Surgical Hand Antiseptic Potential of Oregano Essential Oil

Oregano Esansiyel Yağlarının Cerrahi El Antiseptik Özelliği

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ABSTRACT

Aim: Although the antimicrobial properties of essential oils have been known for a long time they have not been used for surgical hand antisepsis so far. The aim of this study was to test the surgical hand antiseptic properties of Origanum minutiflorum essential oil.

Methods: Surgical hand antiseptics containing 0, 4, 8, 16, and 32% essential oil were compared with povidone-iodine. Fifteen volunteers were assigned for each antiseptic solution. Fingertip imprints were taken on agar plates before treatment (t_0), just after hand treatment (t_1) and three hours after gloving with sterile powder-free surgical gloves (t_2). The bacterial colony counts and bacterial genus number were evaluated.

Results: No antibacterial effect was seen in 0% oregano essential oil group. The results in 32% essential oil group and povidone-iodine group were similar. Bacterial colony counts reduction in povidone-iodine scrub group was greater than the 16% essential oil group at t_1, but the difference between both groups disappeared at t_2. The reductions of bacterial genus number at t_1 in povidone-iodine scrub group was higher than the 4% essential oil group, but it was similar at t_2.

Conclusion: Oregano essential oil has strong and long-acting antiseptic properties on hand pathogens. Hand treatment with 32% oregano essential oil containing hand antisepsis is equivalent to the hand antisepsis with conventional povidone-iodine scrub. Oregano essential oil containing antiseptic solutions have very strong effects in reducing the bacterial genus number even in low concentrations. Oregano essential oil containing hand antisepsis could be suggested for biodegradable and eco-friendly natural microbicidal activities

Keywords: antisepsis, origanum, fatty acids, essential, povidone-iodine

ÖZET

Amaç: Esansiyel yağlar uzun zamandan beri bilinmesine karşın şimdiye kadar el antisepsisinde kullanılmamıştır. Bizim bu çalışmadaki amacımız Origanum minutiflorum (kekik) esansiyel yağının cerrahi el antisepsi özelliğini araştırmaktır.

Yöntemler: %0, 4, 8, 16 ve 32 oranlarında esansiyel yağ içeren solüsyonlar povidon-iyot ile karşılaştırıldı. Herbir solüsyon için 15 kişi kullanıldı. Ekim, parmak ucu izi şeklinde agar besiyerine antisepsi işleminden once (t_0), hemen sonra (t_1) ve üç saatlik pudrasız steril cerrahi eldiven kullanımından sonra (t_2) yapıldı. Bakteriyel koloni sayıları ve cins sayıları değerlendirildi.

Sonuç: Oregano esansiyel yağlarının el patojenleri üzerine güçlü ve uzun etkili antiseptik özelliği vardır. %32 oranında oregano esansiyel yağ içeren solüsyonun antiseptik özelliği geleneksel olarak kullanılan povidon-iyot scrub ile eşdeğerdir. Oregano esansiyel yağ içeren antiseptik solüsyonları düşük konsantrasyonlarda bile bakteriyel cins sayılarını düşürmektedir. Çevre dostu, biobozunur doğal bir mikrobisid olduğundan dolayı mevcut antiseptiklere alternatif olarak kullanılabilir.

Anahtar kelimeler: antisepsi, oregano, yağ asitleri, esansiyel, povidon-iyot

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Date of submission: 25.06.2014 / Date of acceptance: 28.08.2014

Introduction

Despite advanced achievement in surgical techniques, sterilization methods, glove manufacturing techniques, operating room ventilation, barriers and availability of antimicrobial prophylaxis, surgical site infections are still serious and most common postoperative problems with a high substantial cause of mortality and morbidity (1,2). Health care staffs' hands are an important infection transmission pathway of gram positive and gram negative bacteria as well as fungi (3). Larson et al (4) stated that total bacterial counts on the hands of medical staff have ranged from 3.9×10^4 to 4.6×10^6. Their number increases with the duration of clinical activities. Despite advances in the glove manufacturing techniques, as high as 17% glove perforation occurs and many of glove perforations are unnoticed by the surgeons (5,6). Therefore, preoperative antiseptic treatment of the hands of the surgical team prior to surgery is crucial.

When a postoperative infection occurs, it requires a prolonged treatment and extended hospital stay to heal (7). Most of the surgical site infections are preventable when the pre, intra and post-operative phases of care are taken properly (1). The aims of surgical hand antisepsis are to remove or destroy transient microorganisms and reduce the growth of resident microorganisms (8,9). Currently there are two mainly used antiseptic solution groups. The first group consists of povidone-iodine (PVP-I) or chlorhexidine based scrubs. The second group is composed of alcohol based solutions (10,11). Wide varieties of results have been reported in the literature and are not compatible with each other. There are a lot of studies available in the literature indicating that scrubs are superior, equal or inferior to alcohol-based solutions (12-15).

Due to their antimicrobial properties, essential oils represent a high potential source of alternative and environmentally acceptable antiseptic agents for infectious organisms. Anti-bacterial (16), anti-fungal (17), insecticidal (18), herbicidal (19) and nematicidal (20) properties of oregano essential oils have been well known mainly due to the basic constituents including carvacrol, thymol, ϒ-terpinene and p-cymene.

A variety of surgical hand antiseptics are now available, with different active components and application methods. However, none of them are based on essential oils. Also no studies have been reported in the literature testing the essential oil containing surgical hand antisepsis. The objectives of this study were to determine surgical hand antisepsis potential of oregano essential oil and to compare its antimicrobial efficacy with the commercially available 7.5% PVP-I based surgical scrub.

Methods

Aerial parts of Origanum minutiflorum grown in the experimental farm of Mustafa Kemal University (36° 12' 38" N and 36° 10' 44") Turkey were harvested at the flowering stage in 2012 growing season and the plant samples were dried at room temperature. Essential oil of O. minutiflorum was extracted by the hydro-distillation of air-dried plants and analyzed by means of gas chromatography mass spectrometry (GS-MS). Analysis of the essential oil carried out by using Thermo Scientific Focus Gas Choromatograph equipped with MS, auto sampler and TR-5 MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 60 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250°C, MS Ionization source temperature was 220°C, the injection port temperature was 220°C. The samples were injected with 250 split ratio. The injection volume was 1µl. Oven temperature was programmed to from 50°C to 220°C at 3°C /min. The structure of each compound was identified by comparison of their mass spectrum (Wiley) a data was handled through using of Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkane standard solutions C8-C20 (Fluka, product no. 04070) and C21-C40 (Fluka, product no. 04071).

The study protocol was reviewed and approved by local ethical board of Mustafa Kemal University, and informed consent was obtained from each participant. Experienced volunteer surgical staffs and medical students were chosen for the study. Any skin

problems, wounds or infection on the upper extremity any allergic problems to the tested products, use of systemic or topical antibiotics within the last one week were the exclusion criteria. The surgical hand antiseptic treatments and seeding bacteria to the agar plates by fingertip imprints were done in the operating theatre.

Hand antisepsis containing 0, 4, 8, 16, and 32% oregano essential were prepared by dissolving 0, 40, 80, 160, and 320 ml oregano essential oil in 60 ml ethanol and 3 ml Tween 80 in a 1 L liquid sterile soap container, sterile distilled water was added to have until 1 L volume. The commercial PVP-I scrub (7.5% povidone-iodine, Poviiodeks scrubs, Turkey), was used as control since it is the most common used conventional surgical hand antiseptic solution in Turkey. Fifteen volunteers were assigned for each antiseptic solution. Columbia agar with 7% blood in 90 mm petri plates were used for the cultivation of fastidious microorganisms like Staphylococci spp and Diphteroid spp. Two separate agar plates were used for right and left hand fingers. Each treatment was tested per day and the whole of the study was completed in six days.

Two protocols were used for surgical hand antisepsis treatment: I. Three minutes hand treatment with 3x5 ml PVP-I scrub with water, subsequently drying both hands with sterile compress. II. Three minutes hand treatment with 3x5 ml oregano essential oil containing surgical hand antisepsis by hand rubbing followed by one minute waiting until the skin gets dry. For bacterial seeding, finger tips were gently touched on the surface of agar plates for five seconds; before antiseptic hand treatment (t_0), just following antiseptic hand treatment (t_1) and three hours after conventional surgical hand gloving (t_2). Sterile powder-free latex surgical hand gloves were used. Each volunteer continued his routine work with the gloves under nonsterile conditions. At the end of three hours gloves were looked for any perforation and were excluded from the study. The Petri dishes were incubated at the hospital's Microbiology laboratory. After 48 hours of incubation at 36±1°C, applying standard laboratory identification procedures, bacterial colony counts (BCC) and bacterial genus numbers (BGN) were recorded. The volunteers were examined for any allergic symptoms by applying 3 ml of oregano essential oil containing hand antisepsis, 10 days after the first treatment.

To evaluate the antiseptic effects of oregano essential oil groups just after treatment and three hours after treatment; the BCC and BGN values in t_1 and t_2 were subtracted from t_0. The results were compared with PVP-I group.

The relationship between repeated measurements within the group was determined using Wilcoxon Signed Rank Test. The differences among groups were determined using Mann-Whitney U and Kruskal-Wallis tests. The age differences among groups were determined using Kruskal-Wallis test and gender rate was determined using Chi-Square test. All statistical analysis was conducted using SPSS for Windows 13.0 (Statistical Package for Social Sciences), Statistical significance was assumed as $p<0.05$.

Results

The age and gender of the participation were statistically similar in all the groups ($p>0.05$). Fifty essential oil components, representing 99.9% of the essential oil were detected in the essential oil of O. minutiflorum. The (GC/MS) results revealed that carvacrol (67.60%) was the major essential oil constituents followed by p-cymene (5.04%) and γ-terpinene (4.39%). The second dominant essential oil compounds were cyclopentasiloxane, decamethyl (1.59%), cyclohexasiloxane, dodecamethyl (1.48%), cycloheptasiloxane, tetradecamethyl (1.54%), trans-caryophyllene (1.63%), 10-heptadecen-8-ynoic acid, methyl ester (1.27%), borneol (1.96%), cyclononasiloxane, octadecamethyl (1.57%) and cyclodecasiloxane, eicosamethyl (1.13%). There were 39 minor essential components less than 1% were detected.

The BCC and BGN of all groups in T_0 were statistically similar ($p>0.05$). The 0% essential oil group had no significant antiseptic effect on BBC and BGN ($p>0.05$). The BCC and BGN in t_0, t_1 and t_2 are given in Table 1. The amount of reduction in BCC and BGN just following the antiseptic hand treatment (t_0-t_1) and 3 hour after treatment (t_0-t_2) are presented in Table 2.

Table 1. BCC and BGN before surgical hand antisepsis (t_0), immediately after (t_1) and after 3 hour hand gloving with sterile surgical gloves (t_2)

Treatment		t_0			t_1			t_2		
		Left	Right	BGN	Left	Right	BCC	Left	Right	BCC
PVP-I										
	mean±st.dev	343.33±79.88	353.33±63.99	4.6±0.9	66.66±17.59	70.33±27.86	3±0.65	28.33±13.71	29±17.74	1.66±0.61
	min-max	200-400	200-400	3-7	40-100	40-150	2-5	5-50	5-60	1-3
4% Essential oil										
	mean±st.dev	306.66±70.37	313.33±74.32	4.9±0.7	254±53.82	264±50.25	3.8±0.7	174±49.82	175.33±50.83	2.3±0.8
	min-max	200-400	200-400	3-6	180-350	180-350	2-5	100-300	100-300	1-4
8% Essential oil										
	mean±st.dev	335.71±100.82	339.28±88.09	4.5±1.1	267.85±107.93	262.14±103.78	3±0.8	205.71±107.61	220.71±108.09	1.4±0.5
	min-max	100-400	100-400	3-7	80-400	90-400	2-5	80-350	90-350	1-2
16% Essential oil										
	mean±st.dev	320±88.23	296.66±102.58	4.8±1.3	158±61.66	142±64.60	3.1±1	48.33±19.79	40.46±21.30	1.7±0.4
	min-max	150-400	100-400	3-7	80-300	50-300	2-5	15-80	10-75	1-2
32% Essential oil										
	mean±st.dev	292.66±130.46	284.66±96.87	5.6±0.9	53.13±29.75	52.53±32.68	3.8±0.7	14.86±7.12	17.40±12.00	2.1±0.7
	min-max	90-400	120-400	4-7	17-125	15-130	3-5	6-30	2-50	1-3
Control										
	mean±st.dev	320.00±67.61	323.33±49.52	4.2±1	320±67.61	322.66±49.20	4.2±1	318.66±68.22	322±50.45	4.2±1
	min-max	200-400	250-400	2-6	200-400	250-400	2-6	200-400	250-400	2-6

BCC=Bacterial colony count, BGN=Bacterial genus number, PVP-I=Povidone-Iodine

The comparison of essential oil groups to PVP-I group were as follows:

32% essential oil group: The BCC changes in t_1 (t_1 left p=0.0934, t_1 right p=0.170) and t_2 (t_2 left p=0.868, t_2 right p=0.184) were similar. Also, BGN decrease in t_1 (p=0.699) and t_2 (p=0.255) were similar.

16% essential oil group: The BCC reduction on both right and left hands at t1 was significantly higher in PVP-I group (t_1 left p=0.001, t_1 right p=0.000). However, both PVP-I and 16% oregano essential oil groups had similar effects on BCC at t_2 (t_2 left p=0.096, t_2 right p=0.105). At t_1 and t_2, PVP-I and 16% oregano essential oil antiseptic solution had similar effects on BGN (t_1 p=0.727 and t_2 p=0.619).

8% oregano essential oil group: PVP-I had the highest BCC reduction on both right and left hand fingertip imprints at t_1 and t_2 (p<0.001). But both PVP-I and 8% oregano essential oil antiseptic solution had similar effects on the BGN (t_1 p=0.729 and t_2 p=0.747).

4% oregano essential oil group: The BCC reduction effect of 4% oregano essential oil antiseptic solution was significantly lower than the PVP-I at t_1 and t_2 (p<0.001). PVP-I had significantly higher BGN reduction rate at t_1 (p=0.027), however at t_2 both solutions had effected the BGN similarly (p=0.290).

Table 2. The amount of elimination in BCC and BGN after treatment (t_0-t_1) and 3 hour after treatment (t_0-t_2)

		t0-t1			t0-t2			p*		
		Left	Right	BCC	Left	Right	BCC	Left	Right	BCC
PVP-I										
	median	320	310	2	360	350	3	0.001	0.001	0.0001
	min-max	120-350	150-360	1-3	150-395	185-395	2-4			
4% Essential oil										
	median	50	50	1	100	100	2	0.001	0.001	0.0001
	min-max	0-100	0-150	0-3	20-250	20-300	1-5			
8% Essential oil										
	median	60	30	1.5	110	65	3	0.001	0.002	0.001
	min-max	0-200	0-250	1-2	20-300	0-280	2-5			
16% Essential oil										
	median	180	140	2	270	275	3	0.001	0.001	0.002
	min-max	50-300	30-350	1-4	120-385	60-390	1-5			
32% Essential oil										
	median	275	270	2	370	265	3	0.001	0.002	0.001
	min-max	0-370	84-360	1-4	75-390	105-392	1-6			

*Wilcoxon Signed Ranks Test, BCC=Bacterial colony count, BGN=Bacterial genus number, PVP-I=Povidone-Iodine

Discussion

Surgical site infections due to inappropriate preoperative antiseptic treatment of the hands of the surgical team are still the most frequently reported problem in post-operative patients, accounting for 14–16% of all nosocomial infections (21). Different groups of micro-organisms on hands such as bacteria, fungi, viruses or prions can cause a wide variety of infections such as urinary tract, wound, respiratory, blood, bone and skin infections. The most common hand pathogens involved in post-operative surgical site infections are aerobic gram-negative bacteria (E. coli, and Pseudomonas) and aerobic gram-positive bacteria (S. aureus, coagulase-negative Staphylococcus). Preoperative antiseptic treatment of the hands of the surgical team has been repeatedly shown to reduce the level of transient microorganisms on the hands and hand hygiene achieved with antimicrobial solution has been demonstrated to reduce the incidence of post-operative surgical site infection (8).

Most of the studies are focused on the BCC reducing effects of hand antiseptic solutions (10). However in the current study we evaluated both BCC reducing effects and BGN reducing effects of the tested hand antiseptics. Genus identification was not carried out since the main purpose of the present study was to obtain a quantitative rather than a qualitative analysis. Bacterial seeding by fingertip imprints was preferred due to its ease of application. There are several standard protocols to test antimicrobial potential of hand antiseptics. The European standard EN12971 and US standard ASTM E-1115 are well known and most applied standard protocols (10). However, in most of the studies, these two standard protocols were not exactly followed (15,22,23). Our protocol was based on the reduction of BCC and BGN and was close to the European EN12971 standard protocol.

In this study we used PVP-I scrub for control group because it is the most used conventional surgical hand antiseptic solution in our country. Alcohol is thought to be the oldest rapid acting antiseptic and it is used as a topical antiseptic for preoperative skin preparation and surgical scrub purposes (10). In the present study, therefore, we did not combine oregano essential oil with alcohol to test its antimicrobial effects alone. Normally, oregano essential oil cannot dissolve in water. Therefore, we used 60 ml ethyl alcohol and 3 ml Twinn 80 to dissolve oregano essential oil in water.

A variety of surgical hand antiseptics are now available, with different active components and application methods. However none of them are based on essential oils. Also no studies have been reported in the literature testing the essential oils in surgical hand antisepsis. The results of this study has shown that Oregano essential oil offers an alternative method for hand antisepsis due to the high bacterial colony reduction rate, high bacterial genus number reduction and the longevity of its antibacterial effects on hand micro-organism.

In the current study, bacterial colony count similarities at t_0, t_1 and t_2 in the 0% essential oil group showed that colony count reductions were resulted only from the antimicrobial actions of oregano essential oil.

Regarding the reductions in the BGN, the effect of PVP-I scrub group was stronger than the 4% oregano essential oil group at t_1. However there was no significant difference between two groups at t_2. This suggested that oregano essential oil has a long-acting strong antibacterial effect on some certain bacterial strains even in lower doses. Similarly, BCC reduction in PVP-I scrub group was greater than the 16% oregano essential oil group at t_1, but the difference between both groups disappeared at t_2 (Figure 1,2). This could be attributed to the antibacterial longevity of oregano essential oil. There were no significant difference in BCC between PVP-I scrub and 32% oregano essential oil containing hand antisepsis groups at t_1 and t_2. The antibacterial effects of oregano essential oil containing hand antisepsis increased with the increasing essential oil dosage. The results of the current experiment showed that more than 30% oregano essential oil containing hand antisepsis could be used as an alternative to the conventional PVP-I scrub treatment.

Oregano essential oil containing hand antisepsis may have many potential advantages over the currently used hand antiseptics, including broader antimicrobial spectrum, lack of identifiable microbial resistances. The antimicrobial effects of oregano

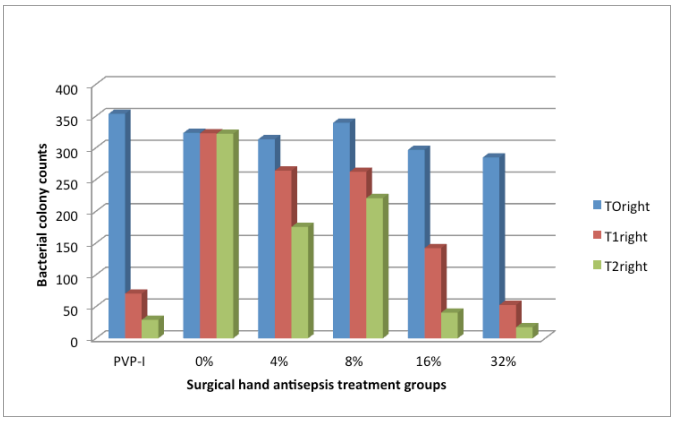

Figure 1. Changes in bacterial colony counts of right hands in all antiseptic treatment groups

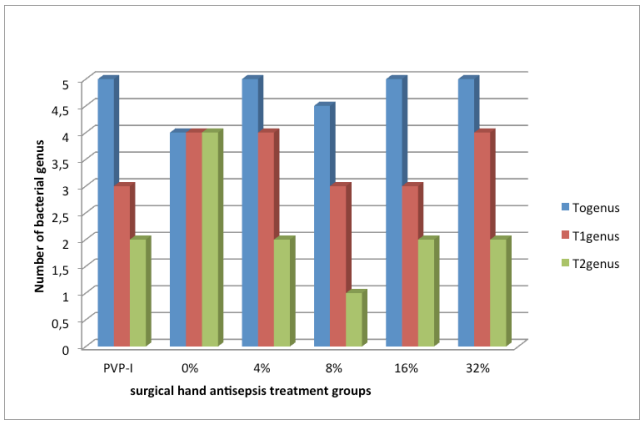

Figure 2: Changes in bacterial colony counts of left hands in all antiseptic treatment groups

essential oil can be attributed to its major components. The essential oil of oregano essential oil contained carvacrol, p-cymene, and γ-terpinene as major components, which showed potent antimicrobial effects. These compounds were found to be the most active components of oregano essential oil. The inhibitory effect of essential oils has been attributed to the most dominant components and not to the other minor components (24,25).

Although alcohol-based hand antiseptics reduce bacterial, fungal, and viral hand contaminations, it is less stable than that of povidone-iodine solutions (26). This disadvantage of alcohol-based hand antisepsis can be improved by use of highly antimicrobial oregano essential oil. Alcohol provides rapid bactericidal activity via dehydration, protein denaturation, interference with metabolism, and cell wall/cell membrane disruption, whereas oregano essential oil provides a more prolonged bactericidal effect via penetrating into the cell and interfere with cellular metabolism (27), disturbing the cellular membrane and reacting with the active sites of enzymes or acting as a H+ carrier, depleting adenosine triphosphate pool (28). Due to the mode of action of either ethyl or isopropyl alcohol they can add extra effects on oregano essential oil. Also various combinations with other active components like chlorhexidine gluconate, mecetronium, zinc etc. must be tested in order to obtain stronger antiseptic solutions. Further studies are needed.

Conclusion

Oregano essential oil has strong and long-acting antiseptic properties on hand pathogens. Hand treatment with 32% oregano essential oil containing hand antisepsis is equivalent to the hand antisepsis with conventional PVP-I scrub. Oregano essential oil containing antiseptic solutions have very strong effects in reducing the BGN even in low concentrations. Oregano essential oil containing hand antisepsis could be suggested for biodegradable and eco-friendly.

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