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Impetigo: green light for essential oils-based natural treatments but not self-formulated

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ABSTRACT

INTRODUCTION. *Staphylococcus aureus* and *Streptococcus pyogenes* are microbial agents of bullous and non-bullous impetigo, respectively. Treatments are based on both topical and oral antibiotics. Adverse effects and the selection of resistant strains are the main drawbacks of the therapy. Essential oils (EOs) could be good candidates for new therapy.

MATERIALS AND METHODS. A bibliographic search was carried out to identify studies based on the efficacy of EOs against Impetigo strains. Nine EOs were selected and tested against nine clinical strains (three of *S. aureus*, three of *S. pyogenes* and three of *Staphylococcus epidermidis*) using the broth microdilutions test. Two of the most active OEs (*L. stoechas and P. graveolens*) were used to formulate four mixtures. An olfactory satisfaction study was conducted on 100 volunteers to identify the most pleasant blend. A commercial juice and a gel of *Aloe vera (A. vera)* were used to formulate topical gels that were analyzed both in solid-phase microextraction coupled to GC-MS to evaluate their chemical composition and using a micro broth-dilution method to confirm the efficacy.

RESULTS. 16 articles and 14 EOs were selected from the literature. Eight EOs were chosen to develop microbiological investigations. Six of the eight EOs showed greater antimicrobial activity on non-bullous impetigo strain (*S. pyogenes*) than on the non-pathogenic one (*S. epidermidis*). *L. stoechas* and *P. graveolens* EOs were chosen to develop formulations for topical use, and a mixture of these, pleasant for both men and women, was selected. Two types of *Aloe vera*-based formulations were developed. The chemical analysis showed the overlapping composition of the two formulations, but only that obtained from the *A. vera* juice maintained its antimicrobial effectiveness.

CONCLUSIONS. *L. stoechas* and *P. graveolens* EOs showed microbiological effectiveness against non-bullous impetigo. However, these EOs must be formulated masterfully avoiding homemade formulations with commercial products containing preservatives.

KEYWORDS

ALOE VERA
STAPHYLOCOCCUS AUREUS
STREPTOCOCCUS PYOGENES
STAPHYLOCOCCUS EPIDERMIDIS
LAVANDULA STOECHAS
PELARGONIUM X GRAVEOLENS

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INTRODUCTION

Impetigo is a primary or secondary highly contagious bacterial infectious skin disease. The bacteria responsible for this infection are *S. aureus* and *S. pyogenes*. Young age, crowding, close contact, and humid heat are the major risk factors. Impetigo can be divided mainly into two different forms: bullous and non-bullous impetigo[1]. Furthermore, depending on the type of impetigo, lesions can occur on healthy skin or on skin damaged by previous inflammatory dermatoses, traumas, penetrating wounds, and rubbing[2,3]. Furthermore, impairment of the skin's barrier function can facilitate the development of the disease, as the skin is more vulnerable to external aggressions. Subjects most affected are children under 10 years old even if manifestations in adults are reported[4]. Generally, the infection begins with well-defined red patches on which vesicles and bubbles containing pus and serum form which then break up and generate crusts[2]. Erythema, de-epithelialization, desquamation and burning are frequent skin manifestations. Scratch the skin in response to itching facilitates the spread of the infection from healthy skin.

To reduce symptoms and clinical manifestations of impetigo, treatments based on oral and/or topical antibiotics are the most prescribed therapies. In case of weak bullose or non-bullose skin manifestations, topical treatments are preferred[1]. Whereas oral or intramuscular treatments are used in more complicated cases. Unfortunately, these treatments are often associated with various adverse effects that limit their use. In addition, prolonged use can promote the development of resistant antibiotic strains (especially *S. aureus* MRSA), representing a serious and global health problem[5] in addition to causing an imbalance of the skin microbiota. In light of these important therapeutic consequences, it becomes necessary to develop new treatments and promote strategies aimed at judiciously reducing the use of existing drugs.

To date, few researches are aimed at identifying new active drugs (e.g., topical ozenoxacin or retapamulin for topical treatments) and almost none identifies natural products that are potentially active in the treatment of impetigo and contextually useful in contrasting antibiotic resistance.

Among natural substances with antimicrobial activity, essential oils (EOs) are increasingly studied by the scientific community. EOs are natural products defined by the European Pharmacopoea and recognized by international organizations such as the European Medicines Agency (EMA) or the European Chemicals Agency, that define them as characterized by the volatile part of aromatic plants, obtained by distillation, steam distillation or pressing, in the case of citrus fruits. They can be obtained from different parts of the plant (leaves, fruits, roots, etc.), and are mainly characterized by volatile hydrocarbons[6]. Although no *in vitro* or *in vivo* studies have so far been conducted on the efficacy of EOs against impetigo, many studies show the efficacy of these natural products against the strains responsible for this disease.

The lack of specific studies and the absence of *ad hoc* products could lead patients to develop self-formulations with or without the support of competent figures. It is known that a common practice in phytotherapy is to mix several natural substances purchased individually. If on the one hand, this practice can be economically and therapeutically advantageous for the patient supervised by a specialized doctor, on the other hand, it may not have the desired effects if the formulated products have not been properly studied, especially in pathologies of microbial aetiology. For this reason, the aims of this research were: (i) identify, through a literature review, the EOs already tested on both bacterial strains responsible for impetigo, (ii) select some EOs in order to identify a formulation active on pathogenic strains and not active on others, (iii) compare the effectiveness of the OEs formulation when inserted into a commercial or newly formulated *Aloe vera* gel.

MATERIALS AND METHODS

2.1 Background investigation

To identify potentially interesting EOs for the treatment of impetigo, a preliminary literature review was done.

Eligible criteria: All articles concerning the use of EOs against impetigo were included in the research. *Information source*: A bibliographic search was performed both in MEDLINE and Embase to conduct the analysis. *Search strategy*: No time limit was considered. Articles in English, Spanish, French, or Italian were selected. The search strategy used a combination of thesaurus terms, i.e., MeSH terms (MH) in MEDLINE and Emtree terms in Embase, and free-text words for each concept. The following search strategy was used in MEDLINE: (*Streptococcus pyogenes*[mh] OR *Streptococcus pyogenes* OR *Staphylococcus aureus* [mh] OR *Staphylococcus aureus* OR *impetigo*[mh] OR *impetigo* OR skin diseases, infectious[mh] OR skin infection OR cutaneous infection OR dermal infection OR infectious skin disease) AND (oils, volatile[mh] OR Essential oil OR volatile oil OR rapid evaporating oil). Thesaurus terms were exploded to include more specific terms automatically in the search and text words were truncated to find singular and plural forms. The same search strategy was run in Embase by replacing MeSH terms with Embase specific thesaurus terms. Additional studies were retrieved by manually searching the reference lists of the relevant articles Data

extraction: Studies that treated the effectiveness and safety of EOs in the treatment of impetigo were included. No contact with authors of articles was necessary. In the case of repeated articles, the most recent was considered. Table 1 provides an overview of the extracted data: (a) the author and the publication year, (b) EOs, (c) the presence of EO quality analysis, (d) the main components, (e) the bacterial strains, (f) the antimicrobial assay, (g) the main results, (h) the presence of statistical analysis, (i) potential biases, (j) the most active essential oil. Assessment of bias: The biases were identified as follows: absence of statistical analysis, absence of binomial nomenclature of aromatic plant, absence of EO quality analysis, absence of quantitative data.

2.2 Bacterial strains, growth medium, and natural products

To develop microbiological analysis, a total of nine clinical strains resulting from skin swabs (3 strains of *Streptococcus pyogenes,* 3 of *Staphylococcus aureus* of which two of *S. aureus* MSSA (methicillin sensitive) and one of *S. aureus* MRSA (methicillin-resistant), and 3 strains of *Staphylococcus epidermidis*) were used. Except for *S. pyogenes,* all strains were grown at 37 °C for 24h in aerobic conditions by using Muller Hilton broth (Mueller-Hinton Broth Oxoid Ltd CM0405, Wade Road, Basingstoke, Hants, RG24 8PW), while *S. pyogenes* was grown in anaerobic conditions. EOs of *Coriandrum sativum, Cinnamomum zeylanicum, Lavandula stoechas, Citrus sinensis, Thymus vulgaris, Syzygium aromaticum, Pelargonium x graveolens, Chamaemelum nobile, Helichrysum italicum (*Pranarôm, Avenue des Artisans, 37 – 7822 Ghislenghien – Belgio) were studied. A juice (ESI s.r.l, Albisola Superiore, SV, Italy) and a commercial gel (Zuccari s.r.l, Trento, Italy) of *Aloe vera* were used to prepare topical formulations.

2.3 Broth micro dilutions susceptibility testing

Broth micro dilutions susceptibility tests, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) international guidelines, were performed. The antibacterial activity of the EOs was evaluated using the culture broth mentioned in the 2.2 paragraph. Due to the hydrophobic characteristics of the EOs, an equal proportion of surfactant (Tween 80, Sigma Aldrich, Saint Louis, MO, USA) and EO were used to create a homogeneous emulsion. All tests were performed on a 96-well plate, and serial dilutions between 4% v/v (40 mL/L) and 0.03% v/v (0.3 mL/L) were tested. Starting from a suspension of 0.5 McFarland, a solution of $5x10^5$ CFU/mL was made. Plates were incubated overnight at 37°C for 24h, and Minimum Inhibitory Concentration (MIC) values were determined as the lowest concentration of EOs corresponding to the complete inhibition of bacterial growth. The Minimum Bactericidal Concentration (MBC) was identified by seeding 5 µL of the contents of each well on Mueller-Hinton Agar plates (Becton Dickson and Company, 7 Loventon Circle, Sparks, MD21152 USA, 225250) and incubating at 37°C for 24 h. MBC was defined as the lowest concentration of EO corresponding to the absence of bacterial growth. Each experiment was repeated almost in triplicate, and positive and negative controls were included.

2.4 GC-MS analyses of EOs and gel formulations

The chemical composition of the *P. graveolens* and *L. stoechas* EOs and the gel formulations were obtained by a Clarus 500 model Perkin Elmer (Waltham, MA, USA) gas chromatograph coupled with a mass spectrometer and equipped with a FID (flame detector ionization). To separate the detected components, a Varian Factor Four VF-1 capillary column was used, and He was the gas carrier at a flow rate of 1.0 mL min-1 in constant flow mode. The GC oven temperature was set as follows: initially at 60 °C and then ramped up to 220 °C at a rate of 6 °C min -1 for 20 min. For MS detection, an electron impact ionization (EI) system was used at 70 eV in scan mode in the range of 35-400 m/z. The volatile components were identified by matching their mass spectra with those stored in the Wiley 2.2 and Nist 02 mass spectra libraries database and by comparison of their linear retention indices (LRIs), with those available in the literature. Relative amounts of the identified compounds were expressed as percentage of the relative peak area to that of the total peak area without the use of an internal standard and any factor correction. All analyses were carried out in triplicate.

2.5 Satisfaction test

A single-blind satisfaction test was carried out on 100 candidates (38 males and 62 females) divided by age (18-25 years old; 26-35 years old; 36-50 years old, over 50). Candidates were asked to choose the best fragrance among four mixtures contained in anonymous test tubes and performed in a non-perfumed vehicle. The following mixtures were tested: Mix1 (2% *Lavandula stoechas*, 0.5 % *Pelargonium x graveolens),* Mix2 *(*2% *Lavandula stoechas*), Mix3 (0.5% *Pelargonium x graveolens),* Mix4 *(*1% *Lavandula stoechas*, 0.5 % *Pelargonium x graveolens).* Each Mix was smelled for 3 seconds, and the right amount of time was waited between samples to avoid interferences.

2.6 Formulation with *A. vera* gel

Two gel formulations based on the EOs selected were developed by using a juice or a commercial gel of *A. vera*. The formulation containing the commercial *A. vera* gel

was obtained by adding only the EOs of Mix1 at the established concentration and obtaining a homogeneous formulation through a blender (Cito Unguetor, Farcom Italia, Genova, Italy). On the contrary, starting from the juice an *ex novo* preparative method was developed to obtain a gel formulation. Carboxymethylcellulose (CMC) was used as jellifying agent. Percentages equal to 2.0%, 3.0% and 4.0% w/w of CMC were used to jellify 20 gr of *A. vera* juice which was mixed with a metal anchor until the complete dissolution of CMC. Subsequently, the EOs of MIX1, at the concentrations reported in paragraph 2.5, were added to *A. vera*. To ensure the solubilization of the EOs, they were first mixed with 5% v/w of Tween80 (Sigma Aldrich, Saint Louis, MO, USA). Before the subsequent tests, both formulations were left to rest at + 4 °C.

2.7 SPME sampling for the chemical characterization of formulations based on *A. vera*

The volatile fraction of "*ex novo*" and "*Zuccari*" gel formulations was investigated by solid phase microextraction (SPME)-GC-MS techniques. About 2 mL of both samples were individually placed into a 7 mL glass vial with PTFE-coated silicone septum. The sampling phase was performed using an SPME device from Supelco (Bellefonte, PA) with 1 cm fiber coated with 50/30 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane), and the operative conditions such as temperature and equilibration time were optimized. Before sampling, the fiber was conditioned at 270 °C for 20 min, and successively it was exposed to the headspace for 15 min at 50°C to capture the volatiles compounds. For the desorption of the components, the fiber was inserted into the GC injector maintained at 250°C. The GC-MS applied parameters are as reported in the previous paragraph *2.4.*

2.8 Antimicrobial effectiveness of formulations based on *A. vera* and EOs

To test if the OEs contained in the formulations maintained their antimicrobial effectiveness, micro broth-dilution tests were repeated following the same protocol described in paragraph 2.3 with only the following differences: (i) no surfactant was added, (ii) only one concentration (50% v/v) of the gel was tested. The test was performed only against strains responsible for non-bullous impetigo (3 strains of *S. pyogenes* and 3 of *S. epidermidis)*. Each experiment was repeated almost in triplicate, and positive and negative controls were included.

2.9 Statistical data

Data are presented as mean ± standard deviation. As suggested by the guidelines, changes in MIC and MBC greater than two dilutions were considered significant.

RESULTS

3.1 Background investigation

Figure 1 presents a flowchart of the search. Literature searches on Medline and Embase resulted in 317 articles. A total of 301 studies were excluded. Specifically, 28 duplicates, 29 articles other than *in vitro* studies or human randomized clinical trials, 127 articles concerning other natural compounds, and 117 articles that did not evaluate possible treatments for both strains responsible for impetigo. Only 16 *in vitro* studies were eligible for the study. Due to the absence of randomized clinical trials concerning the treatment of impetigo with EOs, only articles that evaluated the *in vitro* effectiveness of EOs on *S. aureus* and *S. pyogenes* were considered.

Figure 1. Flow-chart of the study.

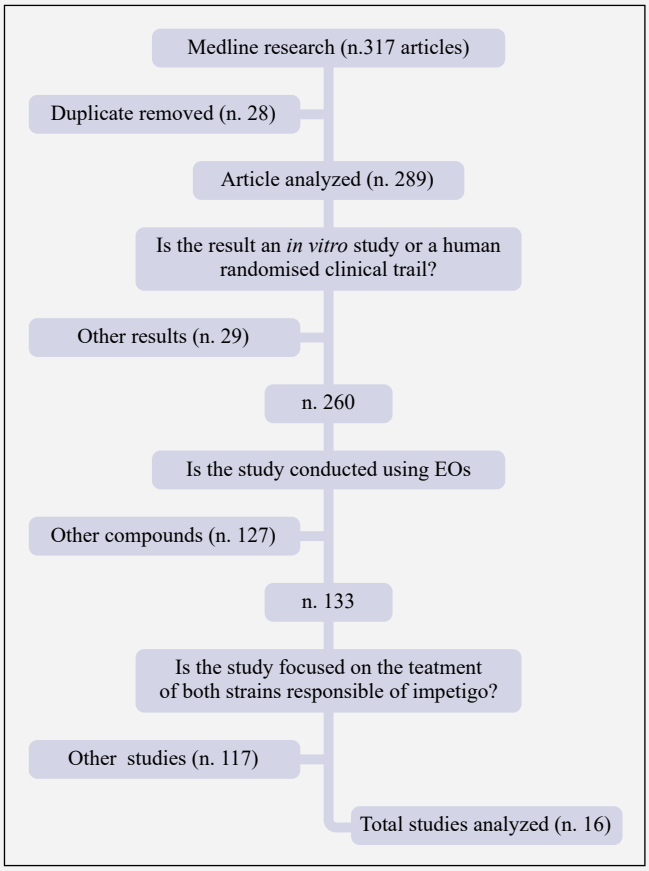

Table 1 summarizes the analysis of the 16 selected articles. Specifically, 11 articles reported the effectiveness of a single EO, and all the other publications were developed by evaluating the antimicrobial activity of several EOs on both *S. aureus* and *S. pyogenes* strains, or on several species, including the two considered. In cases where multiple EOs were tested, it was reported which EO was most active against the selected strains (last column). Of the 16 selected articles, 13 presented the qualitative analysis of EOs, the remaining did not present any kind of anal-

ysis (bias C). Furthermore, two articles did not mention the binomial nomenclature of EOs (bias B). Finally, in 9 studies, the statistical analysis was not reported (bias A). The EOs that showed greater antimicrobial activity against the selected strains were extracted from the following 14 aromatic species: *Thymus vulgaris*, *Bocageopsis multiflora*, *Eremanthus erythropappus*, *Monarda punctata*, *Duguetia lanceolata*, *Coriandrum sativum*, *Hipericum hyssopifolium*, *Cochlospermum planchoni*, *Cinnamomum zeylanicum*, *Citrus sinensis*, *Lavandula stoechas*, *Cryptomeria japonica*, *Syzygium cumini*, *Eremophila maculate*. Based on data obtained from the literature revision, it was decided to investigate the activity of EOs obtained from five of the 14 selected aromatic species (*T. vulgaris, C. sativum, C. zeylanicum*, *C. sinensis*, *L. stoechas*), one EO belonging to the *Syzigium* genus (*Syzygium aromaticum*) and three OEs obtained from aromatic species not selected from literature but characterized by a known eudermic activity (*Pelargonium x graveolens*, *Chamaemelum nobile*, *Helichrysum italicum*).

Table 1. Studies selected from literature and their main results.

Author	Year	EOs	GC	Main Components†	Bacterial Strains	Antimicrobical Assay	Results (MIC or MBC values from MBD assay)		Statistical Analysis	Bias	Most Active EO
							S. pyogenes	*S. aureus*			
Julien Antih	2021	*Thymus vulgaris*	yes	p-Cymene, Thymol, Carvacrol	SS: *H. influenzae*, *S. aureus* and *S. pyogenes*	MBD	MIC: 512 µg/mL	MIC: 512 µg/mL	/	(A)	/
Marcia Bay	2019	*Bocageopsis multiflora, Duguetia quitarensis, Fusaea longifolia, Guatteria punctata*	yes	***B. multiflora:*** cis-Linlool oxide, 1-epi-Cubenol. ***D. quitarensis*:** 4-Heptanol, α-Thujene, (E)-Caryophyllene. ***F. longifolia:*** β-Selinene, cis-β-Guaiene, (Z)-α-Bisabolene. ***G. punctata*:** Germacrene D, (E)-Nerolidol.	SS: *S. mutans*, *S. pyogenes*, *S. MRSA*, *EHEC* and *P. aeruginosa*	MBD	MIC: **B. *multiflora*** 4.68 µg/mL; ***D. quitarensis*:** 37.5 µg/mL; ***F. longifolia*:** NR; ***G. punctata*:** 4.68 µg/mL	MIC: ***B. multiflora*:** 4.68 µg/mL; ***D. quitarensis***: NR; ***F. longifolia:*** 37.5 µg/mL; ***G. punctata:*** NR	ANOVA Tukey-Kramer multiple comparisons test.	/	*B. multiflora*
Marcelo S. Silvério	2013	*Eremanthus erythropappus* (L, B and I)	yes	**E. erythropappus (I):** β-caryophyllene, germacrene-D, α-muurolol. ***E. erythropappus* (L):** β-caryophyllene, germacrene-D, α-copaene. ***E. erythropappus* (B):** α-bisabolol	SS: *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*.	MDB and AD	MIC: **Dry L:** 10 µg/mL, **Fresh L:** 20 µg/mL, I:40 µg/mL, **B:** 250 µg/mL.	MIC: **Dry L:** 310 µg/mL, **Fresh L:** 310 µg/mL, **I:** 125 µg/mL, **B:** 500 µg/mL.	Tukey test	/	/
Behrooz Alizadeh Behbahani	2017	*Allium*	yes	**extraction efficiency of AHEO was 0.06 (v/w)**: 5-chloroorcylaldehyde, methylthiomethyl disulfide, 1-butene, 1-(methylthio)-(Z)*. **extraction-efficiency of AHEO was 0.04 (v/w):** 5-chloroorcylaldehyde, methyl methylthiomethyl disulfide*	SS: *P. aeruginosa*, *E. coli*, *B. cereus*, *B. subtilis*, *S. aureus*, *S. pyogenes*	MBD, DD and WD	MIC: 0.25 mg/mL; MBC: 0.25 mg/mL;	MIC: 0.5 mg/mL; MBC: 1.0 mg/mL;	ANOVA and Duncan's mean comparison test. PLS-DA and PCA.	(B)	/

Table 1. Studies selected from literature and their main results. (continues)

Author	Year	EOs	GC	Main Components†	Bacterial Strains	Antimicrobical Assay	Results (MIC or MBC values from MBD assay)		Statistical Analysis	Bias	Most Active EO
							S. pyogenes	*S. aureus*			
Hong Li	2014	*Monarda punctata* (Flowers)	yes	Thymol, p-cymene	SS: *S. pyogenes*, *S. MRSA*, *S. pneumoniae*, *H. influenzae*. CS: *E. coli*	MBD	/	/	/	(A) (D)	/
Orlando V. Sousa	2012	*Duguetia lanceolata* (barks)	yes	b-Elemene, caryophyllene oxide, and b-selinene	SS: *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*	AD, MBD	MIC: 20 µg/mL	MIC: 60 µg/mL	/	(A)	/
F. Casetti	2011	*Coriandrum sativum L.* (fruits)	no	/	CS: S. MSSA, S. MRSA, *S. viridans*, *S. pyogenes* *E. faecalis*, *E. faecium*, vancomycin resistant *Enterococcus (VRE)*, *E. coli*, *K. Pneumoniae*	AD	/	/	ANCOVA using PROC GLM in SAS. ESTIMATE statement.	(C)	/
Zuhal Toker	2005	*Hypericum hyssopifolium* var. *microcalycinum* and *Hypericum lysimachioides* var. *lysimachioides*	yes	***H. lysimachioides var. lysimachioides:*** Caryophyllene oxide. ***H. hyssopifolium* var. *microcalycinum:*** Caryophyllene oxide, Spathulenol	SS: *E. coli*, *B. brevis*, *B. cereus*, *S. pyogenes*, *P. aeruginosa*, *S. aureus*	DD	/	/	/	(A)	*H. hyssopifolium var. microcalycinum*
Lassina Ouattara	2007	*Cochlospermum planchoni Hook.*f. ex Planch	yes	tetradecen-3-one, tetradecan-3-one, dodecyl acetate, tetradecyl acetate	CS: *E. faecalis*, *P. aeruginosa*, *S. aureus*, *S. pyogenes*. SS: *B. cereus*, *E. faecalis*, *E. coli*, *L. innocua*, *S. enterica*, *S. dysenteria*, *S. aureus*.	MBD, DD	MIC: 0.25 % v/v, MBC: 0.5 % v/v.	MIC: 1.00 % v/v (SS), 8.00% v/v (CS). MBC: 4.0 % v/v (SS), >8.00% v/v (CS).	/	(A)	/
Patrick H. Warnke	2009	Thyme white, Lemon, Lemongrass, Cinnamon, Tea tree, Eucalyptus, Grapefruit, Clove Bud, Lavender, Peppermint, Sage, Kunzea, Sandalwood	no	/	SS: *S. aureus*, *S. epidermidis*, *S. mutans*, *S. pyogenes*, *S. equisimilis*. CS: *S. aureus*, *S. aureus* MRSA, *S. epidermidis*	AD	/	/	/	(A), (B), (C), (D)	/

Table 1. Studies selected from literature and their main results. (continues)

Author	Year	EOs	GC	Main Components†	Bacterial Strains	Antimicrobical Assay	Results (MIC or MBC values from MBD assay)		Statistical Analysis	Bias	Most Active EO
							S. pyogenes	*S. aureus*			
A. Fabio	2007	*Citrus aurantium*, *Citrus bergamia*, *Cinnamomum zeylanicum*, *Syzygium aromaticum*, *Cupressus sempervirens*, *Eucalyptus globulus*, *Foeniculum vulgare*, *Lavandula angustifolia*, *Citrus limon*, *Mentha piperita*, *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*	no	/	CS: *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, *K. pneumoniae*, *H. influenzae*, *S. aureus*, *S. maltophilia*	KBP, MBD	MIC: *C. zeylanicum*: 0.00625 mL/mL. *S. aromaticum*: 0.0125 mL/mL. *T. vulgaris*: 0.0125 mL/Ml. *C. aurantium:* NT. *C. bergamia*: NT. *C. sempervirens:* NT. *E. globulus*: NT. *F. vulgare:* NT. *L. angustifolia:* NT. *C. limon:* NT. *M. piperita:* NT. *R. officinalis:* NT. *S. officinalis*: NT.	MIC: C. zeylanicum: 0.0125 mL/mL; *S. aromaticum*: 0.0125 mL/mL; *T. vulgaris*: 0.0125 mL/mL. *C. aurantium:* NT. *C. bergamia*: NT. *C. sempervirens:* NT. *E. globulus*: NT. *F. vulgare:* NT. *L. angustifolia:* NT. *C. limon:* NT. *M. piperita:* NT. *R. officinalis:* NT. *S. officinalis*: NT.	/	(A), (C)	*Cinnamomum zeylanicum*
Omayma A. Eldashan	2016	Egyptian Navel Orange (*Citrus sinensis* (L.) OSBECK var. *malesy)* (Green B and L)	yes	Sabinene, Limonene	SS: *S. aureus*, *S. pyogenes*, *E. faecalis*, *K. pneumonia*, *E. coli*, *S. typhimurium*	AWD, MBD	MIC: **L:** 1.95 µL/mL. **B:** 31.25 µL/mL.	MIC: **L:** 3.9 µL/mL, **B:** 62.5 µL/mL.	/	(A)	/
Suchawadee Insawang	2019	*Lavandula stoechas 'snowman', L. stoechas 'white lavender', L. stoechas 'major', L. stoechas 'avonview',* and *L. stoechas×viridis 'St. Brelade'*	yes	***L. stoechas 'snowman'*:** Fenchone, Camphor, 1,8-Cineole. ***L. stoechas 'white lavender'*:** Fenchone, 1,8-Cineole. ***L. stoechas 'major':*** Camphor, Fenchone. ***L. stoechas 'avonview':*** Camphor Fenchone. ***L. stoechas×viridis 'St. Brelade:*** 1,8-Cineole, Fenchone	SS: *S. aureus*, *S. epidermidis*, *E. faecium*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. pyogenes*, *S. typhimurium*.	The antibacterial screening was conducted using a method modified from Şerban et al. (2011). microdilution assay and inhibition diameter.	MIC: *Lavandula stoechas 'snowman':* NR *L. stoechas 'white lavender':* NR *L. stoechas 'major':* NR *L. stoechas 'avonview':* NR *L. stoechas×viridis 'St. Brelade':* 6.25 mg/mL	MIC: *Lavandula stoechas 'snowman':* NR *L. stoechas 'white lavender':* NR *L. stoechas 'major':* NR *L. stoechas 'avonview':* 50 mg/mL *L. stoechas×viridis 'St. Brelade':* 6.25 mg/mL	PCA was performed using XLSTAT.	/	*L. stoechas×viridis 'St. Brelade'*

Table 1. Studies selected from literature and their main results. (continues)

Author	Year	EOs	GC	Main Components†	Bacterial Strains	Antimicrobical Assay	Results (MIC or MBC values from MBD assay)		Statistical Analysis	Bias	Most Active EO
							S. pyogenes	*S. aureus*			
Jeng-Dan Cha	2007	*Cryptomeria japonica* (Taxodiaceae)	yes	Elemol, terpinen-4-ol	SS: *S. mutans*, *S. sanguinis*, *S. sobrinus*, *S. ratti*, *S. criceti*, *S. anginosus*, *S. gordonii*, *Actinobacillus actinomycetemcomitans*, *F. nucleatum*, *P. intermedia*, *P. gingivalis*, *E. coli*, *S. aureus*, *S. epidermidis*, *S. pyogenes*.	MBD	MIC: 0.2 mg/mL MBC: 0.2 mg/mL	MIC: 0.2 mg/mL, MBC: 0.4 mg/mL	/	(A)	/
Muhammad Usman Hanif	2020	*Syzygium cumini* (L)	yes	/	CS: *B. subtilis, S. pyogenes, S. aureus, E. coli*.	DD, RMP, MBD	MIC: 1.1±0.6 mg/mL.	MIC: 1.3±0.3 mg/mL	STATISTICA 5.5 software.	/	/
Fadia S. Youssef	2014	*Eremophila maculata* (fresh F, fresh L, fresh S, dried S)	yes	***E. maculata* (F):** sabinene, limonene. ***E. maculata* (L):** sabinene, limonene. ***E. maculate* (Fresh S)** benzaldehyde, α-pinene, palmitic acid, b-myrcene. ***E. maculata* (dried S):** palmitic acid, benzaldehyde, spathulenol.	SS: *B. subtilis*, *S. aureus*, *S. epidermidis*, *S. pyogenes*, *S. agalactiae*, *S. aureus MRSA*, *E. coli*, *K. pneumoniae*, *P.aeruginosa*. CS: *S. aureus* MRSA.	MBD	MIC: 500 µg/mL. MBC: 1000 µg/mL.	MIC: 1000 µg/mL. MBC: 4000 µg/mL.	/	(A)	/

Note. (A) Absence of statistical analysis, (B) Absence of binomial nomenclature of aromatic plant, (C) Absence of essential oil quality analysis, (D) Absence of quantitative data. †Components present at concentrations greater than or equal to 10%, reported in descending order from the most concentrated to the least concentrated. AD: Agar Diffusion, AWD: Agar-Well Diffusion, B: branches, CS: Clinical Strains, DD: Disk Diffusion, EHEC: EnteroHemorrhagic Escherichia coli, EO: Essential Oil, F: flowers, I: inflorescences, KBP: Kirby Bauer paper, L: leaves, MDB: Micro Broth Diffusion, MRSA: Methicillin Resistant Staphylococcus aureus, NR: Not Reported, PCA: principal component analysis, PLS-DA: Partial least squares–discriminant analysis, RMP: Resazurin Microtiter-Plate, S: Stems, SS: Standard Strains, WD: Well Diffusion.

3.2 Broth micro dilutions susceptibility testing

Tables 2 and 3 show the MIC and MBC values obtained values testing the EOs against the selected strains. For most EOs, the mean values of MICs of *S. epidermidis* were not statistically different from those of *S. aureus*.

Whereas, comparing the mean values of MIC of S. *epidermidis* against those of *S. pyogenes* an important difference was observed (higher than two MIC values). In particular, *L. stoechas, T. vulgaris, S. aromaticum, P. graveolens, H. italicum, C. nobile* showed less efficacy against *S. epidermidis* strains and greater against *S. aureus*. Mean values of MBC were respectively: 4.00% v/v ± 0.00% v/v and 0.47% v/v ± 0.08% v/v (*L. stoechas*), 0.25% v/v ± 0.00% v/v and 0.04% v/v ± 0.01% v/v (*T. vulgaris*), 0.29% v/v ± 0.40% v/v and 0.04% v/v ± 0.00% v/v (*S. aromaticum*), 1.29% v/v ± 0.16% v/v and 0.06% v/v ± 0.00% v/v (*P. x graveolens*), <4,00% v/v and 1.11% v/v ± 0.16% v/v (*H. italicum*), and <4.00% v/v and 1.47% v/v ± 0.22% v/v (*C. nobile*). Regarding the MBC values, only *L. stoechas, S. aromaticum* and *P. graveolens* showed an important different activity against *S. epidermidis* and *S. pyogenes*. Mean values of MBC were respectively: <4.00% v/v and 0.92% v/v ± 0.20% v/v (*L. stoechas*), 0.71% v/v ± 0.27% v/v and 0.09% v/v ± 0.03% v/v (*S. aromaticum*), 4.00% v/v ± 0.00% v/v and 0.13% v/v ± 0.02% v/v (*P. graveolens*).

Table 2. MIC values.

Clinical strains (designation)	*C. sativum*	*C. zeylanicum*	*L. stoechas*	*C. sinensis*	*T. vulgaris*	*S. aromaticum*	*P. graveolens*	*H. italicum*	*C. nobile*
***S. epidermidis* (01)**	0.19±0.11	0.21±0.15	4.00±0.00	<4.00	0.25±0.00	0.37±0.00	1.17±0.29	<4.00	<4.00
***S. epidermidis* (02)**	0.08±0.02	0.09±0.00	4.00±0.00	<4.00	0.25±0.00	0.29±0.72	1.50±0.00	<4.00	<4.00
***S. epidermidis* (03)**	0.12±0.05	0.10±0.04	4.00±0.00	<4.00	0.25±0.00	0.23±0.04	1.21±0.26	<4.00	<4.00
Average	0.13±0.04	0.13±0.08	4.00±0.00	<4.00	0.25±0.00	0.29±0.40	1.29±0.16	<4.00	<4.00
***S. aureus* MSSA (02)**	0.14±0.04	0.06±0.00	<4.00	<4.00	0.5±0.00	0.25±0.00	0.75±0.00	<4.00	<4.00
***S. aureus* MSSA (17/01)**	0.37±0	0.06±0.00	<4.00	<4.00	0.5±0.00	0.33±0.07	1.00±0.00	<4.00	<4.00
***S. aureus* MRSA (17/01R)**	0.16±0.04	0.09±0.00	<4.00	<4.00	0.75±0.00	0.25±0.00	0.83±0.14	<4.00	<4.00
Average	0.22±0.02	0.07±0.00	<4.00	<4.00	0.58±0.00	0.27±0.04	0.86±0.08	<4.00	<4.00
***S. pyogenes* (01)**	0.03±0	0.03±0.00	0.58±0.14	4.00±0.00	0.04±0.02	0.04±0.01	0.06±0.00	1.33±0.29	1.67±0.29
***S. pyogenes* (02)**	0.03±0.00	0.03±0.00	0.58±0.14	4±1.50	0.05±0.01	0.05±0.00	0.06±0.00	1.00±0.00	1.50±0.00
***S. pyogenes* (08)**	0.03±0.00	0.03±0.00	0.25±0.00	0.53±0.23	0.035±0.01	0.03±0.00	0.06±0.00	1.00±0.00	1.25±0.43
Average	0.03±0.00	0.03±0.00	0.47±0.08	2.84±0.80	0.04±0.01	0.04±0.00	0.06±0.00	1.11±0.16	1.47±0.22

Note. Values are expressed as mean ± standard deviation (% v/v)

Table 3. MBC values.

Clinical strains (designation)	*C. sativum*	*C. zeylanicum*	*L. stoechas*	*C. sinensis*	*T. vulgaris*	*S. aromaticum*	*P. graveolens*	*H. italicum*	*C. nobile*
***S. epidermidis* (01)**	0.50±0.18	0.05±0.01	<4.00	<4.00	0.63±0.18	0.75±0.00	<4.00	<4.00	<4.00
***S. epidermidis* (02)**	0.28±0.13	0.13±0.00	<4.00	<4.00	0.63±0.18	0.63±0.18	4.00±0.00	<4.00	<4.00
***S. epidermidis* (03)**	0.38±0.00	0.11±0.02	<4.00	<4.00	0.63±0.18	0.75±0.53	4.00±0.00	<4.00	<4.00
Average	0.39±0.09	0.10±0.01	<4.00	<4.00	0.63±0.18	0.71±0.27	4.00±0.00	<4.00	<4.00
***S. aureus* MSSA (02)**	0.28±0.04	0.31±0.00	<4.00	<4.00	0.50±0.00	0.63±0.17	<4.00	<4.00	<4.00
***S. aureus* MSSA (17/01)**	0.38±0.00	0.38±0.00	<4.00	<4.00	0.81±0.26	0.88±0.17	<4.00	<4.00	<4.00
***S. aureus* MRSA (17/01R)**	0.38±0.00	0.5±0.18	<4.00	<4.00	2.00±0.00	1.75±1.06	<4.00	<4.00	<4.00
Average	0.35±0.02	0.40±0.10	<4.00	<4.00	1.10±0.15	1.09±0.51	<4.00	<4.00	<4.00
***S. pyogenes* (01)**	0.11±0.44	0.14±0.00	1.00±0.00	<4.00	0.11±0.23	0.11±0.023	0.13±0.00	3.00±0.00	2.50±0.70
***S. pyogenes* (02)**	0.03±0.00	0.05±0.00	1.25±0.35	<4.00	0.16±0.44	0.10±0.06	0.16±0.04	1.00±0.00	1.50±0.70
***S. pyogenes* (08)**	0.03±0.00	0.03±0.00	0.50±0.00	1.06±0.61	0.05±0.10	0.05±0.00	0.11±0.02	1.00±0.00	2.00±1.40
Average	0.06±0.25	0.07±0.00	0.92±0.20	<4.00	0.11±0.17	0.09±0.03	0.13±0.02	1.67±0.00	2.00±0.40

Note. Values are expressed as mean ± standard deviation (% v/v)

3.3 Satisfaction test

Table 4 shows data obtained from the satisfaction test conducted on 100 volunteers. Both men and women preferred Mix1 (relative percentages equal to 34.21% and 40.32%, respectively). Furthermore, all formulations characterized by the presence of *P. graveolens* were most preferred (absolute percentages were: Mix1=38%, Mix3=34% and Mix4=20%), while the lowest preference was expressed for Mix2 (8%) characterized by *L. stoechas* only.

Table 4. Satisfaction test carried out on the four formulated mixes.

	Age	Mix 1	Mix 2	Mix 3	Mix 4	Total
Men	18-25	4	2	5	3	14
	26-35	3	1	3	3	10
	36-50	1	/	1	2	4
	>50	5	1	2	2	10
	Total	13	4	11	10	38
	%	34.21%	10.52%	28.94%	26.31%	/
Women	18-25	7	1	8	3	19
	26-35	7	2	4	2	15
	36-50	4	1	3	2	10
	>50	7	/	8	3	18
	Total	25	4	23	10	62
	%	40.32%	6.45%	37.10%	16.13%	/
Total	%	38%	8%	34%	20%	100%

3.4 Chemical analysis of EOs

Table 5 shows the chemical composition of EOs performed by the GC-MS technique. In total, sixty-one components were identified of which thirty-nine in *P. graveolens* EO and thirty-one in *L. stoechas* EO. Citronellol (21.4%) followed by cis-geraniol (11.5%) and citronellyl formate (11.1%) were the most abundant compounds in *P. graveolens EO,*

while 1,8-cineole (58.6%) and fenchone (23.5%) were the detected compounds with the highest relative percentages in *L. stoechas* EO.

3.5 Formulation with *Aloe vera* gel

Three types of *A. vera* gel modified with Mix1 were made *ex novo* as described in paragraph 2.6 (Figure 2). The three gels differed in the concentration of carboxymethylcellulose. Depending on the consistency, the gel made with 2% CMC was selected. The formulation made starting from the commercial gel was prepared simply by adding and homogenizing the Mix1 to the gel. Change in the rheology of commercial *Aloe vera* gel was observed after the addition of EOs. Immediately after the formulation and after two weeks, the two formulations were visually homogeneous without any separation phase.

3.6 Chemical composition of formulations

The volatile chemical profile of formulations based on *A. vera* modified with the addition of Mix1 was obtained using the SPME-GC-MS techniques. As shown in Table 6, in both gel formulations, "*Ex novo*" and "*Commercial*", forty-five components were revealed, and they had an almost overlapping qualitative-quantitative composition characterized by fenchone (43.1%; 42.8%) as the main

Figure 2. Aloe gel obtained from the edible juice. (a) 2%, (b) 3% and (c) 4% of carboxymethylcellulose.

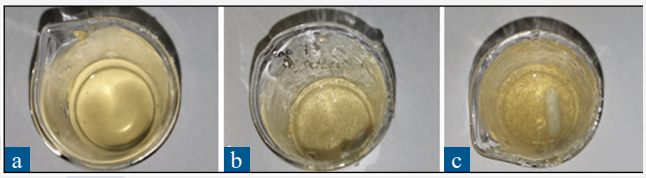

Table 5. Chemical composition of P. graveolens and L. stoechas EOs.

N°	Component†	LRI‡	LRI §	P.g.¶ (%)	L s.* (%)
1	α-pinene	941	943	0.1	0.5
2	camphene	948	946	-	0.3
3	β-pinene	972	969	-	0.1
4	β-myrcene	986	983	0.2	tr
5	α-terpinene	1018	1019	-	0.4
6	p-cymene	1021	1026	-	0.2
7	1,8-cineole	1028	1031	-	59.6
8	γ-terpinene	1059	1062	-	tr
9	cis-linalooloxide	1072	1070	0.2	-
10	cis-sabinene hydrate	1076	1074	-	0.3
11	trans-linalooloxide	1089	1091	0.1	-
12	linalool	1093	1092	0.2	-
13	fenchone	1096	1094	-	23.5
14	chrysanthenone	1105	1103	-	0.1
15	fenchol, exo-	1112	1109,8	-	0.3
16	rose-oxide	1115	1112.7	1.0	-
17	*p*-menthone	1133	1131	2.7	-
18	*I*-menthone	1138	1146	0.4	-
19	camphor	1152	1151	-	9.2
20	myrtenal	1182	1176	-	0.1
21	α-terpineol	1179	1183	0.4	0.2
22	fenchyl acetate	1212	1208	-	0.3
23	(-)-carvone	1222	1218	-	0.2
24	citronellol	1240	1235	41.4	-
25	cis-geraniol	1242	1236	11.5	-
26	linalyl acetate	1255	1252	-	0.1
27	bornyl acetate	1271	1268	-	1.3
28	lavandulyl acetate	1276	1271	-	0.1
29	citronellyl formate	1277	1275	11.1	-
30	myrtenyl acetate	1310	1307	-	1.6
31	geranyl formate	1314	1312	3.1	-
32	α-terpinyl acetate	1341	1344	-	tr
33	citronellol acetate	1355	1351	0.6	-
34	nerol acetate	1368	1363	0.4	-
35	ylangene	1379	1376	0.1	-
36	α-cubebene	1383	1381	0.2	tr
37	α-copaene	1388	1385	0.7	-
38	γ-elemene	1426	1423	0.2	-
39	β-caryophyllene	1430	1426	2.3	0.1
40	(-)-β-bourbonene	1435	*	1.4	-
41	citronellyl propionate	1440	1445	0.8	-
42	guaia-6,9-diene	1450	*	0.3	-
43	β-ylangene	1454	*	0.3	-
44	α-guaiene	1460	1458	1.0	-
45	aromadendrene	1463	1460	0.5	0.1
46	γ-muurolene	1490	1486	0.7	0.2
47	β-bisabolene	1508	1502	1.4	-
48	valencene	1521	1515	0.9	-
49	δ-cadinene	1533	1530	0.4	0.5
50	α-muurolene	1538	1537	2.2	tr
51	β-maaliene	1542	*	0.6	-
52	selina-3,7(11)-diene	1549	1540	-	0.2
53	2-phenylethyl tiglate	1593	1589	1.6	-
54	ledene oxide	1596	*	0.4	-
55	viridiflorol	1599	1594	-	0.2
56	δ-cadinol	1618	1620	-	tr
57	cubenol	1630	1634	0.7	-
58	citronellyl tiglate	1672	1667	0.5	-
59	γ-eudesmol	1680	*	4.6	-
60	citronellol heptnoate	1822	1819	0.1	-
61	palmitic acid	1957	1954	1.1	-
	SUM			96.4	99.7

†the components are reported according to their elution order on apolar column; ‡Linear Retention Indices measured on apolar column; §Linear Retention indices from literature; *LRI not available; P.g. ¶: Percentage mean values of "P. graveolens"components (%); L.s. *: Percentage mean values of "L. stoechas"components; - Not detected; tr: traces (mean value <0.1%).

compound followed by camphor (15.0%; 16.4%) and 1,8-cineole (11.6%; 12.7%) respectively. All other components were present at concentrations below 5%.

3.7 Antimicrobial activities of the formulations

To assess whether the antimicrobial activity of *A. vera* gel formulations was maintained, broth micro dilutions tests were repeated with the strains responsible for non-bullous impetigo. Table 7 summarizes the qualitative data observed after inoculation on solid agar. The gel obtained from *A. vera* juice maintains the differential effectiveness, exerting a greater inhibiting effect on *S. pyogenes* strains compared to those on *S. epidermidis*. On the contrary, the commercial *A. vera* gel does not show the desired effectiveness. Furthermore, tests made with gels alone, without the addition of Mix1, show that the gel obtained from *A. vera* juice does not show antibacterial activity, while the commercial one shows a basal antibacterial activity against all bacterial strains, especially against *S. pyogenes*.

DISCUSSION

Literature revision carried out in this manuscript aimed at identifying articles developed to study the effectiveness of EOs in the treatment of impetigo or their antimicrobial activity against bacteria responsible for this disease. The analysis of articles published until now confirm the absence of human clinical trial and highlight the scientific literature of *in vitro* studies to evaluate the antimicrobial activity of EOs against the strains responsible for impetigo (*S. aureus* and *S. pyogenes*). Several articles showed some fragilities considered important, such as the lack of a chemical analysis, which allows to identify the chemotype of the EO used; the absence of scientific nomenclature, which makes it possible to identify with absolute certainty the aromatic species from which the EO was obtained. However, the selected studies made possible to identify 14 EOs active on both pathogenic strains. Eight of these EOs do not belong to the Italian phytotherapeutic tradition and therefore were excluded from the study, while the remaining six (*T. vulgaris, C. sativum, C. zeylanicum*, *C. sinensis*, *L. stoechas*, *S. aromaticum*) were chosen. Furthermore, three other EOs have been added thanks to their well-known skin tropism and antimicrobial activity (*P. graveolens*, *C. nobile*, *H. italicum*)[7-9]. All eight EOs selected are widely used in the European integrated medical tradition, and some of these have been monographed by the EMA and reported as traditional use[10-14].

The selected EOs were microbiologically tested to identify those showing lower MICs values towards pathogenic strains (*S. aureus* and *S. pyogenes*) than that observed against strains commonly associated with the skin microbiota, such

Table 6. Chemical composition of formulations based on *A. vera* modified with Mix1.

N°	COMPONENT†	LRI‡	LRI§	*Form. A*¶(%)	*Form. B* *(%)
1	α-pinene	938	943	0.9	1.3
2	camphene	941	946	0.3	0.9
3	α-fenchene	942	946	0.1	0.2
4	β-myrcene	985	983	0.3	0.4
5	1,8-cineole	1020	1022	11.6	12.7
6	p-cymene	1023	1026	0.4	0.5
7	cis-β-ocimene	1035	1033	0.8	0.8
8	β-terpinene	1040	1036	0.1	0.1
9	α-ocimene	1045	1042	0.1	0.1
10	trans-β-ocimene	1052	1048	0.1	0.1
11	isoterpinolene	1070	1068	0.1	0.1
12	cis-sabinene hydrate	1062	1069	0.5	0.6
13	fenchone	1098	1094	43.1	42.8
14	chrysanthenone	1106	1103	0.2	7.0
15	fenchol, exo-	1112	1109,8	0.6	0.6
16	rose-oxide	1115	1112,7	0.3	0.3
17	*p*-menthone	1130	1131	1.0	0.5
18	*I*-menthone	1140	1146	1.5	1.1
19	camphor	1153	1151	15.0	16.4
20	borneol	1157	1154	0.1	0.2
21	myrtenal	1182	1176	0.2	0.2
22	myrtenol	1191	1179	0.1	0.2
23	α-terpineol	1186	1183	tr	0.3
24	fenchyl acetate	1212	1208	1.1	2.1
25	(-)-carvone	1220	1218	0.3	0.1
26	citronellol	1241	1235	4.7	0.2
27	lavandulol acetate	1275	1271	0.2	0.1
28	citronellyl formate	1277	1275	3.8	2.2
29	bornyl acetate	1286	1283	3.3	1.9
30	myrtenyl acetate	1312	1307	3.0	1.9
31	citronellol acetate	1355	1351	0.2	0.1
32	α-cubebene	1386	1381	0.7	tr
33	cis-muurola-3,5-diene	1455	1450	0.1	0.1
34	(-)-β-bourbonene	1452	*	0.5	0.4
35	β-caryophyllene	1432	1426	0.9	0.2
36	citronellyl propionate	1440	1445	0.2	0.2
37	β-ylangene	1443	*	0.1	0.1
38	humulene	1455	1454	0.2	0.2
39	aromadendrene	1459	1460	0.4	0.2
40	γ-gurjunene	1483	1479	0.3	0.1
41	β-eudesmene	1486	1483	0.4	0.9
42	δ-cadinene	1528	1530	1.4	1.1
43	α-muurolene	1531	1537	0.4	0.2
44	selina-3.7(11)-diene	1543	1540	0.2	0.2
45	γ-eudesmol	1555	*	0.2	0,1
	SUM			100.0	100.0

†the components are reported according to their elution order on apolar column; ‡Linear Retention Indices measured on apolar column; §Linear Retention indices from literature; *LRI not available; Form. A. ¶: Percentage mean values of "Ex novo" gel components (%); Form. B. *: Percentage mean values of "Zuccari" gel components; tr: traces (mean value <0.1%).

as *S. epidermidis*. The above to develop formulations aimed at rebalancing the skin microbial imbalance present in both bullous and non-bullous impetigo. Microbiological data (Tables 2-3) indicate that no EOs studied has the desired effect on the *S. aureus* - *S epidermidis* balance, while the OEs of *L. stoechas, T. vulgaris, S. aromaticum, P. graveolens, H. italicum, C. nobile* show MICs sufficiently spaced to assume a rebalancing effect towards strains responsible for non-bullous impetigo (*S. pyogenes* – *S. epidermidis*).

Of the OEs potentially useful for the treatment of non-bullous impetigo, those of *L. stoechas* and *P. graveolens* were chosen to continue the research as they are characterized by pleasant floral notes useful in skin formulations, and they are also widely used in several treatments of traditional aromatic medicine.

For a long time, Lavender EO (including *L. stoechas* EO) has been used in traditional medicine for skin treatment, just think that World War I soldiers used it empirically for the treatment of war wounds as a healing and re-epithelizing agent[15], but also as an anti-inflammatory, anti-allergic, anti-nociceptive and antimicrobial for cutaneous treatments[15,16]. Similarly, the traditional use of *P. graveolens* EO is reported for its antimicrobial, anti-pain and restorative activity in the treatment of cracked and sore nipples during breastfeeding9. Sabzghabaee AM *et al*, documented the possible use of gels formulated with 1% of *P. graveolens* EO for the treatment of stomatitis, while Mahboubi M *et al* reported the effectiveness of a cream characterized by a formulation containing this OE for the treatment of diabetic wounds[17,18].

Considering that the pleasant scent of a cutaneous formulation is essential to allow patients to constantly apply it without interruption of therapy, four formulations were in lab-produced and submitted to the olfactory evaluation of 100 volunteers. Two of the four formulations were characterized by a single EO at concentrations of four (*L. stoechas* 2% v/v) and eight (*P. graveolens* 0.5% v/v) times higher than the mean values of MIC observed against *S. pyogenes*. Whereas the other two formulations were a combination of the two EOs and varied only for the concentration of *L. stoechas* for which, given the balsamic-camphorated scent conferred by the presence of 1.8 cineole and camphor, a concentration equal to 2MIC or 4MIC was considered. Furthermore, all four formulations were in line with the International Fragrance Association (IFRA) guidelines concerning the concentrations of warned chemical compounds[19]. Specifically, the warnings and restrictions planned for products included in category 3 (products applied to the face/body with fingertips) were respected. Citronellol and geraniol were among the restricted compounds. The first must not exceed the concentration of 13%, while the second that of 5.1%. Both compounds are present in the *P. graveolens* EO at concentrations of 41.4% and 11.5%, respectively. Therefore, even if using a concentration of *P. graveolens* EO equal to 0.5% v/v (8 times higher than the mean value of MIC of *S. pyogenes*), the two compounds will always be below the threshold level.

Data obtained from the satisfaction survey show that both groups preferred Mix1 (38%) and rejected Mix2 as the least popular fragrance (8%), probably due to the strong camphor scent of *L. stoechas*. Instead, the combination of *P. graveolens* OE with the minimum concentration of lavender has made the fragrance pleasant for both women, who generally prefer floral notes, and men more likely to choose strong fragrances.

To facilitate topical applications, it is important to make treatments more performing for patients. A formulation for cutaneous treatment should be easy to use, easily absorbed by the skin, not greasy and pleasant to the touch. These characteristics are typical for the *A. vera* gels, whose use is already widespread in the world not only for the listed aspects but also for its many therapeutic properties, including anti-inflammatory effects that make it suitable for the treatment of wounds and skin diseases as well as for the promotion of the repair of damaged skin[20]. Furthermore, the *A. vera* gel acts as an enhancer of the transdermal penetration of the active ingredients placed on human skin[21], making it a suitable vehicle for various active ingredients, including EOs.

For the above, it was decided to formulate Mix1 with *A. vera* gel. To obtain the formulations, both a decorticated juice and a commercial gel of *A. vera* were chosen. The first was treated to obtain a new gel formulation, while the second was formulated simply by adding the Mix1 as described in paragraph 2.6. We have chosen to make this comparison because, in phytotherapeutic practices, a specialist doctor can advise the patient to combine two natural products before

Table 7. Antimicrobial effectiveness of *A. vera* gel and EOs formulations based on *A. vera* gel

	A. vera juice		*A. vera* commercial gel	
Clinical strains (designation)	gel w EOs	Gel w/o EOs	gel w EOs	Gel w/o EOs
S. pyogenes (01)	++	++++	+	+
S. pyogenes (02)	++	++++	+	+
S. pyogenes (08)	++	++++	+	+
S. epidermidis (01)	++++	++++	-	+++
S. epidermidis (02)	++++	++++	+++	++++
S. epidermidis (03)	++	+++	-	++

applying them topically. However, there is no scientific evidence in the literature for the equality of the two topical treatments.

Microbiological tests developed with *A. vera* gels modified with the addition of Mix1 show that only the *ex novo* prepared gel maintains the differential activity on physiological and pathological strains. Indeed, this gel shows antimicrobial effectiveness on *S. pyogenes* and is almost inactive on *S. epidermidis*. In comparison, the modified commercial gel loses this activity. The reason for this different behavior is not to be found in the chemistry of formulations because, as shown in table 6, the two formulations are characterized by the same active compounds present in almost overlapping concentrations. On the contrary, the explanation is provided by the result of microbiological tests performed on *A. vera* gels alone without the addition of the Mix1. As shown in table 7, the gel obtained from *A. vera* juice has no antimicrobial activity, while the commercial gel alone inhibits bacterial growth. This data can be explained considering that commercial gels have the addition of preservatives to extend the shelf-life of the product, while the *A. vera* juice is edible; therefore, it is pasteurized but lacks preservatives that interfere with the activity of EOs.

Therefore, if on one hand the *L. stoechas* and *P. graveolens* EOs are good candidates for the local treatment of impetigo, on the other hand it is important that formulations containing EOs are prepared masterfully and not at the moment to avoid the use of formulations for which the effectiveness is not scientifically documented.

CONCLUSIONS

Starting from literature data, EOs effective against microbial strains responsible for bullous and non-bullous impetigo were selected. Microbiological tests developed to select EOs with MIC values against pathogenic strains lower than those against saprophytic ones, allowed to select EOs potentially effective only on non-bullous impetigo. L. stoechas and P. graveolens EOs were chosen for the development a mixture that, through an olfactory satisfaction test, was selected as pleasant for both women and men. To develop an easily absorbed and non-greasy formulation, *Aloe vera* was chosen as the vehicle for EOs. To evaluate whether a masterful formulation or a home-made formulation were better, one *A. vera* gel was produced ex novo starting from edible juice of *A. vera*, while another formulation was made starting from a commercial gel. Only the formulation obtained from the juice of *A. vera* kept the expected efficacy, as the formulations obtained from commercial gels contain preservatives that interfere with the antimicrobial activity of EOs. To our knowledge, this study identifies for the first time a mixture potentially usable in the topical treatment of impetigo and, at the same time, highlights the importance of avoiding the use of formulations made at the moment by mixing commercial products because these formulations lack scientific studies concerning their efficacy and could compromise the success of the therapy.

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Declaration of interests

The authors declare that they have no conflict of interest.

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