties and potential applications in food products, have been reviewed (Bakkali et al., 2008, Burt, 2004).

A number of studies have demonstrated the antimicrobial properties of *Eucalyptus* essential oils against a wide range of microorganisms. These studies, however, are focused on a few *Eucalyptus* species, especially *E. citriodora* (lemon-scented *Eucalyptus*) oil, which has been shown to have a wide spectrum of antifungal activity. Other studies have also mostly focused on the antifungal properties of *Eucalyptus* essential oils (Dhaliwal *et al*., 2004, Fiori *et al*., 2000, Lee *et al*., 2007, Ramezani *et al*., 2002, Somda *et al*., 2007, Tripathi *et al*., 2008), while only a few studies investigated their activity against pathogenic and food spoilage bacteria and yeasts (Chaibi *et al*., 1997, Delaquis *et al*., 2002, Moreira *et al*., 2005, Sartorelli *et al*., 2007).

2.10.2 TEA TREE OIL

The essential oil of *Melaleuca alternifolia*, also named as tea tree oil (TTO), is a complex mixture of terpen hydrocarbons and tertiary alcohols for which an international standard sets levels of 14 components. The main compounds responsible for the antimicrobial activity are terpinen-4-ol and 1,8-cineole (Hammer *et al*., 2006).

Currently, the international standard regulation for TTO sets a minimum content of 30% terpinen-4-ol and a maximum content of 15% 1,8-cineole to meet the European Pharmacopoeia requirements. The mechanisms of action of TTO have not been clearly identified but they seem to be related with the hydrophobic nature of the terpens (Burt, 2004, Bakkali *et al*., 2008).

Melaleuca alternifolia is indigenous to Australia, where it is found growing from Queensland to northeast New South Wales (Cribb, 1981; Penfold and Morrison, 1950) at up to 300 m altitude, on the soil with pH ranging from 4.5 to 7. Other varieties of tea tree oil have been cultivated somewhere else, but *Melaleuca alternifolia* is not produced outside Australia. The short plain between the shore and the dividing range on the north coast of New South Wales (NSW) is the only natural habitat for *Melaleuca alternifolia* in all of Australia. The tree is primarily found in marshes and damp places, where it grows in fairly dense stands with few other species present. This species is best suited to light (sandy), medium (loamy), and heavy (clay) soils, with a preference for well-drained soil. In the shadows, it cannot thrive. This species is vulnerable to

cold, and its leaves have the largest oil content during the warmer months (Orwa *et al*., 2009). Dog dermatitis can be effectively treated with tea tree oil. A cream containing 10% Tea tree oil is effective to treat chronic dermatitis, allergic dermatitis, inter digital pyoderma, acral lick dermatitis and skinfold pyoderma in dogs. (Fitzi *et al.,* 2002). A study reported that daily topical application of tea tree oil for 12 days successfully eradicated warts on the hand of a paediatric patient (Millar and Moore 2008).

2.10.3 LEMON GRASS OIL

Cymbopogon citratus is an aromatic, evergreen, clump-forming, perennial grass producing numerous stiff stems arising from a short rhizomatous rootstock, and growing around 1.5 m tall. It rarely produces owers. The leaves are blue-green in colour, at, erect, linear in shape and give off a characteristic lemon flavour when they are crushed (Avoseh *et al.,* 2015; Haque *et al*., 2018). Lemongrass can be used fresh or dried, powdered, and used as food flavoring. In addition to being served with chicken, fish, beef, and shellfish, it is frequently used in teas, soups, and curries. Numerous studies have demonstrated the great efficacy of infusing lemongrass leaves and other parts to treat nausea, stomach aches, and constipation, as well as to avoid ulcers, promote digestion, and excrete waste (Carbajal *et al*., 1989; Leite *et al*., 1986). Therefore, in many countries lemongrass is used as a medicinal herb (Avoseh *et al*., 2015). The biological activity of lemongrass is due to the presence of the essential oil and phenolic compounds including phenolic acids, flavonoids, and tannins (Olorunnisola *et al*., 2014; Roriz *et al*., 2014; Tavares *et al.,* 2015). Vitamin A, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin C, and folate are among the many essential vitamins that lemongrass contains. It also contains a variety of vital minerals, including calcium, potassium, phosphorous, magnesium, copper, iron, and zinc (USDA National Nutrient Data Base, 2019). Commercial cultivation of *C. citratus* is primarily done for its essential oil, which is principally produced by the plant's leaves (Dijavila *et al*., 2016).

Lemongrass essential oil may be extracted by many different methods like solvent extraction, steam distillation, hydrodistillation (HD), microwave-assisted hydrodistillation (MAHD), and supercritical fluid extraction (SFE) with CO2 . A number of studies have proved that the quality of essential oils depends mainly on its constituents, which is significantly influenced by the extraction techniques (Desai and Parikh, 2015; Schaneberg and Khan, 2002; Wu *et al*., 2019).

Moreover, methods involving heating may induce thermal degradation or hydrolysis of fragile constituents. In solvent extraction, a hydrocarbon solvent (usually n-hexane) is added to the plant material in order to dissolve the essential oil. After filtering the solution and concentrating by distillation, a substance containing resin (resinoid) or a combination of wax and essential oil remains. The method is quite efficient for lemongrass essential oil extraction (Schaneberg and Khan, 2002) and relatively simple but generally requires high volumes of the solvent and sometimes yields unsatisfactory reproducibility. After the extraction operation, the sample is concentrated by evaporation, during which volatiles may be reduced. Moreover, contamination of the essential oil with solvent residues may occur. The Soxhlet apparatus is sometimes used for solvent extraction of lemongrass essential oil (Alhassan *et al.,* 2018).

In Soxhlet extraction, plant material has a continuous contact with refluxing liquid phase, which results in increased extraction efficiency. When compared to the other conventional methods, the most significant drawback of Soxhlet extraction is long heating period at high temperature (usually close to the boiling point of the solvent) which may lead to thermal degradation of fragile compounds.

Both solvent extraction by maceration and Soxhlet extraction require the correct choice of the solvent to obtain a good extraction yield as well as to prevent the loss of volatiles. In recent years, the lemongrass essential oil was successfully extracted from dry and fresh lemongrass leaves using solvent extraction by (Suryawanshi *et al.,* 2016 and Alhassan *et al.,* 2018). The oil yield obtained by Suryawanshi group was 1.85% and that achieved by Alhassan group was 4.5%. In both experiments, n-hexane was used as a solvent. In turn, (Schaneberg and Khan, 2002) reported that sonication-assisted n-hexane extraction allowed obtaining lemongrass essential oil with comparable contents of the main compounds to steam distillation.

Steam distillation is now the most often used technique for obtaining the essential oil from lemongrass. It is mostly used for temperature-sensitive products, such as hydrocarbons, oils, resins, and other substances that may break down at their boiling point and are insoluble in water. Other essential oils are frequently extracted using it as well (Fernandes *et al*., 2019). Using this technique, steam is injected into the dried or fresh plant material, softening the cells and allowing the essential oil to evaporate as vapor. The temperature of the steam should be high enough so that the oil could vapourize, but not so high that it damages the plant material or burns the essential oils. The released essential oil, together with steam molecules, is subsequently cooled in

a condenser and collected. This process enables conducting extraction process below the boiling point(s) of the individual component(s). Essential oils consist of chemical compounds which boiling points often exceed 200°C (Majewska *et al.*, 2019). In the presence of steam, these substances may be extracted at a temperature close to 100°C, at atmospheric pressure. The basic advantage of steam distillation is its simplicity, and low cost of the apparatus. The essential oil's ingredients never break down, therefore its characteristics remain unchanged. Under pressure, this method can also be applied. Lemongrass can be distilled straight from the plant or after it wilts.

Wilting herbage before the distillation process decreases the moisture content and increases oil yield (Majewska *et al.,* 2019). The yields of the essential oil obtained with this method reported in the literature differ substantially, ranging from 0.24% 0.3% (Santin *et al*., 2009), 0.6% (Boukhatem *et al*., 2014), to 0.71% (Kpoviessi *et al*., 2014). Steam distillation is still a leading preparative technique for the extraction of lemongrass oil.

Quality and quantity of lemongrass essential oil are highly dependent on the time of plant harvest, because the composition and the content of the essential oil are strictly connected with the developmental stage of the whole plant, plant organs, and cells (Verma *et al.,* 2015). The harvesting methods usually have little effect on the essential oil yield. The increase in citral content of lemongrass may be influenced by fertilizer application or rhizosphere fungi present in the soil (Shaikh *et al*., 2019). The proportion of young leaves to older leaves during harvesting determines the high citral content and subsequently the quality of the essential oil (Tajidin *et al.,* 2012).

Antibacterial properties of lemongrass essential oil depend on the presence of three main components: geranial, neral, and myrcene (Onawunmi *et al.,* 1984). Geranial and neral individually elicit antibacterial action on Gram-negative and Gram-positive organisms, while myrcene does not show observable antibacterial activity on its own. However, myrcene was observed to generate enhanced bioactivity when it was mixed with either geranial or neral or both.

Naik *et al.,* (2010) investigated the effectiveness of lemongrass essential oil against the selected pathogenic bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* using agar diffusion method and broth dilution method.

In general, Gram-positive bacteria were found to be more sensitive to the oil than the Gram-negative ones, which confirmed findings reported earlier by (Onawunmi *et al.,* 1984). *P. aeruginosa* turned out to be resistant at all the concentrations of lemongrass oil including undiluted solution. *S. aureus* and *B. cereus* were more sensitive to lemongrass essential oil and were inhibited at 0.03% concentration. *B. subtilis* and *E. coli* were inhibited by the oil at a concentration of 0.06%, whereas *K. pneumoniae* at 0.25%. Moreover, the tested microorganisms, particularly the Gram-negative bacteria, turned out to be more susceptible to lemongrass oil than standard antibiotics. Similar results were reported by (Premathilake *et al*., 2018) who investigated such pathogenic bacterial strains as *E. coli*, *B. cereus*, and *S. aureus*. Gram-positive bacterial strains were more sensitive to essential oil of *C. citratus* at all of its concentrations than the Gram-negative strain *E. coli*. Lemongrass essential oil was found to significantly inhibit the growth of such pathogenic foodborne bacteria as *Listeria monocytogenes* and *Salmonella Typhimurium* (Reis- -Teixeira *et al.*, 2019; Mith *et al.,* 2014). Food spoilage bacteria (*Brochothrix thermosphacta* and *Pseudomonas fluorescens*) were also sensitive to lemongrass essential oil (Mith *et al*., 2014). The antibacterial activity of lemongrass essential oil is due to an interaction between the main oil constituents and the bacterial cell membrane. The lipophilic terpenes can modify the fluidity and permeability of the cell membrane or change intracellular pH and ATP concentrations, which results in cell rupture (Shi *et al*., 2016). (Nikaido, 2003) suggested that the higher resistance of Gram-negative bacteria to essential oils is due to the structure of the outer membrane which protects the bacterial cell against extrinsic chemical agents. The outer membrane is one of the main factors contributing to the resistance of Gram-negative bacteria to hydrophobic antibiotics. However, several studies indicate that lemongrass essential oil can successfully inhibit the growth of numerous multidrug-resistant Gram-negative bacterial strains such as *P. aeruginosa*, *E. coli*, *Enterobacter cloaceae*, *Morganella morganii, Proteus mirabilis* or *Burkholderia cepacia* (Bu ková *et al*., 2018; Vasireddy *et al*., 2018).

The antimicrobial activity of lemongrass essential oil is usually higher against fungi than bacteria. (Premathilake *et al*., 2018) found that lemongrass essential oil exhibited fungitoxic activity against *Colletotricum truncatum*, *Fusarium spp*., *Penicillium spp*., and *Crysosporium spp.* All four different concentrations of the essential oil used in this experiment elicited 100% inhibition of *Fusarium spp*., *Penicillium spp.*, and *Crysosporium spp*. *C. truncatum* was also inhibited by the lemongrass essential oil but higher concentrations of the oil were necessary. The essential oil

of *C. citratus* was also investigated towards *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, and *Candida krusei* (da Silva *et al*., 2008). All those strains proved to be sensitive to C. citratus essential oil, which indicates new perspectives in the potential application of lemongrass oil in typical Candida infections. (Tzortzakis and Economakis, 2007) reported a broad antifungal activity of essential oil of *C. citratus* against such food pathogens as *Aspergillus niger*, *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, and *Rhizopus stolonifer*. (Amini *et al*., 2016) demonstrated that lemongrass oil effectively controlled mycelium growth of three *Phytophthora species*: *P. capsici*, *P. drechsleri*, and *P. melonis*. Lemongrass oil produces a fungi toxic effect against postharvest pathogens of the *Aspergillus* genus: *A. flavus*, *A. parasiticus*, and *A. clavatus* (Matasyoh *et al.,* 2011; Bozik *et al.,* 2017). Five mycotoxigenic species isolated from maize samples, including *A. avus*, *A. parasiticus*, *A. ochraceus*, *A. niger*, and *A. fumigatus*, were also found to be sensitive to lemongrass essential oil [Matasyoh et al., 2011]. The highest activity of the oil was observed against *A. niger* with the minimum inhibitory concentration of 15 mg/mL and the highest resistance was observed from A. *flavus* with an MIC of 118 mg/mL. (Sharma *et al*., 2017) recorded an inhibitory effect of lemongrass essential oil against a pathogenic strain of *Fusarium oxysporum*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 list of equipment / apparatus

The following are list of equipment used in the study namely; weighing balance, autoclave, incubator, refrigerator, gas burner, microscope, beakers, petri dishes, measuring cylinder, wire loop, conical flask, micropipettes, L-shaped glass spreader, cork borer, forceps, spiritz lamp, spatula and test tubes.

3.2 Collection of samples

All essential oils (Bergamot oil, Neem oil and Teatree oil) used were gotten from a supermarket opposite Uniben main gate while the rotted carrots were purchased from Ekosodin market, Benin City, Edo state.

3.3 Media preparation

3.3.1 Nutrient agar

Twenty-eight gram (28g) of nutrient agar was dissolved in 1000ml of distilled water in a conical flask according to the manufacturer's instruction and ketoconazole, an antifungal agent was added to inhibit the growth of fungi and the medium was sterilized at 15psi for 15mins using an autoclave. Using the manufacturer standard, four plates were prepared containing 20ml per plate.

3.4 Preparation of the rotted carrot sample

The carrot was cut into smaller pieces and 1g was weighed with a measuring scale. Surface sterilization was then carried out on the carrot. For this procedure, four beakers were rinsed with distilled water and small amount of distilled water was poured into three of the beakers while a small amount of 70% ethanol was poured into the fourth beaker. The weighed 1g of the carrot was rinsed in one of the beakers containing the distilled water. After this, the 1g of carrot was transferred into the beaker containing 70% ethanol for 2 minutes. After sterilizing with ethanol, the carrot was rinsed twice in the remaining beakers containing distilled water and then mashed with a sterilized pestle in an already sterilized mortar.

3.5 Isolation of bacteria from the rotted carrot

Serial dilution of the carrot sample was first carried out. 10ml, 9ml and 9ml of distilled water was measured and poured into three different test tubes labelled 10, 10^{-1} and 10^{-2} respectively. The test tubes were then autoclaved at 121°C for 15 minutes. In the transfer cabinet, the tip of the test tube labelled 10ml was sterilized with flame and the 1g of mashed rotted carrot was transferred into it and mixed well to dislodge the bacteria. From this mixture, 1ml was measured, transferred into the test tube labelled 10^{-1} and mixed well by shaking. After this, 1ml from the test tube labelled 10^{-1} was measured and put into the test tube labelled 10^{-2} and mixed thoroughly. This process was done to reduce the microbial load of the microorganisms in the rotted carrot sample.

For the inoculation, 0.1ml of the serial diluent was measured from the test tube labelled 10^{-1} with a micropipette and inoculated into an already labelled agar plate. A sterilized L-shaped glass spreader was used to distribute the inoculum around the plate. This process was repeated into another agar plate. 0.1ml of the serial diluent from the test tube labelled 10^{-2} was inoculated sinto another agar plate and spread with an already sterilized L-shaped glass spreader. This process was repeated into another agar plate.

3.6 Subculturing of bacterial isolates

The prepared media was poured into petri-dishes and allowed to solidify. Bacteria with different characteristics were picked from the previously cultured plates and streaked on the new medium using a wired loop. This was done to avoid picking two different colonies at a time. The process was carried out very close to the flame for proper sterility.

3.7 Identification of Bacteria

3.7.1 Gram staining

The Gram staining technique was carried out on the basis of the component of the cell wall. Organisms which retained the color of the initial stain are called Gram positive, while those that do not retain the primary stain when decolorized are Gram negative. Gram staining reagent include crystal violet (primary dye stain), iodine (as mordant), 70% alcohol (decolorizer) and safranin (counter stain).

Procedure: A drop of sterile saline water was placed on a clean glass slide. The inoculating wire loop was flamed until red hot. The loop was allowed to cool and a small portion of the organism to be Gram stained was picked, smeared on the drop of saline water on the slide and air dried. The smear was stained with 1% crystal violet for 1 minute and washed with distilled water. Iodine was added as a mordant for one minute. 70% alcohol was added for 30 second, this acted as a decolorizer.

The slide was then rinsed with distilled water and flooded with counter strain safranin for 1 minute, washed off with distilled water and air dried. The slides were observed under the microscope, Gram positive bacteria appeared purple while the Gram negative bacteria appeared pink.

3.7.2. Catalase test

This test was carried out to determine the presence of the enzyme catalase, which catalyzes the release of oxygen from hydrogen peroxide.

Procedure: The pure culture of the test organism was placed on a clean glass slide using a sterile inoculating loop following the addition of a drop of 3% hydrogen peroxide. A positive result was indicated by the production of gas bubbles, while its absence was regarded as a negative.

3.7.3 Indole test

Indole test helps to detect bacteria that have the ability to break down tryptophan for nutritional needs using tryptophanase. Indole can be detected through the use of kovac's reagent.

Procedure: The bacterium were inoculated into test tubes containing 3ml of tryptophan broth and incubated at 37°C for 24 hours. 0.5ml of kovac's reagent was added to the broth and shaken gently. The development of red ring coloration around the surface of the broth indicated a positive recation while the development of a gold ring coloration indicated a negative reaction.

3.7.4 Citrate test

This test detects the ability of an organism to use citrate as the sole source of carbon and energy. Simmons citrate agar was prepared according to the manufacturers guide and 5ml was pipetted into different test tubes. The test tubes were inoculated with the bacteria isolate using a sterile

inoculating loop and incubated at 37°C for 24 hours. Change in color from green to blue indicated a positive reaction while no color change indicated a negative change.

3.8 Antimicrobial Susceptibility Test

Test organisms were subjected to antibacterial susceptibility test using the Agar well diffusion method on prepared media. Petri dishes containing Nutrient Agar were prepared and 100 µl of bacterial suspension respectively were inoculated by spread plating method. Wells 6.0 mm in diameter were made using a cork borer. Each well was properly labeled to represent the positive control, negative control and the different concentrations (100%, 75%, 50% and 25%) of the essential oils (bergamot oil, neem oil and tea tree oil).

The pure essential oils were considered as 100% and used as the stock solution. In order to prepare different tested concentrations of 100µl (75%, 50% and 25% v/v), 75µl, 50µl and 25µl of each pure essential oils were dissolved in 25µl, 50µl and 75µl of Dimethyl Sulfoxide (DMSO) respectively (Najmi, *et al.,* 2023).

100µl of each essential oil and control samples were micro-pipetted into the wells. All the procedures were carried out under a laminar air flow cabinet to preserve sterile conditions. The plates were incubated at 37°C for 24hrs and their antimicrobial activity was measured for each of the three isolates using the meter rule (in millimeters) (Ghavam, *et al.,* 2022). Dimethyl Sulfoxide (DMSO) was used as negative control and antibacterial agent (ampicillin) was used as positive control to compare their antibacterial properties with those of the essential oils.

CHAPTER FOUR

RESULT

Mixed culture of bacteria obtained from the rotted carrot sample is represented in plate 1. The purpose of this culture is to study the bacterial population present in the carrot. Specifically at dilution factor 10^{-1} and 10^{-2}. Plate A and B represents the growth at dilution factor 10^{-1} and plate C and D shows the growth rate at dilution factor 10^{-2}. Plate 2 represents the pure culture isolated from the afore mentioned mixed culture plate by streak plate method, this helps in the identification of the isolates.

Table 1 provides the feature and results of the morphological and biochemical identification of the isolated bacteria respectively.

Table 2, 3 and 4 showcase the antimicrobial properties of the essential oils used against bacteria isolated from rotted carrot.

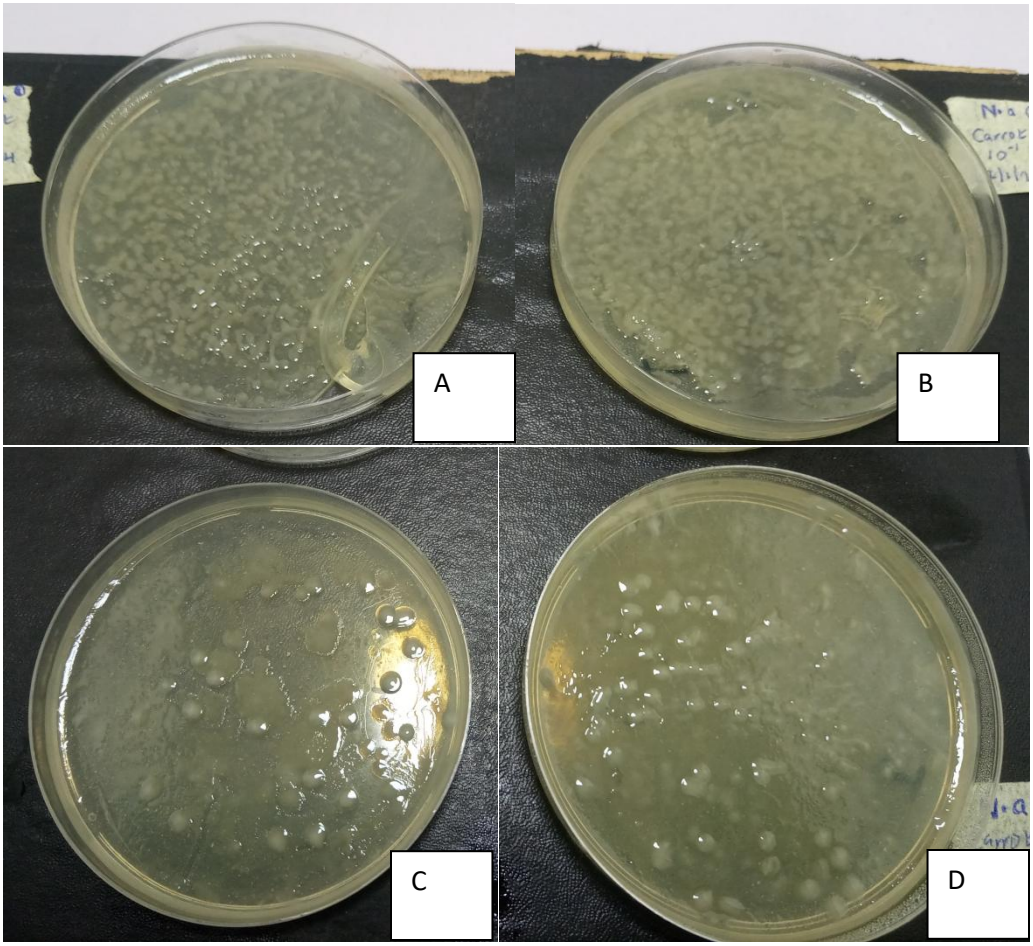

Plate 1: Mixed culture of bacteria isolated from rotted carrot.

Key:

A: Isolated from serial dilution 10^{-1}

B: Isolated from serial dilution 10^{-1}

C: Isolated from serial dilution 10^{-2}

D: Isolated from serial dilution 10^{-2}

Pure cultures were obtained from the mixed cultures of bacteria as represented in Plate 2, this pure cultures were obtained through sub culturing specifically the streak plating method. Plate 2 represents the different isolates gotten from the mixed culture.

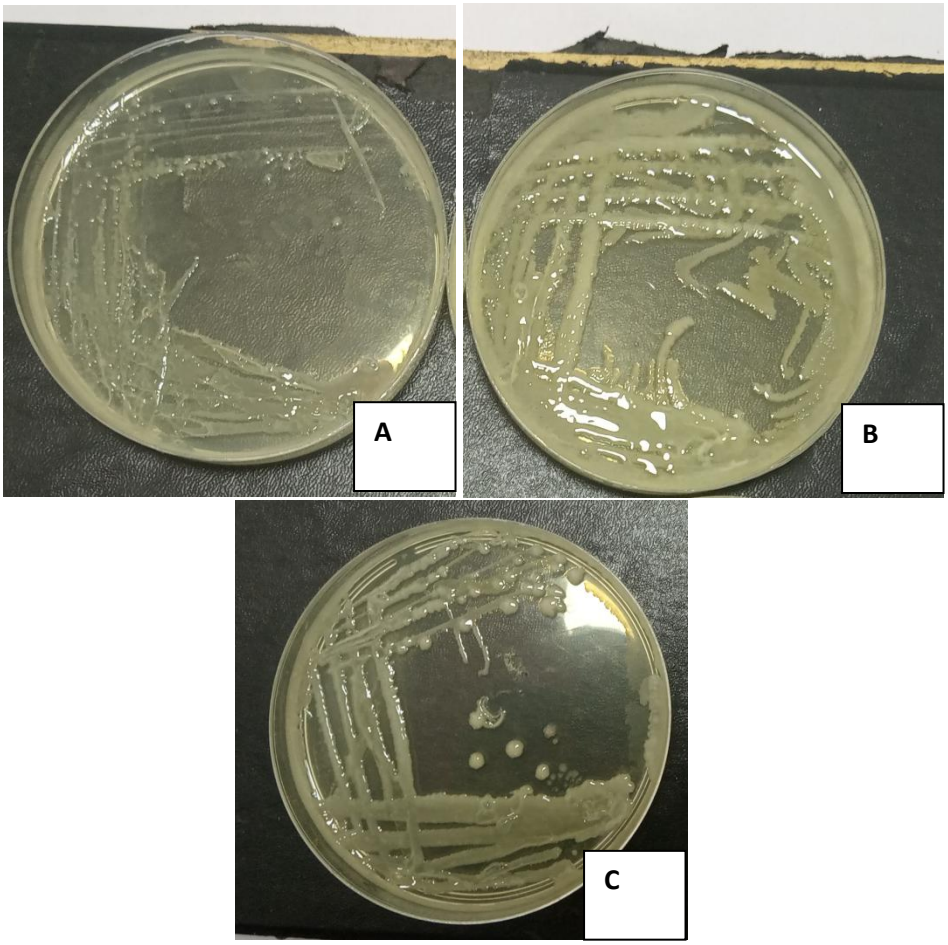

Plate 2: Pure culture of bacteria isolated from the aforementioned mixed cultures.

Legend:

A: *Enterobacter* sp.

B: *Pseudomonas* sp.

C: *Agrobacterium tumenfaciens.*

Table 1: Cultural, morphological and biochemical characteristic of the bacteria isolate

Parameter	Isolate 1	Isolate 2	Isolate 3
Cultural characteristics			
Elevation	Raised	Flat	Raised
Margin	Entire	Entire	Undulate
Color	Cream	Cream	Cream
Shape	Circular	Circular	Circular
Size	Medium	Medium	Medium
Morphology			
Gram stain	-	+	-
Cell type	Rod	Rod	Rod
Arrangement	Disperse	Disperse	Disperse
Biochemical test			
Catalase	+	+	+
Indole	+	-	-
Citrate	+	+	+
Identity	*Enterobacter* sp.	*Pseudomonas* sp.	*Agrobacterium tumenfaciens*

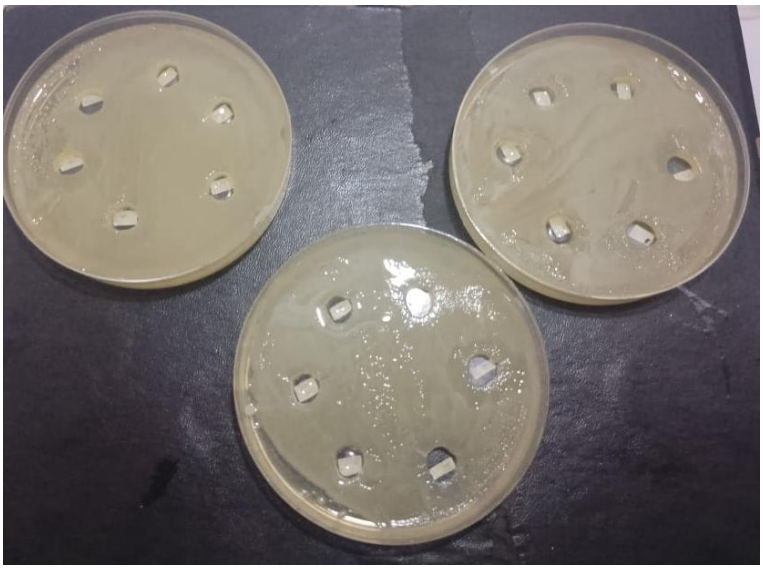

Plate 3: Culture plates showing the antibacterial activities of the essential oils against each bacterial isolate using the agar well diffusion method.

Table 2: Antibacterial activity of Neem oil against the test isolates represented by the zone of inhibition (mm)

ISOLATES (mm)	**Concentration (%)**				**CONTROL**	**CONTROL**
	25	**50**	**75**	**100**	**(AMPICILLIN)**	**(DMSO)**
Enterobacter sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.98 ± 0.03	0.60 ± 0.60
Pseudomonas sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.75 ± 0.75	0.00 ± 0.00
Agrobacterium tumenfaciens	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3: Antibacterial activity of Bergamot oil against the test isolates represented by the zone of inhibition (mm).

ISOLATES (mm)	**Concentration (%)**				**CONTROL**	**CONTROL**
	25	**50**	**75**	**100**	**(AMPICILLIN)**	**(DMSO)**
Enterobacter sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.68 ± 0.08	0.00 ± 0.00
Pseudomonas sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.78 ± 0.48	0.00 ± 0.00
Agrobacterium tumenfaciens	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.90 ± 0.12	0.00 ± 0.00

Table 4: Antibacterial activity of Tea tree oil against the test isolates represented by the zone of inhibition (mm).

ISOLATES (mm)	**Concentration (%)**				**CONTROL (AMPICILLIN)**	**CONTROL (DMSO)**
	25	**50**	**75**	**100**		
Enterobacter sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.20 ± 0.10	0.00 ± 0.00
Pseudomonas sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 0.15	0.00 ± 0.00
Agrobacterium tumenfaciens	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.25 ± 0.25	0.60 ± 0.60

CHAPTER FIVE

DISCUSSION

Carrot (*Daucus carota*) is one of the most important economical root vegetable crops worldwide and the largest source of provitamin A and carotenoids necessary in the human diet (Constance, 1971). The increasing resistance of the microorganisms that causes rot in carrot to conventional chemicals and drugs prompted scientists to search for novel sources of biocides with broad-spectrum activities as stated by Abad *et al.,* (2007). The primary aim of this research endeavor was to meticulously characterize and identify the bacterial isolates obtained from rotted carrot and subsequently ascertain the antibacterial potentials of three distinct essential oils against the aforementioned bacterial isolate.

Given the escalating demand for natural alternatives to synthetic preservatives, essential oils have emerged as a viable substitute owing to their demonstrated ability to impede the growth and proliferation of a wide range of bacterial species. Motivated by this premise, the present study sought to examine and evaluate the antibacterial activities of selected essential oils against the bacterial strains isolated from the rotted carrots. However, despite their well-documented antibacterial properties in previous research projects, it is rather disconcerting to note that none of the selected essential oils, namely Tea Tree Oil (TTO), Bergamot Essential Oil, and Neem Essential Oil (EO), exhibited any discernible zones of inhibition against the isolates obtained from the rotted carrots in this particular investigation.

The bacteria isolated from the rotted carrot were *Enterobacter* sp., *Pseudomonas* sp. and *Agrobacterium tumenfaciens.*

As opposed to the findings in the study of Sultana *et al.,* (2019), Neem oil did not show any zone of inhibition against *Pseudomonas* sp. but there was a clear and distinct zone of inhibition when tested against bacteria like *Staphylococcus aureus* and *Klebsiella* sp., the factor considered in that study is due to the high resistance of *Pseudomonas* sp.

Bergamot oil did not show any antibacterial property when tested against any of the bacterial isolate, this is not in line with the study by Cebi and Eraslan, (2023) who reported Bergamot oil showed prominent antibacterial properties against *Escherichia coli, Listeria monocytogenes* and *Bacillus cereus* at various concentrations such as 0.5 µg/mL, 1 µg/mL, 2.5 µg/mL and 5 µg/mL.

There are several factors to consider as to why the essential oils did not show any clear zone of inhibition against any of the bacterial isolate. The first reason is that the essential oils were not extracted from the original plant source; rather they were purchased in the supermarket as a commercial product which could have affected the integrity of the essential oil's potential. Another factor is the type of bacterial strain that was isolated, bacterial resistance, methodological differences, inactivation or degradation of the essential oils, synergistic or antagonistic reactions and difference in the composition of the essential oils (Hammer *et al*., 1999).

While this study did not reveal any significant antibacterial property of the essential oils, the study by Veira-Brock *et al*., (2017) shows great antibacterial properties when several essential oils were combined together. The study demonstrated the synergistic effect of essential oil blend against highly resistant bacteria. This gives better prospect to the efficiency of the essential oil blend to just a single essential oil used against an individual bacteria strain as shown in this current study.

Conclusion

The isolates employed in the study, coupled with potential bacterial resistance mechanisms, methodological discrepancies, synergistic or antagonistic interactions, inactivation or degradation of the essential oils, and variations in the composition of the essential oils themselves, might all have collectively contributed to the observed lack of potential antimicrobial activity. Further investigations can be done using essential oils against bacterial isolates but it should be extracted from the plant source to avoid destroying its potential antimicrobial properties.

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