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Trachyspermum copticum essential oil: an effective herbal and natural antimicrobial agent against human skin pathogenic bacteria

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ABSTRACT: The toxicity, negative side effects, and antimicrobial resistance of synthetic drugs are noticeable. The essential oil has revived in recent decades with a branch of alternative medicine. The aim of this study was to investigate and compare the antimicrobial effect of essential oil of *Carian copticum* with Body perp disinfectant on human skin. The essential oil was extracted from *Trachchipermum Carian copticum* seeds and also different concentrations of 25, 50, 100, and 200 ppm of essential oils were prepared. To compare the antimicrobial effect of *Carian copticum* essential oil with Body prep disinfectant was performed by a qualitative plate well method and quantitative microdilution method. Initial screening before skin disinfection resulted in the isolation of 29 isolates of *Staphylococcus epidermidis*, 12 isolates of *Staphylococcus saprophyticus*, 2 isolates of *Enterococcus faecalis*, 14 isolates of Micrococcus spp, and 9 isolates of diphtheroid and a total of 66 isolates. According to the microdilution method, the highest MIC belonged to *Staphylococcus epidermidis* with code C5 at 200 ppm and the lowest MIC was related to *Staphylococcus epidermidis* with code K5 and was equal to 25 ppm. The findings of this study showed that the essential oil of *Carian copticum* seed, in comparison with disinfectants (Body prep) that have some side effects, was not only effective in controlling clinical skin bacteria, but also had a good shelf life in competition with Body Prep. The results of the present study showed that the essential seed oil of the *Carian copticum* plant was able to successfully control skin pathogenic bacteria.

Keywords: *Antibacterial activity; Essential oil; Microdilution method; Minimum Inihibitory Concentration (MIC); Minimum Bactericidal Concentration (MBC); Skin Bactria; Trachyspermum copticum.*

INTRODUCTION

There are significant differences between herbal medicines and synthetic medicines, which should be evaluated scientifically and consciously, and the correct culture of prescribing and using them should be established [1]. In recent years, the development of side effects of synthetic drugs, on the one hand, and economic issues on the other hand, has provided the need and importance of research to find drugs with fewer side effects and to prepare for the ability to replace synthetic drugs [2, 3]. Several antiseptic agents are available for preoperative preparation of skin at the incision sites. One of them is the body prep solution that includes chlorhexidine, digluconate, isopropanol, glycerin, citric acid,

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dou/ble distilled water. Chlorhexidine was destroyed a range of Gram positive and Gram negative organisms [4, 5], and binds to the surface layer of the skin, which results in persistent activity [6, 7]. Chlorhexidine has a strong cationic property and exists in three forms: digluconate, acetate and hydrochloride [7-9]. One of the advantages of chlorhexidine is its strong attachment to the position, which causes this substance to be released gradually. The cause of this adhesion is attributed to its cationic property, which binds it to the anionic groups in bacterial glycoproteins, and researchers believe that the mechanism of action of this substance is related to its strong tendency to adhesion and this strong attachment goes back to the bacterial membrane [10, 11]. Glycerin is also used as a skin tissue emollient in this disinfectant to facilitate the opening of skin cells during surgery or injections. Citric acid also acts as an acid with a sour smell and an organic solvent [12]. The antimicrobial properties of organic acids are related to anions activities. Non-ionized organic acids are absorbed by bacteria and after ionization inside the bacterium reduce the pH inside the bacterium. Bacteria increase their activity to counteract this phenomenon, and if the conditions are anaerobic, the process of lowering the pH increases, and eventually the bacterium dies [13, 14]. The anionic part of the acid is trapped inside the bacterium and because it cannot pass through the bacterial wall. It disturbs the anion balance and causes osmotic problems for the cytoplasmic membrane of the bacterium [15]. The use of various disinfectants on the skin, such as saline, alcohols of varying degrees (especially 70% alcohol), and betadine, kills a large number of pathogenic bacteria on the skin surface. Unfortunately, it has also led to some damage, such as the destruction of the skin's natural microflora and adverse effects on the stratum corneum [16]. Essential oils and plant extracts have been reported to be important natural antimicrobial agents. Research on medicinal plants has attracted a lot of worldwide attention [17]. *Carian copticum* with the scientific name of Trachchipermum Capticum is a plant from the Umbelliferae family [18]. Studies have shown that the active ingredients of *Carian copticum* include thymol, semen, α-pinene, dipentene, gamma terpenine, β-pinene, myrcene, carvacrol, carvon, limonene, dilapiol, and oleic acids [19]. The essential oil of this plant is known as juvenile, that has a colorless or brownish appearance and smells like thymol. Its compounds include thymol, caracrol, alpha and β-pinene, trypenene, and paracetamol. In general, most of the components of this essential oil are phenolic compounds, which have both antioxidant and antimicrobial properties. The essential oil of this plant has various applications in the medicine and pharmaceutical industries [20-22]. The antimicrobial and antifungal properties of the essential oil of this plant have significant activities against a variety of microbes. Researchers have shown that *Carian copticum* extracts and essential oils are able to control pathogenic bacteria in humans [23, 24]. The aim of this study was to investigate and compare the antimicrobial effect of *Carian copticum* essential oil with Body perp disinfectant on human skin and to determine the appropriate antibacterial concentrations of the essential oil of this plant in comparison with the antimicrobial power of Body perp disinfectant on the skin of blood donors referred to Najafabad Blood Transfusion Center, Isfahan, Iran.

MATERIALS AND METHODS

Examination of clinical specimens

At the beginning of the experiment, 30 blood donor volunteers who were referred to the Najafabad Blood Transfusion Center were randomly selected for 10 weeks. Each week, 10 volunteers were isolated, before using any kind of skin disinfectant, a culture of the skin of the volunteers was prepared and then, for 30 s and 15 min and finally, 30 min of the essential oil of *Carian copticum* and Body prep on the skin of the 45 volunteers was sprayed and sampling was performed.

The screening and biochemical tests of isolated strains

For each volunteer, 10 tubes contained TSB culture mediums, all culture mediums were examined for turbidity. After 24 hours, identified turbid tubes were cultured on TSA medium and BA agar, incubated for 24 hours at 37°C. The plates were incubated for 24 hours at 37 °C. The bacterial colonies were examined

Table 1. Specifications of standard bacteria used in this study

Bacteria	PTCC	* Risk group	Incubation period (hour)	Incubation temperature (°C)	Culture
Staphlococcus epidermidis	1435	II	24	37	NA
Staphylococcus saprophyticus	1440	II	24	37	NA
Entrococcus faecalis	1394	II	24	37	BA
Entrococcus faecalis	1778	II	24	37	TSA
Entrococcus faecalis	1887	II	24	37	TSA

for macroscopic characterization like (colony color, size or size, and consistency of the colony), and for microscopic evaluation. To detect staphylococci, first Mannitol Salt Agar culture medium used, and so pigment test, growth on Nutrient Agar, Blood Agar medium, catalase, and urease test were examined [25, 26]. Each of the isolates separately were tested for coagulase test, urease, catalase, fermentation of sugars, sensitivity to novobiocin, bacitracin and lysostaphin, pigment production, oxidase, aerobic and anaerobic glucose testing and hemolysis. The standard bacteria were purchased from the Industrial Bacteria and Fungi Collection Center of Iran (PTCC). Specifications and culture conditions of these bacteria are presented in Table (1) according to the information of the website http://ptcc.irost.org.

NA: nutrient agar; **BA**: Blood agar; **TSA**: Trypticase soy agar; the sign * in the table indicates the risk group for the user to work with the bacteria. **Group I**: does not pose any danger to humans and animals. **Group II**: a lower risk to the individual and a limited risk to the community and includes pathogens that can cause disease to humans and animals but are not likely to pose a serious risk to laboratory staff, the community, livestock and the environment. Because exposure to pathogens in the laboratory can pose a serious risk to staff, but in people who become ill due to contamination with this group of bacteria, the existence of effective time and principles of necessary precautions increases the risk of outbreak Reduces. **Group III**: has a high degree of danger that is dangerous to both humans and animals.

Preparation of Carian copticum essential seeds

Carian copticum seeds were purchased from the Agricultural and Natural Resources, Shahid Fazveh Research Station, code 001/017/091, located near Isfahan Province. Then the seeds were washed in several stages with sterile distilled water and completely dried in the oven for 6 days. Novobiocin disks 5µg and bacitracin unit 0.04 made by Padtan Teb Iran were used. To evaluate the antimicrobial activity of the control, erythromycin antibiotic powder was used which was a common antibiotic used to treat skin infections [18, 27].

Preparation of Carian copticum essential oil

First 150 g of *Carian copticum* seeds were washed and placed in sterile balloons with distilled water, up to two-thirds of the balloon volume was filled with water. Then, essential oil was extracted using clevenger apparatus [28]. Several series of dilutions such as 200, 100, 50 and 25 ppm were prepared according to the desired reference. On the other hand, due to the insolubility of essential oils in water solution, it was first necessary to prepare diluent serials from these essential oils with the suitable solvent such as ethanol [29].

The disk diffusion agar method

Evaluation of antimicrobial effect of *Carian copticum* essential oil by qualitative method of plate well. In order to carry out this method, the disk diffusion agar was done. First, the McFarland bacterial suspension (1.5×10^8 CFU/mL) was prepared and cultured on the Müller-Hinton agar medium in four directions by sterile swap. Afterwards, wells with a diameter of 6 mm were created with a distance from each other on the agar medium. In the next step, 80 µL of different concentrations of essential oils were added to each well separately. However, before adding different concentrations of essential oils, positive control (concentration of 0.02 mg/mL of erythromycin antibiotic in a volume of 80 µL) and negative control (80 µL

of physiological serum) were added. Afterwards, 20 µL of molten MHA medium poured. The plates were then incubated for 24 hours in 37 °C and the diameter of non-growth zones was measured. The experiments were performed in three replications [29, 30].

The quantitative microdilution method

Microdilution method was used to measure the Minimum Inhibitory Concentration (MIC) of bacterial growth. In this method, a 96-well ELISA microplate was used. First, 40 bacteria were selected, which included standard and clinically isolated bacteria from the patients' skin. Concentrations of 25, 50, 100 and 200 ppm were prepared from essential oil. In each well, 100 µL of essential oil dilutions were prepared, 95 µL of culture medium and 5 µL of bacterial suspension (1.5×10^8 CFU/mL) were added, so that the final volume of each well became 200 µL. In each test, 100µL of physiological serum was inoculated as a negative control and 100 µL of erythromycin antibiotic at a concentration of 0.02 mg/mL as a positive control. It was immediately placed in the ELISA reader to evaluate the amount of optical density (OD) was read in the 30 seconds, 15 min and 30 min later. The contents of the well, which was considered as MIC and its two higher concentrations were cultured separately to MHA medium, the concentration in which no colony grew after incubation was considered as the Minimum Bactericidal Concentration (MBC) [31, 32].

Statistical analysis

In order to describe the data, frequency distribution tables, statistical graphs and descriptive statistical indices (Mean, Standard Deviation, Minimum and Maximum) were used. In data analysis, the means were compared between several groups of ANOVA and Duncan paired test with soft application. SPSS software version 22 was used. In these tests, ($P\leq0.001$) was considered as a significant difference.

RESULTS

Identification of the bacterial isolates

Preliminary screening before skin disinfection resulted in the isolation of 29 *Staphylococcus epidermidis* isolates, 12 *Staphylococcus saprophyticus* isolates, 2 *Enterococcus faecalis* isolates, 14 Micrococcus spp isolates and 9 diphtheroid isolates, for a total of 66 isolates. The number of clinical D isolates was reduced to 25 specimens after disinfection with Body prep and plant essential oil, including 20 *Staphylococcus epidermidis*, 4 *Staphylococcus saprophyticus* and one isolate *Enterococcus faecalis*. In the present study, 30 volunteers were sampled and each volunteer was assigned 10 tubes containing TSB medium. Therefore, the total number of samples reached 300 samples. Tables (2) to (4) refer to the biochemical properties of the isolates.

The Abundance of isolated bacteria

Before skin disinfection of volunteers, 29 isolates of *Staphylococcus epidermidis*, 12 isolates of *Staphylococcus saprophyticus*, 2 isolates of Enterococcus, 14

Table 2. Biochemical traits to identify species of coagulase-negative bacteria isolated from the skin of volunteers.

	Staphylococcus epidermidis	Staphylococcus saprophyticus
Hypersensitivity to Bacitracin-Pigment	White -R	Lemon -R
Sensitivity to Novobiocin	Susceptible	Resistance
Trehalose Fermentation	-	+
Fermentation of Mannitol	-	-
Urease	+	+
Hemolysis	-	-
Catalase	+	+
Nitrate Reduction	+	-
Anaerobic Glucose	+	-

Table 3. Biochemical traits to identify Enterococcus and micrococcus species isolated from the skin of volunteers.

	Entrococcus faecalis	Micrococcus spp
Coagulase	-	-
Catalase	-	+
Hemolysis	+	
Oxidase	-	+
Motility	-	
Yellow Pigment	-	
Hypersensitivity to Bacitracin	Fermentative Resistance	Oxidative Susceptible
Fermentation of Lactose	+	
Fermentation of Sorbitol	+	
Fermentation of Mannitol	+	

Table 4. Biochemical traits to identify diphtheroid species isolated from the skin of volunteers.

	Diphtheroid spp
Catalase	+
Urease	+
Oxidase	-
Fermentation of Glucose	+
Fermentation of Maltose	+
Fermentation of Mannitol	-
Fermentation of Sucrose	-

Table 5. Percentage of clinical bacteria isolated from the skin of volunteers before skin disinfection of 30 volunteers (Tubes No. 10).

Isolates	Percentage	Abundance
Staphylococcus epidermidis	42%	29
Staphylococcus saprophyticus	%18	12
Enterococcus faecalis	3%	2
Micrococcus	21%	14
Diphtheroid	%13	9
Total	%100	66

isolates of micrococcus and 9 isolates of diphtheroid were isolated and identified. The frequency of isolates along with its percentage was presented in Table (5). Table (6) showed the results of the frequency of clinical bacteria isolated from the skin of volunteers after disinfection with chemical disinfectant body and plant essential oils of *Carian copticum*, which includes 25 isolates. In total, the isolates identified in this part of the study included 20 isolates of *Staphylococcus epidermidis*, 4 isolates of *Staphylococcus saprophyticus* and one isolate of *Enterococcus faecalis*.

The effect of Carian copticum essential oil at different times

The number of isolates and their frequency at different times under the influence of the essential oil of 200ppm are presented in Table (7). The results of Table (7) also showed that there is no significant difference between the different times of the effect of essential oils and body prep ($p < 0.05$) and even over time the number of bacterial isolates isolated from the skin of volunteers increased to some extent.

The effect of Carian copticum essential oil at different concentrations

Tables (8), mean and standard deviation of diameter of growth inhibition transparent zones in millimeters under the influence of different concentrations of *Carian copticum* essential oil on *Staphylococcus saprophyticus* isolates and its standard strain, *Staphylococ-*

Table 6. Percentage of clinical bacteria isolated from the skin of volunteers against body chemical disinfectant and Carian copticum essential oil.

Isolates	Percentage	Abundance
Staphylococcus epidermidis	80%	20
Staphylococcus saprophyticus	%16	4
Enterococcus faecalis	%4	1
Total	%100	25

Table 7. Results of the effect of Carian copticum essential oils and Body prep at different times.

Antimicrobial effect times	Number of isolates (frequency percentage)	30 minutes Number of isolates (frequency percentage)	15 minutes Number of isolates (frequency percentage)	30 seconds Number of isolates (frequency percentage)
Carian copticum	(%24.32) 36	(%23.64) 13	(%.23.07) 12	(%22.50) 9
Body prep	(%22.98) 34	(%25.46) 14	(%25.00) 13	(%.20.00) 8
Total	(%100) 148	(%100) 55	(%100) 52	(%100) 40

cus epidermidis and showed its standard strain and *Enterococcus faecalis* isolates and its standard strains. *Carian copticum* 25 ppm was effective on all isolates and standard strains of *Staphylococcus saprophyticus*

Table 8. Mean and standard deviation of stagnation droplets of Staphylococcus saprophyticus isolates and standard sample (mm) at different concentrations of the Carian copticum essential oil

	Negative control		Body prep		Positive control (Erythromycin)		200 ppm		100 ppm		50 ppm		25 ppm	
Isolates	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
SP1440	0.00	0.00	26.00	6.92	37.33	4.61	36.66	5.77	24.00	3.60	19.33	4.04	16.33	4.04
SPL2	0.00	0.00	32.66	2.08	19.33	1.15	46.33	1.52	25.66	4.04	20.00	.001	15.66	1.15
SPK5	0.00	0.00	28.66	1.15	31.00	1.73	38.66	1.15	28.00	2.00	21.33	1.15	18.33	1.52
SPS5	0.00	0.00	35.33	1.15	34.66	3.05	41.00	1.00	26.00	1.00	27.33	1.15	23.66	1.52
SPU2	0.00	0.00	33.66	1.52	38.66	2.30	28.66	1.15	22.33	1.52	18.66	1.15	16.33	1.52
EN1887	0.00	0.00	41.33	1.15	21.66	2.08	26.04	15.6	30.20	0.57	21.00	2.64	18.00	3.40
EN1394	0.00	0.00	29.00	1.00	19.23	1.15	49.33	1.15	30.66	1.15	21.33	1.52	19.66	1.00
ENY5	0.00	0.00	29.33	1.15	29.00	1.00	48.00	2.00	30.33	0.57	27.33	1.15	22.00	2.00
EN1778	0.00	0.00	40.66	1.15	23.33	1.15	50.33	0.57	36.00	5.29	34.00	2.00	18.66	4.16
SE1435	0.00	0.00	32.33	2.51	30.00	5.00	40.00	1.00	29.66	0.57	23.00	2.64	17.66	2.30
SEC2	0.00	0.00	29.30	1.00	19.33	1.15	40.33	1.52	24.33	0.57	21.33	1.15	19.66	1.52
SEE5	0.00	0.00	29.33	1.15	17.66	2.51	40.66	1.15	28.33	1.52	24.66	0.57	18.66	1.15
SED2	0.00	0.00	19.33	1.15	24.00	1.00	42.00	2.00	32.66	2.51	29.66	1.52	24.00	3.46
SEA3	0.00	0.00	26.66	1.52	22.33	1.52	42.00	2.00	28.00	1.00	26.33	1.52	20.00	2.00
SEB5	0.00	0.00	14.66	0.57	28.66	1.15	49.33	1.15	30.00	1.00	27.00	1.73	21.00	1.00
SED8	0.00	0.00	29.33	1.52	35.00	3.60	40.66	1.15	29.66	1.52	23.66	1.52	21.00	1.00
SEA9	0.00	0.00	29.33	1.15	27.33	2.08	40.66	1.15	20.66	1.15	19.33	1.15	18.00	2.00
SED9	0.00	0.00	29.33	1.15	32.00	2.00	43.33	1.15	27.66	2.51	28.66	1.15	24.00	1.73
SEX5	0.00	0.00	20.66	1.15	29.66	1.52	49.33	1.15	23.66	1.52	20.66	1.15	17.33	2.51
SEA6	0.00	0.00	26.33	1.52	31.00	1.00	30.66	1.15	31.33	2.08	27.33	2.30	21.33	1.15
SEC5	0.00	0.00	20.66	1.15	30.66	3.05	28.66	1.15	22.66	3.05	16.66	2.08	14.33	2.08
SEB6	0.00	0.00	28.33	1.52	32.33	2.51	30.66	1.15	22.33	5.50	17.00	2.64	12.66	3.05
SEB2	0.00	0.00	30.33	0.57	25.33	3.51	40.66	1.15	30.00	2.00	28.00	2.00	25.33	3.05
SEO2	0.00	0.00	19.00	1.00	27.33	2.51	30.66	1.15	28.66	1.15	18.33	1.52	16.33	1.52
SEG5	0.00	0.00	17.33	2.52	30.66	1.15	39.66	1.52	21.33	1.15	18.66	1.15	18.00	2.00
SEA8	0.00	0.00	38.00	2.00	26.66	1.15	40.00	1.00	32.66	3.05	25.66	2.08	23.00	1.73
SEA8	0.00	0.00	38.00	2.00	26.66	1.52	49.00	1.00	32.66	3.05	25.66	2.08	23.00	1.73
SEX6	0.00	0.00	29.66	1.52	30.00	2.00	49.33	1.15	30.66	1.15	25.33	4.16	21.33	2.30
SEV5	0.00	0.00	26.33	1.52	26.66	1.15	48.00	2.00	29.33	1.15	22.00	1.00	19.33	1.15
SEAB5	0.00	0.00	21.00	1.73	29.33	1.15	49.33	1.15	33.33	3.05	20.33	0.57	16.66	1.52

by creating a zone of no growth of maximum 23.66 mm and minimum 16.33 mm. Table (8) also showed that *Carian copticum* essential oil at a concentration of 25 ppm was effective on all isolates and standard strains of *Enterococcus faecalis* by creating a zone of no growth of maximum 23.00 mm and minimum 18.00 mm. Also, the data in below table show that *Carian copticum* essential oil at a concentration of 25 ppm has been effective on all isolates and standard strains of *Staphylococcus epidermidis* by creating a halo of no growth of 25.33 mm and at least 12.66 mm.

SP1440: *Staphylococcus saprophyticus* is standard with code (1440 PTCC). SPL2: Staphylococcus saprophytus isolated from volunteer skin is code L2. SPK5: Staphylococcus saprophyte isolated from volunteer skin is code K5. SPS5: *Staphylococcus saprophyticus* isolated from volunteer skin is code S5. SPU2: Staphylococcus saprophytus isolated from volunteer skin is code U2. EN1887: *Enterococcus faecalis* is standard with code (1887 PTCC). EN1394: *Enterococcus faecalis* is standard with code (1394 PTCC). EN1778: *Enterococcus faecalis* is standard with code (1778

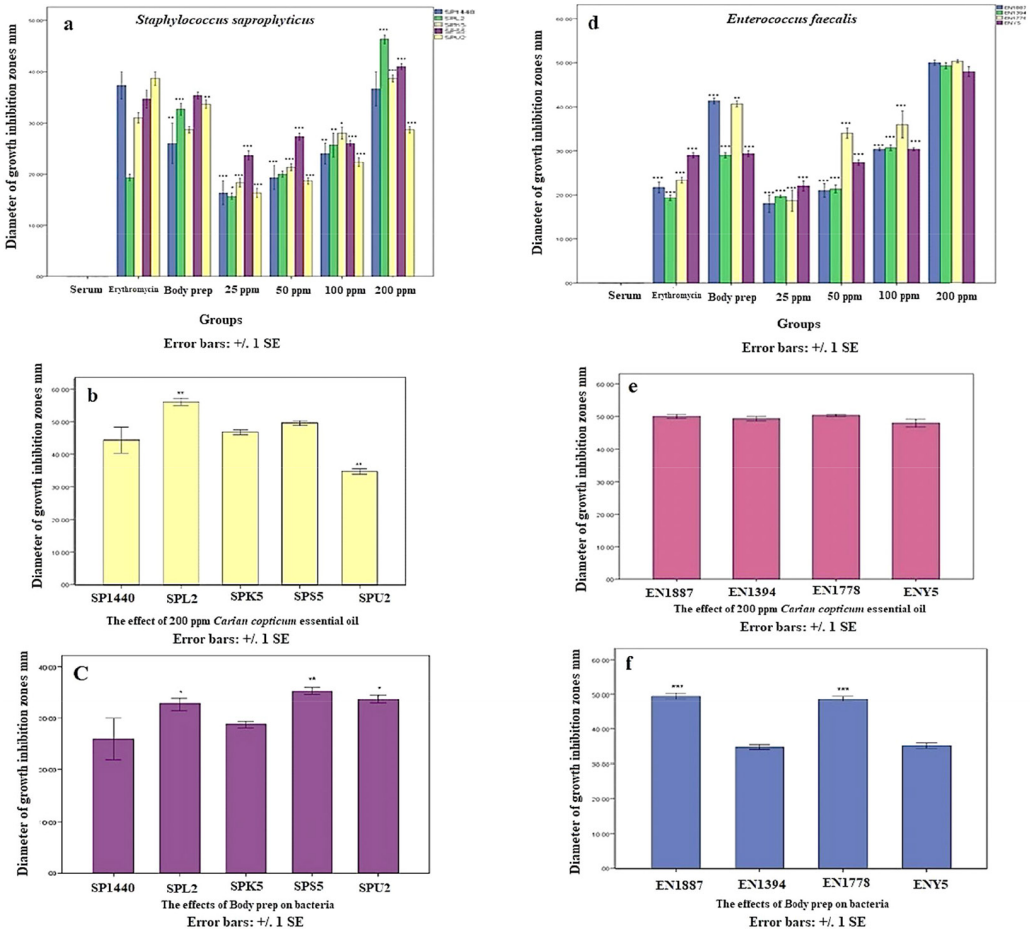

Fig. 1. Comparison of the mean diameter of non-growth halos of Staphylococcus saprophyticus isolates studied in millimeters against different concentrations of Carian copticum essential oil, Body prep and erythromycin antibiotic by plate well method. The sign (*) indicates the difference between the diameter of the growth zone of each bacterium in different groups compared to erythromycin (Fig. 1a); The effect of Carian copticum essential oil with a concentration of 200 ppm on standard and clinical strains of Staphylococcus saprophyticus (Fig. 1b); The mean diameter of growth inhibition halo of Staphylococcus saprophyticus isolates in terms of millimeters in comparison with the standard strain against Body prep, the sign (*) indicates the difference between inhibition of growth by Body prep (Fig. 1c) * P≤0/05, ** P≤0/01. Comparison of mean diameter of non-growth in millimeters of Enterococcus faecalis bacteria against Carian copticum essential oil with different concentrations of Carian copticum essential oil, Body prep and erythromycin antibiotic by plate well method (Fig. 1d); Comparison of the average diameter of growth inhibition halos of standard Enterococcus faecalis bacteria in millimeters at a concentration of 200 ppm (Fig. 1e); The effect of inhibiting the growth of Body Perp on Enterococcus faecalis (P <0.001) (Fig. 1f) * P≤0/05, ** P≤0/01, *** P≤0/001.

PTCC). ENY5: *Enterococcus faecalis* isolated from skin volunteer code Y5. SE1435: *Staphylococcus epidermidis* is standard with the code (1435 PTCC).

The effect of 200 ppm Carian copticum essential oils

Fig. (1) showed a Comparison of the mean diameter of non-growth halos of *Staphylococcus saprophyticus* isolates studied in millimeters against different concentrations of *Carian copticum* essential oil, Body prep and erythromycin antibiotic by plate well method. The statistical results of Fig. 2 was revealed that the effect of *Carian copticum* essential oil with a concentration of 200 ppm on all standard strains of *Enterococcus faecalis* is standard and the only strain isolated from the skin of volunteers did not show a significant difference. Fig. (1) showed a comparison of the average diameter of growth inhibition zones between different bacteria affected by *Carian copticum* essential oil at a concentration of 200 ppm. The highest inhibitory effect of this essential oil was on *Staphylococcus saprophyticus* isolated from volunteer skin with code L2 and the least inhibitory effect of growth was observed on *Staphylococcus saprophyticus* isolated from volunteer skin U2.

Fig. (2) isolates of *Staphylococcus epidermidis*, which are marked in red, showed more growth inhibition against *Carian copticum* essential oil at a concentration of 200 ppm, and isolates of *Staphylococcus epidermidis* with green column less than others affected by *Carian copticum* essential oil with a concentration of 200 ppm. According to Fig. (2), the highest inhibitory effect of *Carian copticum* essential oil with a concentration of 200 ppm was observed on bacterial strains ($P \leq 0.001$). According to this diagram, all *staphylococcus epidermidis* bacteria with standard *Staphylococcus epidermidis* bacteria with code (1435 PTCC) have a significant difference in the diameter of the growth inhibition zone ($P \leq 0.001$).

The MIC and MBC Carian copticum essential oil

Fig. (3) showed the obtained results from MIC and MBC of several concentrations of *Carian copticum* essential oil (in ppm) against bacteria isolated from the skin of Najafabad blood transfusion volunteers and their standard bacteria using microdilution method. The results were interpreted in such a way that the lowest concentration at which the absorption of light at 625 nm reached zero was considered as MIC and the lowest concentration at which the bacterial strain did not form in the solid medium of MHA colony was reported as MBC. For some bacteria the concentrations obtained were the same for MIC and MBC and different for others. According to the results presented, the lowest light absorption to determine the MIC among clinical isolates belonged to *Staphylococcus epidermidis* isolates with code 5 SEK (MIC equal to 25 ppm) and the highest light absorption belonged to *Staphylococcus epidermidis* isolates with codes 5 SEC and 6 SEB (MIC 200 ppm). The lowest and highest MBC values belonged to the bacteria mentioned above with the same values. Also, in standard isolates, the results were as follows: the lowest light absorption to deter-

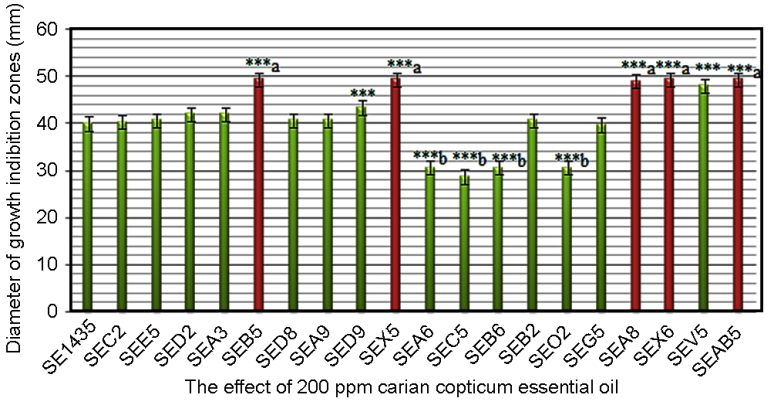

Fig. 3. The effect of 200 ppm Carian copticum essential oil was shown on standard and clinical bacteria of Staphylococcus epidermidis. The letter (a) indicates bacteria that do not differ significantly in growth from Staphylococcus epidermidis (1435 PTCC). The letter (b) indicates bacteria that are significantly different in growth from Staphylococcus epidermidis (1435 PTCC). The sign (***) indicates bacteria that have significantly more growth inhibition (***$p \leq 0.001$) than Staphylococcus epidermidis.

mine the MIC among standard isolates belonged to *Staphylococcus epidermidis* with code SE1435 (MIC equal to 25 ppm) and the highest light absorption belonged to *Staphylococcus saprophyticus* with code SP 1440 (MIC equal to 100 ppm) was obtained. The lowest MBC belonged to the same bacteria mentioned above was obtained from Streptococcus epidermidis with code SE 1435 with the same concentration of 25 ppm. While the highest MBC belonged to *Staphylococcus saprophyticus* strains with SP code 1435, *Enterococcus faecalis* with codes 1778 and EN 1394 and *Staphylococcus saprophyticus* with code SP 1440 with the same concentration of 200 ppm.

DISCUSSION

According to the plate well method, the highest diameter of 33.35 mm growth inhibition zone was related to the essential oil of *Carian copticum* seed on the standard bacterium *Enterococcus faecalis* with code 1778. The K5 code was 25 ppm. While in the standard bacteria studied, the highest MIC belonged to *Staphylococcus saprophyticus* with code 1440 and equal to 100 ppm and the lowest MIC belonged to *Staphylococcus epidermidis* with code 1435 and MIC equal to 25 ppm. The highest MBC belonged to the standard strain of *Staphylococcus saprophyticus* with code 1435. Research by various researchers has shown that the use of herbs in traditional and non-traditional medicine dates back to at least 5,000 years ago. In the present study, micrococcus and diphtheroid isolates were completely eliminated due to the use of body prep and plant essential oil. Researchers have shown that micrococci are also opportunistic pathogens that cause serious infections in patients with weakened immune systems, such as those with AIDS, diabetes, transplants, cancer, and certain patients [33].

In this study, it was recommended that nurses and surgeons before any surgery, it is better to disinfect their hands with decospit instead of ethanol-betadine combination method. This study, considering that no extract or essential oil with two substances. The chemical disinfectant Decocet and ethanol-betadine were

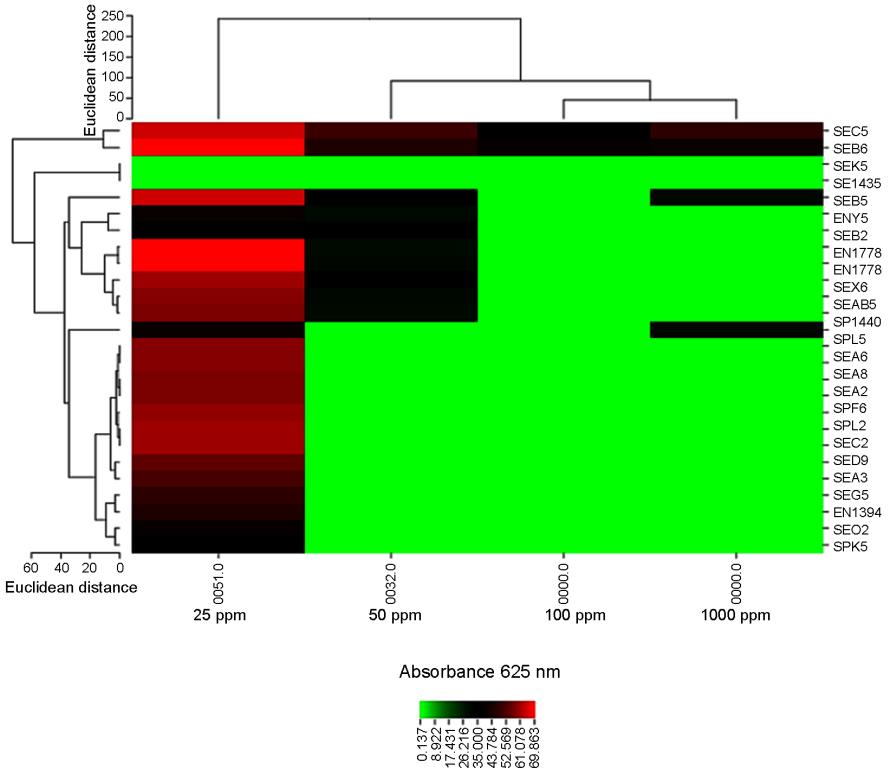

Fig. 3. Heat-map results of different concentrations of Carian copticum essential oil (ppm) on the isolated bacteria by microdilution method (by CIMminer one matrix ncbi nih).

not comparable to the present study, but the idea that the shelf life of these two chemicals after 30 minutes was more effective, contrary to the results of this study according to the table 8 because according to this table in this study, the frequency of bacterial isolates under the influence of two plant essential oils of peppermint and apricots and the chemical Body pard has increased after 30 minutes. The results of this study showed that *Carian copticum* essential oil alone or in combination with antibiotics can be used to treat infections caused by Staphylococcus aureus. This essential oil could enhance the activity of some antibiotics, which made it possible to use this essential oil, especially for the treatment of drug resistance or reducing the dose or toxicity of some drugs [34, 35]. This study was consistent with the present study on the use of *Carian copticum* essential oil on *Enterococcus faecalis*, but in the present study, the best and most effective concentration of *Carian copticum* essential oil was 200 ppm, which prevented bacterial growth better than the antibiotics erythromycin and body prep. On the other hand, according to our study, the highest MIC for the growth of hyperbolic bacteria against *Carian copticum* essential oil in this study was 200 ppm, while in the above study it was 120 ppm. In one study, the synergistic effect of peppermint and *Carian copticum* essential oils with ciprofloxacin, vancomycin and gentamicin on gram-negative and gram-positive bacteria was studied. In this experimental study, the synergistic effect of these two essential oils with antibiotics on Staphylococcus aureus (25923 ATCC), *Enterococcus faecalis* (14990 ATCC) and Listeria monocytogenes (7644 PTCC) was evaluated by broth microdilution method. In this study, *Carian copticum* essential oil at a concentration of 300 ppm inhibited the growth of standard Staphylococcus aureus and in combination with the antibiotic vancomycin, its MIC was 50 to 120 ppm. In addition, its MBC was 240 ppm, indicating a strong effect of this essential oil along with vancomycin against the growth of Staphylococcus aureus. The results of this study showed that *Carian copticum* essential oil alone or in combination with antibiotics can be used to treat infections caused by Staphylococcus aureus. This essential oil could enhance the activity of some antibiotics, which made it possible to use this essential oil, especially for the treatment of drug resistance or reducing the dose or toxicity of some drugs [36].

CONCUSIONS

The use of *Carian copticum* essential oil on *Enterococcus faecalis*, but in the present study, the best and most effective concentration of *Carian copticum* essential oil was 200 ppm, which prevented bacterial growth better than the antibiotics erythromycin and body prep. On the other hand, the highest MIC for the growth of hyperbolic bacteria against *Carian copticum* essential oil in this study was 200 ppm. The results of the present study showed that the essential oil of the *Carian copticum* plant was able to successfully control skin bacteria with a higher shelf life than body prep. Therefore, many health and service centers, hospitals, and clinics and blood transfusion organizations are recommended to use this plant essential oil instead of the disinfectant-chemical Body prep.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests.

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