



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Antimicrobial activity of certain natural-based plant oils against the antibiotic-resistant acne bacteria



Ahmed Esmail ^{a,b,1}, Mervat G. Hassan ^{b,1}, Mahmoud M. Amer ^b, Soheir Abdelrahman ^c, Ahmed M. Hamed ^d, Hagar A. Abd-raboh ^b, Mohamed F. Foda ^{a,e,*}

^a State Key Laboratory of Agricultural Microbiology, College of Plant Science and Technology, College of Life Science and Technology, College of Science, Huazhong Agricultural University, Wuhan 430070, China

^b Botany and Microbiology Department, Faculty of Science, Benha University, Qalubia Governorate 13511, Egypt

^c Clinical Pathology Department, Faculty of Medicine, Benha University, Qalubia Governorate 13511, Egypt

^d Dermatology Department, Faculty of Medicine, Benha University, Qalubia Governorate 13511, Egypt

^e Department of Biochemistry, Faculty of Agriculture, Benha University, Moshtohor, Toukh 13736, Egypt

ARTICLE INFO

Article history:

Received 12 October 2019

Revised 9 November 2019

Accepted 10 November 2019

Available online 20 November 2019

Keywords:

Acne vulgaris

Multidrug-resistant bacteria

Natural oil

Egypt

Minimum inhibitory concentration

GC–MS

ABSTRACT

The unceasing emerging of multidrug-resistant bacteria imposes a global foremost human health threat and discovery of new alternative remedies are necessity. The use of plant essential oil in the treatment of many pathogenic bacteria is promising. Acne vulgaris is the most common skin complaint that fears many people about their aesthetic appearance. In this work we investigated the antibacterial activity of some plant oils against acne-inducing bacteria. Three bacterial isolates were identified from Egypt, biochemically and by means of 16s rRNA gene typing, and were designated as *Staphylococcus aureus* EG-AE1, *Staphylococcus epidermidis* EG-AE2 and *Cutibacterium acnes* EG-AE1. Antibiotic susceptibility test showed resistance of the isolates to at least six antibiotics, yet they are still susceptible to the last resort Vancomycin. *In vitro* investigations of eleven Egyptian plant oils, identified tea tree and rosemary oils to exhibit antibacterial activity against the antibiotic-resistant acne isolates. Inhibition zones of 15 ± 0.5 , 21.02 ± 0.73 and 20.85 ± 0.76 mm was detected when tea tree oil applied against the above-mentioned bacteria respectively, while inhibition zones of 12.5 ± 1.5 , 15.18 ± 0.38 and 14.77 ± 0.35 mm were detected by rosemary oils. Tea tree and rosemary oils exhibited bacteriostatic and bactericidal activity against all the strains with MICs/MBCs ranging between 39–78 mg/L for tea tree oil and 39–156 mg/L for rosemary oil. All the isolates were killed after 4 and 6 h upon growing with 200 mg/L of tea tree and rosemary oils, respectively. Additionally, gas chromatography mass spectrometry (GC/MS) profiling identified and detected a variable number of antimicrobial compounds in both oils.

© 2019 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Acne vulgaris is considered the most common skin complaint that worries many people about their aesthetic appearance and generally impaired quality of life is well informed (Dunn et al., 2011; Gieler et al., 2015; Sarkar et al., 2016). The microbiology

and therapy of acne vulgaris constitute the foremost thrust of current research in the clarification of the causal pathogens and new modes of treatments. *Cutibacterium acnes* (formerly known as *Propionibacterium acnes* (Scholz and Kilian, 2016) are aerotolerant anaerobic gram-negative bacilli. It was originally identified as *Bacilli acnes* (Gilchrist, 1900), because of its ability to generate propionic acid it was named later as *P. acnes*. However recently biochemical and genomic studies resulted in a taxonomical reclassification of *P. acnes* and a novel genus *Cutibacterium* was designated for the cutaneous species (Dréno et al., 2018; Scholz and Kilian, 2016).

Although *C. acnes* bacteria normally commensal on the surface of healthy skin, they anaerobically proliferate deeply within follicles and pores (Dréno et al., 2018) by metabolizing sebum triglycerides from the surrounding skin tissues and use it as a primary

* Corresponding author.

E-mail address: m.frahahat@fagr.bu.edu.eg (M.F. Foda).

¹ These two authors contributed equally to this manuscript.

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

source of energy and nutrients. On the contrary, *Staphylococcus epidermidis* is a gram-positive, facultative anaerobic organism, usually involves superficial infections within the sebaceous unit (Burkhart et al., 1999). Different treatment strategies are to be considered including decreasing hyperkeratinization, microbial colonization, sebum production and inhibiting the inflammation (Zaenglein et al., 2016) which is selected on a case-by-case basis. Moreover, emerging strategies considering the modification of the existing drugs and the progress of new medications targeting the regulatory pathways involved in acne pathophysiology (Tuchayi et al., 2015).

Topical antibiotics and/or chemical peeling agents are being used currently in acne vulgaris treatments, however oral antibiotics, retinoids, or hormones are prescribed daily (Pineau et al., 2019). The most frequently used oral remedies include the tetracyclines (tetracycline, minocycline, and doxycycline), trimethoprim/sulfamethoxazole, and macrolides (erythromycin and azithromycin) (Farrah and Tan, 2016; Leyden, 2003). Acne pharmacotherapies are effective but are associated with adverse events such as mood disorders, antibiotic-resistance and can leave the skin dry with irritation feelings (Eichenfield, 2015). A most important drawback of current therapies is that daily intake of antibiotics, resulted in developing multidrug resistant bacteria (Van den Bergh et al., 2016). Furthermore, many countries have identified increasing the resistance of Acne bacteria to topical macrolides (Walsh et al., 2016) and active antibiotic gel (Mills et al., 2002).

The emergence of multidrug-resistant bacteria has become a foremost health threat, afterward, interests in alternative medicine have been increased rather than traditional antibiotic medications for skin conditions (Neamsuvan et al., 2015). The antibacterial potential of lipids has been long recognized (Nikkari, 1974). A previous study on the therapeutic potential of free fatty acids (FFAs) against methicillin-resistant *Staphylococcus aureus* (MRSA) showed that only oleic acid (C18:1, *cis*-9) disrupted the MRSA cell wall at small doses (Chen et al., 2011).

In Egypt, most of the former studies on Acne vulgaris focused only on the prevalence and the clinical features of patients with Acne, there is no actual search for herbal therapy. Accordingly, we have designed this study to isolate the acne-inducing bacteria and to shed light on the efficacy of using the essential oils of some of the Egyptian domestic medicinal herbs in the treatment.

2. Materials and methods

2.1. Patients and sample collection

Pathological diagnosis examination of Fifty-five patients (20 males and 35 females) at the adolescent age of 17–25 years old, attending Benha university hospital dermatology clinic, with potential inflammatory acne skin lesions, from 2016 to 2018, were included in the study. Patients showed overproduction of sebum and follicular hyperkeratosis which resulted in the development of microcomedones. Inflammatory and non-inflammatory acne lesions were punctured with a hypodermic needle and the contents were collected with a comedone extractor under complete aseptic condition. Patients with pregnancy, Drug addicts, patients with endocrinal problems, adrenal dysfunction and those taking contraceptives were excluded. Basic clinical information (including age, gender, age of onset and duration of disease) and previous consultation records were obtained at the time of patient registration. Three samples were taken from each patient and were then inserted into Thioglycolate broth tube as a transport media and sent immediately to the Microbiology and Immunology department (Benha University, Egypt). The samples were inoculated onto

sheep blood agar medium at 37 °C under both aerobic and anaerobic conditions for 2–7 days.

2.2. Biochemical identification and 16s rRNA gene sequencing

The collected samples were grown both aerobically and anaerobically, only 35 out of the 55 patients showed bacterial growth on the selective media. Biochemical properties (Table S1, [Supplementary data](#)) identified two different groups of the aerobic isolates were found to be gram-positive cocci, non-motile and were arranged in grape-like clusters. Anaerobic isolates were gram-positive polymorphic bacilli and were able to ferment glucose with acid production, the biochemical profile of the anaerobic bacteria (as shown in Table S1) was consistent with those of formerly known as *Propionibacteria*.

The partial 16S rRNA gene sequencing was used to confirm the biochemical identification. Universal 16s rRNA primers 8F (5'-AGAGTTTATCCTGGCTCAG-3') and 1492R (5'-GACGGGCGGTGTGTRCA-3') (Turner et al., 1999) were used to amplify these genes in the aerobic isolates, however, PAS9 (5'-CCCTGCTTTGTGGGGTG-3'), PAS10 (5'-CGCCTGTGACGAAGCGTG-3') and PAS11 (5'-CGACCCCAAAGAGGGGAC-3') (Nakamura et al., 2003) amplified the 16s rRNA of anaerobic bacteria. Sanger sequences were generated at Sigma company Genomics Core Facility (Cairo, Egypt) using an ABI PRISM 3730xl DNA Analyzer (Life Technologies Corporation, CA). Sequences similarity were conducted using the BLASTn search at the NCBI website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), phylogenetic and molecular evolutionary analyses were conducted using MEGA software version X (Kumar et al., 2018).

2.2.1. Isolates accession numbers

Staphylococcus aureus EG-AE1, *Staphylococcus epidermidis* EG-AE2 and *Cutibacterium acnes* EG-AE1 were deposited in the GeneBank database and were assigned the accession numbers MK934843.1, MK937638.1 and MN336167.1 respectively.

2.3. Antibiotic susceptibility test

Antibiotics susceptibility testing was performed using the disc diffusion method (Biemer, 1973) for the following antibiotics (Oxoid, UK); Azithromycin (AZM 15 µg), Amoxicillin + Clavulanic Acid (AMC 20 + 10 µg), Ciprofloxacin (CIP 1 µg), Erythromycin (E 15 µg), Penicillin-G (P 10 µg), Tobramycin (TOB 10 µg), Tetracycline (TEC 30 µg), Cephalexin (CN 30 µg), Clindamycin (DA 2 µg), Chloramphenicol (CL 30 µg), Amikacin (AK 30 µg), Trimethoprim-sulfamethoxazole (SXT 25 µg), Rifampin (RA 5 µg) and Vancomycin (V 30 µg). The results were inferred according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Wayne, 2019).

2.4. Antibacterial activity of some plant oils

Agar well diffusion method (Balouiri et al., 2016; Magaldi et al., 2004; Valgas et al., 2007) was used to evaluate the antimicrobial activity of the following plant oils; Tea tree, Cinnamon, Rosemary, Cactus, Lavender, Basil, Lemon, Thyme, Parsley, Almond and Lupine. The crude oils were purchased from the commercial markets in Egypt. Nutrient agar plates were inoculated by spreading a 100 µl of the actively grown bacteria. A sterile cork was used to punch holes with a diameter of 6–8 mm and 10 µl of the tested oils (200 mg/L) was introduced into the well. The inoculated agar plates were incubated at 37 °C for 24 h. Diameters of the inhibition zones were measured as (mm).

2.5. MICs and MBCs of tea tree and rosemary oils

The MIC of tea tree and rosemary oils were determined using a broth assay (Klančnik et al., 2010) in 96-well microtiter plates (Sigma Aldrich, USA). Fresh cultures of the tested isolates were prepared in Brain Heart Infusion (BHI) broth, inocula of concentrations 2×10^7 cfu/ml were used. A two-fold dilution series of tea tree and rosemary oils were prepared in 1% DMSO to yield final concentrations ranging from 5000 mg/L to 9.7 mg/L. Chloramphenicol was employed as a positive control. After 18 h and 48 h (for *C. acnes*), the optical density at 600 nm was measured with a microplate reader (680 XR reader, Bio-Rad). Bacterial growth was confirmed by adding 10 μ l of a sterile 0.5% aqueous solution of triphenyltetrazolium chloride (TTC, Sigma–Aldrich) and incubating at 36 °C for 30 min. The viable bacterial cells reduced the yellow TTC to pink/red 1,3,5-triphenylformazan (TPF). All assays were performed in triplicate. Streaks were taken from the two lowest concentrations of each oil concentration exhibiting invisible growth and were sub-cultured onto Blood agar media. The plates were incubated at 37 °C for 24–48 h, then inspected for bacterial growth in corresponding to both the oils. MBC was taken as the concentration of the oil that did not exhibit any bacterial growth.

2.6. Time-kill kinetics

This experiment was performed as described previously (May et al., 2000). Prior to the experiment, bacteria were incubated on IsoSensitest broth (ISB; Oxoid, Basingstoke, UK) for 90 mins at 37 °C to ensure that all the bacteria were in the action in the logarithmic growth phase. The initial bacterial concentration was measured as cfu/ml. Each isolate was inoculated into three flasks, one as growth control, one for each type of oil. All flasks were incubated at 37 °C while shaking at 150 rpm. Aliquots were taken at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 h and viable colony counts on blood agar were calculated as cfu/ml.

2.7. Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis of the major essential constituents of the oils of tea tree and rosemary was carried out using the GC Agilent Technologies (Santa Clara, CA) using 6890 N apparatus equipped with the split-splitless injector attached to HP-5 fused silica column (30 m $\times$ 0.25 mm, film thickness 0.25 μ m) and interfaced to flame-ionization detector (FID). 1 μ l of ethanol diluted oil was injected under the following operational conditions: carrier gas was He (1 ml/min); the temperatures were set as follows: injector at 250 °C and detector at 280 °C, while the column temperature was linearly programmed from 40 °C to 260 °C at 4 °C/min. Constituents were identified by comparison of their spectral outcomes with those available on MS libraries (NIST/Wiley), and by comparison of their retention profile (calibrated AMDIS), with the data from the literature (Adams, 2005).

2.8. Ethical approval

Prior to the study, a formal consensus was obtained from all patients at Benha University Hospital.

3. Results and discussion

3.1. Isolation, biochemical characterization and 16S rRNA typing of *acne vulgaris*

The collected samples were grown both aerobically and anaerobically, only 35 out of the 55 patients showed bacterial growth on

the selective media. Biochemical properties (Table S1, [Supplementary data](#)) identified two different groups of the aerobic isolates were found to be gram-positive cocci, non-motile and were arranged in grape-like clusters. Anaerobic isolates were gram-positive polymorphic bacilli and were able to ferment glucose with acid production, the biochemical profile of the anaerobic bacteria was consistent with those of formerly known as *Propionibacteria*.

BLASTn alignments and phylogenetic tree analysis ([Fig. 1](#)) of the assembled 16S rRNA gene sequences showed highest similarities with the previously partially sequenced 16S rRNA of *Staphylococcus aureus*, *S. epidermidis* and *Cutibacterium acnes* on the NCBI website. Bacteria were designated as *Staphylococcus aureus* strain EG-AE1, *Staphylococcus epidermidis* strain EG-AE2 and *Cutibacterium acnes* Strain EG-AE1 and they were detected at frequencies of 21%, 37% and 42% of the subjects in acne patients respectively. Previous investigations on *acne vulgaris* showed that *S. epidermidis* and *C. acnes* are the most significant major groups in patients with acne (Burkhart et al., 2015, 1999; Dhillon and Varshney, 2013), though there are many evidences suggesting potential pathogenic roles of *S. aureus* in *acne vulgaris* inflammation via stimulation of the Toll-like receptor (TLR) 2 and local immunosuppression (Khorvash et al., 2012; Totté et al., 2016).

3.2. Antibiotic susceptibility testing

Qualitative results from the antibiograms ([Table 1](#)) showed that both *S. aureus* EG-AE1 and *S. epidermidis* EG-AE2 were resistant to at least eight antibiotics (Azithromycin, Erythromycin, Penicillin-G, Tetracycline, Clindamycin, Amikacin, Trimethoprim-sulfamethoxazole and Rifampin). *C. acnes* EG-AE1 were found to be resistant to (Azithromycin, Erythromycin, Tetracycline, Clindamycin, Amikacin and Trimethoprim-sulfamethoxazole). Yet, the three isolates were susceptible to Chloramphenicol and Vancomycin.

Antibiotic resistant bacteria have reached a worrying stage all over the world, mainly in the developing countries (Amann et al., 2019; Blomberg et al., 2004; Kunin, 1993; Omulo et al., 2015; Slack, 1989; Williams et al., 2018). Egypt is one of the countries that have less severe restrictions on antibiotic prescription (Awad et al., 2016; Hassan et al., 2011; Ibrahim, 2012; Khalil, 2012; Sabry et al., 2014; Sobhy et al., 2012), the emergence of multidrug-resistant bacteria is unceasing very quickly. A major reason is that antibiotics can be purchased directly from drug retailers and pharmacies without a prescription (Sosa et al., 2010; Istúriz and Carbon, 2000; Llor and Cots, 2009; Volpato et al., 2005). In 2017 the Center for Disease and Control Prevention announced that at least 2 million people in the U.S. are infected with antibiotic-resistant bacteria each year and at least 23,000 of them die.

Antibiotic-resistant *Staphylococci* are a worldwide problem affecting humans, animals and numerous natural environments (Fitzgerald, 2012; Grundmann et al., 2006; Vindel et al., 2014). Similarly, the overall occurrence of *C. acnes* antibiotic resistance has increased dramatically last years, a previous study showed that it rises from 20% in 1987 to 62% in 1996 (Leyden and Levy, 2001), later it was detected the resistance of *C. acnes* to multiple drugs such as Erythromycin, Clindamycin, Tetracycline (Ross et al., 1998), Azithromycin, Minocycline, Doxycycline and Trimethoprim-sulfamethoxazole (Moon et al., 2012; Schafer et al., 2013).

3.3. Antibacterial activity of some plant oils against the *acne bacteria*

The antimicrobial activity of a selection of eleven medicinal plant oils against the isolated bacteria was investigated and the results are summarized in [Table 2](#). Out of the tested oils, only oils of tea tree and rosemary were effective against the *acne bacteria*.

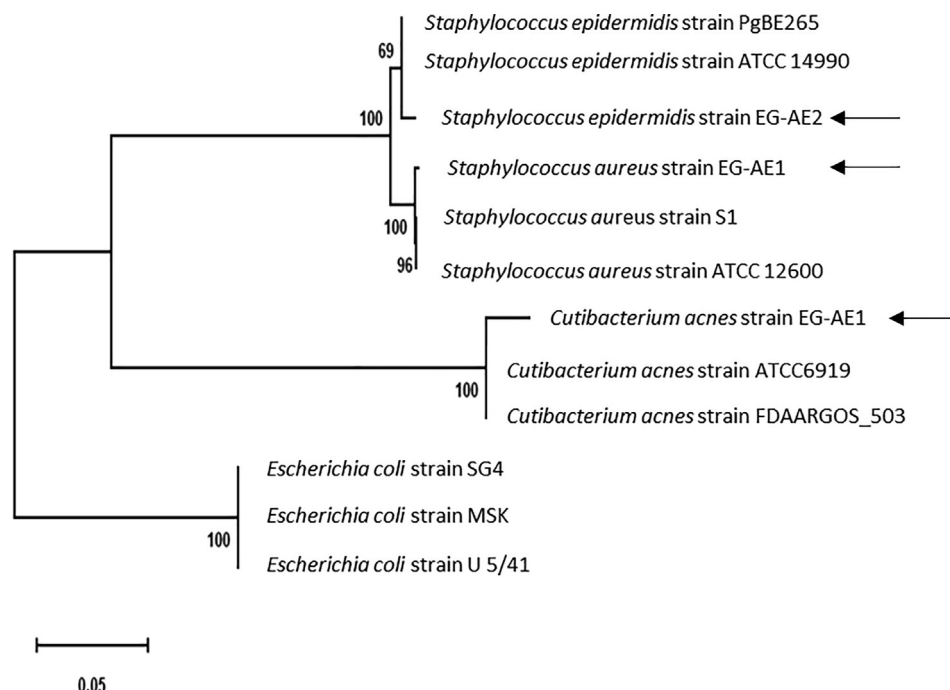


Fig. 1. Phylogenetic tree shows the evolutionary relationships between the 16S rRNA sequences of the isolated acne bacteria with their bacterial concatenated nucleotide sequences of their 16S rRNA. The Maximum Likelihood tree was constructed using the MEGA X software with the Maximum Likelihood algorithm and default setting. The bar length represents 0.05 substitutions per nucleotide site. The new acne isolates in the current study are indicated by black arrows. Branch support was estimated from 1000 bootstrap replicates. Three *E. coli* 16S rRNA sequences serve as outgroups to root the tree.

Table 1

Antibiotic susceptibility pattern of the isolated acne bacteria against a selection of fourteen antibiotics.

Bacteria	AZM	AMC	CIP	E	P	TOB	TEC	CN	DA	CL	AK	SXT	RA	VA
<i>Staph. aureus</i> strain EG-AE1	R*	I*	I	R	R	I	R	I	R	S*	R	R	R	S
<i>Staph. epidermidis</i> strain EG-AE2	R	I	I	R	R	I	R	I	R	S	R	R	R	S
<i>Cutibacterium acnes</i> Strain EG-AE1	R	S	I	R	S	S	R	S	R	S	R	R	I	S

Azithromycin (AZM 15 µg), Amoxicillin + Clavulanic Acid (AMC 20 + 10 µg), Ciprofloxacin (CIP 1 µg), Erythromycin (E 15 µg), Penicillin-G (P 10 µg), Tobramycin (TOB 10 µg), Tetracycline (TEC 30 µg), Cephalixin (CN 30 µg), Clindamycin (DA 2 µg), Chloramphenicol (CL 30 µg), Amikacin (AK 30 µg), Trimethoprim-sulfamethoxazole (SXT 25 µg), Rifampin (RA 5 µg) and Vancomycin (V 30 µg).

* Denotes for Resistant (R), Intermediate (I) and Susceptible (S).

Table 2

Antimicrobial activity of different plant oils on the isolated acne bacteria. Each value is the mean of three readings (mm) ± Standard Error (SE).

Bacteria	Inhibition zones (mm)										
	Tea tree	Cinnamon	Rosemary	Cactus	Lavender	Basil	Lemon	Thyme	Parsley	Almond	Lupine
<i>Staph. aureus</i> strain EG-AE1	15.5 ± 0.50	R	12.5 ± 1.5	R*	R	R	R	R	R	R	R
<i>Staph. epidermidis</i> strain EG-AE2	21.02 ± 0.73	R	15.18 ± 0.38	R	R	R	R	R	R	R	R
<i>Cutibacterium acnes</i> Strain EG-AE1	20.85 ± 0.76	R	14.77 ± 0.35	R	R	R	R	R	R	R	R

* Denotes for Resistant or no inhibition (R).

Tea tree oil was found to be more effective than rosemary oil against *S. aureus*, *S. epidermidis* and *C. acnes* with inhibition zones of 15.5 ± 0.50 mm, 21.02 ± 0.73 mm and 20.85 ± 0.76 mm respectively.

The dreading continuous emergence of multidrug resistance strains led the investigators to develop new alternative treatment options. Of those medicinal plants are promising, medicinal plants are being used since ever in the prevention and treatment of infectious diseases (Bhuchar et al., 2012) and likely, they are safe, cheaper and have low side effects (Rafeian-kopaei, 2012; Singh et al., 2013). It is very common to use medicinal plants in the treat-

ment of acne and skin infectious diseases (Bhuchar et al., 2012). Tea tree (*Melaleuca alternifolia*) oil (TTO) is known since long for many remedial uses and has been claimed as a potential candidate to replace antibiotics particularly in topical applications (Carson et al., 2006). Early studies on the antimicrobial activity of TTO claimed that TTO to be more active against antibiotic-resistant bacteria (Carson et al., 2005). Furthermore, it is added as an active constituent in many topical formulations used for the treatment of cutaneous infections for controlling dandruff, acne, lice, herpes and other skin infections (Pazyar et al., 2013). Previous studies showed that the methanolic extract of *Rosmarinus officinalis* (rose-

mary) has a good inhibition against *S. aureus* (Issabeagloo et al., 2012) and *C. acnes* (Vora et al., 2018).

3.4. Minimum inhibitory (MICs) and Minimum bactericidal concentrations (MBC)

The MIC and MBC of the most effective oils (tea tree and rosemary oils) were examined to evaluate their bacteriostatic and bactericidal properties and were recorded in Table 3. The MICs/MBCs of tea tree oil against *S. aureus* and *S. epidermidis* were found to be 78 mg/L, though the growth of these two isolates were inhibited by 156 mg/L of rosemary oil. Both tea tree and rosemary oils inhibited *C. acnes* at 39 mg/L. Our results showed that, both tea tree and rosemary oils showed potentially bactericidal activity against the tested pathogenic bacteria, however, tea tree oil showed more potency. Previous studies on antimicrobial activity of tea tree oil

reported MICs of 125 mg/L (Carson et al., 2006; Cox et al., 2000) to 2000 mg/L (Banes-Marshall et al., 2001; Carson et al., 2006; Cox et al., 2000).

3.5. Time-kill kinetics of tea tree and rosemary oils

Measurements of killing time by the tea tree and rosemary oils were made for each oil individually and are shown in Fig. 2. Tea tree oil showed greater activity than rosemary oil against all the isolates. *S. aureus*, *S. epidermidis* and *C. acnes* were killed by tea tree oil within 4 h. However, rosemary oil killed all the isolated by 6 h. A previous study on the time of killing of the standard tea tree oil and other chemically different tea tree oils against *Staphylococcus aureus* exhibited variable killing times (May et al., 2000). Apparently, the chemistry of the oils has a great impact on the efficacy and timing of action.

Table 3
Minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations of the tea tree and Rosemary oils against the isolated bacteria. The concentrations were measured as oil (mg/L).

Bacteria	Tea tree oil		Rosemary oil	
	MIC	MBC	MIC	MBC
<i>Staph. aureus</i> strain EG-AE1	78	78	156	156
<i>Staph. epidermidis</i> strain EG-AE2	78	78	156	156
<i>Cutibacterium acnes</i> Strain EG-AE1	39	39	39	39

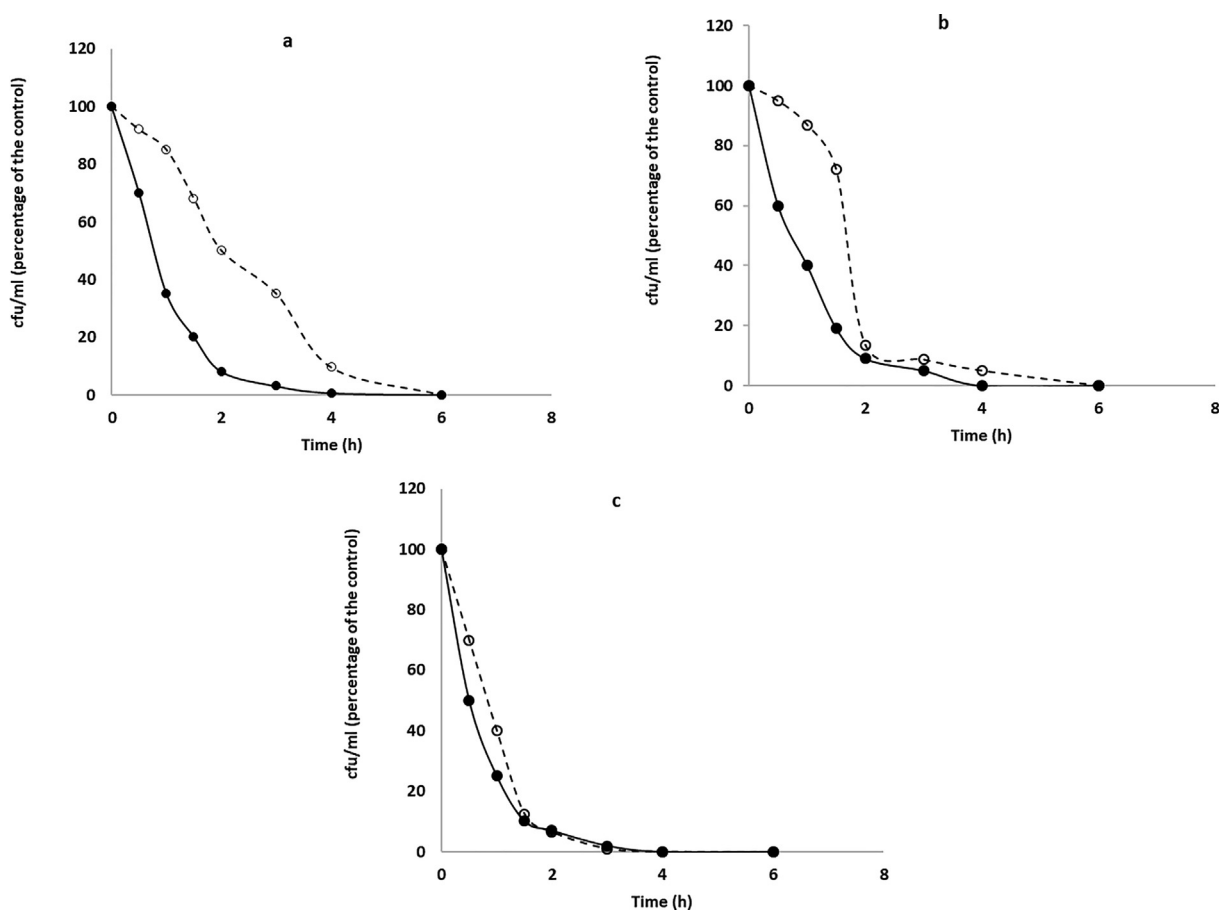


Fig. 2. Time-kill experiment of *S. aureus* (a), *S. epidermidis* (b) and *C. acnes* (c); Relative viable count of the three isolates were measured for 6 h and calculated as cfu/ml (% of the control) against tea tree oil (solid line with filled circle), rosemary oil (dashed line with open circles). Prior to the experiment, all the bacteria were in the action of the logarithmic phase. Aliquots were taken at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 and viable colony counts on blood agar were calculated as cfu/ml.

Table 4

Major constituents of Tea tree and Rosemary oils as identified by GC/MS analysis.

No	List of identified components	Chemical structure	Tea tree oil	Rosemary oil	Known functions
1	Methyl pentanoate	C ₆ H ₁₂ O ₂	+	nd	In fragrances, beauty care, soap, laundry detergents
2	6-Methyl-3,5-heptadien-2-one	C ₈ H ₁₂ O	+	nd	Flavor and fragrance agents
3	α -Pinene	C ₁₀ H ₁₆	+	+	Anti-inflammatory via and seems to be an antimicrobial.
4	2-tert-Butyl-4-isopropyl-5-methylphenol	C ₁₄ H ₂₂ O	+	+	It's an isomer of Cashmeran (known as musk indanone) which is used in fragrances
5	Camphene	C ₁₀ H ₁₆	+	+	Powerful pain relieving, anti-inflammatory and antioxidative properties.
6	Sabinene	C ₁₀ H ₁₆	+	+	Anti-inflammatory agent, antibacterial and antifungal agent
7	Myrcene	C ₁₀ H ₁₆	+	+	Anti-inflammatory, analgesic (pain relief), antibiotic, sedative and antimutagenic
8	13-Methyltetradecanoic acid	C ₁₅ H ₃₀ O ₂	+	+	induces apoptosis or "programmed cell death" of certain human cancer cells.
9	p-Cymene	C ₁₀ H ₁₄	+	+	pain-relieving, anti-inflammatory and analgesic properties
10	α -Terpinene	C ₁₀ H ₁₆	+	+	Fragrance compound, antioxidant
11	γ -Terpinene	C ₁₀ H ₁₆	+	+	A scent in 60%–80% of perfumed hygiene products and cleaning agents as soap, detergents, Shampoos and lotions.
12	Linalool	C ₁₀ H ₁₈ O	+	nd	Anti-anxiety, Antidepressant, Sedative, Anti-inflammatory, Anti-epileptic and Analgesic
13	β -Pinene	C ₁₀ H ₁₆	nd*	+	Antimicrobial and Flavoring Agent.
15	Thymol	C ₁₀ H ₁₄ O	nd	+	Antimicrobial and anti-inflammatory.

+Denotes for detected constituents and not detected (nd).

3.6. GC–MS profiles

GC/MS chromatographs (Figs. 3–4, [Supplementary data](#)) identified and estimated the presence of twelve and eleven major compounds in the ethanolic extracts of tea tree and rosemary oils respectively, the detected compounds are complex mixtures of terpenes and related alcohols. The constituents, as well as their therapeutic functions, are listed in [Table 4](#). Nine compounds were detected in both oils (α -Pinene, 2-tert-Butyl-4-isopropyl-5-methylphenol, Camphene, Sabinene, Myrcene, 13-Methyltetradecanoic acid, p-Cymene, α -Terpinene and γ -Terpinene). Most of those compounds were detected previously in the extracts of tea tree ([Brophy et al., 1989](#)) and rosemary oils ([Jiang et al., 2011](#)).

Interestingly, a web search of the therapeutic functions for the identified compounds displayed antimicrobial, anti-inflammatory and analgesic properties. Besides, some other detected compounds are being used in fragrances, beauty care, soap and laundry detergents. All of this supports the potential use of both tea tree and rosemary oil to be used as antimicrobial agents against acne-inducing bacteria. Interestingly, the efficacy, tolerability and acceptability of a tea tree oil gel and face wash on acne patients showed a significant improvement in mild to moderate acne and that the products were well tolerated ([Malhi et al., 2017](#)). Moreover, a mixed cream formula of 20% propolis, 3% "tea tree oil", and 10% "Aloe vera" was found to be better than a 3% erythromycin cream in reducing erythema scars, acne severity index, and total lesion count ([Mazzarello et al., 2018](#)). However, limited data are available on the cytotoxicity of both oils and more *in vivo* work is to be done to confirm this.

4. Conclusion

This manuscript describes the isolation of three groups of acne-inducing bacteria; *Staphylococcus aureus* EG-AE1, *Staphylococcus epidermidis* EG-AE2 and *Cutibacterium acnes* EG-AE1 from Egypt. The antibacterial effects of some medicinal plant oils were tested against the isolated bacteria, oils of tea tree and rosemary were found to be rich sources for essential oils with many therapeutic uses. Both are considered good candidates to replace antibiotics in acne therapy. For future *in vivo* studies, concentrations up to 78 mg/L and 156 mg/L of the tea tree oil and rosemary respectively to be applied as a topical prescription. Currently, we are investigat-

ing the efficacy of certain types of nanoparticles and phage therapy as new approaches to treat acne bacteria.

Acknowledgment

We gratefully acknowledge the financial supports from the National Nature Science Foundation of China (31750110464); Botany and Microbiology Department, Faculty of Science, Benha University, Egypt, and Huazhong Agricultural University, Talented Young Scientist Program (TYSP Grant No.42000481-7). All authors listed have made a direct, substantial and intellectual contribution to this work therefore approved it for publication.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2019.11.006>.

References

- Adams, R., 2005. Identification of essential oil components by gas chromatography/quadropole mass spectroscopy. *Carol Stream* 16, 65–120.
- Amann, S., Neef, K., Kohl, S., 2019. Antimicrobial resistance (AMR). *Eur. J. Hosp. Pharm.* 26, 175–177. <https://doi.org/10.1136/ejhp-2018-001820>.
- Awad, H.A., Mohamed, M.H., Badran, N.F., Mohsen, M., Abd-Elrhman, A.S.A., 2016. Multidrug-resistant organisms in neonatal sepsis in two tertiary neonatal ICUs, Egypt. *J. Egypt. Public Health Assoc.* 91, 31–38. <https://doi.org/10.1097/01.EPX.0000482038.76692.3>.
- Balouiri, M., Sadiki, M., Ibensouda, S.K., 2016. Methods for *in vitro* evaluating antimicrobial activity: a review. *J. Pharm. Anal.* <https://doi.org/10.1016/j.jpha.2015.11.005>.
- Banes-Marshall, L., Cawley, P., Phillips, C.A., 2001. *In vitro* activity of Melaleuca alternifolia (tea tree) oil against bacterial and Candida spp. isolates from clinical specimens. *Br. J. Biomed. Sci.* 58, 139–145.
- Bhuchar, S., Katta, R., Wolf, J., 2012. Complementary and alternative medicine in dermatology: an overview of selected modalities for the practicing dermatologist. *Am. J. Clin. Dermatol.* <https://doi.org/10.2165/11597560-000000000-00000>.
- Biemer, J.J., 1973. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. *Ann. Clin. Lab. Sci.* 3, 135–140.
- Blomberg, B., Mwakagile, D.S.M., Urassa, W.K., Maselle, S.Y., Mashurano, M., Digranes, A., Harthug, S., Langeland, N., 2004. Surveillance of antimicrobial resistance at a tertiary hospital in Tanzania. *BMC Public Health* 4. <https://doi.org/10.1186/1471-2458-4-45>.
- Brophy, J.J., Davies, N.W., Southwell, I.A., Stiff, I.A., Williams, L.R., 1989. Gas Chromatographic Quality Control for Oil of Melaleuca Terpinen-4-ol Type (Australian Tea Tree). *J. Agric. Food Chem.* 37, 1330–1335. <https://doi.org/10.1021/jf00089a027>.

- Burkhardt, C.G., Burkhardt, C.N., Lehmann, P.F., 2015. Classic diseases revisited: Acne: a review of immunologic and microbiologic factors. *Postgrad. Med. J.* 75, 328–331. <https://doi.org/10.1136/pgmj.75.884.328>.
- Burkhardt, C.G., Burkhardt, C.N., Lehmann, P.F., 1999. Acne: a review of immunologic and microbiologic factors. *Postgrad. Med. J.* <https://doi.org/10.1136/pgmj.75.884.328>.
- Carson, C.F., Hammer, K.A., Riley, T.V., 2006. Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin. Microbiol. Rev.* <https://doi.org/10.1128/CMR.19.1.50-62.2006>.
- Carson, C.F., Messenger, S., Hammer, K.A., Riley, T.V., 2005. Tea tree oil: a potential alternative for the management of methicillin-resistant *Staphylococcus aureus* (MRSA). *Aust. Infect. Control* 10, 32–34. <https://doi.org/10.1071/hl05032>.
- Chen, C.H., Wang, Y., Nakatsuji, T., Liu, Y.T., Zouboulis, C.C., Gallo, R.L., Zhang, L., Hsieh, M.F., Huang, C.M., 2011. An innate bactericidal oleic acid effective against skin infection of methicillin-resistant *Staphylococcus aureus*: a therapy concordant with evolutionary medicine. *J. Microbiol. Biotechnol.* 21, 391–399. <https://doi.org/10.4014/jmb.1011.11014>.
- Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R., Wyllie, S.G., 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J. Appl. Microbiol.* 88, 170–175. <https://doi.org/10.1046/j.1365-2672.2000.00943.x>.
- De J. Sosa, A., Byarugaba, D.K., Amabile-Cuevas, C.F., Hsueh, P.R., Kariuki, S., Okeke, I. N., 2010. Antimicrobial resistance in developing countries. *Antimicrob. Resist. Develop. Countr.* <https://doi.org/10.1007/978-0-387-89370-9>.
- Dhillon, K., Varshney, K.R., 2013. Study of microbiological spectrum in acne vulgaris: an in vitro study. *Sch. J. Appl. Med. Sci.* 1, 724–727.
- Dréno, B., Pécaustings, S., Corvec, S., Veraldi, S., Khammari, A., Roques, C., 2018. Cutibacterium acnes (Propionibacterium acnes) and acne vulgaris: a brief look at the latest updates. *J. Eur. Acad. Dermatol. Venereol.* <https://doi.org/10.1111/jdv.15043>.
- Dunn, L.K., O'Neill, J.L., Feldman, S.R., 2011. Acne in adolescents: Quality of life, self-esteem, mood, and psychological disorders. *Dermatol. Online J.*
- Eichenfield, L., 2015. Evolving perspective on the etiology and pathogenesis of Acne vulgaris. *J. Drugs Dermatol.* 14, 263–268.
- Farrah, G., Tan, E., 2016. The use of oral antibiotics in treating acne vulgaris: a new approach. *Dermatol. Ther.* <https://doi.org/10.1111/dth.12370>.
- Fitzgerald, J.R., 2012. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. *Trends Microbiol.* <https://doi.org/10.1016/j.tim.2012.01.006>.
- Gieler, U., Gieler, T., Kupfer, J.P., 2015. Acne and quality of life - impact and management. *J. Eur. Acad. Dermatol. Venereol.* 29, 12–14. <https://doi.org/10.1111/jdv.13191>.
- Gilchrist, T.C., 1900. A bacteriological and microscopical study of over 300 vesicular and pustular lesions of the skin, with a research upon the etiology of acne vulgaris. *Johns Hopkins Hosptl. Rep.* 9, 400–430.
- Grundmann, H., Aires-de-Sousa, M., Boyce, J., Tiemersma, E., 2006. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet.* [https://doi.org/10.1016/S0140-6736\(06\)68853-3](https://doi.org/10.1016/S0140-6736(06)68853-3).
- Hassan, A.M., Ibrahim, O., El Guinaidy, M., 2011. Surveillance of antibiotic use and resistance in Orthopaedic Department in an Egyptian University Hospital. *Int. J. Infect. Control* 7. <https://doi.org/10.3396/ijic.v7i1.006.11>.
- Ibrahim, O.H.M., 2012. Evaluation of drug and antibiotic utilization in an Egyptian University Hospital: an interventional study. *Intern. Med. Open Access* 02. <https://doi.org/10.4172/2165-8048.1000109>.
- Issabegloo, E., Kermanizadeh, P., Taghizadeh, M., Foroghi, R., 2012. Antimicrobial effects of rosemary (*Rosmarinus officinalis* L.) essential oils against *Staphylococcus* spp. *African J. Microbiol. Res.* 6, 5039–5042.
- Istiriz, R.E., Carbon, C., 2000. Antibiotic use in developing countries. *Infect. Control Hosp. Epidemiol.* 21, 394–397. <https://doi.org/10.1086/501780>.
- Jiang, Y., Wu, N., Fu, Y.J., Wang, W., Luo, M., Zhao, C.J., Zu, Y.G., Liu, X.L., 2011. Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environ. Toxicol. Pharmacol.* 32, 63–68. <https://doi.org/10.1016/j.etap.2011.03.011>.
- Khalil, R.B., 2012. PRM58 Turning the Implausible to the Plausible: Towards a Better Control of Over the Counter Dispensing of Antibiotics in Egypt. *Value Heal.* 15, A169. <https://doi.org/10.1016/j.jval.2012.03.913>.
- Khorvash, F., Abdi, F., Kashani, H.H., Naeini, F.F., Narimani, T., 2012. *Staphylococcus aureus* in acne pathogenesis: a case-control study. *N. Am. J. Med. Sci.* 4, 573–576.
- Klančnik, A., Piskernik, S., Jeršek, B., Možina, S.S., 2010. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *J. Microbiol. Methods* 81, 121–126. <https://doi.org/10.1016/j.mimet.2010.02.004>.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. <https://doi.org/10.1093/molbev/msy096>.
- Kunin, C.M., 1993. Resistance to antimicrobial drugs - A worldwide calamity. *Ann. Intern. Med.* <https://doi.org/10.7326/0003-4819-118-7-199304010-00011>.
- Leyden, J., Levy, S., 2001. The development of antibiotic resistance in *Propionibacterium acnes*. *Cutis* 67, 21–24.
- Leyden, J.J., 2003. A review of the use of combination therapies for the treatment of acne vulgaris. *J. Am. Acad. Dermatol.* 49. [https://doi.org/10.1067/S0190-9622\(03\)01154-X](https://doi.org/10.1067/S0190-9622(03)01154-X).
- Llor, C., Cots, J.M., 2009. The sale of antibiotics without prescription in pharmacies in Catalonia, Spain. *Clin. Infect. Dis.* 48, 1345–1349. <https://doi.org/10.1086/598183>.
- Magaldi, S., Mata-Essayag, S., Hartung De Capriles, C., Perez, C., Colella, M.T., Olaizola, C., Ontiveros, Y., 2004. Well diffusion for antifungal susceptibility testing. *Int. J. Infect. Dis.* 8, 39–45. <https://doi.org/10.1016/j.ijid.2003.03.002>.
- Malhi, H.K., Tu, J., Riley, T.V., Kumarasinghe, S.P., Hammer, K.A., 2017. Tea tree oil gel for mild to moderate acne: a 12 week uncontrolled, open-label phase II pilot study. *Australas. J. Dermatol.* 58, 205–210. <https://doi.org/10.1111/ajd.12465>.
- May, J., Chan, C.H., King, A., Williams, L., French, G.L., 2000. Time-kill studies of tea tree oils on clinical isolates. *J. Antimicrob. Chemother.* 45, 639–643. <https://doi.org/10.1093/jac/45.5.639>.
- Mazzarello, V., Donadu, M.G., Ferrari, M., Piga, G., Usai, D., Zanetti, S., Sotgiu, M.A., 2018. Treatment of acne with a combination of propolis, tea tree oil, and Aloe vera compared to erythromycin cream: two double-blind investigations. *Clin. Pharmacol.* 10, 175–181. <https://doi.org/10.2147/CPAA.S180474>.
- Mills, O.J., Thornsberry, C., Cardin, C.W., Smiles, K.A., Leyden, J.J., 2002. Bacterial resistance and therapeutic outcome following three months of topical acne therapy with 2% erythromycin gel versus its vehicle. *Acta Derm. Venereol.* 82, 260–265. <https://doi.org/10.1080/000155502320323216>.
- Moon, S.H., Roh, H.S., Kim, Y.H., Kim, J.E., Ko, J.Y., Ro, Y.S., 2012. Antibiotic resistance of microbial strains isolated from Korean acne patients. *J. Dermatol.* 39, 833–837. <https://doi.org/10.1111/j.1346-8138.2012.01626.x>.
- Nakamura, M., Kametani, I., Higaki, S., Yamagishi, T., 2003. Identification of *Propionibacterium acnes* by polymerase chain reaction for amplification of 16S ribosomal RNA and lipase genes. *Anaerobe* 9, 5–10. [https://doi.org/10.1016/S1075-9964\(03\)00061-1](https://doi.org/10.1016/S1075-9964(03)00061-1).
- Neamsuvan, O., Kama, A., Salaemae, A., Leesen, S., Waedueramae, N., 2015. A survey of herbal formulas for skin diseases from Thailand's three southern border provinces. *J. Herb. Med.* 5, 190–198. <https://doi.org/10.1016/j.hermed.2015.09.004>.
- Nikkari, T., 1974. Comparative chemistry of sebum. *J. Invest. Dermatol.* 62, 257–267. <https://doi.org/10.1111/1523-1747.ep12676800>.
- Omulo, S., Thumbi, S.M., Njenga, M.K., Call, D.R., 2015. A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: what can be done better? *Antimicrob. Resist. Infect. Control* 4. <https://doi.org/10.1186/s13756-014-0041-4>.
- Pazayr, N., Yaghoobi, R., Bagherani, N., Kazerouni, A., 2013. A review of applications of tea tree oil in dermatology. *Int. J. Dermatol.* <https://doi.org/10.1111/j.1365-4632.2012.05654.x>.
- Pineau, R.M., Hanson, S.E., Lyles, J.T., Quave, C.L., 2019. Growth Inhibitory Activity of *Callicarpa americana* Leaf Extracts Against *Cutibacterium acnes*. *Front. Pharmacol.*
- Rafieian-kopaei, M., 2012. Medicinal plants and the human needs. *J. HerbMed Pharmacol.* [https://doi.org/10.1016/S2222-1808\(14\)60708-8](https://doi.org/10.1016/S2222-1808(14)60708-8).
- Ross, J.I., Eady, E.A., Cove, J.H., Ratyal, A.H., Cunliffe, W.J., 1998. Resistance to erythromycin and clindamycin in cutaneous propionibacteria is associated with mutations in 23S rRNA. *Dermatology*, 69–70. <https://doi.org/10.1159/000017871>.
- Sabry, N.A., Farid, S.F., Dawoud, D.M., 2014. Antibiotic dispensing in Egyptian community pharmacies: an observational study. *Res. Soc. Adm. Pharm.* <https://doi.org/10.1016/j.sapharm.2013.03.004>.
- Sarkar, S., Patra, P., Mridha, K., Ghosh, S., Mukhopadhyay, A., Thakurta, R., 2016. Personality disorders and its association with anxiety and depression among patients of severe acne: a cross-sectional study from Eastern India. *Indian J. Psychiatr.* 58, 378–382. <https://doi.org/10.4103/0019-5545.196720>.
- Schafer, F., Fich, F., Lam, M., Gárate, C., Wozniak, A., Garcia, P., 2013. Antimicrobial susceptibility and genetic characteristics of *Propionibacterium acnes* isolated from patients with acne. *Int. J. Dermatol.* 52, 418–425. <https://doi.org/10.1111/j.1365-4632.2011.05371.x>.
- Scholz, C.F.P., Kilian, M., 2016. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus propionibacterium to the proposed novel genera acidipropionibacterium gen. nov., cutibacterium gen. nov. and pseudopropionibacterium gen. nov. *Int. J. Syst. Evol. Microbiol.* 66, 4422–4432. <https://doi.org/10.1099/ijsem.0.001367>.
- Singh, R., Kaur, N., Kishore, L., Kumar Gupta, G., 2013. Management of diabetic complications: a chemical constituents based approach. *J. Ethnopharmacol.* <https://doi.org/10.1016/j.jep.2013.08.051>.
- Slack, R., 1989. Antibiotic resistance in the tropics. 2. Some thoughts on the epidemiology and clinical significance of bacterial resistance to antimicrobial agents. *Trans. R. Soc. Trop. Med. Hyg.* 83, 42–44. [https://doi.org/10.1016/0035-9203\(89\)90701-3](https://doi.org/10.1016/0035-9203(89)90701-3).
- Sobhy, N., Aly, F., El Kader, O.A., Ghazal, A., Elbaradei, A., 2012. Community-acquired methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections (in a sample of Egyptian population): analysis of mec gene and staphylococcal cassette chromosome. *Braz. J. Infect. Dis.* 16, 426–431. <https://doi.org/10.1016/j.bjid.2012.08.004>.
- Tótté, J.E.E., van der Feltz, W.T., Bode, L.G.M., van Belkum, A., van Zuuren, E.J., Pasmans, S.G.M.A., 2016. A systematic review and meta-analysis on *Staphylococcus aureus* carriage in psoriasis, acne and rosacea. *Eur. J. Clin. Microbiol. Infect. Dis.* <https://doi.org/10.1007/s10096-016-2647-3>.
- Tuchayi, S.M., Makrantonaki, E., Ganceviciene, R., Dessinioti, C., Feldman, S.R., Zouboulis, C.C., 2015. Acne vulgaris. *Nat. Rev. Dis. Prim.* 1, 15029. <https://doi.org/10.1038/nrdp.2015.29>.
- Turner, S., Pryer, K.M., Miao, V.P.W., Palmer, J.D., 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J. Eukaryot. Microbiol.*, 327–338. <https://doi.org/10.1111/j.1550-7408.1999.tb04612.x>.

- Valgas, C., De Souza, S.M., Smânia, E.F.A., Smânia, A., 2007. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.* 38, 369–380. <https://doi.org/10.1590/S1517-83822007000200034>.
- Van den Bergh, B., Michiels, J.E., Wenseleers, T., Windels, E.M., Boer, P., Vanden, Kestemont, D., De Meester, L., Verstrepen, K.J., Verstraeten, N., Fauvart, M., Michiels, J., 2016. Frequency of antibiotic application drives rapid evolutionary adaptation of *Escherichia coli* persistence. *Nat. Microbiol.* 1, 16020. <https://doi.org/10.1038/nmicrobiol.2016.20>.
- Vindel, A., Trincado, P., Cuevas, O., Ballesteros, C., Bouza, E., Cercenado, E., 2014. Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in Spain: 2004–12. *J. Antimicrob. Chemother.* 69, 2913–2919. <https://doi.org/10.1093/jac/dku232>.
- Volpato, D.E., de Souza, B.V., Dalla Rosa, L.G., Melo, L.H., Daudt, C.A.S., Deboni, L., 2005. Use of antibiotics without medical prescription. *Brazilian J. Infect. Dis.* 9, 288–291. <https://doi.org/10.1590/S1413-86702005000400004>.
- Vora, J., Srivastava, A., Modi, H., 2018. Antibacterial and antioxidant strategies for acne treatment through plant extracts. *Inform. Med. Unlocked* 13, 128–132. <https://doi.org/10.1016/j.imu.2017.10.005>.
- Walsh, T.R., Efthimiou, J., Dreno, B., 2016. Systematic review of antibiotic resistance in acne: an increasing topical and oral threat. *Lancet Infect. Dis.* 16, e23–e33. [https://doi.org/10.1016/S1473-3099\(15\)00527-7](https://doi.org/10.1016/S1473-3099(15)00527-7).
- Wayne, P., 2019. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 29th ed. CLSI supplement M100. Clin. Lab. Stand. Institute.
- Williams, P.C.M., Isaacs, D., Berkley, J.A., 2018. Antimicrobial resistance among children in sub-Saharan Africa. *Lancet Infect. Dis.* [https://doi.org/10.1016/S1473-3099\(17\)30467-X](https://doi.org/10.1016/S1473-3099(17)30467-X).
- Zaenglein, A.L., Pathy, A.L., Schlosser, B.J., Alikhan, A., Baldwin, H.E., Berson, D.S., Bowe, W.P., Graber, E.M., Harper, J.C., Kang, S., Keri, J.E., Leyden, J.J., Reynolds, R. V., Silverberg, N.B., Stein Gold, L.F., Tollefson, M.M., Weiss, J.S., Dolan, N.C., Sagan, A.A., Stern, M., Boyer, K.M., Bhushan, R., 2016. Guidelines of care for the management of acne vulgaris. *J. Am. Acad. Dermatol.* 74, 945–973.e33. <https://doi.org/10.1016/j.jaad.2015.12.037>.