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Essential Oil-Mediated Potentiation of Erythromycin Against Resistant and Sensitive *Cutibacterium acnes*: Implications for Antimicrobial Resistance Management

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ABSTRACT

Acne vulgaris, a chronic skin condition, is, in part, caused by the bacteria *Cutibacterium acnes* (C. *acnes*). Antibiotics are commonly used for treatment of this condition, but antibiotic resistance poses an increasing challenge for a successful outcome. This study aims to investigate the synergistic antimicrobial activity of tea tree oil (TTO), chamomile oil (CO), with erythromycin against clinical strains of erythromycin-sensitive and erythromycin-resistant strains of C. *acnes*. Twenty-one clinical samples of C. *acnes* were used. Minimum inhibitory concentrations (MIC) of TTO, CO, and erythromycin alone and in combination were investigated using microdilution and checkerboard assays. Synergistic interactions were evaluated using the Fraction Inhibitory Concentration Index (FICI). The mixture of TTO and CO combined with erythromycin showed a significant 95.97 % ($P<0.05$) reduction in MIC of erythromycin (FICI = 0.13), restoring this antibiotic activity on the erythromycin-resistant C. *acnes*. These results suggest using essential oil combinations simultaneously with conventional topical antimicrobials to treat Acne vulgaris.

Keywords: Tea tree oil, Chamomile oil, C. *acnes*, Synergy, Antimicrobial Activity

1. Introduction

Cutibacterium acnes (C. *acnes*) is an aerotolerant anaerobic Gram-positive pleomorphic diphtheroid, comprising a part of normal skin flora. It grows naturally in pilosebaceous ducts and follicles. C. *acnes* plays an essential role in the pathophysiology of the common skin condition Acne vulgaris. Acne vulgaris is a dermatological condition that initiates when pilosebaceous duct blockage occurs due to excessive sebum and dead skin accumulation. Sebum accumulation arises as a response to hormonal disturbances at puberty. This high lipid content favors the colonization and multiplication of C. *acnes*. This process leads to the release of neutrophil chemotactic factors, forming inflamed pustules called Acne vulgaris [1].

C. *acnes* has also been connected to other serious diseases, such as inflammation of the prostate, infections of the bones and joints, and shoulder arthritis [2]; therefore, proper treatment protocols are essential. Treatment of Acne vulgaris varies between controlling hormonal disturbances, sebum production, and or controlling C. *acnes* growth in inflamed lesions. The latter is done through the use of antibiotics.

Topical protein synthesis inhibitor antibiotics such as clindamycin, Erythromycin, and doxycycline are used to treat Acne vulgaris. However, the misuse of these topical antibiotics has led to the development of resistance towards and cross-resistance between them [3,4]. Thus, some effective antibiotics have been deemed ineffective against C. *acnes*, and natural products in essential oils have emerged as alternatives for C. *acnes* control.

Essential oils (EO) are volatile, natural, fragrant liquids that can be extracted from different parts of the plants (especially leaves and flowers), presenting anti-inflammatory, antiviral, and antibacterial properties [5]. The activity of essential oils' is commonly attributed to the disruption of cell membranes, resulting in bacterial cell death. Essential oils have been shown to exhibit a broad-spectrum antimicrobial activity and can also be used in combination with antibiotics to restore their activities [6].

Tea tree oil (TTO) is produced from the leaves and terminal branches of *Melaleuca alternifolia* (Maiden & Betche) Cheel; it is used in traditional medicine as a topical antiseptic and anti-inflammatory agent and widely formulated into many cosmetic and personal care products [7]. TTO is one of the essential oils to which Gram-positive bacteria are more susceptible. It is commonly used as a natural product in acne treatment. TTO is found in the market as 5% gel (Tattoo, Biamed®). It is widely used with other acne treatments, such as benzoyl peroxide, salicylic acid, glycolic acid, or azelaic acid [8].

On the other hand, chamomile oil (CO) consists of sesquiterpenes such as α-bisabolol oxides A, α-bisabolol oxides B, α-bisabolol and others [9]. These compounds exhibit a bactericidal effect against C. *acnes* in vitro after 20 minutes of application [10]. Herbal combinations are used in traditional Chinese medicine for treatment of several dermatological disorders [11].

Herbal synergism has been long observed and has been experimentally studied. Synergy is the amplified effect of a combination of two drugs compared to the impact of each of these drugs alone, it is usually accompanied by a reduction in these drugs' effective concentration [12]. For example, the MIC of *Emblica officinalis* (*E. officinalis*) was reduced to 250 µg upon mixing it with 250 µg of *Nymphaea odorata* (*N. odorata*), resulting in a larger zone of inhibition (ZOI) by 3.5 ±0. 557 mm.

There are many examples of using combinations of natural products to treat several microbial infections, such as *Melaleuca alternifolia* (Maiden & Betche) Cheel, or tea tree oil (TTO), in combination with *Lavendulla aficionados* that produced a synergistic activity against the fungi *Trichophyton rubrum* [13]. Moreover, combinations of essential oils such as creams containing *Thymus vulgaris*, *Rosa centifolia*, *Rosmarinus officinalis*, and *Saponaria officinalis* were found effective in significantly reducing C. *acnes* sebum lesions after testing on volunteers for four weeks [14].

This study aims to explore a synergistic antimicrobial activity of TTO and chamomile oil against clinical strains of antibiotic-sensitive and resistant strains of C. *acnes* and to investigate whether that effect can potentiate the antibiotic activity against C. *acnes* resistant strains.

2. Materials and Methods

2.1. ANTIBIOTICS, CHEMICALS AND REAGENTS

Antibiotics discs of clindamycin (2 µg), doxycycline (30 µg) and Erythromycin (15 µg) were obtained from Oxoid®, UK. Erythromycin powder (0.85 µg active erythromycin per mg of powder) was kindly provided by Dar Al-Dawa Pharmaceutical Company (Jordan). The powder was first dissolved in glacial acetic acid, followed by dilution with sterile distilled water (dH_2O) to obtain a clear solution. Stock solutions were prepared according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S25, 2015):

$$Weight\ (mg) = \frac{Volume\ (ml) * Concentration\ (\mu g/ml)}{Potency\ (\mu g/ml)}$$

Subsequent dilutions were prepared aseptically in sterile water. Pharmaceutical grade of *Melaleuca alternifolia* (Maiden & Betche) Cheel [15] and *Matricaria chamomilla* (L.) [16] essential oils were purchased from (Eden garden®, USA) and dissolved in dimethyl sulfoxide (DMSO) (AZD Chem®, China).

2.2. QUALITATIVE ANALYSIS OF TTO AND CO USING GAS CHROMATOGRAPHY /MASS SPECTROSCOPY DETECTOR (GC/MS)

2.2.1. GC/MS analysis

An aliquot (1 ml) of 13.44 µg/ml of TTO was dissolved in hexane and 4µl/ml CO was dissolved in cyclohexane. TTO and CO were analyzed using GC/MS using varian®3800 GC with Saturn®2200 MS detector and DB-5 (Agilent®) column. The GC-MS injection volume was 1 µl, injector temperature was 250 °C, column temperature was 240 °C with a temperature rate of 10 °C/min for 20 min, the mobile phase was helium 99.999 with a flow rate of 1 ml/min and the detector trap

temperature was 160 °C, Manifold temperature was 80 °C with transfer line temperature of 230° C and Axial Modulation voltage was 4.4 volts.

Molecular weights of oil components were calculated and compared with cross-references in libraries and existing articles. The retention index and NIST database were used to identify the resultant chromatograms.

2.3. QUANTITATIVE ANALYSIS OF TTO AND CO USING GAS CHROMATOGRAPHY –FLAME IONIZATION DETECTOR (GC-FID)

2.3.1. TTO analysis

Standards and sample preparation

Terpnin-4-ol (external standard) was serially diluted in hexane to produce the following concentrations (44.8, 22.41, 11.2, 5.6, 2.8, 1.4, and 0.7 µg/ml). TTO was serially diluted in hexane to obtain the following concentrations (107.52, 53.76, 26.88, 13.44, 6.72, 3.36 and1.68 µg/ml). Calibration curves were plotted afterward.

GC conditions

Analysis was conducted with CX series with flame ionization detector (Varian®) and 0.25µm*30m*0.32µm capillary column (Kinesis®), injector temperature was 240 °C, column temperature was 230 °C with temperature rate of 10 °C / min, with nitrogen gas as the mobile phase, the flame lonization detector temperature was 240 °C hydrogen and compressed gas were used.

2.3.2. Chamomile oil analysis

Standard and sample preparation

α-Bisabolol (external standard) was serially diluted in cyclohexane to obtain seven concentrations (32, 16, 8, 4, 2, 1 and 0.5 µl/ml). CO was diluted in cyclohexane to obtain seven concentrations (32, 16, 8, 4, 2, 1, and 0.5 µl/ml). Calibration curves were then plotted.

GC conditions

Analysis was conducted with CX series with flame ionization detector (Varian®) and 0.25µm*30m*0.32µm capillary column (Kinesis®), injector temperature was 250 °C column temperature was 230 °C with temperature rate of 10 °C min, the mobile phase was nitrogen gas, the flame lonization detector temperature was 250 °C, Hydrogen and compressed gas were used.

2.4. ISOLATION AND IDENTIFICATION OF C. *acnes* CLINICAL SAMPLES

2.4.1. Bacterial strains

Ethical approval for this study was granted by the Scientific Research Ethics Committee at the University of Petra (Approval No. Q1/11/2021).

Bacterial strains were isolated from 35 clinical samples obtained from patients attending private dermatology clinics in Amman, Jordan. Patients were requested to read and sign a consent form that explained the nature of the research, the sample to be collected, and how it would be collected, Certified nurses or dermatologists collected samples from patients suffering from inflamed acne cysts, using sterile swabs. No specifications for gender or age were requested.

Swabs were placed in sterile Thioglycolate media (Himedia ® India) and transported to the lab. Swabs were incubated for a week at 37°C and subcultured onto Tryptic soy agar (TSA) (Himedia ® India) supplemented with 10% blood. Cultures were incubated at 37°C for seven days under anaerobic conditions using an anaerobic jar (Oxoid®) and Anaerogen kit (Thermo®).

Isolated colonies were Gram-stained and checked for purity microscopically, then subcultured on fresh TSA-blood agar and incubated for an additional seven days. Pure cultures were identified microscopically (Gram stain), macroscopically (colonial morphology), and biochemically using ANA II RapID Kit with indole reagent using ERIC program (Remel®UK).

2.4.2. RapID ANA II test for identification of C. *acnes*

Five to six colonies from pure C. *acnes* isolates were suspended in RapID Inoculation Fluid (1 ml) to achieve a visual turbidity equal to a #3 McFarland turbidity standard (≈9*10^8 CFU). One ml of inoculation fluid was applied into the panel and equally distributed in each well. After four hours of incubation under aerobic condition, the results before and after adding ANA reagent and Indole reagent were recorded and interpreted by ERIC program.

Tests included were URE (Urea), BLTS (ρ-Nitrophenyl-β,D-disaccharide), αARA (ρ-Nitrophenyl-α,L-arabinoside), ONPG (σ-Nitrophenyl-β,D-galactoside), αGLU (ρ-Nitrophenyl-α,D-glucoside), βGLU (ρ-Nitrophenyl-β,D-glucoside), αGAL (ρ-Nitrophenyl-α,D-galactoside), αFUC (ρ-Nitrophenyl-α,L-fucoside), NAG (ρ-Nitrophenyl-n-ac etyl- β,D-glucosaminide) PO4 (ρ-Nitrophenylphosphate), LGY (Leucyl-glycine-β-naphthylamide), GLY (Glycine-β-naphthylamide), PRO (Proline-β-naphthylamide), PAL (Phenylalanine-β-naphthylamide), ARG (Arginine-β-naphthylamide), SER (Serine-β-naphthylamide), PYR (Pyrrolidonyl-β-naphthylamide), and IND (Tryptophane). The software calculates the results obtained. Isolates that achieved a percentage of 99.9% were positively identified as C. *acnes*.

2.5. MICRODILUTION ASSAY FOR MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION OF TEA TREE OIL (TTO) AND CHAMOMILE OIL (CO)

Clinical samples were inoculated in 5 ml sterile Cation adjusted Mueller Hinton broth (CA-MHB) (Himedia India) and the turbidity was adjusted to 0.5 McFarland about $1*10^6$ CFU.

Tea tree oil and chamomile oil Stock solutions were prepared by dissolving 0.8 ml of 100% TTO in 10 ml of DMSO to obtain 8% v/v solution which was then sterilized by filtration through 0.45 µm membrane filter (Millipore®) under aseptic conditions.

Aliquots (50 µl) of freshly prepared sterile CA-MHB were added to all wells of sterile 96 U-shaped bottom microtiter plate. Then 50 µl of TTO or CO test solution was dispensed in wells 1A, 1B, 1C and two-fold serial dilution was performed to the tenth well. The last 50 µl were discarded.

An aliquot of 50 µl of 8% deionized water in DMSO is added to well 1D and then serially diluted to the tenth well to represent solvent control. Bacterial culture and culture media controls were included. 10 µl of the C. *acnes* strain was added to all wells except for wells of the culture media controls. Plates were covered and incubated under anaerobic conditions using Anaerogen® (thermo) at 37 C° for seven days. At the seventh day, the plates were read using the GloMax® Microreader At 600 nm wavelength, and the data obtained were statistically analyzed for mean and standard deviation.

2.6. ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibiotic sensitivity of all C. *acnes* strains was determined using the disc diffusion assay according to the CLSI (2015). Briefly, inoculum for each strain was adjusted to 0.5 McFarland. Mueller Hinton Agar (MHA) plates were seeded with C. *acnes* strains using sterile swabs. Antibiotic discs of Clindamycin, Doxycycline and Erythromycin were then placed on inoculated plates. The plates were incubated for 7 days at 37°C under anaerobic conditions. Zones of inhibition (ZOI) were measured in mm. Results presented are the average of three readings of separate experiments for each strain.

2.7. CHECKERBOARD MICRODILUTION ASSAY FOR SYNERGISTIC ACTIVITY

The synergistic activity of TTO and CO were tested using the checkerboard assay according to Assaf et al. (2013). Briefly, aliquots (50 µl) of CA-MHB were dispensed in all wells of a 96-well sterile plate, 50 µl of stock TTO (4 %) was added to column eleven. Two-fold serial dilutions were performed from column eleven to one. Serial Two-fold dilutions of CO are prepared in tubes and 50 µl of each dilution was added to a row starting from row A. Finally, 10 µl of C. *acnes* strains were added to all wells except for wells in column twelve (broth control). Synergistic interactions were evaluated by calculating the Fraction Inhibitory Concentration Index (FICI) using the following formula:

$$FICI = \frac{MIC\ of\ TTO\ in\ checkerboard\ assay}{MIC\ of\ TTO} + \frac{MIC\ of\ CO\ in\ checkerboard\ assay}{MIC\ of\ CO}$$

The interaction was defined as synergistic if the FIC index was≤ 0.5, additive if it was >0.5 - 1, no interaction if it was 2 and antagonistic if it was >2 (Jain et al. 2011).

Checkerboard assay for synergistic activity of oil combination and Erythromycin

Erythromycin-resistant strains were tested for improved susceptibility to Erythromycin in the presence of the TTO and CO combination. Erythromycin stock solution was prepared to a concentration of 1000 µg/ ml and a concentration of 250 ug/ml was used in the test. Results of the checkerboard assay for TTO and CO were used to prepare the mixture's stock solution. A 125 µl aliquot each of TTO and CO was combined and dissolved in DMSO, then diluted with distilled water to a final volume of 100 ml. From this stock, a test solution was prepared at a concentration of 0.005 µg/ml, which was subsequently used to generate eight serial two-fold dilutions in test tubes.

All wells were filled with 50 µl of CA-MHB, then, 50 µl of Erythromycin test solution was added to column eleven and serially diluted down to column one. An aliquot (50 µl) from each dilution of the eight concentrations of the TTO/CO mixture was added to all wells in a raw starting with row A1 to A11 having the highest concentration down to row H1 to H11 having the lowest concentration. Finally, an aliquot (10 µl) of the inoculum-adjusted C. *acnes* strain was added to all wells.

2.8. STATISTICAL ANALYSIS

Data analysis was conducted using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). The mean ± standard deviation (SD) of the MICs for TTO, CO, and erythromycin across all samples were calculated.

Comparisons between datasets were performed using one-way ANOVA to assess statistical significance, with $p < 0.05$ considered significant. Post hoc analysis was conducted using Dunnett's T3 test in SPSS version 18.0 to evaluate pairwise differences among 12 groups: (1) TTO MIC alone, (2) TTO MIC in TTO & CO, (3) TTO MIC in TTO & erythromycin, (4) TTO MIC in TTO & CO & erythromycin, (5) CO MIC alone, (6) CO MIC in TTO & CO, (7) CO MIC in CO & erythromycin, (8) CO MIC in TTO & CO & erythromycin, (9) erythromycin MIC alone, (10) erythromycin MIC in TTO & erythromycin, (11) erythromycin MIC in CO & erythromycin, and (12) erythromycin MIC in TTO & CO & erythromycin.

3. Results

Results of GC/MS indicated that TTO contains about 45% terponene-4-ol and about 2% 1,8-cineol (Table 1) and (Figure S1 in Supplementary Materials).

Table 1: Major constituents in Tea Tree Oil

	Retention Time	Identity of compound	Mol. Wt.	%	Kovats index*
1	4.705	α- thujene	136	Trace	931
2	4.838	α- pinene	136	2.008	938
3	5.466	Sabinene	136	Trace	976
4	5.510	β – pinene	136	Trace	980
5	5.561	Myrcene	136	Trace	991
6	6.026	α- terpinen	193	8.862	1018
7	6.135	p- cymene	134	2.798	1026
8	6.212	limonene	136	0	1031
9	6.277	1,8-cineol	154	2.339	1033
10	6.644	γ- terpinen	136	16.37	1062
11	7.051	Terpinolene	136	2.862	1088
12	8.547	Terpinene-4-ol	196	45.02	1179
13	8.710	α -terpineol	154	3.094	1189
14	12.116	Aromadendrene	204	1.578	1464
15	12.809	Viridiflorene	204	5.445	1494

*[17]

Chamomile oil contained about 24% α-bisabolol and about 3% bisabolol oxide B (Table 2) and (Figure S2 in Supplementary Materials). A comparison with published data and constituents was also deemed [7,18].

Table 2: Major constituents in Chamomile Oil.

	Retention Time	Identity of compound	Mol. Wt.	%	Kovats index*
1	4.468	α-pinene	136	0.974	938
2	4.830	Sabinene	136	7.296	976
3	5.381	Artemisia ketone	152	0.745	1062
4	5.913	Carvone	150	1.963	1242
5	6.208	Decanoic acid	172	3.638	1373
6	6.423	β-Farnesene	204	18.91	1458
7	6.620	Germacrene\ γ-muurolene	204	4.072	1480\ 1477
8	7.229	Not identified		0.487	
9	7.878	α -Farnesene	204	4.604	1508
10	7.941	Spanthulenol	220	14.28	1576
11	8.250	Caryophyllene oxide	220	1.167	1573
12	8.722	Not identified	-	2.309	-
13	9.391	Not identified	-	2.876	-
14	9.966	Bisabolol oxide B	238	3.157	1655
15	12.803	α-bisabolol	222	23.86	1683

* [17]

3.1. C. *acnes* ISOLATES

Twenty-one clinical samples were successfully identified and confirmed as C. *acnes*. microscopically (Gram-positive, pleomorphic rods) and macroscopically (small whitish colonies on TSB-blood agar). Positive biochemical reactions that resulted in 99.9% identification of C. *acnes* were NAG, LGY, GLY, PRO, ARG SER, and IND, according to the ANA test.

3.2. ANTIBIOTIC SENSITIVITY TESTING

Each of the 21 clinical samples were checked for the sensitivity to the more common antibiotics used in treating C. *acnes*. Results have indicated 40% resistance towards Clindamycin and Erythromycin and 5% towards Doxycycline Table 3.

Table 3: Antibiotic susceptibility for C. *acnes* isolated strains using disc diffusion assay

	Zone of inhibition(mm)		
Sample	**Clindamycin**	**Doxycycline**	**Erythromycin**
1	70± 0	63.7(±0.58)	52.67(±1.15)
2	61.3 (±1.15)	71.7(±2.89)	77(2.64)
3*	3.7(±2.08)	41(±1.73)	1.3(±0.58)
4*	14.3 (±1.15)	36.3(±0.58)	1.7(±1.15)
5	70(±0)	70(±0)	70.3(±0.58)
6	61.3 (±2.3)	62(±0)	70.7(±1.15)
7	57.3 (2.3)	58(±0)	70.7(±1.15)
8	70(±0)	64(±0)	72.7(±2.3)
9*	13.7(±0.57)	41.7(±1.53)	15.7(±0.58)
10*	28.3 (±2.89)	30(±0)	11.7(±1.15)
11	64.3 (±0.58)	68.3(±0.58)	71.3(±1.15)
12*	6(±0)	75(±5)	6(±0)
13	60(±0)	71(±1.73)	75(±8.66)
14*	1.33 (±0.58)	52(±0)	1.3(±0.57)
15*	8(±0)	42(±0)	3(±0)
16	60(±0)	61.7(±2.89)	64.3(±0.58)
17	65(±0)	64.3 (±0.58)	69(±1.73)
18*	5.7(±1.15)	28(±0)	10.3(±0.58)
19	51.7(±2.89)	62(±0)	69.7(±0.58)
20	55(±1.73)	61.7(±2.89)	69.3(±1.15)
21	60(±0)	70(±0)	68.3(±2.89)

*Resistant strains

3.3. MIC DETERMINATION OF ESSENTIAL OILS

Table 4 demonstrates the antimicrobial activity of TTO and CO. on isolates with an average CO MIC of 0.125 and TTO of 0.28 %v/v. supplementary material (S1).

Table 4: Average MIC of TTO and Chamomile oil on isolated strains.

Sample No	**MIC TTO (% v/v)**	**MIC Chamomile oil (%v/v)**
21	0.284214	0.125
STD ±	0.230905	0

3.4. TTO AND CHAMOMILE OIL SYNERGY TEST

Microdilution checkerboard assay was used to determine the MIC of the mixture of two essential oils. Fractional Inhibitory Concentration Index (FICI) was calculated (Table 5). Tea Tree Oil and chamomile Oil synergy was detectable on 61% (13/21) of the strains. The combination of the two essential oils exerted an average reduction in the MIC for TTO by 12% and for CO by 11%. For the synergistic combinations, the MIC for TTO and CO were reduced by 17% and 7%, respectively.

Table 5: Effect of TTO and CO combination on isolated strains

	MIC of each oil(ug/ml)		**MIC of combination(ug/ml)**		**FICI**	**Effect**
Sample No.	**TO**	**CO**	**TO**	**CO**		
1	0.5	0.125	0.0009	0.008	0.06	Synergistic
2	0.5	0.125	0.0019	0.008	0.06	Synergistic
3	1	0.125	0.0009	0.008	0.06	Synergistic
4	0.5	0.125	0.0312	0.008	0.12	Synergistic
5	0.0625	0.125	0.0312	0.008	0.56	Additive
6	0.5	0.125	0.0078	0.125	1.01	Additive
7	0.25	0.125	0.125	0.008	0.56	Additive
8	0.5	0.125	0.0009	0.008	0.06	Synergistic
9	0.06	0.125	0.0009	0.016	0.14	Synergistic
10	0.06	0.125	0.0009	0.008	0.07	Synergistic
11	0.125	0.125	0.0156	0.008	0.18	Synergistic
12	0.25	0.125	0.25	0.008	1.06	Additive
13	0.125	0.125	0.0009	0.008	0.07	Synergistic
14	0.25	0.125	0.125	0.016	0.62	Additive
15	0.25	0.125	0.0039	0.008	0.07	Synergistic
16	0.125	0.125	0.125	0.008	1.06	Additive
17	0.031	0.125	0.0009	0.008	0.09	Synergistic
18	0.125	0.125	0.0019	0.008	0.07	Synergistic
20	0.25	0.125	0.0156	0.008	0.12	Additive
21	0.25	0.125	0.0009	0.008	0.06	Synergistic
Average	0.29	0.125	0.04	0.01		

3.5. EFFECT OF ESSENTIAL OILS ON ERYTHROMYCIN SENSITIVITY IN ERYTHROMYCIN-RESISTANT STRAINS

Eight out of twenty-one clinical samples were Erythromycin resistant. A microdilution assay determined the minimum inhibitory concentration of Erythromycin on erythromycin-resistant strains of C. *acnes*. Results are shown in Table 6.

Improvement in the sensitivity of C. *acnes* towards Erythromycin was detected through a reduction in MIC values of Erythromycin in the checkerboard assay. 40% of samples showed a significant ($P<0.05$) 50.82% reduction in MIC of Erythromycin when combined with TTO. Moreover, MIC of Erythromycin showed a significant ($P<0.05$) 93.54% reduction when combined with CO TTO and CO mixture combined with Erythromycin showed a significant ($P<0.05$) reduction in erythromycin MIC by 95.97 %. The synergistic effect manifested in reducing the effective concentration of all three ingredients when mixed compared to the concentration of each one alone or even in the TTO/CO combinations (Table 6).

Table 6: Minimum inhibitory concentrations of Erythromycin, TTO and CO alone or in combinations on resistant strains.

Sample	Minimum Inhibitory Concentrations (MIC)										
	E	TTO+E			CO+E			TTO+CO+E			
		TTO	E	FICI	CO	E	FICI	TTO	CO	E	FICI
3	7.8	6.1*E-5	0.97	0.1	0.002	0.98	0.14	0.0005	0.00025	0.97	0.2
4	3.9	6.1*E-5	7.81	2	0.002	0.98	0.265	0.0005	0.00025	0.97	0.3
9	15.6	6.1*E-5	7.81	0.5	0.002	0.98	0.0788	0.0005	0.00025	0.488	0.1
10	15.6	6.1*E-5	7.81	0.5	0.002	0.98	0.078	0.0005	0.00025	0.488	0.1
11	15.6	6.1*E-5	15.63	1	0.002	0.98	0.078	0.0005	0.00025	0.488	0.1
14	15.6	6.1*E-5	7.81	0.5	0.002	0.98	0.078	0.0005	0.00025	0.488	0.1
15	15.6	6.1*E-5	7.81	0.5	0.002	0.98	0.078	0.0005	0.00025	0.488	0
18	31.3	6.1*E-5	3.9	0.1	0.002	0.98	0.046	0.0005	0.00025	0.488	0.1
Average	15.1	6.1*E-5	7.44		0.002	0.98		0.0005	0.00025	0.6086	
STD	7.93	0	4.17		0	0				0.223	

4. Discussion

The prevalence of infections caused by antibiotic-resistant bacteria is rising, and the search for new treatment approaches is a crucial area of scientific research. Antibacterial phytotherapeutic approaches with high efficacy and fewer side effects have been extensively investigated as an alternative. This study addresses the use of natural product combinations to control Acne vulgaris. Traditional medicine has long employed herbal extract mixtures in treating certain diseases, which in most cases contained several bioactive constituents that exhibited synergistic effects [19].

Synergistic effects are always sought because they involve minor concentrations of the active agents, suggesting a minimization of the concentration-dependent side effects of the agents used.

Recently, natural essential oils have attracted great interest in phytomedicine due to their potential therapeutic uses in combating various diseases and conditions [20–22]. In our study, TTO (an essential oil well-known for its antibacterial activity, anti-acne properties, and concentration-dependent side effects) was found to be active in lower concentrations when mixed with chamomile oil. Both essential oils were used in minimum concentrations when mixed, resulting in an 8-fold reduction in their MICs against C. *acnes*. (TTO from 0.28 % v/v to 0.037 % v/v, chamomile from 0.125 % v/v to 0.0144 % v/v).

Components of TTO include terpenin-4-ol and 1,8-cineol, which are responsible for its adverse side effects such as irritation, dryness, and elevated induced-histamine inflammation modulators, if used in high concentrations [23]. On the other hand, Chamomile oil includes in its constituents α-bisabolol, which is a relatively safe component even at high concentrations [24]. The use of TTO and chamomile oil in combination reduces the concentration of terpenin-4-ol (from 0.144 % v/v to 0.019 %v/v) and α-bisabolol concentration (from 0.037 % v/v to 0.004 % v / v). This significant decrease in MIC suggests that the combination may result in fewer side effects, making it a promising option for an anti-acne formulation.

Both oils showed potent and significant antimicrobial properties, consistent with existing literature [25,26]. Both tea tree oil (TTO) and chamomile oil are rich in terpenes, primarily monoterpenes, sesquiterpenes, and their corresponding alcohols. Their biological effects are likely attributed to the synergistic interplay among these various constituent[27]. The antimicrobial potency of these oils is primarily linked to their major active constituents: terpinene-4-ol in tea tree oil and α-bisabolol in chamomile oil. Moreover, additional elements such as α-terpineol, α-pinene, and 1,8-cineole may also contribute to TTO's antimicrobial properties [7,28].

Given the heterogeneous composition of volatile oils, their antimicrobial activity can be attributed to the combined effects of multiple components. In the case of TTO and its constituents, these effects manifest in bacterial cells as leakage of intracellular material, disruption of homeostasis, inhibition of respiration, and loss of membrane integrity and function [7]. Furthermore, some researchers reported the higher sensitivity of Gram-positive bacteria (e.g. C. *acne*) to volatile oils compared to Gram-negative ones, due to the presence of cell wall lipopolysaccharides, which inhibit volatile oil from entering the Gram-negative bacteria [29].

On the other hand, the water extract of chamomile did not show anti C. *acnes* effect, which is consistent with the

literature. Researchers have investigated using volatile chamomile oil against C. *acne*, but not water extracts. Chamomile tea has anti-inflammatory, antioxidant, scar healing accelerator, and analgesic effects [10,30].

A previous study conducted in Jordan found that 73% of isolates showed resistance to Erythromycin in C. *acnes* [4]. In our study, 40% of the clinical samples showed erythromycin / clindamycin resistance. The prevalence of resistant strains complicates the treatment regimen. As antibiotics are typically the primary approach for managing inflammatory Acne, the importance of finding effective antibiotic alternatives is growing.

Natural products are excellent sources of natural antimicrobials. Combining these products with useful antibiotics is a suggested approach to overcome bacterial resistance to antibiotics and antimicrobials. Tea Tree Oil has been found to have a synergistic effect with norfloxacin and amphotericin B against *Bacillus cereus*, *B. subtilis*, *Escherichia coli, Staphylococcus aureus*, and several *Candida strains* [31,32]. Moreover, there is evidence of synergistic antifungal properties between a blend of TTO and lavender oil [13] and β-triketones derived from manuka oil [33].

The findings of this study revealed a synergistic interaction between tea tree oil (TTO) and erythromycin, as well as between chamomile oil and erythromycin. Notably, the combined use of both oils with erythromycin exhibited an even stronger synergistic effect, effectively enhancing and restoring the antibiotic's activity.

Previous work has demonstrated that plant-derived compounds can enhance antibiotic activity against C. *acnes*; for instance, kaempferol from *Impatiens balsamina* showed synergistic effects with erythromycin (FICI = 0.266–1.125) and clindamycin (FICI = 0.187–0.562) [34]. In line with these findings, the present study revealed that TTO combined with erythromycin produced a synergistic effect (FICI = 0.488, $P < 0.05$), while chamomile oil displayed an even stronger interaction (FICI = 0.1). Moreover, the erythromycin–CO mixture exerted a highly significant synergistic effect against C. *acnes* (MIC erythromycin = 0.976 ± 0 µg/ml, CO = 0.00195 ± 0 % v/v, FICI = 0.105, $P < 0.05$).

5. Conclusions

Erythromycin-resistant strains were challenged with combinations of TTO, chamomile oil (CO), and erythromycin. A significant increase (3.96-fold) in antimicrobial activity was observed compared to TTO or erythromycin alone. The oils tested in this study may represent a potential source of non-conventional antimicrobials that could enhance antibiotic performance against C. *acnes* infections while reducing the concentration-dependent side effects of TTO. Toxicity studies and formulation development should follow mechanistic investigations to determine the applicability and dosing of such natural compounds in combination therapy. The essential oil combinations evaluated here are promising candidates for topical formulations to treat acne vulgaris. The synergistic effect of TTO and CO resulted in reduced minimum inhibitory concentrations (MICs), with TTO at 0.037% v/v and CO at 0.0144% v/v, whereas chamomile water extract showed weak anti-C. *acnes* activity (MIC > 16% w/v). In resistant strains, erythromycin MIC was reduced by 50.82% when combined with TTO ($P < 0.05$), by 93.54% when combined with CO ($P < 0.05$), and by 95.97% when combined with the TTO–CO mixture ($P < 0.05$). Although the mechanisms of action of TTO and CO differ from that of erythromycin, their strong synergistic activity highlight the potential of these natural products as adjuncts in acne therapy.

Conflicts of Interest Statement

The authors have no conflicts of interest to declare.

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References

1. Suva M. A Brief Review on Acne Vulgaris: Pathogenesis, Diagnosis and Treatment. *Research & Reviews: Journal of Pharmacology*. 2015;4:1-12.
2. Mayslich C, Grange PA, Dupin N. Cutibacterium acnes as an Opportunistic Pathogen: An Update of Its Virulence-Associated Factors. *Microorganisms*. 2021;9(2):303. doi:10.3390/microorganisms9020303
3. Abdelmalek SM, Alowaissi J, Hamadi SA, Akkash L. Impact of Dermatologists' Perceptions about Antibiotic Resistance on Antibiotic Prescribing for Acne. *J Infect Dis Ther*. 2016;4(5). doi:10.4172/2332-0877.1000294
4. Alkhawaja E, Hammadi S, Abdelmalek M, Mahasneh N, Alkhawaja B, Abdelmalek SM. Antibiotic resistant Cutibacterium acnes among acne patients in Jordan: a cross-sectional study. *BMC Dermatology*. 2020;20(1):17. doi:10.1186/s12895-020-00108-9
5. Schelz Z, Molnar J, Hohmann J. Antimicrobial and antiplasmid activities of essential oils. *Fitoterapia*. 2006;77(4):279-285. doi:10.1016/j.fitote.2006.03.013
6. Yap PSX, Yiap BC, Ping HC, Lim SHE. Essential Oils, A New Horizon in Combating Bacterial Antibiotic Resistance. *Open Microbiol J*. 2014;8:6-14. doi:10.2174/1874285801408010006
7. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. *Clin Microbiol Rev*. 2006;19(1):50-62. doi:10.1128/CMR.19.1.50-62.2006
8. Hammer KA. Treatment of acne with tea tree oil (melaleuca) products: a review of efficacy, tolerability and potential modes of action. *Int J Antimicrob Agents*. 2015;45(2):106-110. doi:10.1016/j.ijantimicag.2014.10.011
9. Singh O, Khanam Z, Misra N, Srivastava MK. Chamomile (Matricaria chamomilla L.): An overview. *Pharmacogn Rev*. 2011;5(9):82-95. doi:10.4103/0973-7847.79103
10. Zu Y, Yu H, Liang L, et al. Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. *Molecules*. 2010;15(5):3200-3210. doi:10.3390/molecules15053200
11. Shenefelt PD. Herbal Treatment for Dermatologic Disorders. In: Benzie IFF, Wachtel-Galor S, eds. *Herbal Medicine: Biomolecular and Clinical Aspects*. 2nd ed. CRC Press/Taylor & Francis; 2011. Accessed October 18, 2023. http://www.ncbi.nlm.nih.gov/books/NBK92761/
12. van Vuuren S, Viljoen A. Plant-based antimicrobial studies--methods and approaches to study the interaction between natural products. *Planta Med*. 2011;77(11):1168-1182. doi:10.1055/s-0030-1250736
13. Cassella S, Cassella JP, Smith I. Synergistic antifungal activity of tea tree (Melaleuca alternifolia) and lavender (Lavandula angustifolia) essential oils against dermatophyte infection. *International Journal of Aromatherapy*. 2002;12(1):2-15. doi:10.1054/ijar.2001.0127
14. Moldovan M, Bogdan C, Ursu I, Ionescu Mi, Crișan M. Evaluation of The Efficacy And Characterization of An Anti-Acne Cream Containing Herbal Extracts Anti-Acne Cream Containing Herbal Extracts. *FARMACIA*. 2016;64.
15. WFO. Melaleuca alternifolia Cheel. Published on the Internet; http://www.worldfloraonline.org/taxon/wfo-0000239396. Accessed on: 23 Nov 2023'. Published online 2023.
16. WFO. Matricaria chamomilla var. chamomilla . Published on the Internet; http://www.worldfloraonline.org/taxon/wfo-0000099848. Accessed on: 23 Nov 2023'. Published online 2023.
17. Adams R. *Identification of Essential Oil Components by Gas Chromatography / Mass Spectroscopy*. Vol 16.; 1995.
18. ORAV A, KAILAS T, IVASK K. Volatile constituents of Matricaria recutita L. from Estonia. *Proc Estonian Acad Sci Chem*. 2001;50. doi:10.3176/chem.2001.1.05
19. Wagner H. Synergy research: approaching a new generation of phytopharmaceuticals. *Fitoterapia*. 2011;82(1):34-37. doi:10.1016/j.fitote.2010.11.016
20. Gandhi GR, Vasconcelos ABS, Haran GH, et al. Essential oils and its bioactive compounds modulating cytokines: A systematic review on anti-asthmatic and immunomodulatory properties. *Phytomedicine*. 2020;73:152854. doi:10.1016/j.phymed.2019.152854
21. Johnson SA, Rodriguez D, Allred K. A Systematic Review of Essential Oils and the Endocannabinoid System: A Connection Worthy of Further Exploration. *Evid Based Complement Alternat Med*. 2020;2020:8035301. doi:10.1155/2020/8035301
22. Puvača N, Milenković J, Galonja Coghill T, et al. Antimicrobial Activity of Selected Essential Oils against Selected Pathogenic Bacteria: In Vitro Study. *Antibiotics (Basel)*. 2021;10(5):546. doi:10.3390/antibiotics10050546
23. Khalil Z, Pearce AL, Satkunanathan N, Storer E, Finlay-Jones JJ, Hart PH. Regulation of Wheal and Flare by Tea Tree Oil: Complementary Human and Rodent Studies. *Journal of Investigative Dermatology*. 2004;123(4):683-690. doi:10.1111/j.0022-202X.2004.23407.x
24. Kamatou GPP, Viljoen AM. A Review of the Application and Pharmacological Properties of α-Bisabolol and α-Bisabolol-Rich Oils. *Journal of the American Oil Chemists' Society*. 2010;87(1):1-7. doi:10.1007/s11746-009-1483-3
25. Das S, Horváth B, Šafranko S, Jokić S, Széchenyi A, Kőszegi T. Antimicrobial Activity of Chamomile Essential Oil: Effect of Different Formulations. *Molecules*. 2019;24(23):4321. doi:10.3390/molecules24234321
26. Iacovelli F, Romeo A, Lattanzio P, et al. Deciphering the Broad Antimicrobial Activity of Melaleuca alternifolia Tea Tree Oil by Combining Experimental and Computational Investigations. *International Journal of Molecular Sciences*. 2023;24(15):12432. doi:10.3390/ijms241512432
27. KIM SS, BAIK JS, OH TH, YOON WJ, LEE NH, HYUN CG. Biological Activities of Korean Citrus obovoides

and Citrus natsudaidai Essential Oils against Acne-Inducing Bacteria. *Bioscience, Biotechnology, and Biochemistry*. 2008;72(10):2507-2513. doi:10.1271/bbb.70388

28. Johansen B, Duval RE, Sergere JC. First Evidence of a Combination of Terpinen-4-ol and α-Terpineol as a Promising Tool against ESKAPE Pathogens. *Molecules*. 2022;27(21):7472. doi:10.3390/molecules27217472
29. Nair A, Mallya R, Suvarna V, Khan TA, Momin M, Omri A. Nanoparticles—Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics (Basel)*. 2022;11(1):108. doi:10.3390/antibiotics11010108
30. Kapoor S, Saraf ProfS. Topical Herbal Therapies an Alternative and Complementary Choice to Combat Acne. *Research Journal of Medicinal Plant*. 2011;5:650-669. doi:10.3923/rjmp.2011.650.669
31. Rosato A, Vitali C, De Laurentis N, Armenise D, Antonietta Milillo M. Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. *Phytomedicine*. 2007;14(11):727-732. doi:10.1016/j.phymed.2007.01.005
32. Rosato A, Vitali C, Gallo D, Balenzano L, Mallamaci R. The inhibition of Candida species by selected essential oils and their synergism with amphotericin B. *Phytomedicine*. 2008;15(8):635-638. doi:10.1016/j.phymed.2008.05.001
33. Christoph F, Kaulfers PM, Stahl-Biskup E. In vitro evaluation of the antibacterial activity of beta-triketones admixed to Melaleuca oils. *Planta Med*. 2001;67(8):768-771. doi:10.1055/s-2001-18350
34. Lim YH, Kim IH, Seo JJ. In vitro activity of kaempferol isolated from the Impatiens balsamina alone and in combination with erythromycin or clindamycin against Propionibacterium acnes. *J Microbiol*. 2007;45(5):473-477.