

**Antimicrobial and synergistic activity of essential oils facing isolated bacteria from surgical staff's***Atividade antimicrobiana e sinérgica de óleos essenciais em amostras bacterianas isoladas das mãos de equipe cirúrgica**Actividad antimicrobiana y sinérgica de aceites esenciales contra bacterias aisladas de manos del equipo*Tatiana Areas da Cruz²; Fernanda Rodrigues Torres¹; Monise Fazolin Petrucelli¹; Mariana Heinzen de Abreu¹; Sílvia Sidneia Silva²; Mozart Marins¹; Rene Oliveira Beleboni¹; Ana Lúcia Fachin¹¹ University of Ribeirão Preto, Biotechnology Department, Ribeirão Preto, SP, Brazil.² University of Ribeirão Preto, Nursing Department, Ribeirão Preto, SP, Brazil.**ABSTRACT**

Objective: to evaluate antimicrobial and synergistic activity of essential oils (*Citrus aurantifolia*, *Citrus sinensis*, *Mentha viridis* and *Thymus vulgaris*) against isolated bacteria from surgical staff's hands and bacterial strains. **Method:** the antimicrobial activity of oils was analyzed by microdilution method to determine the Minimum Inhibitory Concentration. Moreover, the combinatory effect of the oil that presented greater effectiveness using gentamycin sulphate through the Fractional Inhibitory Concentration index was tested. The study was performed over the period 2016-2018. **Results:** *T. vulgaris* was effective against *Staphylococcus saprophyticus* with a concentration of 0.0008 $\mu\text{L}\cdot\text{mL}^{-1}$ and against the ATCC standard strains of *Staphylococcus aureus*, *Salmonella choleraesuis*, *Staphylococcus epidermidis* and *Proteus vulgaris* and the bacteria *S. aureus*, *S. epidermidis* and *Bacillus sp.* with MIC $\geq 0.10 \mu\text{L}\cdot\text{mL}^{-1}$. This oil association with gentamycin sulphate showed synergistic activity against *S. epidermidis*. **Conclusion:** *T. vulgaris* essential oil showed expressive antimicrobial activity against ATCC and isolated clinic microorganisms, suggesting broad spectrum of activity.

Descriptors: Anti-Bacterial Agents; Hand Hygiene; Cross Infection; Drug Synergism; *Thymus* Plant.**RESUMO**

Objetivo: avaliar a atividade antimicrobiana e sinérgica de óleos essenciais (*Citrus aurantifolia*, *Citrus sinensis*, *Mentha viridis* e *Thymus vulgaris*) contra bactérias isoladas nas mãos de equipe cirúrgica e cepas de bactérias. **Método:** a atividade antimicrobiana dos óleos foi analisada por método de microdiluição para determinação da Concentração Inibitória Mínima. Também foi testado o efeito combinatório do óleo que apresentou maior efetividade com sulfato de gentamicina através do índice de Concentração Inibitória Fracionada. O estudo foi realizado no período de 2016-2018. **Resultados:** o *T. vulgaris* foi efetivo contra o *Staphylococcus saprophyticus* com uma concentração de 0,0008 $\mu\text{L}\cdot\text{mL}^{-1}$ e contra as cepas padrão ATCC de *Staphylococcus aureus*, *Salmonella choleraesuis*, *Staphylococcus epidermidis* e *Proteus vulgaris* e as bactérias *S. aureus*, *S. epidermidis* e *Bacillus sp.* com CIM $\geq 0,10 \mu\text{L}\cdot\text{mL}^{-1}$. A associação desse óleo com o sulfato de gentamicina mostrou atividade sinérgica contra *S. epidermidis*. **Conclusão:** o óleo essencial de *T. vulgaris* mostrou expressiva atividade antimicrobiana contra microrganismos ATCC e isolados clínicos, sugerindo amplo espectro de atividade.

Descritores: Antibacterianos; Higiene das Mãos; Infecção Hospitalar; Sinergismo Farmacológico; *Thymus* (Planta).**RESUMÉN**

Objetivo: evaluar la actividad antimicrobiana y sinérgica de los aceites esenciales (*Citrus aurantifolia*, *Citrus sinensis*, *Mentha viridis* y *Thymus vulgaris*) contra bacterias aisladas en manos del equipo quirúrgico y cepas bacterianas. **Método:** La actividad antimicrobiana de los aceites se analizó mediante el método de microdilución para determinar la Concentración Inhibitoria Mínima (CIM). También se probó, a través del índice de Concentración Inhibitoria Fraccional, el efecto combinatorio más efectivo del aceite con sulfato de gentamicina. El estudio se realizó entre 2016 y 2018. **Resultados:** *T. vulgaris* fue eficaz contra *Staphylococcus saprophyticus* con una concentración de 0.0008 $\mu\text{L}\cdot\text{mL}^{-1}$ y contra cepas ATCC estándar de *Staphylococcus aureus*, *Salmonella choleraesuis*, *Staphylococcus epidermidis* y *Proteus vulgaris* y las bacterias *S. aureus*, *S. epidermidis* y *Bacillus sp.* con CIM $\geq 0.10 \mu\text{L}\cdot\text{mL}^{-1}$. La asociación de este aceite con sulfato de gentamicina mostró actividad sinérgica contra *S. epidermidis*. **Conclusión:** el aceite esencial de *T. vulgaris* mostró una actividad antimicrobiana pronunciada contra microorganismos ATCC y aislados, lo que sugiere un amplio espectro de actividad.

Descritores: Antibacterianos; Higiene de manos; Infección nosocomial; Sinergia farmacológica; *Thymus* (planta).**How to cite:**

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INTRODUCTION

The control of hospital infections (HI) is considered a great challenge for public health services. The Ministry of Health of Brazil, in ordinance No. 2616/98, defined HI as that infection acquired after patient admission. It manifests during his hospitalization or even after his discharge and can be related to: hospitalization or hospital/outpatient procedures or diagnostic or therapeutic procedures performed during that period.¹ HI can be caused mostly by bacteria, fungi, viruses, among other microorganisms. From this conjunct, bacteria are the most frequent causers of that infections. Moreover, other factors are also associated with its occurrence, such as the patient's immunological state and age. The utilization of invasive methods, such as the catheter placement and surgeries, can be also determinant to HI onset.²

Despite the great technological advance, with the development of more effective treatment methods, more rigorous prophylaxis against diseases, more potent antibiotics and improvement of basic sanitation, the World Health Organization (WHO) points out the bacterial infections as one of the main causes of death around the world.³ According to Centers for Disease Control and Prevention (CDC) in the United States, it is estimated that 23,000 hospital deaths a year are associated with drug-resistant bacterial infections, which costs to health system about 20 billion of dollars annually.⁴ At present, antimicrobial resistance is seen as one of the greatest threats to global health, due to the increase of microorganisms resistant to current antibiotics, hindering the fight against infections

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previously treated with common antimicrobial agents.⁵ The infections caused by microorganisms resistant to antimicrobial treatment often result in longer hospital stays, higher medical costs and increased mortality rates.²

Among sites of hospital institutions, the surgical site infections (SSI) are potential complications associated with every type of surgical procedure and have stood out due to high mortality and morbidity rates presented and to the substantial costs attributed to the treatment. Although SSI are among the most controllable HI, they are the most costly ones to health systems around the world.⁶ In the United States, a WHO report of 2016 about the HI rates based on data of 2014 showed that 3,654 hospitals reported 20,916 SSI among the more than 2 million surgical procedures performed that year. Among SII, *S. aureus* microorganism was the most reported pathogen (30.4%), followed by coagulase-negative staphylococci (11.7%), *Escherichia coli* (9.4%) and *Enterococcus faecalis* (5.9%).⁷⁻⁹

Hand hygiene is considered the most effective isolated action to control HI when performed properly and has as objective remove dirt, organic material and reduce the microbial contamination of transitory flora.⁸ Surgical antisepsis of hands should eliminate the transitory microbiota and reduce the resident microbiota. Furthermore, it should inhibit bacterial growth on the gloved hand. The objective of this preventive measure is to reduce the transmission chance of microbiota of hand-skin bacteria from professional staff to surgical site, especially if the gloves suffer any damage during this type of procedure.⁹

The usage of an efficient antimicrobial agent assists in proper hand-antiseptis process. Essential oils are known for being antiseptic, i.e., they present bactericidal, viricidal and fungicidal activity, with diverse medical proprieties, and they are also used in embalming, food preservation and as antimicrobial agent, analgesic, sedative, anti-inflammatory, spasmolytic and local anesthetic.¹⁰ This fact, combined with the revival of interest in natural therapies and consequent growth of consumer demand for effective and safe natural products, allows the exploration of natural products with therapeutic purpose. The use of plants as a source of medicines is currently predominant in development countries and is considered as alternative solution for health problems, as well as being well-established in some cultures and traditions, especially in Asia, Latin America and Africa.¹¹

Selection of microorganisms resistant in hospital environment can occur due to inappropriate use of antimicrobial agents, many of them inefficient in infection control. Compounds of natural origin can be an alternative to fight against microorganisms resistant to therapy, associated with the right execution of aseptic techniques. However, it is necessary to explore and elucidate better the effects of these compounds in the field literature.

Facing this problematic, the present work aimed to evaluate antimicrobial activity of essential oils facing isolated bacteria samples from professionals' hands of surgical staff and evaluate antimicrobial and synergistic activity of the most effective essential oil with commercial

Antimicrobial and synergistic activity of oils antibiotics against the strains of isolated bacteria.

METHOD

Obtaining the essential oils

The essential oils were obtained from Campo de Ervas Ind. & Com. Ltda.-ME (Ribeirão Preto, SP, Brazil), accompanied by datasheets and serial numbers of each specimen: Lot. No. 116 density (d) = 0.868 (*Citrus aurantifolia*); Lot. No. 116 density (d) = 0.868 (*Citrus sinensis*); Lot. No. 116 density (d) = 0.868 (*Mentha viridis*); Lot. No. 116 density (d) = 0.868 (*Thymus vulgaris*). The essential oils were extracted by hydrodistillation from peel fruit of *C. aurantifolia* and *C. sinensis* and leaves of *M. viridis* and *T. vulgaris*.¹²

Isolating microorganisms from professionals' hands of surgical staff

The sample was composed by fifteen medics from Santa Casa de Misericórdia, hospital located in the city of Guaxupé - MG, Brazil. The inclusion criteria were: absence of contact with chlorhexidine gluconate on the day of collection, due to residual effect; no signals of skin dryness or lesions; no signals of skin mycoses or infections; age between 18 and 55 years, because there are changes in skin microbiota after age 60; healthy and not on medication, especially antibiotics. The study was approved by Human Research Ethics Committee from UNAERP No. 014/2010, consonant with Resolution No. 466/2012 from National Council of Health, and all participants signed the informed consent form.

To perform the collection of microorganisms on professionals' hands, an aseptically open sterile swab was used at the time

of collection. The swab was dampened in buffer solution with neutralizing agent (sodium thiosulfate 0.25%, which accompanies the swab). The cotton was rubbed three times toward each one of the fingers from wrist. Next, from wrist, the cotton of the same swab was rubbed returning again to wrist. Collected microorganisms were transferred to tubes containing 10 mL of buffer solution with neutralizing agent (sodium thiosulfate 0.25%) to inactivate possible residual quantities of sanitizing agents. Time elapsed between the sample collection and its arrive at laboratory did not exceed 24 hours. When necessary, the samples were kept refrigerated until analyzed.¹³ The bacterial isolates were identified through standard procedures.¹⁴

The isolated bacteria from the surgical staff's hands consisted of 4 samples of *Staphylococcus aureus*, 2 samples of *Staphylococcus saprophyticus*, 2 samples of *Staphylococcus epidermidis* and 1 sample of *Bacillus sp.*, with the numeration of samples being the number of professionals. The microorganisms American Type Culture Collection (ATCC) *Salmonella choleraesuis* (ATCC 10708), *Proteus vulgaris* (ATCC 6380), *S. aureus* (ATCC 6538) and *S. epidermidis* (ATCC 12228), along with the isolated samples, were used to evaluate antimicrobial activity of tested essential oils.

Preparing essential oils and positive control

The essential oils were dissolved in ethanol at 10% and, afterward, in Mueller Hinton Broth. The oils were tested over a range of 0.0008 at 100 $\mu\text{L}\cdot\text{mL}^{-1}$. When testing simultaneously, the oils were prepared in 1:1 proportion.

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To the positive control, gentamycin sulfate antibiotic (Ouro Fino[®]) was utilized. It was dissolved in water and, afterward, in culture medium, using initial concentration of 100 $\mu\text{g}\cdot\text{mL}^{-1}$.

The final concentration of ethanol used in antimicrobial tests was 0.5% or less. Solvent controls, sterility and growth control were included in each test.

Evaluation of Minimum Inhibitory Concentration (MIC)

The effect of antimicrobial activity of extracted compounds and essential oils was evaluated by microdilution method in Mueller Hinton Broth, in 96-well plates, as described by CLSI M7-A11.¹⁵ Concentration of bacterial inoculum was adjusted in spectrophotometer using wavelength of 550 nm, within an absorbance range of 0.100 to 0.125. Next, the standard inoculum was diluted 50 times in Mueller Hinton Broth, with 100 μL used per well, equivalent to 1×10^4 CFU. mL^{-1} of each strain of tested bacterium. Sterility controls of culture medium and oils were performed jointly. MIC was determined after 24 hours of growth in incubator at 37.2°C, comparing with the control growth. Gentamycin sulphate antibiotic was used as positive control.

Evaluation of Fractional Inhibitory Concentration Index (FICI)

The combinatory effect of the most effective tested oil with positive control (gentamycin sulphate) was evaluated through FICI, calculated according to the formula of FICI Evaluation, described by Pappalardo.¹⁶

$$A/CIM a + B/CIM b = CIF A + CIF B = ICIF$$

A= MIC of drug a in combination; MIC a= MIC of drug a alone; B= MIC of drug b in combination; MIC b = MIC of drug b alone.

Interaction is defined as:

- Synergistic when $FICI \leq 0,50$;
- Additive when $FICI > 0.50$ and < 1.0 ;
- Indifferent when $FICI > 1$ and < 4 ;
- Antagonistic when $FICI > 4,0$.

RESULTS

Among the evaluated essential oils, the essential oil of *Thymus vulgaris* showed the best antimicrobial activity for ATCC standard strains of *Staphylococcus aureus*, *Salmonella choleraesuis*, *Staphylococcus epidermidis* and *Proteus vulgaris* and against the bacteria *S. aureus*, *S. epidermidis*, *Bacillus sp.* and especially *Staphylococcus saprophyticus* isolated from surgical staff's hands, as shown in Table 1.

Table 1: Minimum Inhibitory Concentration ($\mu\text{L}.\text{mL}^{-1}$) of essential oils against ATCC bacterial strains and isolated samples from surgical staff's hands.

Bacteria	TV	CA	CS	MV	Gent ($\mu\text{g}.\text{mL}^{-1}$)
<i>Salmonella choleraesuis</i> ATCC 10708	$\geq 0,10$	≥ 100	≥ 100	≥ 100	$\geq 0,20$
<i>Staphylococcus aureus</i> ATCC 6538	$\geq 0,10$	≥ 100	≥ 100	≥ 100	$\geq 0,20$
<i>Staphylococcus epidermidis</i> ATCC 12228	$\geq 0,39$	≥ 100	≥ 100	≥ 100	$\geq 0,39$
<i>Proteus vulgaris</i> ATCC 6380	$\geq 0,20$	≥ 100	≥ 100	≥ 100	$\geq 0,78$
1- <i>Staphylococcus aureus</i>	$\geq 0,10$	≥ 100	≥ 100	≥ 100	$\geq 0,20$
2- <i>Staphylococcus aureus</i>	$\geq 0,10$	≥ 100	≥ 100	≥ 100	$\geq 0,20$
3- <i>Staphylococcus aureus</i>	$\geq 0,10$	≥ 100	≥ 100	≥ 100	$\geq 0,20$
4 - <i>Staphylococcus aureus</i>	$\geq 0,10$	≥ 100	≥ 100	≥ 100	$\geq 0,20$
5- <i>Staphylococcus saprophyticus</i>	$\geq 0,0008$	≥ 100	≥ 100	≥ 100	$\geq 6,25$
6- <i>Staphylococcus saprophyticus</i>	$\geq 0,39$	≥ 100	≥ 100	≥ 100	$\geq 6,25$
7- <i>Staphylococcus epidermidis</i>	$\geq 0,39$	≥ 100	≥ 100	≥ 100	$\geq 6,25$
8- <i>Staphylococcus epidermidis</i>	$\geq 0,78$	≥ 100	≥ 100	≥ 100	$\geq 6,25$
9- <i>Bacillus sp.</i>	$\geq 0,39$	≥ 100	≥ 100	≥ 100	$\geq 6,25$

Note: TV: *Thymus vulgaris*, CA: *Citrus aurantifolia*, CS: *Citrus sinensis*, MV: *Mentha viridis*, Gent: gentamycin sulphate.

Regarding FICI Evaluation, in which *T. vulgaris* oil was associated with gentamycin sulphate to evaluate synergistic activity, this

association presented an additive action only for *S. epidermidis* (ATCC 12228), as presented in results in Table 2.

Table 2: Evaluation of Fractional Inhibitory Concentration Index.

Bacteria	TV	Gent ($\mu\text{g}.\text{mL}^{-1}$)	TV+Gent	ICIF	Activity
<i>Salmonella choleraesuis</i> ATCC 10708	$\geq 0,10$	$\geq 0,20$	$\geq 3,125$	4,68	Antagonistic
<i>Staphylococcus aureus</i> ATCC 6538	$\geq 0,10$	$\geq 0,20$	$\geq 6,25$	4,68	Antagonistic
<i>Staphylococcus epidermidis</i> ATCC 12228	$\geq 0,39$	$\geq 0,39$	$\geq 0,10$	0,52	Additive
<i>Proteus vulgaris</i> ATCC 6380	$\geq 0,20$	$\geq 0,78$	$\geq 0,39$	2,45	Indifferent
7- <i>Staphylococcus epidermidis</i> (mãos)	$\geq 0,39$	$\geq 6,25$	$\geq 6,25$	4,68	Antagonistic
8- <i>Staphylococcus epidermidis</i> (mãos)	$\geq 0,78$	$\geq 6,25$	$\geq 6,25$	4,68	Antagonistic

Note: TV: *Thymus vulgaris*, Gent: gentamycin sulphate, FICI: Fractional Inhibitory Concentration Index.

The presented results related to MIC demonstrated that *T. vulgaris* essential oil presented expressive antimicrobial activity in comparison to the other evaluated essential oils and facing gentamycin sulphate, used as positive control in the study. When the results of FICI Evaluation were verified, the association of this essential oil with gentamycin antibiotic showed synergistic activity only for *S. epidermidis* (ATCC 12228).

DISCUSSION

Thymus vulgaris species, popularly known as “white thyme”, is a plant that belongs to Lamiaceae family. Diverse compounds identified by chemical analysis through CG-EM¹⁷ identified in its constitution major compounds such as thymol (49.24%), ρ -cymene (25.48%) and γ -terpinene (7.46%). Its application as food preservative or antiseptics is broadly studied.¹⁷ Based on scientific literature, pharmacological studies point out this species as insecticide and larvicide,¹⁸ antimicrobial¹⁹ and antifungal.²⁰

T. vulgaris vegetal extracts present good antimicrobial activity against pathogens, suggesting that such extracts could be used to treat various types of infections caused by microorganisms.²¹

Many of herbal medicines have favorable effectiveness through the additive or synergistic action of various chemical compounds, acting on one or various targets.²² Data showed that the combination of *T. vulgaris* and *Pimpinella anisum* oils presented additive effect against most of tested pathogenic bacteria. *Pseudomonas aeruginosa* was inhibited using the highest concentration of combined essential oils.²³

Studies carried out in 2010 by disk diffusion methods and agar dilution resulted in a semiquantitative antimicrobial activity between two essential oils (*T. vulgaris* and *Eucalyptus globulus*) against methicillin-resistant *Staphylococcus aureus* (MRSA). *T. vulgaris* oil presented the largest inhibition zones, with these results confirmed by MIC values (variation: 0,1-4 v/v%).²⁴

In the present study, the essential oils of *Citrus sinensis*, *Citrus aurantifolia* and *Mentha viridis* did not present antimicrobial activity and were not considered for use in hand hygiene. However, the scientific literature has reported antimicrobial, anti-inflammatory, antipyretic,²⁵ antiemetic, soothing and antiallergic²⁶ activities.

Hand hygiene is an isolated action considered as the first important technique in control of HI and SSI. It is realized that, due to the resistance of bacteria to current antibiotics, there is a great preoccupation in the hospital environment about the subject constantly associated with the use of antibacterial agents, such as soaps and alcoholic solutions. Then, the search for new antimicrobial agents is extremely important,⁸ such as *T. vulgaris* essential oil, that could be added to products for hand hygiene.

Facing the presented results, it is recommended that further studies be carried out about the best way for using *T. vulgaris* essential oil in commercial products and in association with other types of essential oils that can be utilized for antiseptics and bacterial control in hospital environment. It is important that tests with other bacterial species and *in vitro* and *in vivo* cytotoxicity tests to evaluate the safe and effectiveness of this compound be performed.

CONCLUSION

Thymus vulgaris essential oil showed expressive antimicrobial activity as well as broad spectrum of activity against Gram-positive and Gram-negative ATCC microorganisms and against isolated bacterial strains on professionals' hands

Antimicrobial and synergistic activity of oils of surgical staff. In addition, synergistic activity of *T. vulgaris* essential oil with gentamycin sulphate antibiotic demonstrated additive activity only for a type of Gram-positive bacterial strain (*Staphylococcus epidermidis* ATCC 12228).

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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