

Potential of Chinese-Thai herbal extracts as natural sources of antioxidants, anti-inflammatory, antimicrobials, and antifungal compounds

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ABSTRACT

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This study explores the biological effects of three Chinese-Thai medicinal extracts: *Alpinia galanga* (L.) Willd. rhizome extract (AE), *Morus alba* L. stem extract (ME), and *Camellia oleifera* Abel. seed cake extract (CE). Their total phenolic content (TPC) and total flavonoid content (TFC) were measured using the Folin–Ciocalteu assay and the aluminium chloride colorimetric assay, respectively. The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays, with anti-inflammation assessed by the heat-treating albumin protocol. Additionally, the antibacterial and antifungal effects were tested using a broth microdilution assay. The results showed that AE provided the highest percentage yield (26.57%), with ME having the highest TPC (35.84±1.27 mg gallic acid equivalent (GAE)/g) and TFC (23.3±1.48 mg quercetin equivalent (QE)/g). Although AE had the lowest TPC and TFC, it exhibited the highest antioxidant activity (Trolox equivalent antioxidant capacity: 61.39±4.4 mg/g; Ascorbic acid equivalent antioxidant capacity: 7.93±0.27 mg/g). AE showed the highest percentage inhibition of 14.86±1.99 on protein denaturation. Moreover, AE demonstrated the greatest efficacy in inhibiting the growth of *S. epidermidis* and *S. aureus*. Conversely, CE had the strongest antifungal action against *T. mentagrophytes*, *T. rubrum*, and *M. gypseum*. This finding suggests that utilizing these extracts can contribute to improving health and well-being.

Keywords: biological activity; extract; galanga; mulberry; tea seed cake

1. INTRODUCTION

The overgrowth of several fungi, mainly dermatophytes (e.g., *Trichophyton*, *Microsporum*, and *Epidermophyton* species), yeasts (especially *Candida* species), and moulds, causes fungal skin infections, which are common dermatological diseases. Dermatophytes are the primary

cause of superficial fungal infections, affecting the skin, hair, and nails, and are responsible for diseases like athlete's foot, ringworm, and scalp infections. Yeasts, especially *Candida albicans*, are often implicated in mucocutaneous infections such as thrush and diaper dermatitis and can also cause more severe systemic infections in immunocompromised individuals. Moulds,

although less common, can also cause skin infections, particularly in immunosuppressed individuals. Itching, erythema (redness), scaling, flaking, and soreness are common symptoms of fungal skin infections, often leading to significant cosmetic concerns and a reduction in the quality of life. These infections can be persistent, recurrent, and challenging to cure, particularly in people with impaired immune systems or when treatment is not received. Untreated fungal infections can occasionally result in more serious illnesses like extensive dermatophyte infections or subsequent bacterial infections (Hani et al., 2024).

Reactive oxygen species (ROS) and other free radicals (oxidants) are usually generated in the human metabolism process and quenched by the endogenous antioxidant system. Besides being responsible for the formation of free radicals in the body, their numbers can be increased by the stimulation of environmental factors such as ultraviolet (UV) radiation, pollution, and cigarette smoking. An imbalance between oxidants and antioxidants in favour of oxidants is termed 'oxidative stress,' which leads to oxidative damage. Antioxidants are substances that may inhibit some oxidative processes, thereby preventing or delaying oxidative stress. Natural resources, especially fruits and vegetables, play an important role in the antioxidant system by providing essential antioxidants such as vitamin E, vitamin C, β -carotene, minerals, and several polyphenolic compounds (Zujko & Witkowska, 2023).

The plant kingdom is a rich source of organic compounds, many of which are used for medicinal purposes. In traditional systems, many crude drug substances are beneficial for treating diseases or disorders. Microorganisms, infections, parasites, organisms, prions, worms, and helminths are associated with causing irresistible sicknesses (Pavithra et al., 2024). One of the plant families extensively found in South and Southeast Asia is Zingiberaceae. More than 30 genera and more than 300 species can be found in Thailand. They have long been utilized in medications and cosmetics.

The rhizomes of galangal (*Alpinia galanga*), ginger (*Zingiber officinale*), plai (*Zingiber cassumunar*), and turmeric (*Curcuma longa*) are among the most often used medicinal herbs in Thailand. These plants have several applications (Laokor & Juntachai, 2021). The potential for treating fungal diseases with plants and their extracts is enormous, and some exhibit broad-spectrum antifungal activity. Due to their significant therapeutic qualities, galangal plant species are the most extensively researched (Reka et al., 2023).

Mulberry trees are deciduous plants belonging to the genus *Morus* (family Moraceae), with more than 17 species growing in tropical climates. These include *Morus alba* L., *Morus rubra*, *Morus nigra*, *Morus australis*, *Morus atropurpurea*, *Morus cathayana*, *Morus notabilis*, and *Morus mesozygia*. The most common species are *Morus alba* (*M. alba*, white mulberry) and *Morus nigra* (blackberry). A significant amount of research has been conducted, and several reviews have been published on the species. Various parts of *M. alba*, including the roots, stems, leaves, and fruit, have long been used in China as herbal medicines due to their pharmacological effects (Chang et al., 2021). Mulberry is reported to have benefits in the world of health for its antimicrobial, astringent, hypoglycemic, anti-atherosclerotic, ophthalmic, antifungal, and diuretic activities. The antimicrobial and antifungal activities of mulberry are interesting to study, and recent research

reveals that anthocyanins can protect against human pathogenic bacteria (Hidayatunnikmah et al., 2023).

Camellia plants, native to Eastern Asia, have been cultivated worldwide and comprise more than 200 species. *Camellia oleifera* Abel. (*C. oleifera*), a species of tea, is planted for oil-rich seeds. *C. oleifera* is one of the four largest woody oil crops in the world, and *C. oleifera* seed cake is rich in active ingredients such as tea saponin, polysaccharide, protein, and polyphenol (Yu et al., 2022). Due to the presence of a variety of different compounds, tea seed cake has been incorporated into health, food, and cosmetic products. These seeds are shown to have enormous medicinal potential and are a rich source of many secondary metabolites. Numerous activities, including antioxidant, antihyperlipidemic, antifungal, and anticancer properties, have been demonstrated for the saponins of *C. oleifera*. In Chinese traditional medicine, tea seed oil has been used in regimens for the treatment of fungal infection (Tian et al., 2017).

Based on some of the theoretical studies above, the purpose of this study is to highlight the pharmaceutical and cosmetic potential of selected plant byproducts as sources of functional compounds. Regarding traditional use, the *A. galanga* rhizome is safe since it is an edible part. Numerous studies have explored its benefits, including bioactive components, biological activity, and antioxidant properties (Malik et al., 2016). However, little evidence has been reported of its inhibitory effect on pathogenic organisms, including bacteria, fungi, viruses, and parasites, which can cause a variety of skin diseases, specifically dermatophytes. The retention of its antipathogenic properties is not fully understood and remains under investigation. The *M. alba* stem is widely used in Chinese traditional medicine for its antimicrobial properties. Previous research has mostly demonstrated biological activity from the leaf and fruit parts of *M. alba* (Memete et al., 2022). Nevertheless, the scientific exploration of the *M. alba* stem is so far limited, especially its antimicrobial properties against pathogen-related diseases. Additionally, tea seed cake is rich in saponin with antimicrobial properties. Antimicrobial evidence of *C. oleifera* seed cake affecting some pathogenic organisms, like gram-positive bacteria and dermatophytes, is limited. This present research aims to evaluate extracts of *A. galanga* rhizome, *M. alba* stem, and *C. oleifera* seed cake, focusing on antioxidation, anti-inflammation, antimicrobial, and antifungal activities to elucidate their therapeutic potential.

2. MATERIALS AND METHODS

2.1 Materials

All solvents were purchased from Labscan (Dublin, Ireland). Folin-Ciocalteu solution, gallic acid, quercetin, Trolox, 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 2,2-diphenyl-2-picrylhydrazyl (DPPH), and diclofenac sodium were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate, ferric chloride, ferrous sulfate, sodium acetate, aluminium trichloride (AlCl_3), ascorbic acid, and potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) were purchased from Merck (Darmstadt, Germany). Fetal bovine serum (FBS) was obtained from Biochrom AG (Berlin, Germany). Mueller-Hinton agar (MHA), Mueller-Hinton broth (MHB), trypticase soy agar (TSA), Sabouraud dextrose agar (SDA), brain heart infusion (BHI), brain

heart infusion broth (BHIB), and Dey-Engley neutralizing broth were purchased from GL Biochem (Shanghai, China). Levofloxacin antimicrobial susceptibility discs were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Ciclopirox was supplied from Sigma-Aldrich (St. Louis, MO, USA), with vancomycin purchased from Thermo Fisher (Waltham, MA, USA).

2.2 Preparation of plant materials

Three Chinese-Thai herbal materials were prepared, namely *A. galanga* (L.) Willd. rhizome, *M. alba* L. stem, and residual seed cake *C. oleifera* Abel. Briefly, *A. galanga* rhizomes were collected from the agricultural field in Samutprakarn Province, Thailand. The stems of *M. alba*, used in traditional Chinese medicine, were gifted by the Faculty of Traditional Chinese Medicine at Huachiew Chalermprakiet University, Thailand. The residual seed cake of *C. oleifera* was purchased from commercial suppliers in Thailand. All plants were cleaned with tap water and allowed to dry. They were then cut or chopped into small pieces and dried in a hot air oven set at 50–60°C and blended into a fine powder. The dried powders of *A. galanga* rhizome and the seed cake of *C. oleifera* were kept at -20°C for further use, while the *M. alba* stem was kept in a desiccator at room temperature.

2.3 Preparation of plant extraction

The dried plant materials were extracted by heat-assisted maceration for 24 h in three cycles (Neimkhum et al., 2021). Briefly, each dried powder extract was mixed with absolute ethanol (*A. galanga* rhizomes and *M. alba* stems) or methanol (*C. oleifera* seed cake) at a ratio of 1:5 w/v. The extraction was conducted with the assistance of heating using a water bath set at 50°C. As for the extraction process, the extract mixture stood for 24 h and was then filtered through Whatman® No.1 under a vacuum pump to obtain the filtrate. After that, the residue was re-extracted with fresh absolute extraction solvent twice and pooled, followed by removing the extraction solvent to dryness using a rotary evaporator. The obtained *A. galanga* rhizome extract (AE), *M. alba* stem extract (ME), and *C. oleifera* seed cake extract (CE) were stored in a refrigerator at 4°C for further analysis. The extraction yield was calculated by the following equation: Yield (%) = $(W_e / W_d) \times 100$, where W_e represents the weight of the obtained extract after removing the extraction solvent and W_d the weight of the original dry plant material (Sharma & Cannoo, 2016).

2.4 Determination of the total phenolic content (TPC) of extracts by the Folin-Ciocalteu method

The TPC of each extract was determined using the Folin-Ciocalteu method according to Neimkhum et al. (2021) with some modifications. Briefly, the Folin-Ciocalteu reagent was diluted 10-fold with deionized water. The 20 µL of samples at 1 mg/mL were mixed with a reagent (100 µL) and then incubated at room temperature for 5 min. Sequentially, 80 µL of sodium carbonate solution (7.5% w/v) was added and allowed to react in the dark at room temperature for 2 h. The TPC in the sample was then determined using a microplate reader at a wavelength of 760 nm. The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of each extract. Gallic acid was used as a positive control and calculated for the construction of a standard curve.

2.5 Determination of the total flavonoid content (TFC) of extracts by aluminium chloride colorimetric assay

Each extract was subjected to the aluminium chloride colorimetric technique according to Chaiyana et al. (2019) with some modifications for the flavonoid content measurement. Briefly, an aluminium trichloride reagent was dissolved in methanol to create a concentration of 2% w/v. The resulting solution was prepared by mixing 100 µL of the sample solution (1 mg/mL) and 100 µL of the reagent. It was then allowed to react for 10 min at room temperature. Its absorbance was subsequently measured at a wavelength of 415 nm using a microplate reader. The results were exhibited in terms of milligram quercetin equivalent (QE) per gram of each extract. Quercetin was used as a positive control and calculated for the construction of a standard curve.

2.6 Evaluation of antioxidant activity of extracts

2.6.1 ABTS assay

The power of the radical scavenging property of each extract was tested using the ABTS assay according to Chaiyana et al. (2019). The ABTS^{•+} radical reagent was obtained by generating the ABTS solution (7.0 mM) with potassium persulfate (2.45 mM) in an equal volume. The generating solution was protected from the light and then allowed to stand in a refrigerator for 15 h before use. After that, a concentration of 1 mg/mL of each extract (20 µL) was reacted with the ABTS^{•+} reagent (180 µL) and allowed to incubate at room temperature for 5 min. The radical scavenging capacity of each extract against ABTS^{•+} radical was determined by measuring its absorbance at 750 nm using a microplate reader. The results were expressed as a Trolox equivalent antioxidant activity (TEAC) since Trolox was used as a positive control and calculated for the construction of a standard curve.

2.6.2 DPPH assay

The antioxidant potential of each extract was assessed by its DPPH[•] radical scavenging capacity according to the method previously described by Chaiyana et al. (2017). Briefly, 20 µL of each extract (1 mg/mL) was reacted with 180 µL of DPPH[•] radical solution (0.004% w/v) and incubated in the dark at room temperature for 30 min. Absorbance of the resulting mixture was determined at 520 nm using a microplate reader. The radical scavenging capacity was calculated by the following equation: Inhibition (%) = $[1 - (A_s / A_b)] \times 100$, where A_b represents the absorbance of the DPPH[•] radical solution without the test sample and A_s the absorbance of the DPPH[•] radical solution with the test sample. Ascorbic acid was used as a positive control and calculated for the construction of the standard curve. The results were reported as ascorbic acid equivalent antioxidant activity (AEAC).

2.7 Evaluation of the anti-inflammatory activity of extracts

Anti-inflammation was analyzed by a protein denaturation assay using the modified method of Aidoo et al. (2021). Briefly, the samples, namely 800 µL of each extract (5 mg%) and a positive control (diclofenac sodium), were mixed with 160 µL of 10% w/v bovine serum albumin (BSA) and 1.12 mL of phosphate buffer saline pH 6.4, then

incubated at $37 \pm 2^\circ\text{C}$ for 20 min. Afterwards, each sample mixture was heated at $85 \pm 3^\circ\text{C}$ for 30 min to denature the protein and then cooled at room temperature. The turbidity was measured by a UV-Vis spectrophotometer at a wavelength of 660 nm, and the percentage inhibition of protein denaturation was calculated according to the following equation: $\text{Inhibition (\%)} = [1 - (A_s / A_b)] \times 100$, where A_b represents the absorbance of the BSA solution without the test sample and A_s the absorbance of the BSA solution with the test sample.

2.8 Evaluation of the antibacterial and antifungal activities of extracts

2.8.1 Microorganisms used and growth conditions

This study was performed using *Staphylococcus epidermidis* (*S. epidermidis*) ATCC12228 and *Staphylococcus aureus* (*S. aureus*) ATCC6538 obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) for antibacterial activity. Dermatophytes such as *Trichophyton mentagrophytes* (*T. mentagrophytes*) (Robin) Blanchard ATCC MYA-4439, *Trichophyton rubrum* (*T. rubrum*) ATCC MYA 4438, and *Microsporum gypseum* (*M. gypseum*), provided by the Medical Mycology Laboratory, Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Thailand, were used for antifungal activity. The microorganisms were cultured and grown in nutrient media.

2.8.2 Antibacterial activity of extracts

According to the modified protocol of the Clinical and Laboratory Standards Institute (CLSI) 2016, the antibacterial activity of the extracts and reference antibiotic (levofloxacin) was tested. Both the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were examined using a 96-well microplate. The susceptibilities of the isolates to extracts were determined by the broth microdilution method with 2-fold dilutions. Briefly, a series of 2-fold dilutions of each extract in final concentrations of 0.392 to 200 mg/mL was prepared in MHA. Bacterial strains were initially cultured in MHA and added to each well of a 96-well plate to reach the acquired colonies at a density of 10^5 CFU/mL. The tested 96-well microplate was then incubated at $35 \pm 2^\circ\text{C}$ for 18–24 h before interpreting.

2.8.3 Antifungal activity of extracts

The antifungal activity of the extracts and reference antibiotics (ciclopirox and vancomycin) was analyzed as outlined in CLSI M38-A2. The MIC and minimum fungicidal concentration (MFC) of each extract were determined using the broth microdilution technique in a 96-well microplate. Briefly, aliquots containing 5×10^3 conidia/mL of dermatophytes were mixed with a series of 2-fold dilutions of each extract (the final concentrations ranging from 0.025 to 100 mg/mL) in equal volume. Subsequently, the tested microplate was incubated at 37°C for four days before reading.

2.9 Statistical analysis

All data were presented as a mean $\pm$ standard deviation (S.D.). Individual differences were evaluated by t-test or one-way analysis of variance (ANOVA). In all cases, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ indicated statistical significance.

3. RESULTS AND DISCUSSION

3.1 Plant extracts

In this study, yields were calculated based on dried plants. As shown in Figure 1, AE had the highest yield at 26.57%, followed by CE (24.78%) and ME (21.02%), respectively. All extracts were observed to exhibit the same characteristics of the viscous semi-solid state (Figure 1).

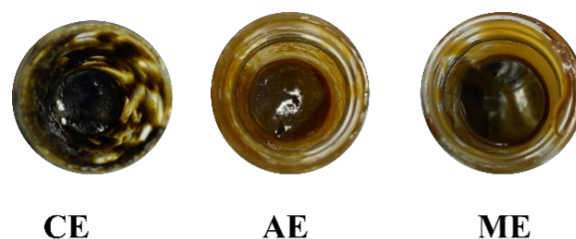


Figure 1. Physical appearances of extracts: *C. oleifera* seed cake extract (CE), *A. galanga* rhizome extract (AE), and *M. alba* stem extract (ME)

3.2 Total phenolic and flavonoid content of extracts

Phenolic compounds, important secondary metabolites in many plants, have been reported to be safe and beneficial for human health (Tungmunnithum et al., 2018). Additionally, ethanol and methanol, used as extraction solvents in the present study, were found to be suitable for extracting phenolics and flavonoids due to their hydrophilicity (Dhar et al., 2017). All extracts possessed higher amounts of phenolic acids than flavonoids. ME had the highest TPC with 35.84 ± 1.27 mgGAE/g and TFC with 23.3 ± 1.48 mgQE/g, followed by AE (TPC with 26.48 ± 0.61 mgGAE/g and TFC with 9.02 ± 0.05 mgQE/g) and CE (TPC with 20.57 ± 1.51 mgGAE/g and TFC with 19.2 ± 0.63 mgQE/g). In a previous report, the ethanolic rhizome extract of *A. galanga* presented TPC and TFC of 34.21 and 28.79 mg/g, respectively (El-Hadidy et al., 2020), higher than those found in this present study. The ethanolic stem extract of *M. alba* in the previous study showed a TPC of 35.0 mg/g, aligning with the present study, while TFC was previously reported as 7.9 mg/g, three times less than that found here. In the case of the methanolic extract from residual seed cake of *C. oleifera*, the TPC values of the *Camellia* seed kernel and shell were previously reported as 23.99 mg/g and 20.81 mg/g, respectively (Luo & Fei, 2019), correlating with the results of the present study. Furthermore, the TFC value was 11.88 mg/g, marginally higher in this investigation than in the prior report (Tsai & Lin, 2019). Moreover, in the present study, both ethanol and methanol, which were found to have a high polarity index, were more effective at extracting phenolic acids than flavonoids from these plant materials. The difference in the amount of phenolic acids and flavonoids was due to the polarity of the extracting solvents, plant species, and their solubility in the solvent used for the extraction method. It is likely that the chemical structures of phenolic acids and flavonoids, particularly the presence and arrangement of hydroxyl groups and other functional groups, significantly influence their solubility in different solvents (Vittaya et al., 2022). According to previous research by Lim et al. (2024), aqueous ethanol (70%) was more effective than aqueous

methanol (70%) for phenolic acid extraction. Similarly, according to other studies, both ethanol and methanol extracts have shown higher amounts of phenolic compounds than flavonoids (Dirar et al., 2019; El Mannoubi et al., 2023)

3.3 Antioxidant activities of extracts

The antioxidant ability of the extracts was evaluated using two different protocols, as shown in Table 1. The ABTS and DPPH scavenging methods are based on the electron transfer mechanism (Apak et al., 2016). The experimental results indicated that the AE showed similar DPPH radical scavenging activity compared to a previous report but yielded higher ABTS radical scavenging activity. Specifically, the previous study reported an AEAC value of 6.23 mg/g extract for the ethanol galangal extract in the DPPH assay (Osiriphun et al., 2022). The observed difference in antioxidant activity between DPPH and ABTS assays is likely due to their different mechanisms and sensitivities to various antioxidant compounds (Platzer et al., 2021). DPPH is a stable free radical, primarily soluble in organic solvents, while ABTS can be used in both aqueous and organic solutions and is more sensitive to a wider range of antioxidant compounds, including hydrophilic (Xiao et al., 2019). The higher TEAC value in the ABTS assay suggests that the extract might contain compounds more effective at scavenging the ABTS radical cation than the DPPH radical. Although AE exhibited lower TPC than ME, it seems likely that the potency of the antioxidant depends on the number and position of free OH groups (Panche et al., 2016). On the other hand, the extraction yield results indicated that AE was the highest, aligning with the antioxidant results. The statement highlights that various bioactive compounds found in *A. galanga* rhizome, including saponins, tannins, anthocyanins, sterols, triterpenoids, and anthraquinones (Malik et al., 2016), contribute to its antioxidant properties alongside the phenolic and flavonoid compounds. This suggests a synergistic effect where multiple compounds, rather than just phenolics and flavonoids, are responsible for the observed antioxidant activity (Mateos-Maces et al., 2020).

Table 1. ABTS and DPPH scavenging capacity of the extracts

Extract	ABTS TEAC (mg/g extract)	DPPH AEAC (mg/g extract)
CE	23.66 ± 1.82 ^a	6.42 ± 0.25 ^a
ME	24.51 ± 9.09 ^a	7.36 ± 0.07 ^b
AE	61.39 ± 4.40 ^b	7.93 ± 0.27 ^c

TEAC represents scavenging capacity of extract-ABTS. AEAC represents the scavenging capacity of extract-DPPH. The letters *a*, *b*, and *c* indicate significant differences from each experiment at *p* < 0.05 after analysis by one-way ANOVA followed by Tukey's test.

3.4 Anti-inflammatory activity of extracts

In this study, the anti-inflammatory effects of AE, ME, and CE were determined using the denaturation of the BSA method, measured at a concentration of 5 mg%. The highest inhibition was observed in AE. Both ME and CE had a lower percentage of inhibition than AE, as presented in Table 2. The reference drug of diclofenac sodium showed a 50% inhibitory concentration (IC₅₀) at 0.40 mg/mL. The thermal inhibition of protein denaturation is one of the methods for screening *in vitro* anti-inflammation, usually compared to the non-steroidal anti-inflammatory drug

(NSAID) (Chaiya et al., 2022). The anti-inflammatory effects might be due to the presence of flavonoids and phenolic compounds, active ingredients in the extracts (Rauf et al., 2015). However, many kinds of chemical constituents in natural products have been reported to reduce protein denaturation, not only total phenolic compounds and flavonoids, but also tannins, cardiac glycosides, eicosane, naphthalene, alkaloids, triterpenoids, and saponins. Normally, biomolecules of *A. galanga* ethanolic extract are phenylpropanoid (e.g., 1'-acetoxychavicol acetate (ACA) and its derivative), a small amount of volatiles (e.g., chavicol, eugenol, and methyl eugenol), alkaloids, carbohydrates, steroids, terpenoids, proteins, phenols, and tannins, while chemical constituents of *M. alba* consist of steroids, saponins, alkaloids, and tannins. Therefore, the content of chemical compounds in each plant influences the anti-inflammatory effect via the inhibition of protein denaturation (Hussain et al., 2017; Verma & Sharma, 2022; Gonfa et al., 2023).

Table 2. Percentage of protein denaturation inhibition in the extracts at a concentration of 5 mg%

Extract	Concentration (mg%)	%Inhibition
CE	5	6.19 ± 0.72 ^b
ME	5	6.06 ± 1.50 ^b
AE	5	14.86 ± 1.99 ^a

Different letters refer to statistically significant differences at *p* < 0.05 after analysis by one-way ANOVA followed by Tukey's test.

3.5 Antibacterial and antifungal activities of extracts

In this study, the antibacterial and antifungal activities of extracts were investigated using the broth microdilution assay against gram-positive bacteria, e.g., *S. aureus* ATCC6538 and *S. epidermidis* ATCC12228, and dermatophytes, e.g., *T. mentagrophytes* ATCC MYA 4439, *T. rubrum* ATCC MYA-4438, and *M. gypseum*, commonly pathogenic microorganisms of skin disease. The results of this study revealed that AE was the most active against bacteria since it yielded the lowest MIC and MBC, as represented in Tables 3 and 4. Besides, AE was also more effective in inhibiting the growth of *S. epidermidis* than *S. aureus*. Additionally, ME had the ability to prohibit the growth of bacteria. However, CE could not interrupt the growth of both bacteria. According to the study findings, AE was more effective as an antimicrobial agent against *S. aureus* and *S. epidermidis* compared to the results of a previous study, where MIC exhibited values of more than 31.68 and 15.84 mg/mL, respectively (Na Nongkhai et al., 2024). According to a previous study by Srihaphon et al. (2020), MIC values for *M. alba* stem extract against both bacteria were reported to be higher than those found in the current experiment. Specifically, the previous study showed MIC values exceeding 25.0 mg/mL. However, no prior reports appear to specifically mention antimicrobial activity against both bacteria targeted by *C. oleifera* seed cake extract. For antifungal activity, the CE possessed the highest antifungal activity against *T. mentagrophytes*, *T. rubrum*, and *M. gypseum* with the lowest MIC and MFC values. According to Prastiyanto et al. (2021), *A. galanga* rhizome extract showed antifungal activity against *T. rubrum* by creating an inhibition zone, while its MIC and MFC were not determined in that study. There was



no evidence of antifungal activity against these dermatophytes from extracts of *M. alba* stem extract and *C. oleifera* seed cake. It should be noted that the findings of this present study provide the first evidence of their antifungal properties. This finding also aligns with several studies reporting that herbal medicinal plants contain bioactive constituents, like phenolic acids and flavonoid compounds, providing several biological functions such as antibacterial and antifungal properties (Rahman et al., 2021; Simonetti et al., 2020). Interestingly, although ME

exhibited the highest total phenolic and flavonoid content, AE demonstrated the most potent antibacterial activity, and CE showed the strongest antifungal activity. While phenolic and flavonoid compounds are known to contribute to antibacterial activity, it is not solely their responsibility. Other plant-derived secondary metabolites like tannins, terpenoids, and alkaloids also exhibit antibacterial properties (Khameneh et al., 2019). Additionally, saponins are known to have antifungal properties (Abdallah et al., 2023).

Table 3. MIC of the extracts against microorganisms

Isolates	CE (mg/mL)	ME (mg/mL)	AE (mg/mL)	CP (µg/mL)	VC (µg/mL)
MIC					
<i>S. aureus</i> ATCC6538	>200	12.5	6.25	NA	NA
<i>S. epidermidis</i> ATCC12228	>200	12.5	1.56	NA	NA
<i>T. mentogrophytes</i> ATCC MYA 4439	0.05	6.25	0.2	0.5	0.03
<i>T. rubrum</i> ATCC MYA-4438	0.025	6.25	0.1	0.5	0.0078
<i>M. gypseum</i>	0.05	6.25	0.1	0.5	0.03

CP; ciclopirox, VC; vancomycin, NA; not available

Table 4. MBC and MFC of the extracts against microorganisms

Isolates	CE (mg/mL)	ME (mg/mL)	AE (mg/mL)	CP (µg/mL)	VC (µg/mL)
MBC					
<i>S. aureus</i> ATCC6538	NA	50	100	NA	NA
<i>S. epidermidis</i> ATCC12228	NA	50	25	NA	NA
<i>T. mentogrophytes</i> ATCC MYA 4439	0.1	12.5	0.2	32	0.12
<i>T. rubrum</i> ATCC MYA-4438	0.1	12.5	0.1	>32	>0.5
<i>M. gypseum</i>	0.1	25	0.2	32	>0.5

CP; ciclopirox, VC; vancomycin, NA; not available

4. CONCLUSION

The extracts from *A. galanga* rhizome, *M. alba* stem, and *C. oleifera* seed cake demonstrated potential for use in healthcare products due to their antioxidant, anti-inflammatory, antibacterial, and antifungal properties. These extracts also contain polyphenols and flavonoids, key phytochemicals known for promoting health and well-being. According to the present study, they had the ability to inhibit the growth of pathogenic microorganisms, particularly dermatophytes. Future research should focus on exploring the benefits of specific compounds, particularly those derived from medicinal plants, with an emphasis on their potential roles in pharmaceutical and cosmetic formulations. A comprehensive research approach is needed to assess the safety, efficacy, and side effects of targeted compounds, encompassing both preclinical and clinical trial phases. To gain a better understanding of bioactive compounds in natural sources, therefore, further research should examine the combination of these Chinese-Thai herbal extracts, which are incorporated into topical dosage forms such as creams or solutions, and investigate the antimicrobial and antifungal effects in volunteers. Such a study should evaluate the potential therapeutic benefits of the formulations and explore how they work against pathogens. By focusing on the synergistic effect of herbal extracts, safer and more effective treatments can be developed for skin-related diseases.

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AUTHOR CONTRIBUTIONS

N.W. created the approach, visualization, research, and funding acquisition in addition to writing the original draft. T.K., S.P., C.S., and N.W. executed the conceptualization, experiments, formal analysis, and contributed to the interpretation of data and discussion. W.P. and M.H. coordinated the project and provided equipment for the experiments. N.W., T.K., S.P., and C.S. reviewed and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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