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Antibacterial Activity of Some Essential Oils Extracts Against Pathogenic Bacteria

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ABSTRACT: Essential oils and many other plant extracts have been researched as possible therapies for the prevention of some infectious diseases and their future uses. The purpose of this study is to investigate the negative and positive bacterial anti-bacterial activity of cinnamon, thyme and clove essential oil strains against gram negative and positive bacteria. The disk diffusion method was used to determine the antimicrobial effects of these essential oils in vitro. Minimum inhibitory level (MIC) was determined by the use of serial broth dilution procedure, and the minimum MBCs was defined as the lowest sample concentration, contributing 99.9% to the killing of the initial inoculum. Antibacterial activity was graded as either bactericidal or bacteriostatic according to the proportion: MB/MIC. The strong antimicrobial activity of cinnamon is 25 mm against all microbial measurements. The thyme and clove essential oils showed the antibacterial role of inhibition regions of 18-21 and 14-16 mm. This essential oil was resistant to both gram-positive and gram-negative bacteria. Basic cinnamon oil has the most spectacular bactericidal activity, according to MICs and MBC data. Essential oil may also be used as a natural antibiotic to cure a number of infectious diseases caused by bacteria and as a preservative.

INTRODUCTION

In culture, medical oils have been used. The medical treatments offered by those who market therapeutic oils range from skin medicines to cancer cures and mostly based primarily on the tradition of the use of essential oils for this purpose. Indeed, over recent decades the interest in essential oil has re-emerged with aromatherapy, a branch of alternative medicine, which claims to cure essential oils and others. The emergence of microorganisms' resistance to traditional chemicals and medicines has encouraged scientists to explore alternative sources of biocidal substances with wide range activities (Abad *et al*, 2007). It has been known for a long time that the antimicrobial function of essential oils has been extensively studied in in vitro for different pathogens and fungi (Kalemba and Kunicka, 2003). In cancer therapy, certain oils have been used (Sylvestre *et al*, 2006). In food preservation (Faid *et al*, 1995), aromatherapy (Buttner *et al*, 1996) and the scent industries (Van de Braak and Leijten, 1999) many other oils have been used. The principal constituents of essential oils, including sugars, phenols, alcohols, ethers, aldehydes and ketones, take care of the biochemical function of aromatic, medicinal and fragrant plants. Although, it is poorly known the mechanism by which essential oils are actively involved with the antibiotic industry, the main purpose tends to be bacterial cell membrane (Di Pasqua *et al*, 2007). Essential oils like cinnamon, thyme and clove are commonly used in different parts of the world by people for various purposes (Berche *et al*, 1991). The aim of this study was to see how these essential oils performed *in vitro* against four-gram negative bacteria and two-gram positive bacteria.

MATERIALS AND METHODS

Hot extraction method of Clevenger apparatus

In 1928 Joseph Franklin Clevenger, named the Clevenger device. Various models are available. A special glassware element, as is seen above the oval bottom flake is the most common element. The flask holds the heated water and the plant to be extracted, which are inconsistently broad. The vapor is streaming into a little office on the right with a condenser (Fig. 1). The water floats with oil and is finally returned to the warm bottle through the diagonal tubing. After a few hours of extraction, the oil content in the desk can be directly assessed (Sabahat and Perween, 2008). In 400 ml distilled water, the plant weight was approximately 200 grams. Around 2-3 ml oils were collected of three plants after six hours.

Figure: 1- Clevenger apparatus.

Test bacteria

Test microorganisms used in this study included gram-positive and negative bacteria screened and isolated from urinary tract infection and wound at Ramadi Hospital, Iraq.

Culture preparation

Re-cultivation of nutrient agar activated bacteria, which were then incubated for 24 hours. When sterilized tubes containing the heart infusion are placed in the incubator for 24-72 hours at 37^0C. It was 37°C. Spectrophotometers were used to quantify the bacteria, and 27 percent of them had a wave length of 580 nanometers, while 100 percent of the nutrient broth used in the preparation of these bacteria had a wave length of 580 nanometers (Paul *et al*, 2013).

Essential oils activity test

A well diffusion method was used to screen for antibacterial activity (Khashan, 2018). 0. 1 ml of normal bacterial inoculum was seeded onto Mueller-Hinton agar plates. A sterile glass spreader was used to fairly scatter the inoculums around the dish. In the incubator, the plates were able to dry for 20 minutes at 37^0C. Standard wells were cut on the surface of the plates using a regular crack edge of 9 mm scale and 0.1 ml of each oils concentration was presented as a monitor in a well of distal water. The disks were inoculated and incubated at 37^0C for 24 hours, with the inhibited diameter area measured to the closest distance (mm).

RESULTS AND DISCUSSION

Table 1 summarizes the antimicrobial function of three major oils for bacterial organisms. These findings revealed that cinnamon oil displays higher antibacterial activity against all measured microorganisms, with the highest 25 mm inhibition zones in both *E. coli* and *S. aureus* and lowest inhibition effect in both *E. coli* and *S. aureus* found in clove (14-16 mm).

Clove essential oil produced the lowest inhibitory zone in all bacteria, ranging from (14 to 16) mm, there for cinnamon gives a highest zone of inhibitory effect (25 mm) against *E. coli* and *S. aureus*, while the same clove produced an inhibition zone in bacteria ranging from (18 to 21) mm. As seen in Fig. 2, the minimum inhibition zone was 14 mm against *E. coli* and the maximum inhibition zone was 25 mm against *S. aureus*.

In this study, cinnamon oil was found to have MIC and MBC values of 3–6 mg/mL and 5–10 mg/mL, respectively. The lower MIC was detected in *Escherichia coli*, while the higher MIC was found in *Staphylococcus aureus*. In contrast, *E. coli* was found to have the lowest MBC value, while *Staphylococcus aureus* had the highest MBC value. Clove essential oil had a MIC concentration of 3-6 mg/ml. MBC and 5-10 mg/ml. Thyme oil had a MIC of 3-5 mg/mL, while MBC had a MIC of 10 mg/mL. *Staphylococcus aureus* had the highest MIC rating, while *Escherichia coli* had the lowest. MBC for thyme essential oil was observed to be lower in *Escherichia coli*, but higher in *Staphylococcus aureus*. The MBC/MIC (Table 2) ratio showed that essential oils made from thyme and clove had a bactericidal effect against the bacteria examined. Both gram-positive and gram-negative bacteria have been shown to be killed by cinnamon essential oil.

Table 1: Antimicrobial activity oils (10 microliter/disc.) against bacterial strains tested by disc diffusion, inhibition zones in millimeters.

Bacteria	Clove	Thyme	Cinnamon
E. coli	14	18	25
S. aureus	16	21	25

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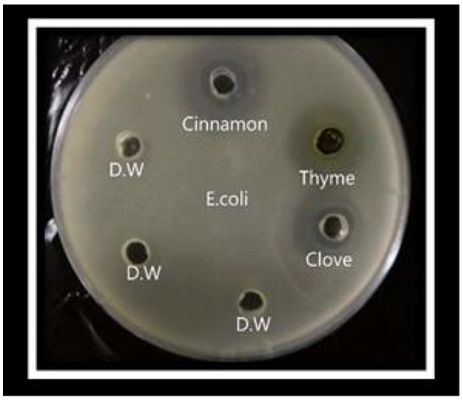

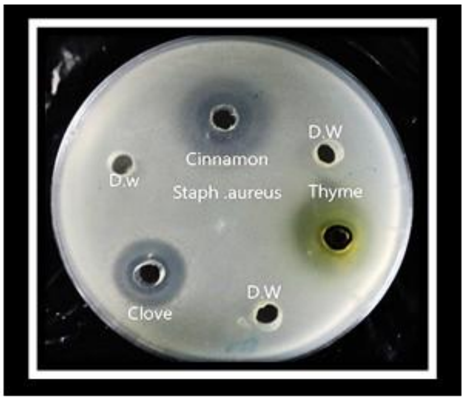

Figure:2- Inhibition zone of essential oils, cinnamon, thyme and clove against *E.coli* and *Staph. aureus.*

Table 2: MIC and MBC values of selected oils against bacterial strains tested.

Bacteria	Clove			Thyme			Cinnamon		
	MIC	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**
Escherichia coli	3.5	6	1.7	5	10	2	36	2	
S. aureus	5	10	2	3	10	3.3	5	10	2

Cinnamon, thyme and clove essential oils were shown to have antibacterial action against microbial strains in an *in vitro* study, but their efficacy differed. There have been several studies on the antimicrobial action of plant compounds against a variety of bacteria, including bacterial pathogens (Friedman *et al*, 2002; Tassou *et al*, 1995). Both gram-positive and gram-negative bacteria are immune to this essential oil. With inhibition zones of 14–16 mm and 18–21 mm, respectively, thyme and clove essential oils have good antibacterial efficacy, as seen in Fig. 2. These findings are similar to those of Lopez *et al* (2005). Clove oil has been shown to protect against Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Listeria monocytogenes*) and Gram-negative bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Listeria monocytogenes*) bacteria present in food (Lopez *et al*, 2005) (*E. coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis* and *P. aeruginosa*). Clove oil had the greatest effectiveness against *V. cholerae*, according to Sabahat and Perween (2008), with a mean inhibition zone diameter of 23.75 mm.. Thyme essential oil has a higher antibacterial efficacy against *E. coli*, according to Deise *et al* (2014). The antimicrobial properties of thyme essential oil and thymol have been studied in several studies. Thyme extract and essential oil had good antibacterial effectiveness against *E. coli*, according to Ivanovic *et al* (2012). Important thyme oil has also been shown to have antimicrobial action against *E. coli* and other nutrient bacteria (Silva *et al*, 2013). Cinnamon essential oil has the most antibacterial activity against any of the bacteria tested. The findings of this study corroborate that of Seenivasan *et al* (2006), who found that cinnamon oil had potent antibacterial properties against a variety of bacteriaFor the three oils (thyme, clove and cinnamon), the mean inhibitory and bactericidal concentrations (MIC, MBC) were 3–6 and 2.5–20, respectively. Cinnamon essential oil has antibacterial effects against Gram-positive and Gram-negative bacteria, with MIC and MBC values of 2.5 to 5 and 5 to 10 mg/ml, respectively, against gram-positive and gram-negative bacteria. Smith-Palmer *et al* (2001) found a reduction in *Listeria monocytogens* from 0.6 log CFU/ g to less than 0.1 log CFU/g in medium and high fat cheese with low concentrations of clove essential oil. Cinnamon, clove and rosemary oils, on the other hand, have been shown to have high and persistent inhibitory action against a variety of pathogens in many studies (Matan *et al*, 2006). Many experiments have shown that essential oil properties are responsible for the behavior of oils, and that their hydrophobic nature helps lipids to be isolated, destroyed, and made more permeable by the bacterial cell membrane's cell structure (Knobloch *et al*, 1986; Sikkma *et al*, 1994). Significant bacterial cell leakage or the escape of vital molecules and ions will lead to death (Denyer and Hugo, 1991). According to this report, cinnamon oil has the most antibacterial properties. This essential oil can be used to cure a variety of infectious diseases caused by pathogen germs as a natural antibiotic. The current study, in conjunction with previous study, is intended to validate cinnamon's antibacterial properties.

Conflict of interests: There is no conflict of interest.

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