Phytochemicals and antimicrobial properties of Thai edible plant extracts and their prebiotic-like effects

Sarita Sangthong [a,b], Santi Phosri [c], Nont Thitilertdecha [a,b], Nara Yaowiwat [a,b], Phanuphong Chaiwut [a,b], Mayuramas Wilai [a,d], Namfa Sermkaew [e,f], Satyajit D. Sarker [g], Lutfun Nahar [h,**], Tinnakorn Theansungnoen [a,*]

[a] School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand
[b] Green Cosmetic Technology Research Group, School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand
[c] Department of Chemical Engineering, Faculty of Engineering, Burapha University, Chonburi, 20131, Thailand
[d] Phytocosmetics and Cosmeceuticals Research Group, Mae Fah Luang University, Chiang Rai, 57100, Thailand
[e] School of Pharmacy, Walailak University, Nakhon Si Thammarat, 80160, Thailand
[f] Drug and Cosmetic Excellence Center, Walailak University, Nakhon Si Thammarat, 80160, Thailand
[g] Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK
[h] Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, The Czech Academy of Sciences, Šlechtitelů 27, 78371, Olomouc, Czech Republic

ABSTRACT

In this study, the phytochemicals and antimicrobial activities of ethanolic extracts of Thai edible plants, green tea, red cotton tree flower, fingerroot and ginger were evaluated. The plant extracts were taken for evaluation of antimicrobial activities against *Cutibacterium acnes* DMST 14916, *Staphylococcus epidermidis* TISTR 518, and *Staphylococcus aureus* TISTR 746. The minimum inhibitory concentrations (MICs) of green tea, fingerroot, and ginger extracts against *C. acnes* DMST 14916 were 3.92, 0.49, and 7.85 mg cm^{-3}, respectively and the minimum bacteriostatic concentrations (MBCs) were 3.92, 0.49, and 7.85 mg cm^{-3}, respectively. The MICs and MBCs of fingerroot extract against *S. epidermidis* TISTR 518 were 0.12 and 0.49 mg cm^{-3}, respectively, while those against *S. aureus* TISTR 746 were 0.12 and 0.98 mg cm^{-3}, respectively. Red cotton tree flower extract showed no anti-microbial activity against the acne-causing bacteria. By scanning electron microscopy (SEM) evaluation, the bacterial cells treated with the plant extracts revealed visible shrinkages compared to the smooth cell surfaces of the controls. The phytochemicals in the plant extracts were analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). Well-known antimicrobial compounds like azelaic acid, embelin and kaempferol 3-rutinoside-4′-glucoside were identified in all extracts. The cytotoxic effects of the plant extracts on human cell lines were further investigated. The green tea extract was slightly toxic to HaCaT cells found at the initial concentration of 62.5 mg cm^{-3}, but not toxic to MRC-5 cells. The fingerroot and ginger extracts had no cyto-toxicity on HaCaT cells, but promoted the MRC-5 cell proliferation. The combination effects of the plant extracts were prebiotic-like and indifferent effects. Regarding all results, the ethanolic extracts of green tea, fingerroot, and ginger could be used individually as natural anti-acne ingredients capable of further product development to improve human skin health.

1. Introduction

Acne vulgaris, commonly known as acne, is a skin condition that occurs in the pilosebaceous unit of the skin follicles, and particularly prevalent in adolescents and young adults [1]. Acne can cause skin problems ranging from mild abnormal lesions to severe permanent acne scars, and sometimes leads to long-lasting psychosocial effects, such as depression, social isolation, and even suicidal tendency [2]. *Cutibacterium acnes*, an anaerobic Gram-positive bacterium, is a common cause in acne pathogenesis and important for acne progression and severity [1,

3]. In a previous study, common aerobic Gram-positive bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus* were also isolated from patients with acne [4]. Moreover, *C. acnes* and *S. epidermidis* were reported to promote pus formation and inflammatory lesions of acne [3,5].

Owing to the multifactorial pathogenicity, acne progression and severity influenced by acne-causing bacteria are treated by many drugs, particularly antibiotics. Topical antibiotics have been used to treat mild and moderate acne lesions, while oral antibiotics are used for severe acne lesions. However, some antibiotics, such as erythromycin, clindamycin and tetracycline, have been found to induce resistances of some bacterial strains [6,7]. The increasing antibiotic resistance of *C. acne* to topical macrolides (erythromycin and azithromycin) has been found in many countries, which is the leading cause of therapeutic failure of acne treatment [8]. To overcome the failure and antibiotic resistance, novel antimicrobial agents have been discovered and developed from many natural sources, particularly herbs and plants [9]. Plants and their extracts are the most-used natural resources to address human health problems; they have been widely tested for many pharmacological and biological activities, such as antioxidant, anti-inflammatory, and antimicrobial activities [9–11]. Plant-derived antimicrobial agents are considered an alternative approach for the treatment of bacterial infections because these agents may be capable of killing bacteria with a different mode of action than conventional antibiotics [12,13].

Camellia sinensis (L.) Kuntze var. *Assamica*, commonly known as green tea, is a 2–5 feet shrub of the family Theaceae, cultivated in many Asian countries, especially in the Chiang Rai province of Thailand. Green tea leaves have antioxidant, antiviral, antitumoral, and antimicrobial properties [14,15]. The biological important components of green tea are the polyphenols, most importantly the flavonoids. The main flavonoids in tea are the catechins, which possesses antimicrobial property [16]. Extracts of green tea were found to be antimicrobial against Gram-positive bacteria including *Micrococcus luteus*, *Bacillus subtilis*, *Streptococcus pneumonia*, and *S. aureus* [17]. *Bombax ceiba* L., commonly known as silk cotton tree or red cotton tree, belongs to the family Bombacaceae, which is widely found in Africa, Australia, temperate Asia, and Tropical Asia [18]. This plant has been used for traditional medicines, especially in Ayurvedic medicine. The bark gum of *B. ceiba* could cure bleeding piles, body burns, blood diseases, dysentery, influenza, pulmonary tuberculosis, enteritis, menorrhagia, and burning sensation. Bark extracts of this species exhibited antimicrobial properties against *Pseudomonas aeruginosa*, *Xanthomonas maltophilia*, *B. subtilis*, *Escherichia coli*, and *Candida albicans* [19]. *B. ceiba* flowers also possess pharmacological properties such as antioxidant, anti-inflammatory, and antimicrobial activities [20]. *Zingiber officinale* Rosc., known as ginger, is a member of the family Zingiberaceae that has been used not only as spices but also as traditional herbal medicine. Ginger can be used to treat diarrhoea, cough, and bacterial infections [21]. Ginger extracts possess many pharmacological properties, such as antioxidant, and antibacterial activities. The aqueous extracts of ginger possess great antioxidant activity which can potentially aid in the management of dyslipidemia [22]. Ginger can inhibit the growth of bacteria, such as *E. coli*, *B. subtilis*, and *Staphylococcus* sp [23,24]. *Boesenbergia rotunda* (L.) Mansf., commonly known as fingerroot or Chinese keys, is a perennial herb of the family Zingiberaceae, widely distributed in many Asian countries, particularly in India, Sri Lanka, Southern China, and Southeast Asia [25]. The rhizome of *B. rotunda* has been utilized for food ingredients, such as spices, flavouring agents, and dyes, as well as for traditional herbal medicines [26]. This plant could exhibit antifungal, antiviral, anti-inflammatory, anticancer, hepatoprotective, antiparasitic, and antibacterial activities [27].

Although many pharmacological activities of the above plants, particularly antimicrobial activity, were evaluated, to the best of our knowledge, the antimicrobial activity against acne-causing bacteria and phytochemical analysis of the ethanolic extracts of the plants have not been completely investigated. Therefore, the present study aimed to evaluate the antimicrobial activity against acne-causing bacteria of selected Thai plant extracts including green tea, red cotton tree flower, ginger, and fingerroot extracts, and to identify their phytochemicals performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

2. Materials and methods

2.1. Chemicals and reagents

Nutrient agar medium (Catalog No. 1HMD-M001), nutrient broth medium (Catalog No. 1HMD-M002, brain heart infusion broth medium (Catalog No. 1HMD-M210), brain heart infusion agar medium (Catalog No. 1HMD-M211), resazurin sodium (Catalog No. 1HMD-RM125-1g), and ethanol (Catalog No. 1HMD-ML013) were purchased from HiMedia Laboratories Private Limited (Maharashtra, India). CO2 charged atmosphere bag (Catalog No. 1SCB-3.880.400) was purchased from schuett-biotec GmbH (Göttingen, Germany). RPMI1640 medium (Catalog No. 1IVG2-11875-093) and phosphate-buffered saline (PBS) (Catalog No. 1IVG4-10010-023) were purchased from Thermo Scientific (Massachusetts, U.S.A). Methylene blue (Catalog No. KA1137-2) was manufactured by Elago Enterprises Pty Ltd., (N.S.W., Australia). 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) (Catalog No. ACRO158990050), acetonitrile (Catalog No. 9829-88) and Water (Catalog No. 9831-P04) were purchased from VWR international limited (Lutterworth, UK). Formic acid (Catalog No. AAB-A117-50) was manufactured by Thermo Fisher Scientific (Pardubice, Czech Republic).

2.2. Bacterial strains and plant samples

Bacteria including *C. acnes* DMST 14916, *S. aureus* TISTR 746, and *S. epidermidis* TISTR 518 were obtained from the Biology and Biotechnology Laboratory, the Scientific & Technological Instruments Center, Mae Fah Luang University, Chaing Rai, Thailand. Plants used in this study included green tea (*C. sinensis*), red cotton tree flower (*B. ceiba*), fingerroot (*B. rotundo*) and ginger (*Z. officinale*). The selected plants were purchased from local markets in Chiang Rai and Chiang Mai, Thailand.

2.3. Preparation of plant extracts

The dried plant parts were placed into a tray dryer at 60 °C for 6 h and then pulverized using a hammer mill grinder. The extraction condition of the dried plant powders was modified slightly from the previous study [28]. Briefly, the plant powders (20 g) were soaked separately in 95 % ethanol (0.2 dm^3) in 0.5-dm^3 flasks with complete covers. The sample flasks were taken to an incubator shaker at room temperature, overnight. The samples were filtered through a Whatman No.1 paper. The filtrates were evaporated to eliminate the solvent by a rotary evaporator at 45–50 °C. The crude extracts of plants were kept at 4°C in a freeze for further use.

2.4. Antimicrobial assay

The antimicrobial activity of plant extracts against selected bacteria was evaluated by the broth micro-dilution method, which was modified from previous studies [29,30]. Briefly, bacterial cells were grown to log phase (OD_{600nm} = 0.5-0.8) and diluted to the cell density at approximately 10^6 cells cm^{-3} (OD_{600nm} = 0.001). The bacterial cells were incubated in the presence of plant extract concentrations (0.03-15.69 mg cm^{-3}) at 37 °C for 24 h for *S. epidermidis* TISTR 518 and *S. aureus* TISTR 746, and under an anaerobic condition at 37 °C for 72 h for *C. acnes* DMST 14916. Triplicate tests were performed. The minimal inhibitory concentration (MIC) values were measured by a resazurin dye solution technique [31,32]. The maximum bactericidal concentration (MBC) values were further investigated on the bacteria by a standard colony plate count technique.

2.5. Scanning electron microscopy (SEM)

The antimicrobial effects of the plant extracts on *C. acnes*, *S. epidermidis* and *S. aureus* were further investigated by SEM with a modified method of Lau et al., 2004 [33]. Briefly, *C. acnes*, *S. epidermidis* and *S. aureus* were cultured in anaerobic and aerobic conditions, to log phase (OD_{600nm} = 0.5-0.8). Bacterial cells in the cultures were harvested by taking to the centrifugation at approximately $3500 \times g$ for 3 min and washing twice with PBS (pH 7.4). The bacterial cells were re-suspended and diluted to a cell density of approximately 10^8 cells cm^{-3} (OD_{600nm} = 0.1). The diluted cells were incubated in the presence of plant extracts at 10xMICs for 5 min and 60 min under the same conditions. Cells without any treatment were controls. Each bacterial sample (10 mm^3) was smeared on a cover slide and fixed by moving it through a fire, three times. The bacterial cells were dehydrated by adding 0.1 cm^3 of ethanol solution with a series at 30 %, 50 %, 70 %, 90 %, 90 %, 100 %, and 100 %, respectively, for 30–60 min in each solution. The dehydrated bacterial cells were coated with gold-palladium and observed with a Field Emission Scanning Electron microscope (TESCAN MIRA4, Brno, Czech Republic).

2.6. LC-MS/MS analysis

The phytochemical composition of the selected Thai herbal extracts was determined by LC-MS/MS analysis as the previous study [34]. Briefly, the samples of extracts (500 ppm) were prepared by dilution in the absolute methanol and then filtered through a 0.2 μm NYL filter, and filled into a vial. The samples were individually injected to the LC system operated by a combination of Agilent 1290 UHPLC (Agilent Technologies, Santa Clara, CA, USA) and Agilent Poroshell EC-C18 (2.1 mm × 150 mm, 2.7 μm) column with Agilent Poroshell EC-C18 (4.6 mm × 5 mm, 2.7 μm) guard column. The LC separation was achieved at a flow rate of 0.2 cm^3 min^{-1} under a time and gradient programme in which mobile phase A was composed of 0.1 % (v/v) formic acid in water and mobile phase B was composed of 0.1 % (v/v) formic acid in acetonitrile. For MS acquisition, the data was obtained with an Agilent G6454B Q-TOF Mass Spectrometry (Agilent Technologies, California, USA). The operation system of the mass spectrometer used a Dual AJS ESI ion source. The capillary voltage (VCap) and nozzle voltage of both ion modes was set at 4000 V and 500 V, respectively. The voltages of the fragmentor, skimmer1, and OctopoleRFPeak were kept at 150 V, 65 V, and 750 V, respectively. The scan range was adjusted to 100–1100 *m/z* at the scan rate of 1.00 spectra/sec. Agilent reference mass solution containing internal reference compounds with *m/z* 112.98558700 and *m/z* 1033.98810900 for the negative mode, and *m/z* 121.05087300 and *m/z* 922.00979800 for the positive mode was infused into the MS by Agilent 1260 isocratic pump. For MS/MS acquisition, the data was achieved by setting at the same scan source parameters of the MS acquisition, as well as at 10, 20, or 40 eV of collision energy.

2.7. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

The cytotoxic activities of selected Thai plant extracts were investigated on human cell lines by MTT assay [35]. The samples of the plant extracts were prepared in the various concentrations. HaCaT (approximately 1×10^4 cells/well) and MRC-5 cells (approximately 1×10^4 cells/well) were seeded onto 96-well plates in new RPMI-1640 medium for 24 h of incubation. Cells were treated with the herbal extracts and then incubated under a humidified condition of 5 % CO_2 at 37 °C for 24 h. A 0.15-cm^3 MTT solution (0.5 mg cm^{-3}) was added to each sample well and incubated for 1 h. A 0.1-cm^3 Dimethyl sulfoxide (DMSO) solution was added to the same wells to solubilize the formazan salt product. The solubilized solutions were taken for measurement at 550 nm, the cell viability of each sample was determined by comparing the absorbance values of extract-treated and untreated cells.

2.8. Methylene blue staining

The morphological evaluation of the plant extracts on HaCaT and MRC-5 cells was carried out by the methylene blue staining method [36]. Briefly, cells treated with various concentrations of the rhizome extracts for different time intervals. The treated cells were washed with ice-cold phosphate-buffered saline (PBS), fixed with 50 % ice-cold ethanol. The cells were stained by methylene blue staining solution (0.2 %, w/v) for 30 s. The solution was aspirated, and the cells were washed thrice with ice-cold water. The cell samples were dried and observed under a light microscope.

2.9. Micro-checkerboard assay

The combination effects of selected Thai plant extracts were investigated by the micro-checkerboard technique [37]. The sample concentrations of the plant extracts were prepared with two-fold dilution by starting at the least 2xMICs of each extract according to the recommendations of the National Committee on Clinical Laboratory Standards (NCCLS). All extract samples were individually added to a 0.1-cm^3 *C. acnes* solution (approximately 1x10^6 cfu cm^{-3}) in each well of 96-well plates, and then incubated at an anaerobic condition for 72 h at 37 °C. The interaction for each combination was determined by calculating the fractional inhibitory concentration (FIC) of each extract sample and then summarizing their FIC to ΣFIC as follows:

ΣFIC = FICA + FICB, where FICA is the MIC of sample A in the combination divided by the MIC of sample A alone, and FICB is the MIC of sample B in combination divided by the MIC of sample B alone. The interaction of each combination is considered as being synergistic for ΣFIC values of ≤0.5, additive (>0.5–1.0), indifferent (≥1.0–≤4.0) or antagonistic (ΣFIC >4.0) [38].

2.10. Statistical analysis

The differences between control and sample groups were evaluated by the statistical software Statistix ver. 9.0. Comparisons among groups were determined by analysis of variance using ANOVA test. Significant difference analysis between control and sample groups were carried out via the Student's t-test, and the significance was considered when P-value was less than 0.05 (*$P < 0.05$*).

3. Results

3.1. Yields of crude extracts of selected Thai plants

The dried parts used of selected Thai edible plants, green tea leaves, red cotton tree flowers, fingerroot rhizomes, and ginger rhizomes, as shown in Fig. 1, were powdery grinded and taken for the ethanol extraction. The yield of green tea extract was 38 ± 2 %, while that of red cotton tree flower extracts was 5.5 ± 0.4 %. The crude fingerroot and ginger extracts yielded 12.2 ± 0.5 % and 12.3 ± 0.2 %, respectively.

3.2. Antimicrobial activity of selected Thai plant extracts

The antimicrobial activity of the selected Thai plant extracts was determined by the broth-microdilution assay on three bacteria, *C. acnes* DMST 14916, *S. epidermidis* TISTR 518, and *S. aureus* TISTR 746. The extracts of green tea, fingerroot, and ginger exhibited antimicrobial activity against *C. acnes* DMST 14916 (Table 1). The fingerroot extract could also inhibit *S. epidermidis* TISTR 518 and *S. aureus* TISTR 746. The MICs and MBCs of green tea extract against *C. acnes* were 3.92 mg cm^{-3} and 3.92 mg cm^{-3}, respectively. The MICs and MBCs of fingerroot extracts against *C. acnes* were 0.49 mg cm^{-3} and 0.49 mg cm^{-3}, while those of ginger extract were 7.85 mg cm^{-3} and 7.85 mg cm^{-3}, respectively. The MICs and MBCs of fingerroot extracts against *S. epidermidis* were 0.12 mg cm^{-3} and 0.49 mg cm^{-3} while those against *S. aureus* were 0.12 mg

Fig. 1. Dried parts used of selected Thai edible plants. Dried leaves of green tea (a), dried flowers of red cotton tree, dried rhizomes of fingerroot (c), and dried rhizomes of ginger (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1

Minimum inhibitory concentration (MIC) and minimum bacteriostatic concentration (MBC) of selected Thai plant extracts against *C. acnes* DMST 14916, *S. epidermidis* TISTR 518, and *S. aureus* TISTR 746.

Plant extracts	*C. acnes* DMST 14916		*S. epidermidis* TISTR 518		*S. aureus* TISTR 746	
	MIC (mg cm^{-3})	MBC (mg cm^{-3})	MIC (mg cm^{-3})	MBC (mg cm^{-3})	MIC (mg cm^{-3})	MBC (mg cm^{-3})
Green tea extract	3.92	3.92	Nd	Nd	Nd	Nd
Red cotton tree extract	Nd	Nd	Nd	Nd	Nd	Nd
Fingerroot extract	0.49	0.49	0.12	0.49	0.12	0.98
Ginger extract	7.85	7.85	Nd	Nd	Nd	Nd

Nd = Not detected at the concentration ranged from 0.03 to 15.69 mg cm^{-3}

cm^{-3} and 0.98 mg cm^{-3}, respectively. The extract of red cotton tree flower showed no antimicrobial activity on these bacteria at the tested concentrations.

3.3. Antimicrobial effects on bacterial cells observed by SEM

The antimicrobial effects of the selected plant extracts on bacterial cells were investigated using SEM. The antimicrobial effects of the extracts on *C. acnes* DMST 14916, *S. epidermis* TISTR 518 and *S. aureus* TISTR 746 are shown in Figs. 2 and 3. As shown in Fig. 2, control cells of *C. acnes* are untreated with any extract which show smooth cell surfaces without obvious damages (Fig. 2a). The SEM images of *C. acnes* treated with extracts of ginger, fingerroot, and green tea at 10xMICs for 60 min are displayed in Fig. 2b-d, respectively. As shown in the figures, most cell surfaces of *C. acnes* treated with each extract have visible shrinkages (black arrows). The SEM images of Fig. 3a and c are control cells of *S. epidermidis* and *S. aureus*, respectively, which have smooth cell surfaces. Cells of *S. epidermidis* and *S. aureus* treated with fingerroot extract (10xMICs) showed slight shrinkages after 60-min incubation (brown arrows) (Fig. 3b and d).

3.4. Cytotoxic activities of selected Thai plant extracts

The cytotoxicity of plant extracts was evaluated by the MTT assay on

human skin cells (Fig. 4). The effects of the plant extracts on HaCaT and MRC-5 cells were compared to those of untreated cells (controls). The green tea extract exhibited cytotoxicity against HaCaT cells at 62.5 μg cm^{-3} (30 ± 2 %) (Fig. 4a), while the ginger and fingerroot extracts showed no cytotoxicity reached the concentration of 1000 μg cm^{-3} ($P>0.05$) (Fig. 4c and e). The extract of green tea had no toxicity on MRC-5 cells reached the concentration of 1000 μg cm^{-3} (Fig. 4b) while the extracts of ginger and fingerroot could promote the cell viability beginning at 62.5 μg cm^{-3} ($P<0.05$) as shown in Fig. 4d and f, respectively.

The cell morphological changes were visible in methylene blue stained HaCaT cells (Fig. 5) and MRC-5 cells (Fig. 6) treated with the different concentrations of the plant extracts (62.5 μg cm^{-3} -1000 μg cm^{-3}). As shown in Fig. 5b-f, the treated HaCaT cells with the green tea extract were abnormal of cell morphological shapes compared to the untreated cells (Fig. 5a). The morphological abnormality of the HaCaT cells were more visible observed depending on the dose-manner of the green tea extract. The morphological appearance of HaCaT cells treated with the fingerroot extract (Fig. 5g-k and ginger extracts were found to be normal shapes similar to the untreated cells (Fig. 5a). The MRC-5 cells treated with the green tea extract (Fig. 6b-f), fingerroot extract (Fig. 6g-k), and ginger extract (Fig. 6l-p) were present in spindle shaped morphology similar to the untreated cells (Fig. 6a).

3.5. LC-MS/MS analysis of selected Thai plant extracts

The phytochemical analysis of plant extracts was performed by LC-MS/MS. The LC-MS chromatograms were shown as Fig. S1 (available as Supplementary data). Twenty major compounds in green tea, fingerroot, and ginger extracts were identified as shown in Tables 2-4, respectively. Among the major phytochemicals, there were thirteen similar compounds present in all extracts, including (5*R*,6*S*)-5,6-epoxy-7-megastigmen-9-one, (9*R*,10*S*,12*Z*)-9,10-dihydroxy-8-oxo-12-octadecenoic acid, 12-oxo-2,3-dinor-10,15-phytodienoic acid, 3,5-dinitroguaiacol, 3-hydroxyphenyl-valeric acid, 6,8-di-C-glucosylgenkwanin 2‴-*O*-xyloside, azelaic acid, D-sorbitol, embelin, kaempferol 3-rutinoside-4′-glucoside, myrsinone, pseudouridine and thyrotropin releasing hormone. Five other compounds were also found in some extracts, such as 3′,4′,5′-trimethoxycinnamyl alcohol, 3-deaza-2′-deoxyadenosine, iso-orientin 7-*O*-rhamnoside, kelampayoside A and *n*-acetyldopamine. 1-Hexanol arabinosylglucoside and biflorin were found only in the green
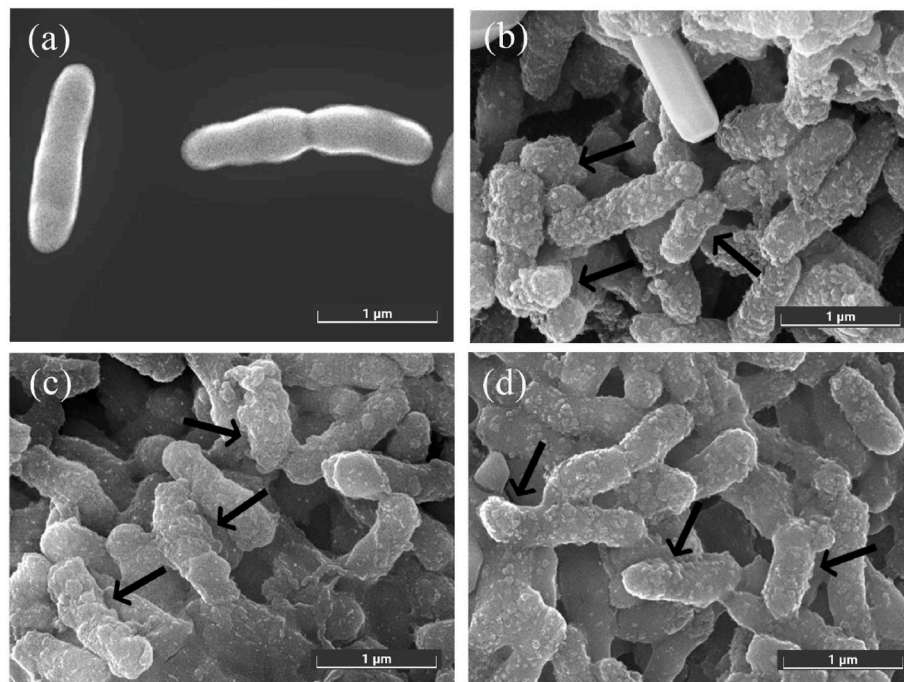
Fig. 2. SEM images of *C. acnes* DMST 14916 after incubation with plant extracts at 10 × MICs for 60 min. The bacterial cell density was used at approximately 1 × 10^8 cells cm^{-3}. Untreated bacterial cells were used as a control (a). Bacterial cells were treated with extracts of ginger (b), fingerroot (c), and green tea (d).
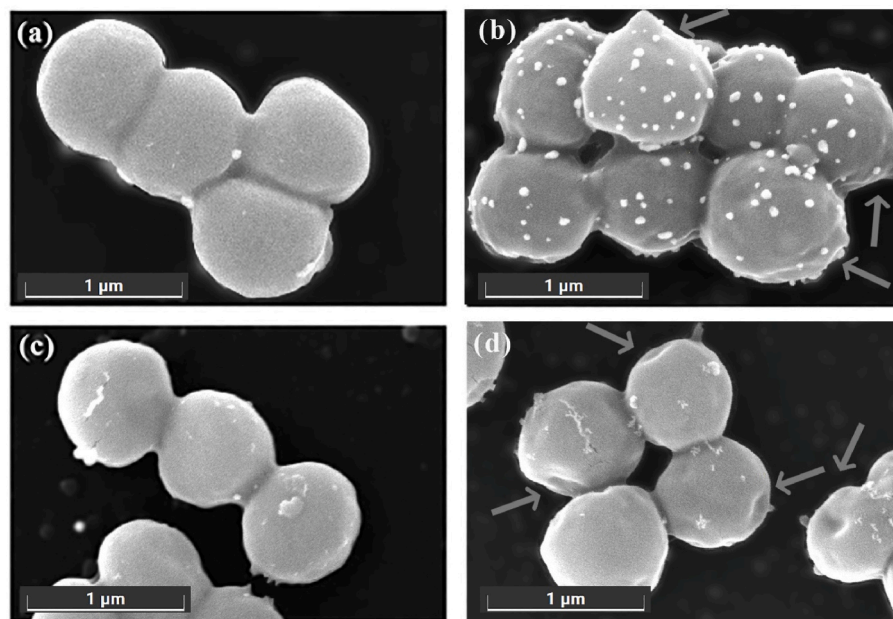

Fig. 3. SEM images of *Staphylococcus epidermidis* TISTR 518 and *Staphylococcus aureus* TISTR 746 after incubation with fingerroot extracts at 10 × MICs for 60 min. The bacterial cell density was used at approximately 1 × 10^8 cells cm^{-3}. Cells of *S. epidermidis* (a) and *S. aureus* (c) were treated without any sample as controls. Cells of *S. epidermidis* (b) and *S. aureus* (d) were treated with fingerroot extracts.

tea extract. Five different compounds present only in the fingerroot extract were 3,4-dihydroxybenzaldehyde, 5,7,2′,5′-tetrahydroxyflavone, dehydrocurdione, emodin 8-glucoside, and hydrojuglone glucoside. Four compounds in the ginger extract were 4,5-dihydrovomifoliol, actinonin, butyl butyryllactate, and sapidolide A.

3.6. Combination effects of selecting Thai plant extracts

The combination effects of the selected plant extracts on *C. acnes*

DMST 14916 were evaluated using the micro-checkerboard assay. The results of the combination effects are shown in Table 5-7 The combination effects of green tea and fingerroot extracts on *C. acnes* were prebiotic-like at the ratios of 1:2 to 1:16 and indifferent at the ratios of 1:0.25 to 1:1 (Table 5), while those of green tea and ginger extracts were prebiotic-like at the ratios of 1:1 and 1:0.062 to 1:0.125 and indifferent at the ratios of 1:0.25 to 1:0.50 (Table 6). The combination effects of fingerroot and ginger extracts on *C. acnes* were indifferent at the ratios of 1:16 to 1:32 and prebiotic-like at the ratios of 1:0.5 to 1:8 (Table 7). The
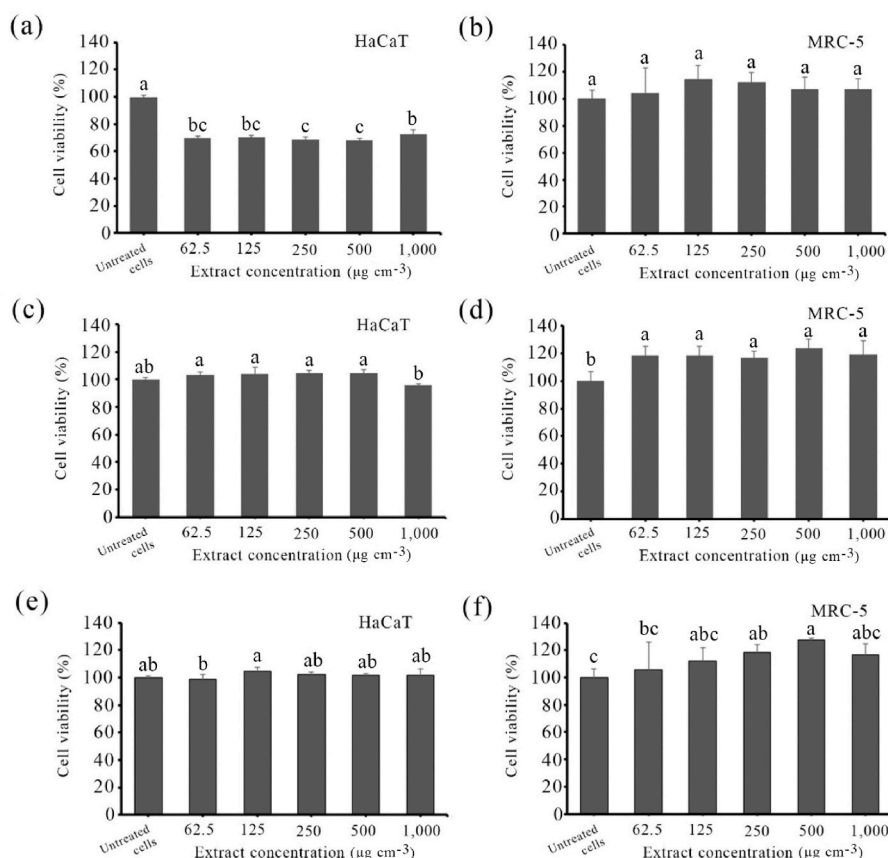
Fig. 4. Cytotoxicity activities of ethanolic extracts of selected Thai plants on HaCaT and MRC-5 cell lines determined by MTT assay. Cytotoxicity of green tea (*Camillia sinesis* var. assamica) (a and b), fingerroot (*Boesenbergia rotundo* (L.) Mansf.) (c and d), and ginger (*Zingiber officinale* Roscoe) (e and f) tested on HaCaT and MRC-5, respectively.
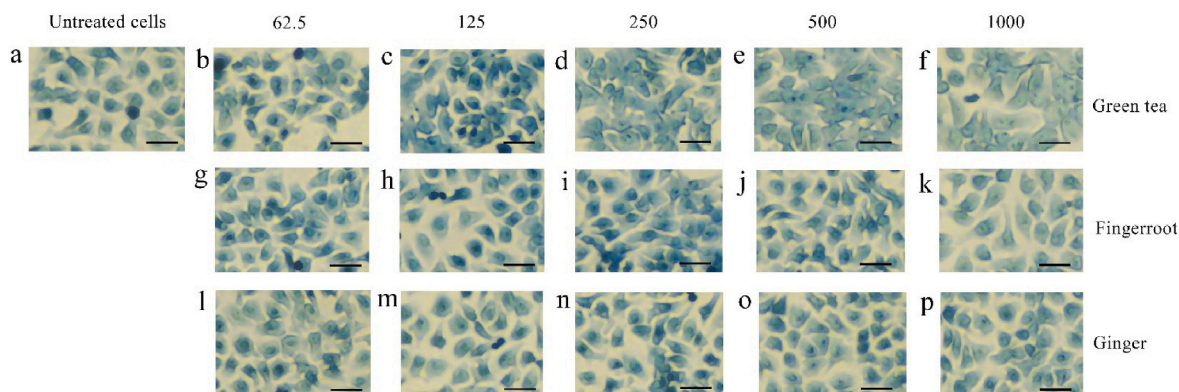

Fig. 5. Microscopic examination of morphology of HaCaT cells after treatment with the ethanolic extracts of Thai edible plants obtained by methylene blue staining technique. HaCaT cells were treated without any sample as controls or untreated cells (a). The cells were treated with the extracts of green tea (*Camillia sinesis* var. assamica) (b-f), fingerroot (*Boesenbergia rotundo* (L.) Mansf.) (g-k), and ginger (*Zingiber officinale* Roscoe) (l-p) in a concentration range of 62.5 - 1000 µg cm^{-3}, respectively. Scale bar: 20 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

combination effects of the selected plant extracts, such as synergistic and additive effects, on *C. acnes* DMST 14916 were not found.

4. Discussion

Green tea leaf, red cotton tree flower, fingerroot rhizome, and ginger rhizome are cultivated plants that are routinely used in many Thai foods and beverages. To develop natural cosmeceutical products for acne treatment, these plants were selected and extracted with ethanol. The extracts were evaluated for antimicrobial activity and their combination

effect, skin-related cytotoxicity, and LC-MS based phytochemical analysis. Among the plant extracts, the ethanolic extracts of green tea, fingerroot, and ginger exhibited antibacterial activity against *C. acnes* DMST 14916 with MIC values of 3.92 mg cm^{-3}, 0.49 mg cm^{-3} and 7.85 mg cm^{-3}, respectively. From previous studies, the methanolic extract of green tea was found to possess antibacterial property against *C. acnes* MTCC* 1951, and the MIC and MBC of the green tea extract were 1.25 mg cm^{-3} and 2.50 mg cm^{-3}, respectively [39]. Panduratin A and iso-panduratin A in fingerroot extract displayed antibacterial activity against *C. acnes* and their MIC values were found to be 2 and 4 µg cm^{-3},
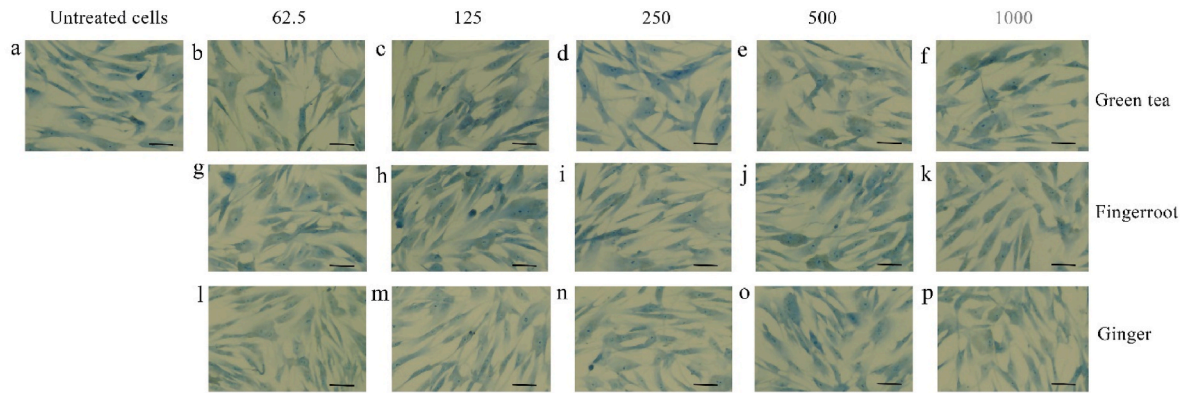
Fig. 6. Microscopic examination of morphology of MRC-5 cells after treatment with the ethanolic extracts of Thai edible plants obtained by methylene blue staining technique. MRC-5 cells were treated without any sample as controls or untreated cells (a). The cells were treated with the extracts of green tea (*Camillia sinesis* var. assamica) (b-f), fingerroot (*Boesenbergia rotundo* (L.) Mansf.) (g-k), and ginger (*Zingiber officinale* Roscoe) (l-p) in a concentration range of 62.5 - 1000 μg cm^{-3}, respectively. Scale bar: 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Identified phytochemicals in green tea extract analysed by LC-QTOF-MS-MS.

No.	t_R (min)	m/z	Ion species	MS/MS fragments	Formula	Tentative identification	Mass	Match score
1	1.770	181.0719	(M − H)-	59.0145, 89.0245, 181.0717	$C_6H_{14}O_6$	D-Sorbitol	182.0792	99.68
2	2.421	243.0622	(M − H)-	130.9661, 174.9560, 244.0642	$C_9H_{12}N_2O_6$	Pseudouridine	244.0695	99.80
3	8.892	477.1607	(M − H)-	59.0137, 431.1545	$C_{20}H_{30}O_{13}$	Kelampayoside A	478.1680	98.58
4	9.160	353.0873	(M − H)-	191.0555, 353.0867	$C_{16}H_{18}O_9$	Biflorin	354.0946	98.80
5	10.361	395.1918	(M − H)-	119.0347, 349.1845	$C_{17}H_{32}O_{10}$	1-Hexanol arabinosylglucoside	396.1991	99.19
6	13.288	739.2091	(M − H)-	285.0391, 430.0935, 593.1494, 739.2080	$C_{33}H_{40}O_{19}$	6,8-Di-C-glucosylgenkwanin 2‴-O-xyloside	740.2161	99.20
7	15.671	755.2032	(M − H)-	112.9849, 285.0402, 755.2022	$C_{33}H_{40}O_{20}$	Kaempferol 3-rutinoside-4′-glucoside	756.2105	99.06
8	16.312	593.1504	(M − H)-	112.9860, 285.0400, 484.1726, 593.1497	$C_{27}H_{30}O_{15}$	Isoorientin 7-O-rhamnoside	594.1577	95.14
9	17.123	187.0971	(M − H)-	59.0130, 125.0965, 187.0960	$C_9H_{16}O_4$	Azelaic acid	188.1045	98.79
10	18.142	327.2172	(M − H)-	85.0299, 171.1029, 327.2170	$C_{18}H_{32}O_5$	(9R,10S,12Z)-9,10-Dihydroxy-8-oxo-12-octadecenoic acid	328.2245	96.89
11	18.440	213.0153	(M − H)-	80.0136, 197.9911	$C_7H_6N_2O_6$	3,5-Dinitroguaiacol	214.0224	97.10
12	18.51	223.0974	(M − H)-	68.9959, 153.0890	$C_{12}H_{16}O_4$	3′,4′,5′-Trimethoxycinnamyl alcohol	224.1048	97.23
13	19.33	193.087	(M − H)-	79.0192, 193.0876	$C_{11}H_{14}O_3$	3-Hydroxyphenyl-valeric acid	194.0942	98.46
14	19.712	361.1627	(M − H)-	71.0131, 236.1053, 361.1621	$C_{16}H_{22}N_6O_4$	Thyrotropin releasing hormone	362.1701	99.49
15	19.737	293.1754	(M − H)-	71.0139, 236.1045	$C_{17}H_{26}O_4$	Myrsinone	294.1827	98.50
16	19.822	207.1387	(M − H)-	107.0512, 165.0941, 191.1061	$C_{13}H_{20}O_2$	(5R,6S)-5,6-Epoxy-7-megastigmen-9-one	208.1460	98.52
17	20.239	293.1756	(M − H)-	99.0082, 193.1594, 293.1754	$C_{17}H_{26}O_4$	Embelin	294.1829	99.58
18	20.312	194.0823	(M − H)-	79.0199, 179.0565, 194.0812	$C_{10}H_{13}NO_3$	n-Acetyldopamine	195.0896	99.95
19	20.473	263.1647	(M − H)-	145.0658, 160.0890	$C_{16}H_{24}O_3$	12-Oxo-2,3-dinor-10,15-phytodienoic acid	264.172	97.29
20	25.252	249.099	(M − H)-	61.9890, 249.8886	$C_{11}H_{14}N_4O_3$	3-Deaza-2′-deoxyadenosine	250.1063	97.04

respectively [40]. Glycolic extracts of ginger were used for antibacterial evaluation on *C. acnes* ATCC 6919, and it was shown that the ginger extract (100 %) could inhibit the bacterial growth with an MIC value of 12.5 mg cm^{-3} [41]. Moreover, panduratin A was highly antimicrobial against both *Staphylococci* and *Enterococci* isolates [40]. These reports indicated that variations in extraction techniques and/or protocols of the plant materials as well as differences in bacterial strains could contribute to variable antibacterial efficacy against acne-causing bacteria.

The SEM is a useful to tool for analysing surfaces, including cell surfaces. In this study, from the SEM images, it was clear that after incubation with each extract the cell surfaces of *C. acnes* treated with the green tea, fingerroot, and ginger extracts were extremely rough, while those of *S. epidermis* and *S. aureus* treated with the fingerroot extract had obvious shrinkages and collapses. From previous studies, *C. acnes* ATCC 11827 was incubated with the fraction 3 of Surian (*Toona sinensis*) leaf extract and the destruction of cell surfaces was observed after SEM analysis [42]. *S. aureus* ATCC 6538 treated with ethanolic leaf extract of *Perilla frutescens* var. *acuta* leaf was observed by SEM [43]. The cell surfaces of the bacteria appeared disrupted and the lysis of membrane

integrity, wrinkled abnormalities and cleft formation, and abnormal breaking of cell were observed compared to the control cells [43]. Moreover, *S. epidermidis* TISTR 518 treated with the aqueous extract of *Aquilaria crassna* leaves revealed swelling and distorted shapes after 24 h of incubation [44].

The cytotoxic effects of green tea, fingerroot, and ginger extracts were evaluated on human keratinocyte (HaCaT) and fibroblast (MRC-5) cells by MTT assay. Green tea extracts showed slightly cytotoxic effects on HaCaT cells while ginger and fingerroot extracts had no toxicity on the cells. The extract of green tea had no toxicity on MRC-5 cells while the ginger and fingerroot extracts could promote the cell proliferation. In a previous study, green tea polyphenols were reported to show dual effects on human keratinocyte HaCaT cells in a dose-dependent manner. The low concentrations of green tea polyphenols (0.7–140 μg cm^{-3}) could demonstrate the promotional effect on cell viability of HaCaT cells, while the high concentrations (280–1400 μg cm^{-3}) were toxic to the cells [45]. The result indicated that in the present work, polyphenols in the green tea extract could influence the cytotoxicity on HaCaT cells. Moreover, the aqueous extract of green tea was used for cytotoxic assay on human fibroblast 3T3 cells. The green tea extract could cause 50 %

Table 3
Identified phytochemicals in fingerroot extract analysed by LC-QTOF-MS-MS.

No.	t_R (min)	m/z	Ion species	MS/MS fragments	Formula	Tentative identification	Mass	Match score
1	1.728	181.0721	(M − H)-	59.0144, 101.0245, 181.0716	$C_6H_{14}O_6$	D-Sorbitol	182.0794	99.17
2	2.424	243.0622	(M − H)-	110.0245, 200.0561	$C_9H_{12}N_2O_6$	Pseudouridine	244.0695	98.58
3	8.462	137.0243	(M − H)-	81.0339, 92.0261, 137.0243	$C_7H_6O_3$	3,4-Dihydroxybenzaldehyde	138.0316	99.73
4	11.340	337.0925	(M − H)-	93.0343, 191.0556	$C_{16}H_{18}O_8$	Hydrojuglone glucoside	338.0998	97.10
5	13.257	739.2091	(M − H)-	284.0335, 430.0907, 593.1503, 739.2078	$C_{33}H_{40}O_{19}$	6,8-Di-C-glucosylgenkwanin 2‴-O-xyloside	740.2162	99.49
6	15.634	755.2032	(M − H)-	285.0403, 755.2007	$C_{33}H_{40}O_{20}$	Kaempferol 3-rutinoside-4′-glucoside	756.2105	98.80
7	16.306	593.1505	(M − H)-	178.9959, 285.0382, 593.1501	$C_{27}H_{30}O_{15}$	Isoorientin 7-O-rhamnoside	594.1577	98.69
8	17.022	187.0973	(M − H)-	57.0344, 125.0972, 187.0970	$C_9H_{16}O_4$	Azelaic acid	188.1046	99.56
9	17.553	431.0979	(M − H)-	68.9960, 151.0033, 285.0390, 431.0983	$C_{21}H_{20}O_{10}$	Emodin 8-glucoside	432.1051	98.98
10	18.130	327.2174	(M − H)-	85.0296, 171.0999, 327.2171	$C_{18}H_{32}O_5$	(9R,10S,12Z)-9,10-Dihydroxy-8-oxo-12-octadecenoic acid	328.2247	99.31
11	18.405	285.0402	(M − H)-	216.9904, 285.0407	$C_{15}H_{10}O_6$	5,7,2′,5′-Tetrahydroxyflavone	286.0474	99.00
12	18.500	223.0975	(M − H)-	112.9856, 223.0969	$C_{12}H_{16}O_4$	3′,4′,5′-Trimethoxycinnamyl alcohol	224.1048	99.13
13	18.514	213.0153	(M − H)-	197.9915, 213.0150	$C_7H_6N_2O_6$	3,5-Dinitroguaiacol	214.0224	98.68
14	19.296	193.0870	(M − H)-	178.0623, 193.0867	$C_{11}H_{14}O_3$	3-Hydroxyphenyl-valeric acid	194.0942	99.57
15	19.686	293.1760	(M − H)-	71.0140, 236.1049, 293.1751	$C_{17}H_{26}O_4$	Myrsinone	294.1832	98.66
16	19.863	207.1388	(M − H)-	92.9958, 207.1384	$C_{13}H_{20}O_2$	(5R,6S)-5,6-Epoxy-7-megastigmen-9-one	208.1461	98.77
17	20.229	293.1757	(M − H)-	99.0088, 193.1600, 293.1763	$C_{17}H_{26}O_4$	Embelin	294.1830	98.92
18	20.249	361.1629	(M − H)-	193.1590, 293.1755, 361.1635	$C_{16}H_{22}N_6O_4$	Thyrotropin releasing hormone	362.1702	94.79
19	20.476	263.1651	(M − H)-	162.1044, 263.1649	$C_{16}H_{24}O_3$	12-Oxo-2,3-dinor-10,15-phytodienoic acid	264.1723	98.65
20	21.345	233.1544	(M − H)-	94.0799, 233.1542	$C_{15}H_{22}O_2$	Dehydrocurdione	234.1617	98.93

Table 4
Identified phytochemicals in ginger extract analysed by LC-QTOF-MS-MS.

No.	t_R (min)	m/z	Ion species	MS/MS fragments	Formula	Tentative identification	Mass	Match score
1	1.780	181.0719	(M − H)-	59.0142, 181.0715	$C_6H_{14}O_6$	D-Sorbitol	182.0792	99.79
2	2.504	243.0621	(M − H)-	88.0397, 110.0863, 159.0642	$C_9H_{12}N_2O_6$	Pseudouridine	244.0694	98.92
3	8.869	477.1611	(M − H)-	161.0458, 269.1044, 431.1559	$C_{20}H_{30}O_{13}$	Kelampayoside A	478.1683	99.00
4	10.606	384.2500	(M − H)-	112.9855, 338.2441	$C_{19}H_{35}N_3O_5$	Actinonin	385.2574	98.65
5	13.246	739.2092	(M − H)-	284.0319, 593.1503, 739.2078	$C_{33}H_{40}O_{19}$	6,8-Di-C-glucosylgenkwanin 2‴-O-xyloside	740.2163	99.54
6	15.682	755.2033	(M − H)-	285.0399, 658.9635, 755.2032	$C_{33}H_{40}O_{20}$	Kaempferol 3-rutinoside-4′-glucoside	756.2105	97.97
7	17.030	187.0972	(M − H)-	125.0974, 187.0972	$C_9H_{16}O_4$	Azelaic acid	188.1045	99.32
8	18.089	265.1078	(M − H)-	207.0647, 265.1070	$C_{14}H_{18}O_5$	Sapidolide A	266.1149	97.10
9	18.153	327.2176	(M − H)-	85.0295, 183.1396, 327.2181	$C_{18}H_{32}O_5$	(9R,10S,12Z)-9,10-Dihydroxy-8-oxo-12-octadecenoic acid	328.2247	98.25
10	18.250	215.1285	(M − H)-	153.1283, 197.1180, 215.1285	$C_{11}H_{20}O_4$	Butyl butyryllactate	216.1358	97.46
11	18.391	213.0151	(M − H)-	80.0132, 197.9917	$C_7H_6N_2O_6$	3,5-Dinitroguaiacol	214.0223	98.11
12	18.517	225.1494	(M − H)-	167.1055, 225.1490	$C_{13}H_{22}O_3$	4,5-Dihydrovomifoliol	226.1566	98.18
13	19.319	193.0871	(M − H)-	178.0640, 193.0871	$C_{11}H_{14}O_3$	3-Hydroxyphenyl-valeric acid	194.0943	98.82
14	19.699	293.1756	(M − H)-	71.0141, 148.0524, 221.1542, 293.1745	$C_{17}H_{26}O_4$	Myrsinone	294.1829	99.33
15	19.706	361.1629	(M − H)-	221.1545, 293.1749, 361.1631	$C_{16}H_{22}N_6O_4$	Thyrotropin-releasing hormone	362.1703	99.53
16	19.828	207.1388	(M − H)-	92.9955, 207.1388	$C_{13}H_{20}O_2$	(5R,6S)-5,6-Epoxy-7-megastigmen-9-one	208.1461	98.95
17	20.241	293.1756	(M − H)-	99.0080, 193.1592, 293.1763	$C_{17}H_{26}O_4$	Embelin	294.1829	97.98
18	20.306	194.0822	(M − H)-	179.0593, 194.0822	$C_{10}H_{13}NO_3$	n-Acetyldopamine	195.0895	99.86
19	20.497	263.1651	(M − H)-	205.1583, 263.1651	$C_{16}H_{24}O_3$	12-Oxo-2,3-dinor-10,15-phytodienoic acid	264.1723	98.99
20	24.308	249.0987	(M − H)-	61.9888, 186.0999	$C_{11}H_{14}N_4O_3$	3-Deaza-2′-deoxyadenosine	250.1060	96.33

Table 5
Combination effects of green tea extract (GT) and fingerroot extract (FR) on *C. acnes* DMST 14916.

Variable	Fingerroot extract (FR) (mg cm⁻³)	Green tea extract (GT) (mg cm⁻³)	Ratio (FR:GT)	Results	Combination effects
MICs alone	0.49	3.92	-	-	-
In combination	0.49	7.85	1:16	Growth	Prebiotic-like effect
	0.49	3.92	1:8	Growth	Prebiotic-like effect
	0.49	1.96	1:4	Growth	Prebiotic-like effect
	0.49	0.98	1:2	Growth	Prebiotic-like effect
	0.49	0.49	1:1	Inhibition	Indifferent effect
	0.49	0.25	1:0.50	Inhibition	Indifferent effect
	0.49	0.12	1:0.25	Inhibition	Indifferent effect

cell deaths on the 3T3 cells at a final concentration of 727.2 μg cm⁻³ [46]. The result was not correlated to our cytotoxic result of green tea extract on human fibroblast MRC-5 cells. In a previous study [47], fingerroot extract and its active compound, panduratin A, were used to test cytotoxic activity against HaCaT cells. The fingerroot extract with different concentrations of 5–20 μg cm⁻³ had no toxicity to the HaCaT

Table 6
Combination effects of green tea extract (GT) and ginger extract (GG) on *C. acnes* DMST 14916.

Variable	Ginger extract (GG) (mg cm⁻³)	Green tea extract (GT) (mg cm⁻³)	Ratio (GG:GT)	Results	Combination effects
MICs alone	7.85	3.92	-	-	-
In combination	7.85	7.85	1:1	Growth	Prebiotic-like effect
	7.85	3.92	1:0.50	Inhibition	Indifferent effect
	7.85	1.95	1:0.25	Inhibition	Indifferent effect
	7.85	0.98	1:0.125	Growth	Prebiotic-like effect
	7.85	0.49	1:0.062	Growth	Prebiotic-like effect

Table 7
Combination effects of fingerroot extract (FR) and ginger extract (GG) on *C. acnes* DMST 14916.

Variable	Fingerroot extract (FR) (mg cm⁻³)	Ginger extract (GG) (mg cm⁻³)	Ratio (FR:GG)	Results	Combination effects
MICs alone	0.49	7.85	-	-	-
In combination	0.49	15.69	1:32	Inhibition	Indifferent effect
	0.49	7.85	1:16	Inhibition	Indifferent effect
	0.49	3.92	1:8	Growth	Prebiotic-like effect
	0.49	1.96	1:4	Growth	Prebiotic-like effect
	0.49	0.98	1:2	Growth	Prebiotic-like effect
	0.49	0.49	1:1	Growth	Prebiotic-like effect
	0.49	0.25	1:0.5	Growth	Prebiotic-like effect

cells, while its concentrations of 30–40 µg cm⁻³ were significantly toxic to the cells. Panduratin A with concentrations of 3–20 µM was extremely toxic to the HaCaT cells [47]. The results indicated that the cytotoxic effects of fingerroot extract on HaCaT cells could be linked to the amount of panduratin A present in the extract. The ethanolic extract of the fingerroot was used to investigate the cytotoxic effect on human skin fibroblast cells. The fingerroot extract at concentrations of 0.1–100 µg cm⁻³ could promote the proliferation of the human skin fibroblast cells (105.57 - 111.51 %). Its concentration at 1000 µg cm⁻³ was slightly toxic to the cells (93.62 %) [48]. Furthermore, the ginger phenylpropanoids and quercetin showed limited cytotoxic effects on human fibroblast BJ and keratinocyte HaCaT cells because their half maximal inhibitory concentration (IC_{50}) values showed over maximum tested concentrations 50 µg cm⁻³ and 50 µM, respectively [49]. The result indicated that the high concentration of ginger could exert cytotoxic effects on human keratinocyte and fibroblast cells.

In this study, some major phytochemicals in the green tea, fingerroot, and ginger extracts were identified by LC-MS/MS technique (Tables 2-4). The identified compounds were searched for their antimicrobial profiles from the published literature. It was found that three compounds including azelaic acid, kaempferol 3-rutinoside-4′-glucoside and embelin (Fig. 7) in all extracts examined in the present study could contribute to the antimicrobial effects [50]. Azelaic acid exhibited antibacterial activity on many acne-causing bacteria, such as *C. acnes* ATCC 6919, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, and *S. epidermidis* ATCC 14990 [50]. Embelin displayed significant antibacterial activity highly against *P. aeruginosa*, *Streptococcus pyogenes*, *Shigella flexneri*, *Shigella sonnei*, and *S. aureus* [51]. Kaempferol and related compounds could have antibacterial, antifungal, and antiprotozoal activities [52]. It was also observed that biflorin in green tea extract, 3,4-dihydroxybenzaldehyde in the fingerroot extract, actinonin in the ginger extract were reported to have antimicrobial activity [53–55].

From the previous study undertaken by Milutinovic et al., 2021 [56], it was apparent that the plant extracts rich in polyphenols could function
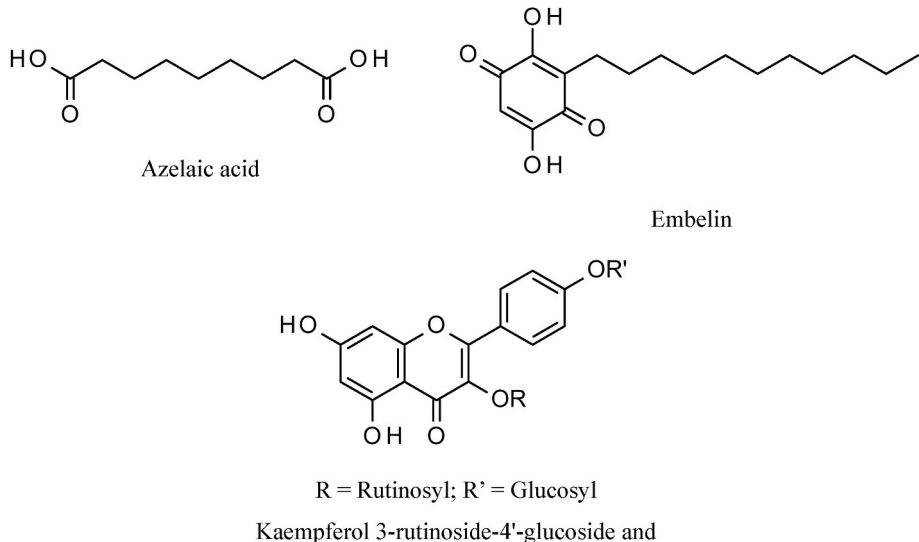

Azelaic acid

Embelin

R = Rutinosyl; R' = Glucosyl

Kaempferol 3-rutinoside-**4'**-glucoside and

Fig. 7. Three major common compounds (identified by LC-MS/MS) with antimicrobial activity present in the tested Thai plants.

as effective modulators affecting the growth of intestinal probiotics and pathogens. The extracts of medicinal plants including yarrow (*Achillea millefolium* L.), winter savory (*Satureja montana* L.), St. John's wort (*Hypericum perforatum* L.), and willow gentian (*Gentiana asclepiadea* L.) were found to promote the growth of *Saccharomyces boulardii* (prebiotic effect), while inhibiting the growth of *Candida* yeast (antimicrobial activity) [56]. Moreover, the prebiotic effect of polyphenols from green tea, and ginger were reported. Natural selenium containing green tea extract was found to promote the growth of *Lactobacillus rhamnosus* [57]. Ginger extract possessed the high prebiotic effects on *Lactobacillus* and *Bifidobacterium* [58]. These results indicated that polyphenols in plant extracts could stimulate the growth of microbial cells. Therefore, it could be assumed from the current study that polyphenols in the green tea and ginger extracts could produce either prebiotic-like or indifferent effect in their combinations on *C. acnes*.

5. Conclusion

The present study revealed that the ethanolic extracts of green tea, fingerroot, and ginger possess antimicrobial activity against *C. acnes* DMST 14916. The fingerroot extract also showed the antimicrobial effect against *S. aureus* TISTR 746, and *S. epidermidis* TISTR 518. In LC-MS/MS analysis, azelaic acid, kaempferol 3-rutinoside-4′-glucoside, and embelin were found and assumed to be the main compounds in all extracts that offered the antimicrobial activities based on the literature information. The fingerroot and ginger extracts showed no noticeable cytotoxicity on human keratinocyte HaCaT and fibroblast MRC-5 cells at the tested concentrations. The green tea extract appeared to be slightly toxic on HaCaT cells, but was not toxic to MRC-5 cells. Furthermore, the combination effects of the extracts of green tea, fingerroot, and ginger were prebiotic-like or indifferent depending on different ratios used. Therefore, the ethanolic extracts of green tea, fingerroot, and ginger could be used individually as natural anti-acne ingredients capable of further product development to improve human skin health.

CRediT authorship contribution statement

Sarita Sangthong: Methodology, Formal analysis, Data curation. **Santi Phosri:** Methodology, Investigation, Formal analysis, Data curation. **Nont Thitilertdecha:** Methodology, Formal analysis, Data curation. **Nara Yaowiwat:** Methodology, Formal analysis, Data curation. **Phanuphong Chaiwut:** Visualization, Validation, Supervision. **Mayuramas Wilai:** Visualization, Validation, Supervision. **Namfa Sermkaew:** Writing – original draft, Visualization. **Satyajit D. Sarker:** Writing – review & editing, Writing – original draft, Visualization. **Lutfun Nahar:** Writing – review & editing, Writing – original draft, Visualization, Supervision. **Tinnakorn Theansungnoen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101152.

References

[1] H.C. Williams, R.P. Dellavalle, S. Garner, Acne vulgaris, Lancet 379 (2012) 361–372, https://doi.org/10.1016/S0140-6736(11)60321-8.

[2] U. Gieler, T. Gieler, J.P. Kupfer, Acne and quality of life - impact and management, J. Eur. Acad. Dermatol. Venereol. 29 (Suppl 4) (2015) 12–14, https://doi.org/10.1111/jdv.13191.

[3] B. Dréno, S. Pécastaings, S. Corvec, S. Veraldi, A. Khammari, C. Roques, *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: a brief look at the latest updates, J. Eur. Acad. Dermatol. Venereol. 32 (Suppl 2) (2018) 5–14, https://doi.org/10.1111/jdv.15043.

[4] N.I. Muhammed, R.A. Dabbagh, Isolation and identification of microorganisms in acne patients, Zanco J. Med. Sci. 20 (2016) 1330–1336, https://doi.org/10.15218/zjms.2016.0028.

[5] A.R. Shalita, W.L. Lee, Inflammatory acne, dermatol, Clinic 1 (1983) 361–364, https://doi.org/10.1016/S0733-8635(18)31022-2.

[6] J.I. Ross, A.M. Snelling, E.A. Eady, J.H. Cove, W.J. Cunliffe, J.J. Leyden, P. Collignon, B. Dréno, A. Reynaud, J. Fluhr, S. Oshima, Phenotypic and genotypic characterization of antibiotic-resistant *Propionibacterium acnes* isolated from acne patients attending dermatology clinics in Europe, the U.S.A., Japan and Australia, Br. J. Dermatol. 144 (2001) 339–346, https://doi.org/10.1046/j.1365-2133.2001.03956.x.

[7] H. Ashkenazi, Z. Malik, Y. Harth, Y. Nitzan, Eradication of *Propionibacterium acnes* by its endogenic porphyrins after illumination with high intensity blue light, FEMS Immunol. Med. Microbiol. 35 (2003) 17–24, https://doi.org/10.1111/j.1574-695X.2003.tb00644.x.

[8] T.R. Walsh, J. Efthimiou, B. Dréno, Systematic review of antibiotic resistance in acne: an increasing topical and oral threat, Lancet Infect. Dis. 16 (2016) e23–e33, https://doi.org/10.1016/S1473-3099(15)00527-7.

[9] N. Vaou, E. Stavropoulou, C. Voidarou, C. Tsigalou, E. Bezirtzoglou, Towards advances in medicinal plant antimicrobial activity: a review study on challenges and future perspectives, Microorganisms 9 (2021) 2041, https://doi.org/10.3390/microorganisms9102041.

[10] L. Nahar, S.D. Sarker, Medicinal natural products – An introduction, in: L. Nahar, S.D. Sarker (Eds.), The Annual Reports in Medicinal Chemistry, first ed., Elsevier, 2020, pp. 1–44.

[11] M.J. Rodríguez-Yoldi, Anti-inflammatory and antioxidant properties of plant extracts, Antioxidants 10 (2021) 921, https://doi.org/10.3390/antiox10060921.

[12] J.N. Eloff, It is possible to use herbarium specimens to screen for antibacterial components in some plants, J. Ethnopharmacol. 67 (1999) 355–360, https://doi.org/10.1016/s0378-8741(99)00053-7.

[13] M. Rahman, S.D. Sarker, Antimicrobial natural products, in: L. Nahar, S.D. Sarker (Eds.), The Annual Reports in Medicinal Chemistry, first ed., Elsevier, 2020, pp. 77–113.

[14] P. Namita, R. Mukesh, K. Vijay, *Camellia Sinensis* (Green Tea): A review, Global J. Pharmacol. 6 (2012) 52–59. https://www.academia.edu/37122079/Camellia_Sinensis_Green_Tea_A_Review. (Accessed 28 March 2024).

[15] P. Subramaniam, U. Eswara, K.R. Maheshwar Reddy, Effect of different types of tea on *Streptococcus mutans*: an in vitro study, Indian J. Dent. Res. 23 (2012) 43–48, https://doi.org/10.4103/0970-9290.99037.

[16] W.C. Reygaert, The antimicrobial possibilities of green tea, Front. Microbiol. 5 (2014) 434, https://doi.org/10.3389/fmicb.2014.00434.

[17] A. Farooqui, A. Khan, I. Borghetto, S.U. Kazmi, S. Rubino, B. Paglietti, Synergistic antimicrobial activity of *Camellia sinensis* and *Juglans regia* against multidrug-resistant bacteria, PLoS One 10 (2015) e0118431, https://doi.org/10.1371/journal.pone.0118431.

[18] P.H. Chaudhary, M.G. Tawar, Pharmacognostic and phytopharmacological overview on *Bombax ceiba*, Sys. Rev. Pharm. 10 (2019) 20–25, https://doi.org/10.5530/srp.2019.1.4.

[19] S.S. Shah, S.S. Shah, A. Iqbal, S. Ahmed, W.M. Khan, S. Hussain, Z. Li, Report: Phytochemical screening and antimicrobial activities of red silk cotton tree (*Bombax ceiba* L.), Pak. J. Pharm. Sci. 31 (2018) 947–952. https://www.academia.edu/36495269/Phytochemical_screening_and_antimicrobial_activities_of_red_silk_cotton_tree_Bombax_ceiba_L. (Accessed 28 March 2024).

[20] M. Safdar, S. Aslam, M. Akram, A. Khaliq, A. Ahsan, A. Liaqat, M. Mirza, M. Waqas, W.A. Qureshi, Bombax ceiba flower extract mediated synthesis of Se nanoparticles for antibacterial activity and urea detection, World J. Microbiol. Biotechnol. 39 (2023) 80, https://doi.org/10.1007/s11274-022-03513-z.

[21] I. Indrawati, M. Miranti, I.R. Mayfi, Antibacterial activity of ethanolic extracts of rhizome from three ginger varieties against acne-isolated bacteria, Nusantara Biosci. 9 (2017) 92–96, https://doi.org/10.13057/nusbiosci/n090116.

[22] A. Fadhlina, N.F.A. Alias, H.I. Sheikh, N.H. Zakaria, F.A.A. Majid, M.A.S. Hairani, D. Hudiyanti, Role of herbal tea (*Camellia sinensis* L. Kuntze, *Zingiber officinale* Roscoe and *Morinda citrifolia* L.) in lowering cholesterol level: A review and bibliometric analysis, J. Agric. Food Res. 13 (2023) 100649, https://doi.org/10.1016/j.jafr.2023.100649.

[23] M. Afzal, D. Al-Hadidi, M. Menon, J. Pesek, M.S. Dhami, M. Ginger, an ethnomedical, chemical and pharmacological review, Drug Metab, Pharma 18 (2001) 159–190, https://doi.org/10.1515/dmdi.2001.18.3-4.159.

[24] S. Mahyari, B. Mahyari, S.A. Emami, B. Malaekeh-Nikouei, S.P. Jahanbakhsh, A. Sahebkar, A.H. Mohammadpour, Evaluation of the efficacy of a polyherbal mouthwash containing *Zingiber officinale, Rosmarinus officinalis* and *Calendula officinalis* extracts in patients with gingivitis: A randomized double-blind placebo-controlled trial, Compl. Ther. Clin. Pract. 22 (2016) 93–98, https://doi.org/10.1016/j.ctcp.2015.12.001.

[25] T. Eng-Chong, L. Yean-Kee, C. Chin-Fei, H. Choon-Han, W. Sher-Ming, C.T. Li-Ping, F. Gen-Teck, N. Khalid, N. Abd Rahman, S.A. Karsani, S. Othman, R. Othman, R. Yusof, *Boesenbergia rotunda*: From ethnomedicine to drug discovery, Evid. Based Complement Alternat, Méd. 2012 (2012) 473637, https://doi.org/10.1155/2012/473637.

[26] O. Ongwisespaiboon, W. Jiraungkoorskul, Fingerroot, *Boesenbergia rotunda* and its aphrodisiac activity, Pharm. Rev. 11 (2017) 27–30, https://doi.org/10.4103/phrev.phrev_50_16.

[27] A.M. Rosdianto, I.M. Puspitasari, R. Lesmana, J. Levita, Bioactive compounds of *Boesenbergia* sp. and their anti-inflammatory mechanism: A review, J. Appl. Pharmaceut. Sci. 10 (2020) 116–126, https://doi.org/10.7324/JAPS.2020.10715.

[28] P. Kumnerdkhonkaen, S. Saenglee, M.A. Asgar, G. Senawong, K. Khongsukwiwat, T. Senawong, Antiproliferative activities and phenolic acid content of water and ethanolic extracts of the powdered formula of *Houttuynia cordata* Thunb. fermented broth and Phyllanthus emblica Linn. Fruit, BMC Compl. Alternative Med. 18 (2018) 130, https://doi.org/10.1186/s12906-018-2185-x.

[29] S. Pata, N. Yaraksa, S. Daduang, Y. Temsiripong, J. Svasti, T. Araki, S. Thammasirirak, Characterization of the novel antibacterial peptide leucrocin from crocodile (*Crocodylus siamensis*) white blood cell extracts, Dev. Comp. Immunol. 35 (2011) 545–553, https://doi.org/10.1016/j.dci.2010.12.011.

[30] Y. Wu, Y. Qiang, K. Cao, W. Zhang, G. Zhang, Inhibitory effect of the antimicrobial peptide BLP-7 against *Propionibacterium acnes* and its anti-inflammatory effect on acne vulgaris, Toxicon 184 (2020) 109–115, https://doi.org/10.1016/j.toxicon.2020.06.006.

[31] S.D. Sarker, L. Nahar, Y. Kumarasamy, Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals, Methods 42 (2007) 321–324, https://doi.org/10.1016/j.ymeth.2007.01.006.

[32] M. Elshikh, S. Ahmed, S. Funston, P. Dunlop, M. McGaw, R. Marchant, I.M. Banat, Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants, Biotechnol. Lett. 38 (2016) 1015–1019, https://doi.org/10.1007/s10529-016-2079-2.

[33] S.K. Lau, P.C. Woo, G.K. Woo, A.M. Fung, M.K. Wong, K.M. Chan, D.M. Tam, K. Y. Yuen, *Eggerthella hongkongensis* sp. nov. and *Eggerthella sinensis* sp. nov., two novel *Eggerthella* species, account for half of the cases of *Eggerthella* bacteremia, Diagn. Microbiol. Infect. Dis. 49 (2004) 255–263, https://doi.org/10.1016/j.diagmicrobio.2004.04.012.

[34] S. Phosri, K. Kiattisin, A. Intharuksa, R. Janon, T. Na Nongkhai, T. Theansungnoen, Anti-aging, anti-acne, and cytotoxic activities of *Houttuynia cordata* extracts and phytochemicals analysis by LC-MS/MS, Cosmetics 9 (2022) 136, https://doi.org/10.3390/cosmetics9060136.

[35] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63, https://doi.org/10.1016/0022-1759(83)90303-4.

[36] S. Chockalingam, S.S. Ghosh, Amelioration of cancer stem cells in macrophage colony stimulating factor-expressing U87MG-human glioblastoma upon 5-fluoro-uracil therapy, PLoS One 8 (2013) e83877, https://doi.org/10.1371/journal.pone.0083877.

[37] N. Reuk-ngam, N. Chimnoi, N. Khunnawutmanotham, S. Techasakul, Antimicrobial activity of coronarin D and its synergistic potential with antibiotics, BioMed Res. Int. 2014 (2014) 581985, https://doi.org/10.1155/2014/581985.

[38] S. van Vuuren, A. Viljoen, Plant-based antimicrobial studies-methods and approaches to study the interaction between natural products, Planta Med. 77 (2011) 1168–1182, https://doi.org/10.1055/s-0030-1250736.

[39] P. Nand, S. Drabu, R. Gupta, Phytochemical and antimicrobial screening of medicinal plants for the treatment of acne, Indian J. Nat. Prod. Resour. 3 (2012), 28-22, https://nopr.niscpr.res.in/bitstream/123456789/13803/1/IJNPR%203(1)%2028-32.pdf. (Accessed 28 March 2024).

[40] A. Chahyadi, R. Rika Hartati, K.R. Wirasutisna, L. Elfahmia, *Boesenbergia pandurata* Roxb., An Indonesian medicinal plant: Phytochemistry, biological activity, plant biotechnology, Procedia Chem. 13 (2014) 13–37, https://doi.org/10.1016/j.proche.2014.12.003.

[41] L.A.D. Silva, L.P. Ramos, T.A. Silva, S.A.B. Lapena, C.E.R. Santos, A.A. Hasna, A. Bressane, L.D. Oliveira, Effect of combining *Zingiber officinale* and *Juglans regia* extracts on *Propionibacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis*: Antibiofilm action and low toxicity, An. Acad. Bras. Cienc. 94 (2022) e20201133, https://doi.org/10.1590/0001-3765202220201133.

[42] E. Febriyani, S. Falah, D. Andrianto, T. Lastini, Identification of active compounds and anti-acne activity from extracts and fractions of surian (*Toona sinensis*) leaves planted in Sumedang, West Java, Indonesia, Biodiversitas 19 (2018) 1406–1412, https://doi.org/10.13057/biodiv/d190429.

[43] D.H. Kim, Y.C. Kim, U.K. Choi, Optimization of antibacterial activity of *Perilla frutescens* var. acuta leaf against *Staphylococcus aureus* using evolutionary operation factorial design technique, Int. J. Mol. Sci. 12 (2011) 2395–2407, https://doi.org/10.3390/ijms12042395.

[44] S. Kamonwannasit, N. Nantapong, P. Kumkrai, P. Luecha, S. Kupittayanant, N. Chudapongse, Antibacterial activity of Aquilaria crassna leaf extract against *Staphylococcus epidermidis* by disruption of cell wall, Ann. Clin. Microbiol. Antimicrob. 12 (2013) 20, https://doi.org/10.1186/1476-0711-12-20.

[45] L.Y. Wu, X.Q. Zheng, J.L. Lu, Y.R. Liang, Protective effect of green tea polyphenols against ultraviolet B-induced damage to HaCaT cells, Hum. Cell 22 (2009) 18–24, https://doi.org/10.1111/j.1749-0774.2008.00063.x.

[46] M. Mehrabani, B. Amirheidari, N. Ahmadinia, M. Forooghi-Nematollahi, S. Sabouri, Antioxidant and cytotoxic effects of aqueous extract of Iranian green tea (*Camellia sinensis* L.), Iran, J. Pharmaceut. Sci. 18 (2022) 77–86, https://doi.org/10.22037/ijps.v18.41381.

[47] S.W. Woo, D.B. Rhim, C. Kim, J.K. Hwang, Effect of standardized *Boesenbergia pandurata* extract and its active compound panduratin A on skin hydration and barrier function in human epidermal keratinocytes, Prev. Nutr. Food Sci. 20 (2015) 15–21, https://doi.org/10.3746/pnf.2015.20.1.15.

[48] Y. Sudjaroen, P. Thongmuang, Phytochemical screening and biological activity of fingerroot (*Boesenbergia rotunda* L.) rhizome on skincare application, J. Pharm. Negat. Results 13 (2022) 1414–1418, https://doi.org/10.47750/pnr.2022.13.04.198.

[49] E. Schadich, J. Hlaváč, T. Volná, L. Varanasi, M. Hajdúch, P. Džubák, Effects of ginger phenylpropanoids and quercetin on Nrf2-ARE pathway in human BJ fibroblasts and HaCaT keratinocytes, BioMed Res. Int. 2016 (2016) 2173275, https://doi.org/10.1155/2016/2173275.

[50] M.A.T. Blaskovich, A.G. Elliott, A.M. Kavanagh, S. Ramu, M.A. Cooper, In vitro Antimicrobial activity of acne drugs against skin-associated bacteria, Sci. Rep. 9 (2019) 14658, https://doi.org/10.1038/s41598-019-50746-4.

[51] M. Chitra, C.S. Devi, E. Sukumar, Antibacterial activity of embelin, Fitoterapia 74 (2003) 401–403, https://doi.org/10.1016/s0367-326x(03)00066-2.

[52] A. Periferakis, K. Periferakis, I.A. Badarau, E.M. Petran, D.C. Popa, A. Caruntu, R. S. Costache, C. Scheau, C. Caruntu, D.O. Costache, Kaempferol: Antimicrobial properties, sources, clinical, and traditional Applications, Int. J. Mol. Sci. 23 (2003) 15054, https://doi.org/10.3390/ijms232315054.

[53] N. Syafni, D.P. Putra, D. Arbain, 3,4-Dihydroxybenzoic acid and 3,4-dihydroxy-benzaldehyde from the fern *Trichomanes chinese* L.: Isolation, antimicrobial and antioxidant properties, Indones. J. Chem. 12 (2012) 273–278, https://doi.org/10.22146/ijc.21342.

[54] G.G. Wisintainer, E.R. Simões, T.L. Lemos, S. Moura, L.G. Souza, A.M. Fonseca, M. O. Moraes, C. Pessoa, M. Roesch-Ely, J.A. Henriques, Biflorin: An o-naphthoquinone of clinical significance, An. Acad, Bras. Cienc 86 (2014) 1907–1914, https://doi.org/10.1590/0001-3765201420140085.

[55] D. Jung, S.J. Yum, H.G. Jeong, Characterization and evaluation of antimicrobial activity of actinonin against foodborne pathogens, Food Sci. Biotechnol. 26 (2017) 1649–1657, https://doi.org/10.1007/s10068-017-0190-3.

[56] M. Milutinović, S. Dimitrijević-Branković, M. Rajilić-Stojanović, Plant extracts rich in polyphenols as potent modulators in the growth of probiotic and pathogenic intestinal microorganisms, Front. Nutr. 8 (20121) 688843, https://doi.org/10.3389/fnut.2021.688843.

[57] A.L. Molan, J. Flanagan, W. Wei, P.J. Moughan, Selenium-containing green tea has higher antioxidant and prebiotic activities than regular green tea, Food Chem. 114 (2009) 829–835, https://doi.org/10.1016/j.foodchem.2008.10.028.

[58] K. Narendra Babu, R. Hemalatha, U. Satyanarayana, Md Shujauddin, N.K. Himaja, K. Bhaskarachary, B. Dinesh Kumar, Phytochemicals, polyphenols, prebiotic effect of *Ocimum sanctum, Zingiber officinale, Piper nigrum* extracts, J. Herb. Med. 13 (2018) 42–51, https://doi.org/10.1016/j.hermed.2018.05.001.