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college of science

biology department

Research project

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Dedication

We dedicate this project to God Almighty our creator, our strong pillar, our source of inspiration, wisdom, knowledge and understanding. He has been the source of our strength throughout this program.

We also dedicate this to our parents who encouraged we all the way and whose encouragement have made sure that we give it all it takes to finish that which we have started. May the blessing of God be with them now and always “Amin”

Aim of study

1. to investigate the antibacterial activity of two types of oils that extracted by classical method and that extracted by solvent against both Gram-positive and Gram-negative bacteria species.
2. To discover antibiotic against pathogen.
3. For treatment infectious disease caused by bacterial pathogen and other opportunities.

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Comparison The Antibacterial Activity Of Two Extracted Oils Of Thyme (*Thymus vulgaris L.*) Against *staphylococcus aureus* And *Escherichia coli* In Vitro

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Abstract

Thyme (*Thymus vulgaris L.*) is a valuable medicinal plant used in the manufacture of antiseptics, skin toners and several traditional medicines for treating cough and cold. In the present study, antibacterial activity in the leaf extracts of Thymus vulgaris L. Oils were extracted by olive oil(classical method) and petroleum ether against pathogenic bacteria like *Escherichia coli* and *Staphylococcus aureus* was evaluated by in vitro agar well diffusion method. Results showed that the oil that was extracted by petroleum ether was found to possess strong antibacterial activity compared by classical method. With diameter of the inhibition zone of 17 to 35 mm against Staphylococcus and 13 to 26 mm against *E.coli* respectively, when compared with the oil extracted by classical method showed maximum inhibition zone diameter of 25mm against Escherichia coli and21mm against *Staphyloccous aureus*.

Keywords

Thymus vulgaris L., *Staphyloccous aureus*., antibacterial activity, leaf extracts, inhibition zone

Introduction

There are many microorganisms in humans that typically do not cause disease (normal flora). The problem is, some bacteria responsible for disease often come from normal flora, causing infections (Parisa et al., 2019). Most healthy individuals carry *Staphylococcus aureus* on their skin and mucous membranes (mostly the nasal area) in the environment and on their bodies, The bacteria normally do not cause infections on healthy skin; however, if they enter the bloodstream or internal tissues, they may cause pneumonia, respiratory tract infections, surgical site infections, prosthetic joint infections, and nosocomial infections, all of which can be serious (Gordon et al., 2020). The incidence rate of bacteremia caused by *staphylococcus aureus* estimated about 20 to 50 per one hundred thousand each year and the mortality rate due to infection is 10 to 30 percent (van Hal et al., 2012). A licensed vaccine for the prevention of *Staphylococcus aureus* infections doesn't exist, and several candidate vaccines have failed clinical trials. As a result of these failures, uncertainties remain in developing a successful vaccine, including understanding the mechanisms of protective immunity, difficulty identifying ideal vaccine antigens, and debate regarding which population to target for vaccination (Kleinhenz et al., 2022). *Escherichia coli* is a common flora that often colonizes the large intestine. It is a gram negative bacterium that appears as bacilli. 90% of urinary tract infections, gastrointestinal infections, and systemic infections in people are caused by the major bacterium *Escherichia coli* (Parisa et al., 2019).

The discovery of antibiotics in the twentieth century is considered one of the greatest discoveries of the century. Antibiotics were powerful weapons that helped mankind battle microbial pathogens and successfully control numerous infections. Some community-acquired infections, such as *Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae*, and other *Enterobacter* species, developed remarkable resistance to discovered antibiotics, and so scientists had to develop new antibacterial drugs. As reported by the Food and Drug Administration (FDA), the prevalence of multidrug resistant (MDR) bacteria is steadily increasing, while efforts to develop new antibiotics are rapidly decreasing. According to FDA, we are facing a serious health crisis, while the approval rate for new antibiotics has declined by 56 percent over the past three decades (Abdallah Emad Mohamed et al., 2020).

Increasing resistance of bacteria has led to increased interest in the development of new antimicrobial agents. The extracts, oils, and derivatives of plants are known to be effective against microorganisms, and they have been used for centuries to combat bacteria, fungi, and viruses (Vasconcelos et al., 2018).

Thymol and carvacrol are mainly present in the essential oils of thyme and oregano(Ismail et al., 1990 and Lang NP et al., 2015), many studies showed their antimicrobial properties against both Gram-positive and Gram-negative bacteria species(Adriaens PA et al.,2000). Eugenol is a major component (approximately 87%) of leaves and buds from Thyme (Murakami et al., 2018). This component is largely used perfumes and in mouthwashes as dental analgesic and has been well recognized, for its antimicrobial activities (Sajadi FS et al., 2015). Menthol is a terpenoid and the active principle of essential oils from the mentha species, such as peppermint and horse mint (James P et al., 2017). However, to the best of our knowledge, there are no available data about the antibacterial activity of these compounds against nosocomial infection-bacteria. Therefore, the objective of the present work was to investigate the antibacterial activity of two types of oils that extracted by classical method and that extracted by solvent against both gram-positive and gram-negative bacteria species

Material and method

Collection of plant

Leaves of *Thymus vulgaris*L. were collected in safen mountains (Erbil-Kurdistan),dried and powdered (2023).

Preparation of oil using olive oil as solvent (oil 1):

10g of powder of Thymus vulgarisL. was weighted with the digital scale and transferred into 100ml conical flasks. 15ml of olive oil was added. The conical flask were closed by foil paper and placed in dark place for 5 days. The crude oil were then filtered by passing the extract through Whatman no.1 filter paper. The oil were stored in refrigerator at 40c in small and sterile plastic test tube (Gordon,1980**).**

Preparation of oil extract by using petroleum ether as solvent (oil 2):

10g of powder of Thymus vulgarisL. Was weighted with the digital scale and transferred into 100ml conical flasks. 15ml of olive oil was added. The conical flask were closed by foil paper and placed in dark place for 5 days. The crude oil were then filtered by passing the extract through Whatman no.1 filter paper, and then concentrated at 80c by using water bath. The oil was stored in refrigerator at 40c in small and sterile plastic test tube (Gordon, 1980)

Determination of antibacterial activity

The *staphylococcus aureus* and *E.coli* were obtained from college of science ,Department of biology, Microbiology laboratory; Bacterial culture were activated by transferring at least 4-5 well isolated colonies of bacterial culture to a plastic petri dish (standard size) containing 18ml of sterilized nutrient agar, incubated at 37c for 12-18h. Nutrient agar prepared and poured into petri dish to a depth 4mm then in each plate, three hole were made each of 7mm in diameter using a sterile cork borer. The plate were streaked by sterile loop which transfer bacterial colonies and different amount of extract were added to these hole with sterile micro pipettes, The plate were incubated at 37c for 18-24h, The diameter of inhibition zone were measured in millimeter using a ruler(Yassine et al.,2019).

Result and Discussion

The present study, gives an account on the antagonistic activities of *Thymus vulgaris* L. oil extracts against species of gram negative *E.coli* and gram positive bacteria *staphylococcus aureus*. The above mentioned plant extract has a powerful antimicrobial activity that inhibit or at less stop the growth of microbial populations of different bacteria categories; so those oil extract may be used as food additives and food preservatives to control such microbial population and conserve human and animal health table (1)..

Table 1: Antibacterial activity of (oil1) extract of *Thymus vulgaris*L against *staphylococcus aureus* and *E.coli* by pore plate method.

Amount of extract (microliter)	Radius of inhibition in mm	
	E.coli	*Staphylococcus aureus*
6	7	11
8	11	15
10	15	19
12	18	22
14	21	26

The oil of *Thymus vulgaris* L that was extracted by petrolume ether (oil2) and tested against (Gram-positives and gram-negatives) as showed in table (2). It exhibited strong antibacterial activity against two tested bacteria and recommended as a natural preservative in the food industry.

Table 2: Antibacterial activity *Thymus vulgaris* L. (oil2) against *staphylo coccus aureus* and *E.coli* by pore plate method.

Amount of extract (microliter)	Radius of inhibition in mm	
	E.coli	*Staphylococcus aureus*
6	13	17
8	15	22
10	19	25
12	22	32
14	26	35

It was published that the essential oils of *Thymus vulgaris*L showed remarkable inhibitory effect against the MDR-pathogens, namely *Escherichia coli*, and *Staphylococcus aureus*; Moreover, it was observed that there is a considerable synergistic inhibition of that essential oil with streptomycin (Haidari M et al., 2018)

Usually, Gram-negative bacteria are more resistant to plant extracts, oils, and their constituents than Gram-positive bacteria, because the cell wall of Gram-negative bacteria is more complex (Mehta et al.,2020). Porin proteins serve as hydrophilic trans membrane channels for small hydrophilic solutes, which easily pass through the outer membrane of Gram-negative bacteria; however, it is hard for hydrophobic antibiotics to penetrate the cell and this is one reason that makes Gram-negative bacteria more resistant (Cavali et al., 2017). The Gram-positive bacteria cell wall allows hydrophobic molecules to easily penetrate and act on both the cell wall and in the cytoplasm (Mehta et al.,2020) as shown in figure(1).

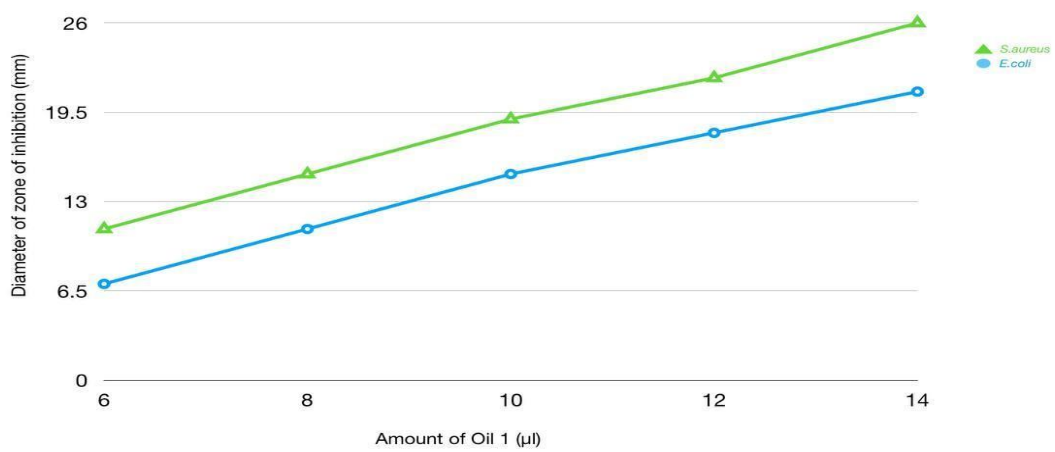

Fig1: Comparison the activity of Oil 1 against *S.aureus* and *E.coli*

The results showed that expressed a significant antibacterial activity against the two strains studied. The oils showed the high antibacterial activity against *S.aureus* than *E. coli* .These oils, especially essential oils can be used as antibacterial agents for the treatment of various infectious diseases caused by these germs on the other hand, Gram-negative bacteria were more resistant than Gram-positive refers to the structure of their outer membrane. Thus, the outer membrane of Gram-negative is richer in lipo-polysaccharides and proteins than those of Gram-positive that make it more hydrophilic, which prevents the hydrophobic terpenes from adhering to these bacteria (Buyton et al., 2017 and Cavali et al., 2019).

As shown in figure (2)there is a significant deference between grame positive and grame negative bacteria against (oil2) may be due to accumulation of numerous molecules inside the cell can perturb its selective permeability, potentially reaching a critical level to induce cell death (Dametres et al.,2020).

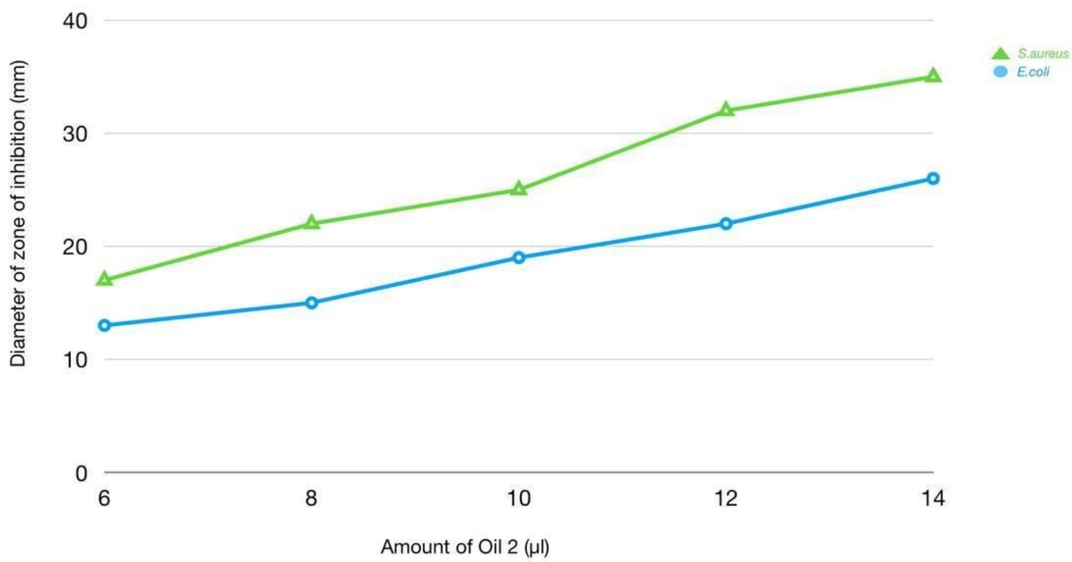

Fig2: Comparison the activity of Oil 2 against *S.aureus* and *E.coli*

It is clear from the results figure (3,4) that(oil2) extract more active than (oil1) extracts of this plant against these two types of bacteria may be due to the essential oils that dissolved in petrolume ether that *E. coli* cells possessed a reduced negative charge after exposure to essential oil and bacterial cells experienced irreversible membrane damage caused by the acidification and protein denaturation of the cell membrane owing to the accumulation of the components of the oil, which allowed the access of antibiotics to PBPs and the induction of cell death(Samaranak et al.,2018).

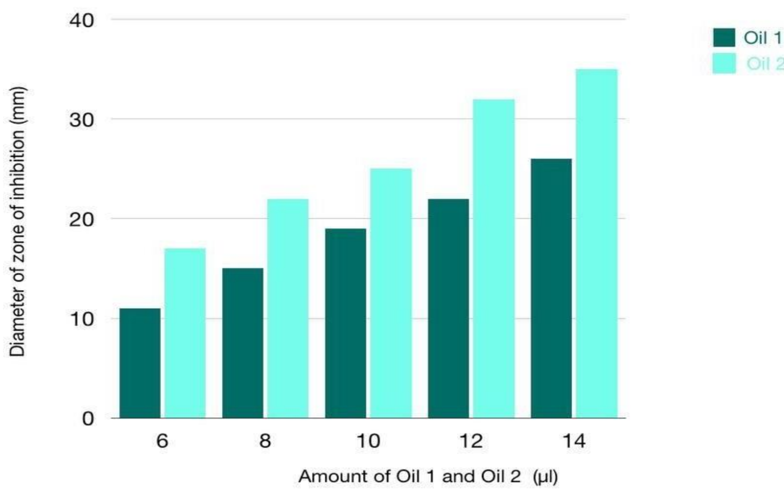

Fig3: Comparison the activity of Oil 1 and Oil 2 against *S.aureus*

Nevertheless, some low molecular weight phenolic compounds can adhere to these microorganisms thanks to their functional groups. The mechanisms by which the Aromatic and phenolic compounds can inhibit the microorganisms involve different mechanisms. Thymol and Carvacol have a hydroxyl group, which play a major role in their antibacterial activities (Razaq et al., 2006). They are able to alter the cell outer membrane and combine with the charged groups of membrane via increasing its permeability (Abdulbaqi et al., 2016 and Parvekar et al., 2020).

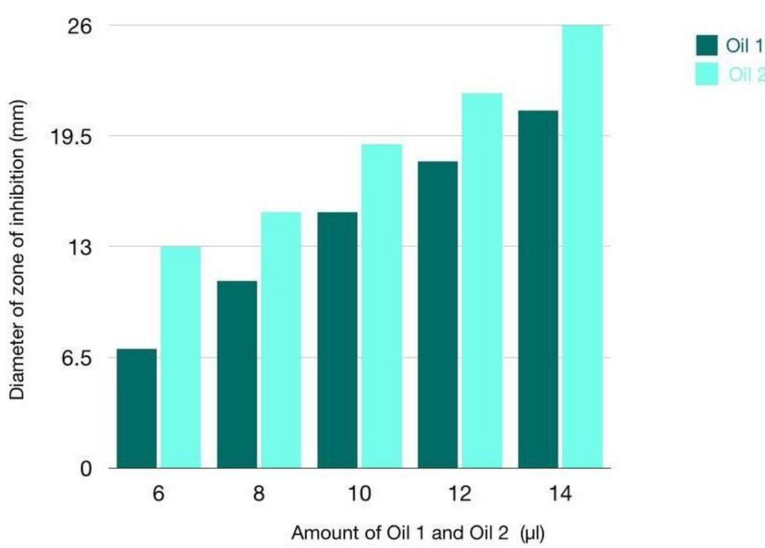

Fig4: Comparison the activity of Oil 1 and Oil 2 against *E.coli*

The present investigation reveals a real solution for antimicrobial resistance by using a natural herbal extract . The above mentioned plant extract has a powerful antimicrobial activity that inhibit or at less stop the growth of microbial populations of different bacteria categories; so those extract may be used as food additives and food preservatives to control such microbial population and conserve human and animal health.

5. Conclusion

1. Thymus vulgaris L has a powerful wide-spectrum antibacterial agent which attributed to its phytochemical constituents particularly thymol.
2. The Thymus vulgaris L. oil was more effective against Staphylococcus aureus than E.coli and could be used as a natural active agent to produce oral health care products.
3. intensive and in-depth chemical, biological and pharmacological studies are required in order to isolate the antibacterial molecules and evaluate the safety, dosage and possible side effects

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