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Effect of Essential Oils on the Beneficial Bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and Pathogenic Bacteria *Pseudomonas* spp. and *E. coli*

Rania Ahmed, Zienb El-Naage , D. A. El-Wakil
Jazan University, Jazan, Kingdom of Saudi Arabia
Corresponding Author: Rania Ahmed

Abstract: *Aromatic volatile oils are considered important materials as a natural preservative. It has a dampening effect of the growth of many microbes as well as have the perverse effect of some types of pathogenic bacteria to humans. This study was conducted on 4 types of these essential oils extracted from the region of Jizan in Saudi Arabia, a grain of oil Baraka and clove oil and cinnamon oil and mustard oil. The aim was to study the effect of these oils on the beneficial bacteria (initially yogurt), Lactobacillus bulgaricuuocs and Streptococcus thermophilus. The results showed that Nigella sativa oil and cinnamon had their inhibitory effect, followed by mustard oil was the effect on these bacteria using the same concentrations of these oils, clove oil is less of these oils. Also studied the effect of these volatile aromatic oils on pathogenic bacteria to humans E. coli and Pseudomonas spp the result confirm the study the effectiveness of black bean against pathogenic bacteria (as stated in the word of profit Mohamed be upon him in the black seed).*
Keywords: *Aromatic volatile oils, beneficial bacteria**,** pathogenic bacteria**.***

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I. Introduction

1- Medicinal and Aromatic Plants.

The attractiveness of medicinal and aromatic plants is constantly increasing due to increased consumer demand and interest in these plants for culinary, medical and other human activities. As consumers become more informed about food, health, and nutrition issues, they also become aware of the benefits of medicines and aromatic and their potential applications. Plants and their products, these plants produce a large variety of secondary metabolism, among them, essential oils. Despite its rich and complex composition, the use of aromatic oils is still extensive and is limited to cosmetics and perfume fields. It is worth developing a better understanding of the chemistry and biological properties of these extracts and their new individual components and valuable applications in human health, agriculture and the environment. Essential oils can be used as an effective alternative or supplement to industrial compounds of the chemical industry, without producing the same secondary effects [1].

Medicinal and aromatic plants form a large part of natural plants and are an important resource in various fields such as pharmaceutical, flavor, perfume, perfumery and cosmetic industries [2]. Medicinal plants and herbs were used in antiquity. Our ancestors knew the importance of these medicinal plants because they were extracted from important chemicals Significant and significant importance to their physiological and therapeutic effects on humans and animals. It has spread throughout the world, especially on the Nile River in the era of the Pharaohs and Mesopotamia [3,4]. After the emergence of Islam, the basic science became full of scientific materials and field experiments, especially in the branches of medicine and plant drugs [5].

Essential oils obtained from faucets are aromatic in nature due to a mixture of chemicals of multiple variations that belong to various chemical families, turbines, aldehydes, alcohol, esters, phenols, ethers and ketones. Essential oils have the enormous business potential on the world market because of its unique flavor and fragrance properties and also biological activities [6,7]. Essential oils that work in aromatherapy and treat many diseases, including cardiovascular disease, diabetes, Alzheimer's disease and cancer. Antimicrobial antimicrobial oils and their chemical components by many researchers in the past, moreover, studies have shown the synergistic effect of any two or more Ingredients of essential oils against various human pathogens. Recently, the proliferation of anti-microbial drug resistance. The researchers have led to the discovery of lead antimicrobial lead molecules to treat various human pathogens. Some synthetic drugs currently available do not prevent many pathogenic microbes [8]. In addition, the use of synthetic chemicals to control pathogenic microorganisms is limited due to its carcinogenic effects, acute toxicity, and potential environmental hazards. In this regard, the exploitation of essential oils to combat the epidemic of multi-drug pathogenic organisms can be useful to combat various infectious diseases, thus, this review of antibacterial, antifungal detail, the anti-viral potential of essential oils extracted from the therapeutic and possible significance mechanism involved in the reservation of Human pathogenic microorganisms [9].

2. Chemical Composition of Essential Oils.

Essential oils have the potential to prevent the growth of a variety of pathogens due to the presence of natural compounds produced by plants. Most importantly, the unique aroma and other essential biologically active oil properties depend on their chemical components. In MAP, aromatic oils usually accumulate in the amino channels or cavities, glandular gland disease and sometimes in skin cells. Essential oils and their chemical components show the most active activity when they are present in oxygen or active form. In general, the chemical composition of aromatic oils is relatively complex and notes between 20 to 60 different biologically active ingredients of these essential oil aspirations. Many of these compounds are classified for many cooking characteristics. Typically, the chemical characterization of many essential oils reveals that there are only 2-3 main ingredients in (20-70%) compared with other ingredients present in trace amounts. Most essential oils consist of Terpenes or terpenoids and other aromatic aliphatics with low molecular weights. Terpenes or terpenoids are synthesized in the cytoplasm of the cell pathway through the mephalonic acid. Terpenes consist of isoprene units and are generally represented by the chemical formula $(C_5H_8)_n$. Turbines can be annular, monoclonal, helical, or triglycerides. Due to the diversity of their chemical structures, turbines are classified in several groups such as monotropin ($C_{10}H_{16}$), systarapetin ($C_{15}H_{24}$), deuterbus ($C_{20}H_{32}$), and traitrapens ($C_{30}H_{40}$) [10]. The main ingredient (~ 90%) of biologically active essential oils consists of monotropin. Some major hydrocarbons include monotropin (p-semen, lymon, α-benin, α-terpenin), monoproteins monoproteins (alcohol, carvacrol, iginol, thimol), reedbins, acetate, alkomarin, benzofuran). Chemical components of essential vegetable oils Some factors that may affect these components include geographical location, and this chemical difference is directly related to changes in antimicrobial activity against various pathogenic microorganisms. Although essential oils can be recovered using fermentation, extraction, or evaporation, commercial production is preferred to achieve through a tightening process. Similarly, the antimicrobial efficiency of essential oils depends on the type of microbes that should be discouraged as well as the assessment, including biological availability and mitigation [11].

3. Antimicrobial Effects of Essential Oils.

Antimicrobial effects of essential oils derived from MAP are the basis for large-scale applications in various income-generating sectors such as pharmaceuticals, cosmetics, perfumes, agro-industries and health industries. In the next section, we extensively discussed the antimicrobial, antifungal, and antiviral effects of essential oils obtained from MABS. Many antibiotics are available to treat various bacterial pathogens. However, increased resistance to multiple drugs has increased the severity of diseases caused by bacterial pathogens. In addition, reduced immunity in host cells and the ability of bacteria to develop drug resistance associated with biofilm increased the number of bacterial infections in life-threatening humans. Thus, bacterial infection remains a major cause of human death, even today [12] .In addition, the use of many anti-bacterial agents in higher doses may cause toxicity in humans. This has led researchers to explore new key molecules against bacterial strains. In this regard, essential vegetable oils and key chemical ingredients are potential candidates as antibacterial agents. Several types of essential oils and chemical components have been reported from the Mabs schemes to possess a wide range of bacterial inhibitory potential. The effect of antibacterial activity of essential oils may prevent the growth of bacteria (bacteria) or destructive cells (microbicides). However, it is difficult to distinguish between these procedures. In this respect, antibacterial activity is measured more frequently, such as minimal microbial concentration (MEPC) or a less inhibitory concentration (MEC). A rapid antimicrobial examination of aromatic oils is usually performed using the agar diffusion technique, where essential oils are added to paper tablets or holes placed in agar that have been standardized with a bacterial strain. After incubation, it represents an antimicrobial inhibitory region [13].

II. Materials and Methods

In vitro study.

1-Essential Oils: Essential oils of 5 herbs, Cinnamon (*Cinnamomum zeylanicum*). Clove (*Syzygium aromaticum* L.). Mint (*Mentha piperita*), Black cumin (*Nigella sativa*). Thyme (*Thymus vulgaris*) was isolated from growing plants in Jazan, Saudi Arabia. The essential oils were extracted in the chemical laboratory.

2-Bacterial Species: Four bacterial species belonging to Gram- negative and Gram positive was tested. Bacteria strains were isolated from Hospitals and identified in Egypt. Lactobacillus bulgaricus, *Streptococcus thermophiles*, Pseudomonas aeruginosa and *E. coli*.

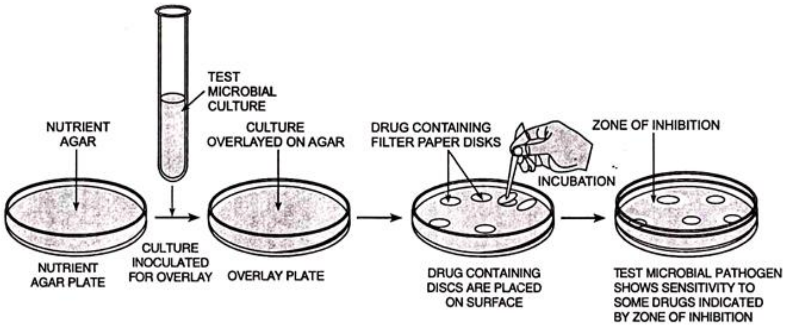

Figure (1): standard disc diffusion method

3- Screening Of Antimicrobial Activity:

Screening of antibacterial activity was performed by the standard disc diffusion method [14]. Fifty sterilized disc of filter paper (6 mm diameter) were soaked in 1 ml of oil, separately for 2 min and then and then used for screening. The potency of each disk was 10 µL (each 50 discs of filter paper absorbed 0.5 Ml. Nutrient agar was used as base medium and Nutrient broth was used for the preparation of the inoculums (Fig.1). A sterile cotton swab was dipped into the bacterial test suspension to inoculate entire surface of a nutrient agar plate. Discs of oil were placed on the surface of inoculated plates with the help of sterile forceps. After incubation inhibition zone diameters of 4-5 discs for each oil were measured to the nearest millimeter (mm).

III. Results and Discussion

In vitro study : The antimicrobial activity of essential oils against bacteria varies depending on the source of essential oil and strain bacteria. Essential oils used in this study exhibited antibacterial activity against some tested bacteria with different degree of inhibition (Table 1, 2, 3 ,4) and Figure (2,3,4,5).

Table (1): Antimicrobial activity of Cinnamon oil

Tested Bacteria		Inhibition zone (mm)*
Normal Flora (Benefit Bacteria)		
Gram-Positive	*Lactobacillus bulgaricus*	0.0
	Streptococcus thermophilus	0.0
Pathogenic Bacteria		
Gram-Negative	*Pseudomonas aeruginosa.*	6.8
	E. coli	6.7

*Diameter of paper disc is 6 mm.
*Negative control (sterile water).
* Positive control Penicilin, Amoxicillin, Azithromycine, Tetracycline.

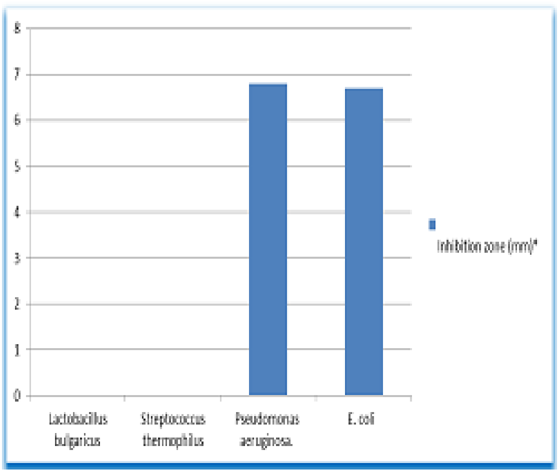

Figure (2): Antimicrobial activity of Cinnamon oil

Table (2): Antimicrobial activity of Clove oil

Tested Bacteria		Inhibition zone (mm)*
Normal Flora (Benefit Bacteria)		
Gram-Positive	*Lactobacillus bulgaricus*	0.0
	Streptococcus thermophilus	0.0
Pathogenic Bacteria		
Gram-Negative	*Pseudomonas aeruginosa.*	2.1
	E. coli	2.2

*Diameter of paper disc is 6 mm.
*Negative control (sterile water).
* Positive control Peniciliin, Amoxicillin, Azithromycine, Tetracycline

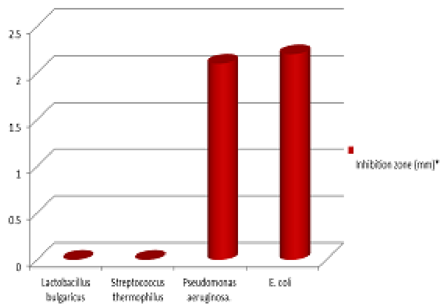

Figure (3): Antimicrobial activity of Clove oil

Table (3): Antimicrobial activity of Mint oil

Tested Bacteria		Inhibition zone (mm)*
Normal Flora (Benefit Bacteria)		
Gram-Positive	*Lactobacillus bulgaricus*	0.0
	Streptococcus thermophilus	0.0
Pathogenic Bacteria		
Gram-Negative	*Pseudomonas aeruginosa.*	3.0
	E. coli	5.5

*Diameter of paper disc is 6 mm.
*Negative control (sterile water).

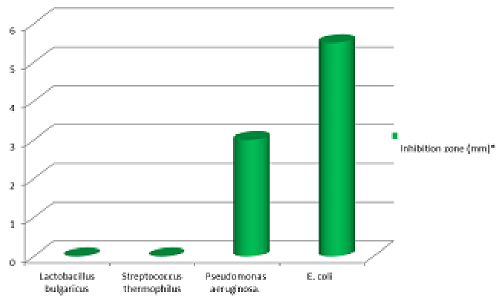

Figure (4): Antimicrobial activity of Mint oil

Table (4): Antimicrobial activity of Cumin oil

Tested Bacteria		Inhibition zone (mm)*
Normal Flora (Benefit Bacteria)		
Gram-Positive	*Lactobacillus bulgaricus*	0.0
	Streptococcus thermophilus	0.0
Pathogenic Bacteria		
Gram-Negative	*Pseudomonas aeruginosa.*	0.0
	E. coli	0.0

*Diameter of paper disc is 6 mm.
*Negative control (sterile water).

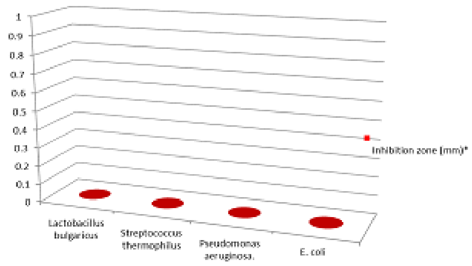

Figure (5): Antimicrobial activity of Cumin oil

Table (5): Antimicrobial activity of Thyme oil

Tested Bacteria		Inhibition zone (mm)*
Normal Flora (Benefit Bacteria)		
Gram-Positive	*Lactobacillus bulgaricus*	0.0
	Streptococcus thermophilus	0.0
Pathogenic Bacteria		
Gram-Negative	*Pseudomonas aeruginosa.*	12.8
	E. coli	5.8

*Diameter of paper disc is 6 mm.
*Negative control (sterile water).

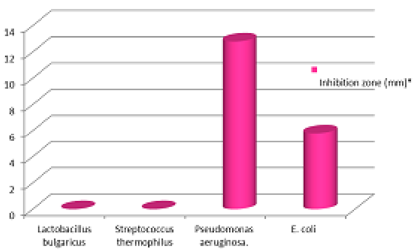

Figure (6): Antimicrobial activity of Thyme oil

Based on the diameter of inhibition zone, Thyme oil had the strongest antibacterial activity against all tested bacteria except *E. coli*. and *Pseudomonas aeruginosa*. These results agree with those obtained by [15,16]. Table (6) and Fig (7). The concentration of active components (carvacrol, thymol, cumin and y-terpinene) in Essential oil varied widely depending on the species of the plant. The inhibitory effects of thyme oil against bacteria are due to the interaction with bacterial cell membrane. These results agree with those obtained by [17,18].

Table (6): Antimicrobial activity of essential oil against pathogenic bacteria.

Essential oils	Pathogenic Bacteria	
	Pseudomonas aeruginosa.	*E. coli*
Cinnamon oils	6.8	6.7
Clove oils	2.1	2.2
Mint oils	3.0	5.5
Black cumin oils	0.0	0.0
Black Thyme oils	12.8	5.8

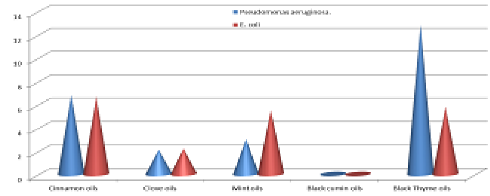

Figure (7): Antimicrobial activity of essential oil against pathogenic bacteria.

Essential oils used in this study indicate that the absence of any antibacterial effect against *Lactobacillus bulgaricus* and *Streptococcus thermophiles.* Table (7) and Fig (8). The inhibitory effects of thyme oil against bacteria are due to the interaction with bacterial cell membrane. Fig (9). These results agree with [19, 20].

Table (7): Antimicrobial activity of essential oil against benefit bacteria

Essential oils	Beneficial Bacteria	
	Lactobacillus bulgaricus	*Streptococcus thermophilus*
Cinnamon oils	*0.0*	*0.0*
Clove oils	*0.0*	*0.0*
Mint oils	*0.0*	*0.0*
Black cumin oils	*0.0*	*0.0*
Black Thyme oils	*0.0*	*0.0*

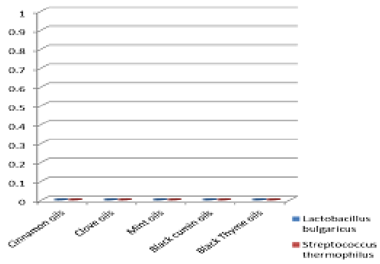

Figure (8): Antimicrobial activity of essential oil against pathogenic bacteria.

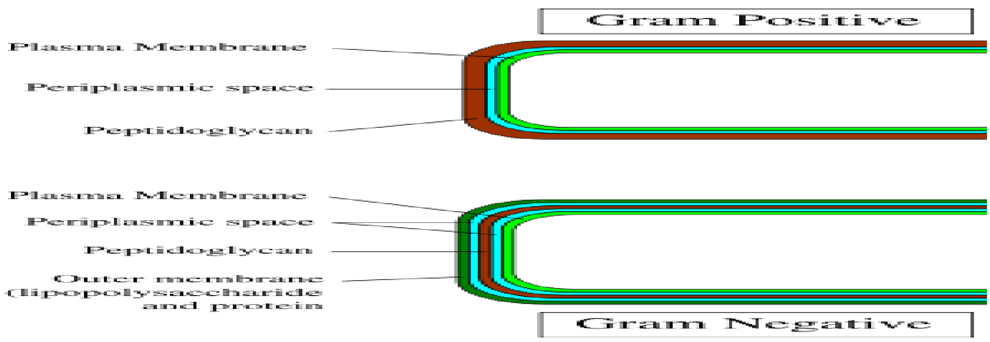

Figure (9): Cell wall in gram positive and gram negative bacteria.

IV. Conclusion

This study has shown that the most of the tested essential oils gave an antibacterial effects on gram negative bacteria except Cumin oil. This study indicates that the absence of antibacterial effect against beneficial bacteria and the most potent oils were Thyme oil.

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Corresponding author: E-mail: academic4@yahoo.com.

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