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Antibacterial efficacy of Thymol, Carvacrol, Eugenol and Menthol as alternative agents to control the growth of nosocomial infection-bacteria

Yassine El Atki, Imane Aouam, Fatima El kamari, Amal taroq, Abdelkader Gourch, Badiaa Lyoussi, Abdelfattah Abdellaoui

Laboratory of Physiology Pharmacology and Environmental Health, Department of Biology, Faculty of Sciences Dhar Mehraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco.

Abstract

Resistance of infection nosocomial-bacteria to common antibiotics has been developed in different parts of the world and continues to increase. It is important to investigate the novel and efficient antibacterial agents, among which, the major compounds of essential oils would be suitable sources. In the current study, we evaluated the antibacterial activity of thymol, carvacrol, eugenol and menthol against four bacterial strains responsible for nosocomial infections such as *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Staphylococcus aureus*. This activity was assessed using disc diffusion method and micro-dilution assay for determinate minimum inhibitory concentration (MIC).

The results showed that thymol, carvacrol and eugenol expressed a significant antibacterial activity against the four strains studied. Thymol showed the high antibacterial activity against *S.aureus* and *E. coli* with MIC value of 0.35 mg/ml. Menthol demonstrated a low activity against all tested bacterial with a MIC value greater than 6 mg/ml. These compounds, especially thymol and carvacrol, can be used as antibacterial agents for the treatment of various infectious diseases caused by these germs, which have developed resistance to antibiotics in Centre Hospital University of Fez, Morocco.

Keywords: Antibacterial effect; major compounds; nosocomial infections.

INTRODUCTION

Nosocomial infections are known as a serious threat to global health in the 21st century. and they are characterized by high morbidity and mortality rates [1]. In 2007, About 1.4 million people suffer from nosocomial infections [2]. In Morocco, The incidence of nosocomial infections in the reanimation units is high and dominated bacteria that are increasingly resistant to antibiotics [3] . The solution to this problem is therefore crucial and requires the search of new alternatives. Essential oils (EOs) are aromatic complex mixtures of volatile compounds extracted from different parts of plants such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots [4]. several studies have shown significant antibacterial activity of essential oils (EOs) from some medicinal and aromatic plants against resistant microbial strains [5–7] . It reported that the antibacterial effect of EOs is related to the presence of phenolic compounds, such as thymol, carvacrol, and eugenol, which are recognized as the main components of some EOs [8].

Thymol and carvacrol are mainly present in the essential oils of thyme and origano [9,10], many studies showed their antimicrobial properties against both Gram-positive and Gram-negative bacteria species [10-12]. Eugenol is a major component (approximately 87%) of leaves and buds from clove [7]. This component is largely used perfumes and in mouthwashes as dental analgesic and has been well recognized, for its antimicrobial activities [13]. Menthol is a terpenoid and the active principle of essential oils from the mentha species, such as peppermint and horse mint [14]. 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 thymol, carvacrol, eugenol, and menthol against four nosocomial infection-bacteria.

MATERIALS AND METHODS

Bacterial Strains

In this study the antibacterial activity of thymol, carvacrol, eugenol, and menthol was tested against, Gram-positive *Staphylococcus aureus* (*S. aureus)* and Gram-negative bacteria included Escherichia *coli* (*E. coli), Klebsiella pneumoniae (K. pneumoniae)* and *Pseudomonas aeruginosa (P. aeruginosa)*. All strains tested were isolated in a hospital environment from clinical patients in reanimation service (CHU Fez, Morocco). The inoculum suspension was obtained by taking colonies from 24 H cultures. The colonies were suspended in sterile 0.9% aqueous solution of NaCl and shacked for 20 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (10^8 CFU/ml) [15].

Agar disc diffusion Assay

The agar disc diffusion assay was determined in triplicate according to the experiment described by Furtado and Medeiros (1980) [16]. The suspensions of microorganisms (1–5 10^8 CFU/ml) were flood inoculated onto the surface of Mueller Hinton (MH) agar plates. Sterile 6 mm diameter filter discs (Whatman paper N° 3) were impregnated with 10µg/disc of the compound and were put on to the surface of the inoculated Mueller Hinton agar. The plates were incubated at 37°C for 18 h. Antibacterial effect was evaluated by measuring the inhibition zones against the tested bacterial strains. The standard drugs for comparison were the antibiogram discs of Imipenem (IMP)

Vancomycyn (VA), Cefaclor (CEC), Nifrofurantoin (F), Kanamycin (K).

Determination of the Minimum Inhibitory Concentration (MIC).

The minimum inhibitory concentration (MIC) was performed using a microdilution assay in 96-well plates according to the experiment of the *National Committee for Clinical Laboratory Standards* (NCCLS, 1999)[17] with some modifications; the different concentrations of compounds are prepared in a suspension containing 0.2% agar in sterile distillated water in order to disperse the compounds without adding solvent or detergent [18]. They are carried out by successive dilutions 1/2 ranging from 45 to 0.351 mg/ml. The concentrations obtained in the well were between 11.25 and 0. 087 mg/ml. Bacterial suspensions were prepared in the same manner described previously and diluted in MH broth and plated in 96 well plates at a density of 1-5 × 10^6 CFU/ml. Compounds were added at different concentrations at the corresponding wells to determine MIC values. Finally the plates were incubated at 37°C for 18-24 h, bacterial growth was visually by adding to each well 20μl of 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution (1%), with additional incubation for 1 h. MIC was defined as the lowest concentration that does not produce a red color [15].

RESULTS AND DISCUSSIONS

In this work, the antibacterial activity of the thymol, carvacrol, eugenol and menthol has been evaluated in vitro against four bacterial species responsible for nosocomial infections contracted at the University Hospital Center of Fez, Morocco. Several publications from our laboratory have previously reported the antibacterial activity of some essential oils against bacterial strains under consideration in the present study [6,7,10,12,19]. To the best of our knowledge, there are no available data about the antibacterial effect of some major compounds from EOs (thymol, carvacrol, eugenol and menthol) against nosocomial infection bacteria. Table 1 summarized the inhibition zone diameter of these compounds, thymol, carvacrol and eugenol showed a wide antibacterial spectrum, against tested strains with the inhibition zone diameters varying from 12 to 34 mm. Moreover, the thymol had the highest inhibitory activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae* with inhibition diameters values of 34.5 , 28, 17 and 22 mm respectively. Interestingly, these diameters were sometimes higher than those obtained with standard antibiotics used as controls. The present data indicated that the *E. coli* was the most sensitive of the strains tested to the components. However, all compounds, especially menthol, showed a low activity against *P. aeruginosa*. Table 2 summarized the MIC values of these compounds against the tested strains. Thymol exhibited a significant antimicrobial activity against *E. coli* and *S. aureus* with the same MIC value of 0.351 mg/ml. Carvacrol had the same MIC against *E. coli*, *S. aureus* and *K. pneumoniae* (0.703 mg/ml). Thymol and carvacrol possess the same MIC and present a low activity against *P. aeruginosa*, which was only inhibited at a concentration of 1.06 mg/ml. The antibacterial effect of eugenol was higher than that of menthol. However, both eugenol and menthol showed a lower antibacterial effect against all tested strains (Table 2). Otherwise, Didry *et al* tested the antimicrobial activity of thymol, carvacrol, cinnamaldehyde and eugenol on seven oral bacteria. These components showed an inhibitory activity against all tested microorganisms [20]. Abbaszadeh *et al* founded that thymol, carvacrol, eugenol and menthol are a good alternative agents to control the growth of food-fungi [21]. Another study demonstrated antibacterial effect of eugenol, carvacrol, and thymol against *Salmonella Enteritidis* and *Campylobacter jejuni* in chicken cecal [22]. In 2015 Falsafi *et al*., evaluated the antibacterial activity of *Satureja bachtiarica* Bunge essential oil and its constituents against ten *helicopter pylori* clinical isolates. The results showed that thymol antibacterial activity was lower than those of carvacrol [23]. In order to examine the antimicrobial effect against foodborne pathogens (*E. coli* O157:H7, *S. thyphimurium*and *L. monocytogenes)*, recently, Moon and Rhee (2016) combined soy sauce with carvacrol, thymol, eugenol, trans-cinnamaldehyde, β-resorcylic acid and vanillin, The authors concluded that thymol and carvacrol inhibited all the tested bacteria and acted in a synergistic interaction with soy sauce to increase the antimicrobial effect [24]. Karapmar, *et al* (1987) evaluated the antibacterial property of thymol, eugenol, menthol and anethole against foodborne bacteria (*Salmonella typhimurium, Staphylococcus aureus and Vibrio parahaemolyticus*), the results showed that thymol and eugenol were more effective than anethole and menthol [25]. A study conducted by Pemmaraju *et al*, investigated the activity of thymol, eugenol and menthol against *C. albicans* MTCC 227. Thymol and eugenol showed antimicrobial effect at a concentration of 0.12 %, while menthol showed it at a concentration of 0.25% [26]. On the other hand, Gram-negative bacteria were more resistant than Gram-positive thanks 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 [27,28]. 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 Carvacrol have a hydroxyl group, which play a major role in their antibacterial activities [29]. They able to alter the cell outer membrane [30] and combine with the charged groups of membrane, via increasing its permeability [31]. In addition, carvacrol had ATPase inhibitory propriety which causes dissipation of the motive force of the proton, and can subsequently inhibit other enzymes [32]. Eugenol, by its hydrophobic structure, is able to penetrate lipopoly-saccharides of Gram-negative bacteria outer membrane, insert into phospholipid bilayer and alter the structure and permeability of cell membrane.

Table 1: Inhibition zone diameter (mm) of thymol, carvacrol, eugenol and menthol

Bacterial strains		*E. coli*	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*
Major compounds	Thymol	34.5 ± 1	28 ± 0.6	17± 0.5	22 ± 0.3
	Carvacrol	27 ± 1	24 ± 0.4	15± 0.3	21 ± 1
	Eugenol	15± 0.0	15 ± 0.1	12± 0.3	16 ± 0.4
	Menthol	8 ± 0.5	8.5 ± 0.1	NI	8 ± 0.2
Antibiotics	F	19	20	20	22
	CE	NI	14	NT	NI
	IMP	28	39	12	25
	K	17	17	NT	24
	VA	NI	14	13	NI

Inhibition zone includes diameter of disk (6 mm); NI: No inhibition; NT: Not tested; IMP: Imipenem; VA: Vancomycyn; CEF: Cefaclor; F: Nifrofurantoin; K: Kanamycin.

Table 2 Minimal inhibitory concentration (mg/ml) of thymol, carvacrol, eugenol and menthol

Bacterial species	*E. coli*	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*
Thymol	0.351	0.351	1.406	0.703
Carvacrol	0.703	0.703	1.406	0.703
Eugenol	5.625	5.625	>6	5.625
Menthol	>6	>6	NT	>6

NT: Not tested

Moreover, the hydroxyl group of eugenol binds to membrane proteins, affects the membrane features and disorders the of cytoplasmic membrane function [33]. In addition, Alteration of membrane structure and function may make macromolecules easy to transport through membrane. Therefore, the ability of permeabilizing cell membrane makes thymol, carvacrol and eugenol a potential synergistic agents against antibiotic resistance bacteria, because they can be able to facilitate the absorption of antibiotics.

CONCLUSION

The objective of this work was to evaluate the antibacterial activity of thymol, carvacrol, eugenol and menthol against nosocomial infection-bacteria. Thymol, carvacrol and eugenol showed an important antibacterial activity against all tested bacteria. The diameters of the inhibition zones and minimal inhibitory concentration varied between samples and between bacterial strains. Thymol and carvacrol gave significant results expressed by the lowest MIC. Therefore, these compounds, can be used as alternative agents for the treatment of various infectious diseases caused by these germs, which have developed resistance to antibiotics in Centre Hospital University of Fez Morocco.

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