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Potent Anti-*Cutibacterium acnes* Activity of the Essential Oils Derived from *Allium sativum, Gardenia jasminoides*, and *Hedychium coronarium* Cultivated in Thailand

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Plant oils derived from medicinal herbs have furnished bioactive synergistics, as well as antibacterial and antifungal properties. The study was to evaluate the antibacterial activity of essential oils (EOs) against *Cutibacterium acnes* and to analyze the chemical compositions of the effective oils. The experiment was conducted using a completely randomized design with duplications. Three EOs obtained from *Allium sativum* (garlic), *Gardenia jasminoides* (gardenia), and *Hedychium coronarium* (*hydechium*) cultivated in Thailand were tested for antibacterial activity using an agar well diffusion assay and the macro-dilution method. The chemical compositions of the selected oil were determined by gas chromatography and mass spectrometry (GC-MS). To compare the data, Duncan's multiple range test (DMRT) was performed, and significance was determined at the $p < 0.05$ level. The results showed that garlic oil had the highest potent inhibitory zone on *C. acnes*, according to the findings. As a bactericidal, garlic oil had MIC and MBC values of 0.39 and 0.78 mg/mL, and even a MIC index (MBC/MIC) of 4. Diallyl thiosulfinate (allicin) (20.53%), 1,3-dithiane (12.53%), cyclic octaatomic sulfur (5.02%), 1,4-dihydro-2,3-benzoxathiin-3-oxide (4.73%), and carvone (3.40%) were the major components of garlic oil. The results suggest that garlic oil might be used to develop topical anti-acne treatments.

Keywords: antibacterial activity, *Cutibacterium acnes*, essential oil, garlic

INTRODUCTION

Acne is frequently a chronic inflammatory condition of the human skin, suggesting that oilier skin can rapidly increase by digesting facial fat to give fatty acids, which cause irritation at the hair follicle area. It has infected individuals of different ages and genders all over the world. The disorder can have psychological implications, along with a loss of self-confidence and depression in severe cases. The important factors of acne are induced such as hormones, genetics, chronic diseases, surrounding environment, medications, cosmetics, hygiene, human

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skin type, and microbes that occur in the epidermis of human hair follicles and skin (Hou *et al.* 2019). The most common bacteria found in acne vulgaris and lesions are *C. acnes* (syn. *Propionibacterium acnes*) and *Staphylococcus epidermidis* (Jusuf *et al.* 2020). *C. acnes* is a morphologically Gram-positive anaerobic bacillus that produces non-producing spores. It is frequently detected in patients' clinical samples. Acne treatments have been used with inorganic compounds mixed with topical agents (Jusuf *et al.* 2020). However, topical agents such as azelaic acid, benzoyl peroxide, and salicylic acid have been shown to have a significant effect on and accumulation in human cells (Choi *et al.* 2020). So, alternatively, natural medicines derived from medicinal plants have increased the number of studies.

Essentials oils (EOs) are plant secondary metabolites obtained by hydro-distillation (Owen *et al.* 2017). Various EOs have recently been shown to exhibit broad-spectrum antibacterial properties against phytopathogens and human pathogens, which is interesting for both health and economic considerations. The EOs and solvent extracts from *Panax ginseng*, *Syzigum aromaticum*, *Aniba rosaeadora*, *Cucuma longa*, *Zingiber montanum*, *Andrographis paniculata*, *Garcinia mamgostana*, *Mangifera indica*, *Rabdosia rosthornii*, *Selaginella involvens*, and *Passiflora edulis* have also been reported for anti-*C. acnes* (Bussaman *et al.* 2015; Joo *et al.* 2008; Jusuf *et al.* 2020; Kim *et al.* 2021; Kubo *et al.* 2004; Poomanee *et al.* 2018; Pothitirat *et al.* 2010). The EOs are increasingly being used by researchers to examine and protect the skin against acne-causing bacteria. The EOs from tropical areas, including Thailand, possess active antibacterial activity against *C. acnes*, which is sufficient for ongoing investigations. Many aromatic plant cultivars – especially those in the families of Alliaceae, Rubiaceae, and Zingiberaceae – are widely distributed in the central provinces of Thailand and have long been used in Thai traditional medicine. Because the active components of the plants have been characterized, this study examined the anti-acne capability of three aromatic plants used in Thai traditional medicine: *Allium sativum* (Alliaceae), *Gardenia jasminoides* (Rubiaceae), and *Hedychium coronarium* (Zingiberaceae). On GC-MS analysis, the major compositions of EO from fresh and dried rhizomes of *H. coronarium* were 1,8-cineole, α-pinene, and α-terpineol, which were evaluated against *Trichoderma* sp., *Candida albicans*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* plus the active compounds suberic acid, triparanol, ginkgolide C, and swietenine (Joy *et al.* 2007; Panigrahy *et al.* 2020). The two major phytochemicals isolated from *G. jasminoides* – genipin and crocin – have been reported to exhibit substantial antioxidant properties, hypoglycemic effects, reduction of inflammation, and restoration of barrier function and T-helper 2-mediated immune response (Xiao *et al*. 2017; Park *et al*. 2022). Sulfur-containing compounds found in *A. sativum* bulbs include ajoenes, diallyl thiosulfinate (allicin), alliin, 2-vinyl-4h-1,3-dithiin, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and allyl methyl sulfide – all of which have been associated with antiprotozoal, antioxidant, anti-inflammatory, plus anticancer, antifungal, and antibacterial activities (Batiha *et al*. 2020).

The objectives of the study were to evaluate the *in vitro* antibacterial activity against *C. acnes* from three essential oils derived from *A. sativum*, *G. jasminoides*, and *H. coronarium* cultivated in Thailand. The selected oil was also analyzed for its chemical composition by GC-MS analysis. Furthermore, the minimum inhibitory concentration of the most effective oil from Thai plant cultivars *in vitro* activity is a potential new basic guideline for acne therapies in terms of lotions, solutions, and washes.

MATERIALS AND METHODS

Plant Materials

A. sativum (garlic bulbs), *G. jasminoides* (gardenia flowers), and *H. coronarium* (*hydechium rhizomes*) were purchased from the Or Tor Kor Market, Bangkok, Thailand in 2020. All plants tested were identified and confirmed by the herbarium of the Kasin Suyathabandhu Herbarium (HCU-Herbarium) at the Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

Hydro-distillation

Ten (10) kg of each plant was cleaned and cut into small pieces before even being packed into a round-bottom flask in a Clevenger-type apparatus for 3 h. Following that, the oil was extracted with diethyl ether, filtered, and the droplets were removed with sodium sulfate anhydrous. A rotary evaporator was then used to condense the oil. After that, each essential oil was weighed and placed in a fraud paper-wrapped container for oxidation resistance (kept at 0–4 °C) (Dziri *et al.* 2014).

Gas Chromatography–Mass Spectrometry Analysis (GC-MS)

The GC-MS analysis was carried out using an Agilent 6890 NGC and an Agilent 5973 MS. The samples were analyzed using an HP-5MS fused-silica capillary column with dimensions of 30 m x 0.250 mm (inner diameter, ID) and a film thickness of 0.25 m. Helium was used as the carrier gas, with a consent flow of 1 mL/min, an injector temperature of 270 °C, and a spitless ratio of 1:100. The

temperature of the column oven was designed to rise from 60–280 °C in 1 min and then remain isothermal for 25 min. The temperature of the ion source was 250 °C, the temperature of the transfer line was 280 °C, and the ionization energy was 70 eV. Electron-impact mass spectra were collected from 20–550 amu.

The chemical components of the oil were identified using a comparison of their retention times and mass spectra with the Wiley 7N electronic libraries, which were performed by the Central Instrument Facility, Faculty of Science, Mahidol University, Bangkok, Thailand.

Bacterial Strain

C. acnes was supplied by the Center of Excellence in Natural Products Chemistry, Department of Chemistry, Chulalongkorn University.

Antibacterial Assay

The antibacterial activity was conducted using the agar diffusion assay (Barry *et al.* 1988). Briefly, the bacterial inoculum was maintained in nutrient agar (NA) at 37 °C for 24 h in anaerobic conditions. After that, 2–3 colonies were chosen for transferring to be cultured in nutrient broth (NB) under anaerobic conditions at 37 °C for 24 h, and the number of cells was counted at an absorbance of 595 nm of 1.5 x 10^8 CFU/mL (McFarland No. 0.5) standardization and adjusted by 0.85% w/v sterile sodium chloride solution. The NAs (19 mL for each tube) had been melted and combined with the adjusted cell suspension (1 mL) and then poured onto the test plates, allowing them to set. The test plates were made with a 5-mm cork borer and loaded with 55 µL of oil with a final concentration of 10 mg/mL (dissolved in 10% dimethyl sulfoxide, DMSO) in such wells, compared with 10% DMSO (negative control) and 1 mg/mL erythromycin (positive control). The plates were incubated in anaerobic conditions overnight at 37 °C, and the inhibition zones were measured.

Determination of the Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The NCCL's procedure was used to assess the susceptibility of macro-dilution broth (Vandenbossche *et al.* 2002). The chosen oil was dissolved in 10% DMSO. Serial dilutions of the oil from 100 mg/mL to 0.00 mg/mL were generated 10-fold in 10 experiment tubes with sterile NBs (1 mL) as diluents. Each dilution was piped with 1 mL of test bacteria at the standard concentration (1.5 x 10^8 CFU/mL). The 1% erythromycin and the DMSO (10%) were employed as positive and negative controls, respectively. The conduits were incubated at 37 °C for 24 h. The MIC was comprised of the lowest oil extract concentration, which demonstrated no significant visible growth. After 24 h of incubation, the MIC values were determined. Duplicates of the bacterium were carried out, even with results expressed as quantitative variables. Each MIC broth's test media (10 mL) was disseminated over NB panels. The plates were incubated in anaerobic conditions for 24 h at 37 °C. A MBC of the extract that indicated no bacterial growth in agar plates was observed (Singh *et al.* 2011). The MIC indices were calculated using MBC/MBC values to indicate the mode of action, including bactericidal (MIC index < 4) and bacteriostatic (MIC index ≥ 4) (Sreepian *et al*. 2022).

Statistical Analysis

The data set was analyzed using the SPSS software for Windows version 20.0. A comparison with the DMRT was performed, and significance was observed at the $p < 0.05$ level. The experiment was designed as a general linear model with duplication within a completely randomized design.

RESULTS

Percentage Yields

The percentage yield (%w/w) of *A. sativum*, *G. jasminoides*, and *H. coronarium* gave 1.12, 1.09, and 1.05, respectively, are represented in Table 1.

Table 1. Yields of essential oils.

Plant species	% yield (w/w)	Color characteristics
Allium sativum	1.12	Pale-yellow clear liquid
Gardenia jasminoides	1.09	Pale-yellow clear liquid
Hedychium coronarium	1.05	Pale-yellow clear liquid

Antibacterial Activity

The antibacterial activity of essential oils performed against *C. acnes* is presented in Table 2. The size of

Table 2. Antibacterial activity of essential oils against *C. acnes*.

Samples (10 mg/mL)	Inhibition zone (mm[a] ± SD)
Allium sativum	52.25 ± 3.89[a]
Gardenia jasminoides	29.10 ± 2.68[b]
Hedychium coronarium	19.63 ± 1.24[c]
1% erythromycin (1 mg/mL, positive control)	22.75 ± 2.05[c]
10% DMSO (negative control)	0.00[d]

[a]Values for inhibition zones are represented as mean ± SD of duplications and mean values with different superscript letters in each column are significantly different ($p < 0.05$, DMRT).

Table 3. MIC and MBC plus MIC index of garlic oil against *C. acnes*

Treatment	Concentration (mg/mL)		MIC indices	Indication[a]
	MIC	MBC		
Garlic oil	0.39	0.78	2.00	Bactericidal
1% erythromycin	< 0.01	< 0.01	1.00	Bactericidal

[a]Indication based on the MIC indices – MBC/MIC; bactericidal – MIC indices < 4 and bacteriostatic ≥ 4.

inhibition zones to indicate relative antibacterial activity is not typically quantitative and sufficient. Garlic oil at 10 mg/mL displayed the significantly highest inhibition zone, followed by gardenia oil and hydechium rhizome oil. Therefore, the garlic oil was selected for the MIC/MBC values and subjected to chemical constituents.

The MIC values were determined in NB using the macro dilution technique. The garlic oil and 1% erythromycin had MIC (MBC) values of 0.39 (0.78) and 0.01 (0.01) mg/mL, respectively (Table 3). Due to its bactericidal properties, garlic oil was suitable for subjecting to phytochemical investigations.

Chemical Compositions of the Garlic Oil

GC/MS analysis of the hydro-distillation of the garlic oil gave 59 components, accounting for 99.8% of the total oil (Figure 1). Diallyl thiosulfinate (syn. = allicin) was the most abundant of the 59 compounds, amounting to 20.53%, followed by 1,3-dithiane (12.53%), cyclic octaatomic sulfur (5.02%), 1,4-dihydro-2,3-benzoxathiin-3-oxide (4.73%), and carvone (3.40%) (Table 4).

Table 4. Chemical compositions of Thai garlic oil.

RT[a]	RI[b]	Compound	% area[c]
4.86	978	1,3-dithiane	12.53
6.20	1052	Diallyl sulfide (DAS)	0.14
6.76	1091	Diallyl thiosulfinate (allicin)	20.53
6.90	1101	Allyl (E)-1-propenyl disulfide	0.34
6.99	1107	Benzyl alcohol	0.70
7.23	1124	Methyl allyl-trisulfide	0.10
7.57	1148	Methyl propyl trisulfide	2.53
7.85	1167	Methyl (E)-1-propenyl trisulfide	2.84
8.32	1200	3-vinyl-1,2-dithiocyclohex-4-ene	3.17
8.52	1214	2-vinyl-4H-1,3-dithiine	1.85
8.60	1220	1,2-dithiole	0.53
8.65	1224	3-dithiin-2-vinyl-4H-1	0.66
8.82	1236	Dimethyl trisulfide	0.16
8.91	1243	1.4-dimethyl tetrasulfide	0.26
9.01	1250	l-menthone	0.22
9.37	1276	Diallyl tetrasulfide	0.41

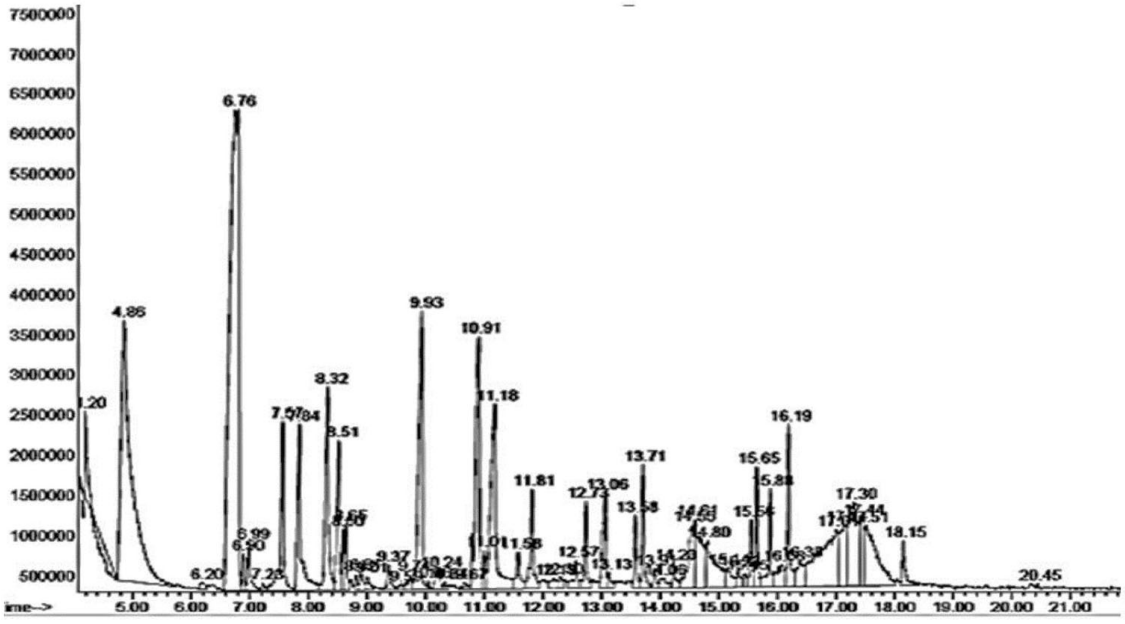

Figure 1. GC/MS chromatogram of Thai garlic oil.

Table 4. Chemical compositions of Thai garlic oil.

9.59	1292	p-menthan-3-one	0.12
9.73	1302	Diallyl trisulfide (DATS)	0.29
9.93	1317	Allyl propyl trisulfide	4.47
10.05	1325	Allyl (E)-1-propenyl trisulfide	0.06
10.24	1339	Pulegone	0.42
10.34	1346	Eugenol	0.08
10.67	1370	5-methyl-1,2,3,4-tetrathiane	0.15
10.91	1388	α-limonene	4.72
11.01	1395	Menthyl acetate	0.41
11.18	1408	1,4-dihydro-2,3-benzoxathiin 3-oxide	4.73
11.58	1440	[(E)-1-propenyl] 2-thiopent-3-yl disulfide	0.65
11.82	1459	Aromadendrene	1.36
12.18	1487	Fluorosilyl-bis(hexamethylcyclo-trisilzane)	0.32
12.31	1498	α-bisabolene	0.18
12.57	1518	γ-cadinene	0.52
12.73	1531	2,4,7,9-tetramethyl- 5decyn4,7diol	0.91
13.06	1557	Dially tetrasulphide	1.40
13.13	1563	(E)-1-allyl-2-(prop-1-en-1-yl) disulfane	0.24
13.57	1598	Disulfide, dipropyl	0.74
13.71	1610	Di-(propen-1'-yl)-sulfide-*cis*	1.22
13.90	1626	Di-(propen-1'-yl)-sulfide-*trans*	0.27
14.06	1640	2,4-dimethylthiophene	0.30
14.23	1655	Trisulfide, methyl-2-propenyl	0.36
14.55	1684	4-methyl-1,2,3,5,6-pentathiepane	1.77
14.61	1689	2,4-dimethylthiophene	2.14
14.80	1706	4-methyl-1,2,3-trithiolane	2.10
15.14	1736	Allyl methyl disulfide	0.52
15.33	1752	ά-bisabolol oxide	0.32
15.45	1763	Disulfide, methyl 1-propenyl-*cis*	0.21
15.55	1772	3-vinyl-3,6-dihydro-1,2-dithiine	0.72
15.65	1780	1-thia-2-(2,3-dithia-5-hexenyl)-5-cyclohexane	1.05
15.88	1801	1-monolinoleoylglycerol-tri-methylsilyl ether	1.11
16.08	1828	Avocadynofuran	0.76
16.19	1842	Disulfide, methyl 1-propenyl-*trans*	1.73
16.38	1868	2-vinyl-4H-1,3-dithiine	0.86
17.01	1951	Cyclic octaatomic sulfur	5.02
17.15	1970	(Z)-methyl-isoprenyl cinnamate	1.97
17.30	1990	Carvone	3.40
17.44	2011	2-oxazolidinethione	1.29
17.51	2023	Cyclooctasulfur	3.43
18.15	2134	Hexadecanoic acid, methylester	0.64
20.45	2582	Allyl methyl tri-sulfide	0.05
Total			**99.98**

Note: [a]RT – retention time (min); [b]RI – retention indices calculated against a homologous series of *n*-alkane standards (C_{10}–C_{36}); [c]relative area (%)

DISCUSSION

The percentage yield of garlic oil was higher than that of Satyal *et al.* (2017) but lower than that of Yasmin *et al.* (2020).The percentage yield in the case of gardenia flower oil was greater than in earlier investigations (Chaichana *et al.* 2018; Chekki *et al.* 2014). In particular, the amount of hedychium rhizome oil obtained was larger than in prior studies (Noriega *et al.* 2019; Santos *et al.* 2010). Temperature and precipitation levels were used to assess the climate at various locations. EO yields have been shown to be reduced by higher average temperatures and radiation (Luro *et al*. 2020). The garlic oil showed the best bacterial tests and was similar to Chekki *et al.* (2014). The anti-acne activity of garlic oil was higher than that of the other plant extracts and oils (Bussaman *et al.* 2015; Hou *et al.* 2019; Jusuf *et al.* 2020; Kim *et al.* 2021; Kubo *et al.* 2004; Owen *et al.* 2017; Poomanee *et al.* 2018). The antimicrobial properties of essential oils are ascertained by their chemical compositions and quantity (Saharkhiz *et al.* 2015). The Thai cultivar of the garlic oil in our study displayed higher activity than other cultivars from other countries in topical usage because the Thai cultivars displayed a higher percentage of allicin content than other countries (Genatrika *et al.* 2020; Saptarini and Herawati 2017). Moreover, the garlic oil performed higher anti-acne activity than other studied essential oils cultivated in Thailand, such as citronella oil, clove oil, coriander oil, galanga oil, ginger oil, guava leaf oil, holy basil oil, jasmine oil, kaffir lime oil, lavender oil, lemongrass oil, galangal oil, michelia oil, plai oil, sweet basil oil, tea tree oil, turmeric oil, guava leaf oil, ylang ylang oil, thyme oil, and cinnamon oil (Athikomkulchai *et al.* 2018; Julianti *et al.* 2017; Luangnarumitchai *et al.* 2007; Matiz *et al.* 2015). The ethanol extracts of Nepalese wild mushrooms such as *Inonotus andersonii, I. clemensiae, I. cuticularis, I.* sp., and *Cyclomyces setiporus,* which had the most inhibitory impact on *C. acnes,* had less anti-*C. acnes* activity than in our study (Tamrakar *et al.* 2017). Garlic oil and extracts have been reported for their ability to control Gram-positive and Gram-negative bacteria, as well as fungal infections such as *Staphylococcus aureus*, *S. epidermidis*, *P. aeruginosa,* Streptococci spp., and *Escherichia coli* in the gel formulation (Khairan *et al.* 2019; Abiy and Berhe 2016; Genatrika *et al.* 2020; Bayati 2018; Bhatwalkar *et al.* 2021; Sorlozano-Puerto *et al.* 2018). In the case of MIC results,

the garlic oil showed inhibition close to that derived from kaffir lime leaf oil, clove oil, citronella oil, and lemongrass oil, which have been reported (Luangnarumitchai *et al.* 2007). The garlic oil's MIC index was estimated at below 4, suggesting that it was bactericidal and that the oil might interrupt the bacterial cell wall and cell membrane synthesis and could also damage DNA synthesis. Allicin's antibacterial activity is thought to be due to its chemical interaction with thiol-containing enzymes like thioredoxin reductase, RNA polymerase, and alcohol dehydrogenase, which oxidize protein cysteine or glutathione residues under physiological conditions (Batiha *et al.* 2020). Moreover, the modes of action of garlic oil as a synergistic agent with ampicillin in inhibiting cell wall synthesis have been reported (Eja *et al.* 2007). The bulbs of the garlic oil had good activity against MSSA (methicillin-sensitive *S. aureus*) and MRSA (methicillin-resistant *S. aureus*) as Gram-positive bacteria, as indicated by the higher MIC and MBC values (Low *et al.* 2021). Previous reports identified the main component, which contrasted with another investigation that found allyl methyl tri-sulfide to be more common than allicin (Ashraf *et al.* 2019). Interestingly, the oil from our study's GC/MS analysis could analyze more compositions than in previous studies (Chekki *et al.* 2014; Dziri *et al.* 2014; Satyal *et al.* 2017; Wang *et al.* 2019; Zhang and Wang 2019). However, the allicin amount of 20.53% was lower than that reported by Chekki *et al.* (2014) and Wang *et al.* (2019), which was equivalent to those of Zhang and Wang (2019) and Dziri *et al.* (2014). According to Bhatwalkar *et al.* (2021), organosulfur compounds derived from garlic were found to have antibacterial action against several Gram-positive bacteria. Furthermore, allicin has an antifungal effect on yeast cells – affecting DNA replication, mitochondrial translation, and chromatid cohesion (Khameneh *et al.* 2021). Garlic allergies are uncommon, but in those who are susceptible, they can cause skin irritation, rhinoconjunctivitis, asthma, urticaria, and other symptoms (Kao *et al.* 2004). The primary allergens in garlic, allicin, DADS, and allylpropyl disulfide (Farrell and Staughton 1996), are thought to cause the most allergic symptoms, with DAS being the most allergenic compound when applied topically. In addition, the garlic oil and allicin might well have strong antibacterial properties against *C. acnes*, which should be used to develop topical antiacne therapeutics in the form of lotions, solutions, and washes for human skin.

CONCLUSION

According to the findings, essential oils extracted from *A. sativum*, *G. jasminoides*, and *H. coronarium* displayed significant antibacterial action against *C. acnes*. Garlic oil had the most potent antibacterial action against *C. acnes*. Diallyl thiosulfinate (allicin) was the main component of garlic oil. Garlic oil might be utilized to influence the development of topical formulations for medicinal usage in the future. Furthermore, the anti-acne MIC of topical garlic essential oil grown in Thailand could be used for new alternative topical treatments on humans, such as healing acne wounds and reducing allergic skin.

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STATEMENT ON CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

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