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Photoprotective effect, antiacne-causing bacterial activity and inhibitory effect against tyrosinase, elastase and hyaluronidase in extracts of Trang peppercorn (*Piper nigrum*)

Thanakorn Damsud [1,*], Chutinut Sujarit [2], Preecha Phuwapraisirisan [3]

[1]Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Thung Song, Nakhon Si Thammarat, 80110, Thailand
[2]Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang 92150, Thailand
[3]Center of Excellence in Chemistry of Natural Products, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

ABSTRACT: Natural substances are highly appealing due to their minimal side effects and widespread use in today's world. Plants serve as a source of natural substances, offering a variety of substances suitable for product development in the cosmetic industry. The aim of the study was to test the effect of Trang peppercorns grown in Thailand to identify potent compounds for UV protection, acne-causing bacteria prevention, skin whitening, and anti-aging properties. Ethanol extracts of Trang peppercorn were investigated for their photoprotective effect, antiacne-causing bacterial activity, together with inhibitory effect against tyrosinase, elastase and cell viability of B16F10 melanoma cells after 72 h treatment. The samples exhibited an SPF greater than 4, with the sample indicating the most significant photoprotective effect. Trang pepercorn extracts showed that against antiacne-causing bacteria, *Propionibacterium acnes* and *Staphylococcus epidermidis* RP are good sources of tyrosinase (IC_{50} 98.63±4.11 μg/mL), elastase (42.32% inhibition at 500 μg/mL), and hyaluronidase (53.14% inhibition at 500 μg/mL). In the cytotoxicity test, there were IC_{50} values of 81, 107, 258 and 143 μM, respectively. The total findings provide relevant information on Trang peppercorn, suggesting its potential usefulness as a material for the cosmetic industry.

1. INTRODUCTION

The skin care cosmetics industry has recently seen major growth and development. This global industry has garnered substantial attention (Smit et al., 2009). Many types of ultraviolet radiation (UV) produced by sunshine, including UVA, UVB, and UVC, have the potential to harm the outer layer of the skin, leading to sunburn and increasing the risk of developing skin cancer (Aziz et al., 2023; Garbe et al., 2024). Furthermore, it has the ability to stimulate the production of damaging metalloproteinase enzymes, which break down collagen and elastin, leading to the development of wrinkles, sagging skin, and faster aging of the skin. Moreover, the stimulation of melanogenesis caused by UV radiation could possibly lead to an increase in the synthesis of melanin pigment, which in turn causes skin hyperpigmentation and tanning (Laronha & Caldeira, 2020; Malta et al., 2023). Prolonged exposure to UV radiation may produce free radicals, which trigger the production of pro-inflammatory cytokines by skin cells already present in the body. This can result in cellular harm or the development of serious skin disorders, including skin cancer (Nakai & Tsuruta, 2021).

Peppercorn (*Piper nigrum*) is well recognized in Thailand for its culinary use as a spice and its therapeutic properties. For this reason, the herb. *P. nigrum* is often used as a spice by several nations, including Thailand, because of its strong and intense flavor characteristics. Its chemical composition contains numerous types of compounds, including phenolics, lignans, terpenes, chalcones, flavonoids, alkaloids, and steroids. Due to the presence of flavonoids and phenolic compounds (Ashokkumar et al., 2021; Feitosa et al., 2024), *P. nigrum* exhibits antioxidant properties and may serve as a readily available source for natural radical scavenging activity in the nutritional and cosmetic industries. Perfumers have used *P. nigrum* not just for its fragrance but also as a flavoring

* Corresponding author.
E-mail address: thanakorn.d@rmutsv.ac.th (Thanakorn Damsud)

agent, preservative, and medicinal component. This species has substantial commercial, economic, and medicinal potential due to its associated physiological activity (Yousuf et al., 2022). Moreover, *P. nigrum* and piperine indicate a diverse array of pharmacological effects, encompassing antitumor, antidepressant, antiasthmatic, antihypertensive, and antiplatelet properties, along with anti-inflammatory, hepatoprotective, anticonvulsant, antioxidant, antidiarrheal, analgesic properties, immunomodulatory, and antimicrobial effects (Ashokkumar et al., 2021).

Trang pepper belongs to the Piperaceae family. There are three sorts of pepper: white, black, and red. This kind of pepper is cultivated in the Trang province region of southern Thailand. Its defining attribute is its piquant flavor and its odor is distinct and strong. The peculiar growing conditions and environment of Trang Province have a significant impact on the specific features of Trang pepper. As a result, Trang pepper has gained recognition as a distinguished agricultural product across the country due to its registration as a Geographical Indication (GI) (Liamnimitr et al., 2023). This pepper processing includes the separation of poor-quality Trang pepper using a floating technique, which is necessary owing to the low density of the seeds. Consequently, it leads to the generation of agricultural waste. The first chemical composition analysis of the buoyant seeds indicated a 2% concentration of piperine, which holds promise for possible future uses. In the current study, we performed a study on black (BP), red (RP), white (WP) and floating (FP) Trang peppercorns grown in Thailand to identify potent compounds for UV protection, acne-causing bacteria prevention, skin whitening, and anti-aging properties. The research examined the efficacy of these peppercorns in terms of UV radiation protection, acne treatment, tyrosinase inhibition, elastase inhibition, and hyaluronidase inhibition. Furthermore, the detrimental effects of the three most potent crude extracts on B16F10 melanoma cells were investigated.

2. MATERIAL AND METHODS

2.1. Chemicals and reagents

Ethanol, methanol, hydrochloric acid, hydrochloric acid, Folin-Ciocalteu reagent, aluminum chloride, sodium carbonate, sodium hydroxide, sodium chloride, phosphate buffer, dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany). Elastase form porcine pancreas, quercetin, tyrosinase form mushroom, ascorbic acid, kojic acid, hyaluronidase form bovine test, tyrosinase form mushroom, hyaluronic acid sodium salt form rooster comb, L-3,4-dihydroxypenlylalanine (L-DOPA), tannic acid, gallic acid, N-Succinly-Ala-Ala-Ala-p-nitroanilide, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, penicillin, streptomycin and 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Peppercorn preparation

Trang peppercorns (BP, RP, WP and FP) were obtained from the Ban Suan Heritage Company in Yan Ta Khao District, Trang Province, Thailand. The specimens were collected in June 2023, at coordinates 7°23′12″N 99°40′0″E. Specimens (PCMU 0024378) were stored at the Herbarium of the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University.

2.3. Peppercorn extraction

The dried and ground fruits of Trang peppercorn (200 g) were extracted by maceration using 95% ethanol (1 L) for a period of 72 h. The solvent was removed under reduced pressure using rotary evaporator with waterbath temperature of 45 °C. This step was repetedly performed twice. The combined extracts were finally concentrated to dryness and kept in dark until use.

2.4. Phytochemical content

The total phenolic (TPC) and total flavonoid contents (TFC) of the ethanol extract of Trang peppercorn were quantified using the established technique suggested by Damsud et al. (2023). The obtained concentration are expressed in mg of rutin equivalents (QE)/g of extract for TFC and mg of gallic acid equivalents (GAE)/g of extract for TPC. The piperamides were analyzed using liquid chromatography with high resolution tandem mass spectrometry (LC-HRMS/MS), following an approach described by Luca et al. (2021). The data is calculated in mg of piperine equivalents (PE) /g of extract.

2.5. Photoprotective activity

The maximum wavelength (λmax) was calculated following this standard approach. The extracts were diluted in ethanol (Synth-BRA) to a concentration of 500 μg/mL and measured with a spectrophotometer (BMG Labtech, Germany) over a range of wavelengths from 260 to 400 nm. In addition, the Sun Protection Factor (SPF) was determined using in vitro methods. In order to evaluate the sun protection factor (SPF), dried extracts were diluted in ethanol at concentrations of 0.6, 0.8, and 1 μg/mL, as the absorbance was measured within the wavelength range of 290 to 320 nm. The average value SPF was calculated using the methods proposed by De et al. (2018).

$$SPF_{spectrophotometric} = CF \times \times I(l) \times A\ (l)$$

The character CF shows the adjustment factor, which has an amount of 10. EE (λ) denotes the erythematogenic effect of light at a particular wavelength (λ). I (λ) describe the magnitude of sunlight at a specific wavelength (λ), while A (λ) defines an absorbance measurement of a sample prepared using spectrophotometry at a specific wavelength (λ).

2.6. Tyrosinase inhibition assay

The experiment was conducted for inhibiting tyrosinase following the method according to Chummalee et al. (2024) by utilizing L-DOPA as the substrate. Trang peppercorn extracts, with concentrations ranging from 0.1 to 50 mg/mL, were added

to a solution of phosphate buffer (pH 6.8, 100 μL) in a 96-well plate. After that, the mixture was pre-incubated at a temperature of 37 °C. The spectrophotometer detected the concentration of dopa-chrome at a wavelength of 475 nm, with kojic acid serving as a positive control. The percentage inhibition against tyrosinase was determined using the formula: $[(A_{control} - A_{sample}) / A_{control}] \times 100$ where A $(_{sample})$ indicates the absorbance of the sample extracts and A $(_{control})$ indicates the absorbance of the test using the buffer instead of the inhibitor (sample). The IC_{50} value was calculated using the dose-inhibition curve.

2.7. Elastase inhibition assay

The Trang peppercorn extract's efficiency to inhibit elastase was determined using the porcine pancreatic elastase (PPE) enzyme inhibitory assay, as previously defined by (Liyanaarachchi et al., 2018), with some modifications. A 96-well plate was loaded with 0.1 M Tris-HCl buffer (pH 8.0). The PPE was mixed in sterile water to develop a stock solution with a concentration of 300 units. The AAAPVN substrate was dissolved in a buffer solution. The Trang peppercorn extract was incubated with the enzyme for 25 min prior to the addition of the substrate to initiate the reaction. Thus, the final reaction mixture (250 μL) contained a buffer, AAAPVN (10 μg/mL), PPE (0.001 units) and peppercorn extract (10 mg/mL). The maximum velocities (V_{max}) were measured at a wavelength of 410 nm during a duration of 25 min, with measures obtained each 30 s. Quercetin was utilized for the positive control, although Tris-HCl was used as the blank. The percentage inhibitor of the elastase enzyme is calculated with the following equation: $[(V_{max(control)} - V_{max(sample)}) / V_{max(control)}] \times 100$ where $V_{max(sample)}$ refers to the velocity of the sample extracts, whereas $V_{max(control)}$ represents the velocity of the assay when using the buffer instead of the inhibitor $_{(sample)}$. The IC_{50} value was determined by analyzing the dose-inhibition curve.

2.8. Hyaluronidase inhibition assay

The measurement of hyaluronidase inhibitory activity was conducted using the method described by Livanaarachchi (Liyanaarachchi et al., 2018), with slight modifications. A solution consisting of 10 μL of type-1-S bovine testes hyaluronidase (4200 units/mL) diluted in 0.1 M acetate buffer (pH 3.5) was combined with a solution containing 50 mg/mL of Trang peppercorn extract dissolved in 5% DMSO. The mixture was then incubated in a water bath at 37 °C for 10 min. The Ca^{2+} activated hyaluronidase was exposed to 100 μL of sodium hyaluronate solution in 0.1 M acetate buffer (pH 3.5). It was then placed in a water bath at 37 °C for 40 min. The reaction mixture was supplemented with 10 μL of a 0.9 M sodium hydroxide and 50 μL of a 0.2 M sodium borate. Additionally, the preparation underwent incubation within a vigorously boiling water boiler at a duration of 3 min. Following the reduction in temperature to ambient levels, a 50 μL solution of p-dimethylaminobenzaldehyde was combined with the reaction mixture. Next, the mixture was incubated in a water bath set at a temperature of 37 °C for duration of 10 min. The control group obtained 50 μL of a 5% DMSO solutions instead of the Trang peppercorn extract. The measurement of absorbance was conducted at a wavelength of 585 nm. The percentage enzyme inhibition was determined by using the following formula: $[(A_{control} - A_{sample}) / A_{control}] \times 100$ where A $(_{sample})$ represents the absorbance of the sample extracts, whereas A $(_{control})$ represents the absorbance of the test conducted using the buffer instead of the inhibitor $(_{sample})$. Tannic acid serves as a benchmark or point of comparison. The IC_{50} value was determined by analyzing the dose-inhibition curve.

2.9. Cell culture

Cell lines of B16F10 melanoma were obtained from the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, as previously documented (Han et al., 2015), These cell lines were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified 95% air and 5% CO_2 atmosphere.

2.10. Cell viability assay

In 96-well plates, B16F10 melanoma cells were planted at a density of 1 x 10^4 cells/well. The cells were exposed to different concentrations of peppercorn extracts for 72 h. after a 24 h period. Following the incubation time, 20 μL of the MTT test solution (10 mg/mL in normal saline solution) was added to each well, and the cells were incubated for an additional 4 h at 37 °C. To dissolve the formazan crystals, 200 μL DMSO was applied. The wavelength of absorbance at 540 nm was measured using a microplate reader. The cell survival rate was calculated using the following equation: (A × 100)/B where A measures the difference in absorbance between the sample and the control, and B represents the absorbance in the absence of the sample (Han et al., 2015).

2.11. Antimicrobial activity

The agar disc diffusion technique was employed to ascertain the zone of inhibition of Trang peppercorn extract. A microbial solution comprising 1 × 10^8 colony-forming units per milliliter (CFU/mL) of *P. acnes* DMST 14916 and *S. epidermidis* DMST 15055 was applied over Brain Heart Infusion (BHI) and Trypic soy agar, respectively. A 20 μL of Trang peppercorn extract was applied onto a paper disc with a diameter of 6 nm, and then put on the agar. Regarding both of the bacteria, a concentration of 100 μg/disc of amoxicillin in methanol was employed as a positive control. The agar plate was incubated at 37 °C for 72 h under anaerobic conditions and 24 h under normal conditions for *P. acnes* and *S. epidermidis*, respectively. The activity was determined by measuring the diameter of the inhibitor zone on the examined organism. MIC (Minimum Inhibitory Concentration) values of Trang peppercorn extract were evaluated using broth microdilution. The positive control for *P. acnes* was the clindamycin disc with a concentration of 2

μg/disc, whereas the positive control for *S. epidermidis* was the tetracycline disc with a concentration of 30 μg/disc. DMSO was used as a negative control (Nakyai et al., 2021).

2.12. Statistical analysis

The experiment was performed three times. The average value was shown together with the mean ± standard deviation (S.D.). Significant results were detected using the GraphPad Prism and IBM SPSS Statistics 22 software, using a p-value level of less than 0.05 for the one-way analysis of variance (ANOVA).

3. RESULTS

3.1. Phytochemical content

The Trang peppercorn extracts used in this study were prepared from BP, RP, WP, and FP using ethanol as the solvent. Subsequently, an analysis was conducted to examine the phytochemical composition in term of TPC and TFC, and the amount of piperamide. Among the peppercorn extracts evaluated in Table 1, RP exhibited the highest levels of TPC (55.34±0.18 mg GAE/g of extract) and TFC (16.02±0.45 mg QE/g of extract). A statistically significant difference was observed with a confidence level of $p<0.05$. The quantity of piperamides was determined using the LC-HRMS/MS method, resulting in a total of 17 compounds shown in Table 2. Analysis showed a range of components in all four kinds of Trang peppercorn extract. BP exhibited the highest total piperamide content, followed by WP, RP, and FP, with the values of 593.58±6.82, 503.42±5.74, 244.90±4.04, and 191.49±3.89 mg PE/g extract, respectively. However, WP showed the highest value for piperine, followed by BP, FP, and RP, with values of 317.71±0.71, 294.70±0.57, 156.76±0.64, and 146.76±0.79 mg PE/g extract, respectively. Both the total piperamides and piperine readings exhibited significant statistically differences with a confidence level of $p<0.05$.

3.2. Photoprotective activity

A comprehensive analysis of UV absorption was conducted on all four variants of Trang peppercorn extract, using wavelengths ranging from 260 to 400 nm. This range covers UVA (320 to 400 nm), UVB (290 to 320 nm), and UVC (100 to 320 nm). The results revealed that all extracts demonstrated the ability to absorb UV feels from the UVA range. BP demonstrated the maximum efficacy in absorbing UV radiation. In contrast, RP demonstrated more efficacy in absorbing UVB compared to UVA in Figure 1A). The measured SPF values varied between 4.98 and 9.53 (Figure 1B). BP obtained a highest SPF value of 9.53 at a concentration of 1 μg/mL, which was significant at a confidence level of $p<0.05$.

3.3. Tyrosinase inhibitory activity

The results of inhibiting the tyrosinase enzyme activity in all four varieties of Trang peppercorn extract are shown in Table 1. It was revealed that the highest inhibitory efficacy was found in RP, followed by WP, with IC_{50} values of 98.63±4.11 and 101.03±1.13 μg/mL, respectively. The results are not statistically different at a confidence level of $p<0.05$ and are comparable to the IC_{50} values of kojic acid and ascorbic acid, which are positive controls with values of 82.14±0.70 and 70.25±2.41 μg/mL, respectively.

3.4. Elastase and hyaluronidase inhibitory

Studies on inhibiting the activity of hyaluronidase and elastase from all four types of Trang peppercorn extract are shown in Table 1. It was found that the highest percentage of inhibition of both elastase and hyaluronidase was given by RP, equal to 42.32% and 53.14%, respectively, when compared to the positive control quercetin with an IC_{50} value of 21.32±4.32 μg/mL. The percentage of inhibition by tannic acid was 95.20%, while the other types of Trang peppercorn extract were unable to inhibit the activity of hyaluronidase and elastase.

3.5. Antimicrobial

The results of extracting all four types of Trang peppercorn using disc diffusion techniques are presented in Table 3. It was observed that RP exhibited the most effective inhibitory effect on *P. acnes*, with a value of 4.87±0.35 cm, while BP demonstrated the strongest inhibitory effect on *S. epidermidis*, with a value of 14.75±0.29 cm. Furthermore, it was noted that the four types of Trang peppercorn extracts were considerably less effective than clindamycin and tetracycline, which exhibited inhibition values of 61.54±0.23 cm and 22.45±0.24 cm, respectively, at the 0.05 level of confidence. Minimal inhibition concentration (MIC) values were determined, revealing that RP had an MIC value against *P. acnes* of 27.41 mg/mL, a significant difference ($p<0.05$), while the MIC values against *S. epidermidis* for BP and RP were not significantly different at the confidence level ($p<0.05$), measuring 4.58 and 4.32 mg/mL, respectively.

3.6. Effect of Trang peppercorn extract on cell viability

Cell viability was evaluated using the MTT assay following a 72-h treatment. The results are shown as the percentage of viability compared to the control (0 μM). In addition, it may be used to determine the cytotoxicity of potential pharmaceuticals and dangerous compounds, since these chemicals possess the ability to either enhance or impede cell viability and growth. Based on Figure 2, BP, RP, WP, and FP demonstrated cytotoxicity against B16F10 melanoma cells at concentrations of 40, 120, 20, and 100 μM. After 72 h of treatment, cell viability decreased to approximately 80% of the control group (Figure 2). In contrast, kojic acid did not have any cytotoxic effects on the cell morphology at different concentrations. WP possessed the highest level of toxicity, while BP, FP, and RP shown levels of toxicity with IC_{50} values of 81, 107, 258 and 143 μM, respectively.

4. DISCUSSION

Currently, there is a notable increase in the discovery of natural compounds derived from medicinal plants for

Table 1
Total phenolic content (TPC), total flavonoid content(TFC) and tyrosinase, elastase, hyaluronidase inhibitory of Trang peppercorn.

Sample	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	Tyrosinase IC_{50} (μg/mL)	Elastase IC_{50} (μg/mL)/inhibition (%)[1]	Hyaluronidase Inhibition (%) [1]
BP	40.31 ± 1.00^{c}	15.84 ± 0.30^{a}	129.02 ± 3.55^{b}	NI	NI
RP	55.34 ± 0.18^{a}	16.02 ± 0.45^{a}	98.63 ± 4.11^{c}	42.32[1]	53.14[1]
WP	43.14 ± 0.15^{b}	4.57 ± 0.03^{b}	101.03 ± 1.13^{c}	NI	NI
FP	20.31 ± 0.13^{d}	2.21 ± 0.01^{c}	204.03 ± 4.67^{a}	NI	NI
Kojic acid	-	-	82.14 ± 0.70^{d}	-	-
Ascorbic acid	-	-	70.25 ± 2.41^{e}	-	-
Tannic acid	-	-	-		95.20[1]
Quercetin	-	-	-	21.32±4.32	-

The total phenolic content (TPC) is represented as mggallic acid equivalents (GAE)/g extract, while the total flavonoids content(TFC) is represented as mg quercetin equivalents (QE)/g extract. The meanvalues ± S.D. of three determinations is presented. As the level of significance is defined at p<0.05, values in the same column that are identified with an exclusive after letter appear to be statistically different. NI-no inhibition at 500 μg/mL, 1%inhibition at 500 μg/mL. GAE= Gallic acid equivalent; QE= quercetin equivalents; IC_{50}= Inhibitory concentration at 50%; BP= Black peppercorn; RP= Red peppercorn; WP= white peppercorn; FP= floating peppercorn; NI= No inhibition

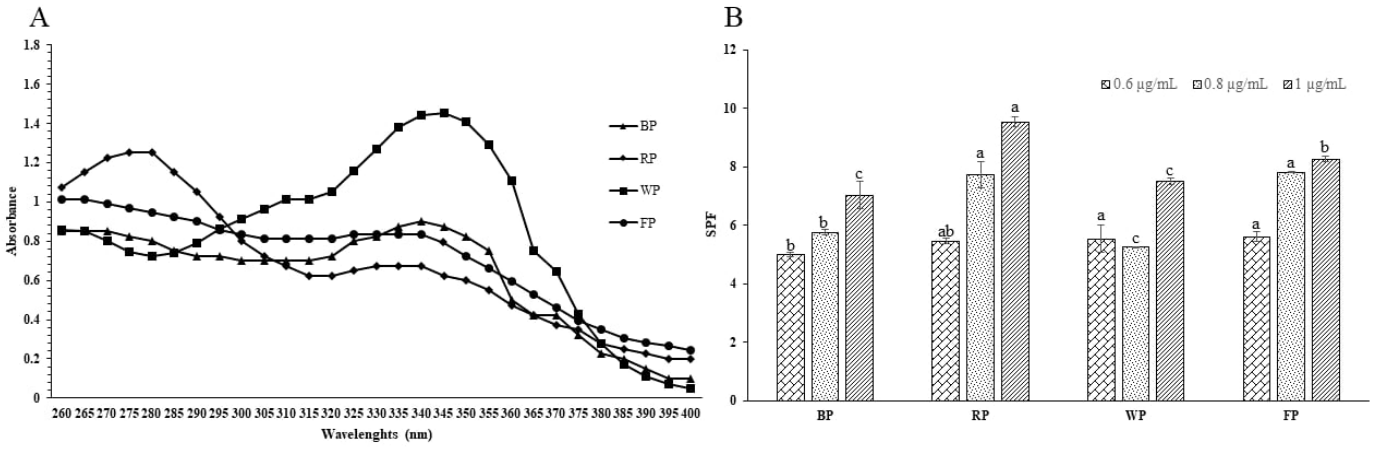

Figure 1. UV wavelength absorption spectrum (260 to 400 nm, A) and sun protection factor (SPF, B) of Trang peppercorn extract. BP= black peppercorn; RP= red peppercorn; WP= white peppercorn. $^{a-c}$Data are presented as mean ± SD and the same letters indicate non-significant differences at the same concentration level.

the purpose of manufacturing cosmeceutical products. The manufacturing method is environmentally sustainable and provides customer safety. Additionally, it lowers the use of chemicals. This research evaluates the performance of Trang peppercorn extract obtained from its production procedure in its use in cosmetics for the purposes of skin lightening, anti-aging, and promoting skin health. Trang pepper seeds have a high quantity of phytochemicals. They affect growth because of their influence on metabolism. The chemical composition consists of a variety of biologically active compounds, such as piperamides, lignans, flavonoids, and essential oils. These findings were similar to the results previously reported by Luca et al. (2021), in which RP revealed highest levels of TPC and TFC values of 41.41 mg GAE/g and 5.42 mg QE/g of extract (Luca et al., 2021), respectively. The high TPC and TFC content of RP may be contributed to by its production process, which involves collecting the seeds when the fruits are ripe and red, followed by the drying process utilizing a relatively mild temperature range of 45-60°C to prevent Maillard reaction. On the other hand, the production of WP, BP, and FP employed a higher temperature range of 70-85 ºC. Temperatures exceeding 80 °C may induce changes in phytochemicals, potentially leading to a loss of their biological potential (Syeunda & Awika, 2024; Ye et al., 2024). The distinctive characteristics of Trang peppercorn, including its spicy taste and pungent odor, are contributed to by substances in the piperamides group, such as piperine or other alkaloids. The amount of piperine in black pepper (276.70 and 293.07 mg PE/g extract) was previously investigated by Luca et al. (2021). The piperine content of BP was found to be 317.71±0.71 mg PE/g extract, which is higher than that of a previous report (Luca et al., 2021). This disparity may be accounted for by factors such as the type of breed and variables involved in providing water and nutrients to plants, such as the climate and terrain.

For people to obtain comprehensive skin protection, many photoprotective formulations that only comprise of chemically produced sunscreens are insufficient. Hence, plant extracts that are abundant in bioactive components are being extensively

Table 2
Piperamide content (mg PE/g extract) in the ethanolic extracts obtained from Trang peppercorn.

No.	Compound	Trang peppercorn			
		BP	RP	WP	FP
1	Piperolactam C	ND	ND	ND	ND
2	Piperlongumine	ND	ND	ND	ND
3	Piperyline	15.77±0.74^{a}	8.74±0.02^{b}	8.20±1.06^{b}	3.73±0.48^{c}
4	Piperlonguminine	4.90±0.81^{a}	6.50±0.40^{a}	1.85±0.85^{b}	0.84±0.55^{b}
5	Piperine	294.70±0.57^{b}	146.76±0.79^{d}	317.71±0.71^{a}	156.76±0.64^{c}
6	Piperettines	71.78±0.76^{a}	23.33±0.17^{c}	64.71±0.71^{b}	ND
7	Piperolein A	14.74±0.69^{b}	4.04±0.28^{c}	20.89±0.80^{a}	2.37±0.06^{d}
8	Pellitorine	21.93±0.68^{a}	9.33±0.17^{c}	25.63±0.22^{b}	10.83±0.54^{d}
9	Pipercallosine	3.22±0.28^{b}	2.08±0.09^{c}	5.31±0.14^{a}	1.39±0.21^{d}
10	Dehydropipernonaline	22.58±0.23^{a}	2.51±0.23^{b}	15.31±0.14^{c}	2.51±0.52^{b}
11	Pipernonaline	2.36±0.07^{a}	ND	ND	ND
12	Neopeollitorine B	5.51±0.39^{a}	ND	1.49±0.36^{c}	ND
13	Retrofractamide B	17.59±0.37^{a}	12.56±0.47^{b}	5.51±0.39^{c}	ND
14	Piperolein B	15.84±0.08^{a}	1.28±0.05^{d}	12.51±0.05^{b}	2.57±0.30^{c}
15	Piperundecalidine	8.50±0.40^{a}	4.60±0.54^{b}	ND	1.23±0.03^{d}
16	Guineensine	45.67±0.45^{a}	10.63±0.53^{b}	10.66±0.17^{b}	5.61±0.40^{a}
17	N-Isobutly-2,4,12-octadecatrienamide	48.53±0.30^{a}	12.57±0.30^{b}	13.67±0.16^{c}	3.67±0.16^{d}
	Total piperamides	593.58±6.82^{a}	244.90±4.04^{b}	503.42±5.74^{c}	191.49±3.89^{d}

BP= black peppercorn; RP= red peppercorn; WP= white peppercorn; ND= Not Detected. The data is presented as mg piperine equivalent (PE)/g extract and indicates the mean values ± S.D. of three determinations. Values within the same row that have specific superscript letters are statistically significant at a significance level of p<0.05.

Table 3
Antiacne-causing bacteria activities of Trang peppercorn extract.

Sample	Inhibition zone (cm)		MIC (mg/mL)	
	P. acnes DMST 14916	*S. epidermidis* DMST 1550	*P. acnes* DMST 14916	*S. epidermidis* DMST 1550
BP	3.30±0.21^{c}	14.75±0.29^{b}	23.27^{d}	4.58^{b}
RP	4.87±0.35^{b}	10.47±0.25^{c}	27.41^{c}	4.32^{b}
WP	2.74±0.23^{d}	9.27±0.24^{d}	35.32^{b}	5.02^{a}
FP	1.21±0.02^{e}	1.02±0.01^{e}	48.31^{a}	2.14^{c}
Clindamycin[1]	61.54±0.23^{a}	-	0.125^{e}	-
Tetracycline[2]	-	22.45±0.24^{a}	-	0.5^{c}

BP= black peppercorn; RP= red peppercorn; WP= white peppercorn. The data was presented as the mean ± S.D. Values within the same colum that have specific superscript letters are statistically significant at a significance level of p<0.05. [1] Clindamycin (2 μg/disc) was used as a positive control for *P. acnes*. The positive control for *S.epidermidis* was [2] Tetracycline (30 μg/disc). MIC = Minimum Inhibitory Concentration.

used (Prasanth et al., 2020). The results indicate the photochemical potential of the extract, and these findings exceed the standards provided by the Food and Drug Administration Thailand for sunscreen products (Phadungsaksawasdi & Sirithanabadeekul, 2020). Piperine and its derivatives, methyl piperate, ethyl piperate, including peperic acid, isobutyl piperate, propyl piperate, and isopropyl piperate, have been the subject of a study report. These derivatives were prepared with a 5% oil-in-water emulsion, and their SPF values ranged from 3.15 to 2.73. The values ranged from 16.37 to 1.8 and from 9.68 to 1.71, with the UVA/UVB ratios of all substances falling within the range of 0.860 to 0.967. Additionally, Choochana et al. (2015) found piperine and its derivatives to be non-toxic to skin cells. Moreover, the flavonoids and phenolic substances found in high levels in RP, as shown in Table 1, have been identified as effective in both absorbing UV and possessing antioxidant properties that protect against UV and help eliminate free radicals that occur when the skin is damaged. The inflammation of the skin is also prevented by prolonged exposure to sunlight (Oliveira et al., 2021).

The tyrosinase enzyme plays a crucial role in the production of human skin color, including the development of skin disorders. Investigation has shown that inhibiting a function of the tyrosinase enzyme may lead to lightening the skin and a decrease in skin irregularity. Compounds such as flavonoids, phenolics, stilbenes, triterpenes, and sterols that are obtained from plants have been shown to efficiently inhibit the reactions of tyrosinase. Nevertheless, piperine, the primary chemical compound found in pepper, is considered to possess the capacity to suppress tyrosinase activity. Furthermore, we conducted a comprehensive examination of piperine. The computational docking approach proved to be effective against tyrosinase, demonstrated by docking scores of -7.04 and it was classified as non-toxic. The molecular dynamics simulations demonstrated

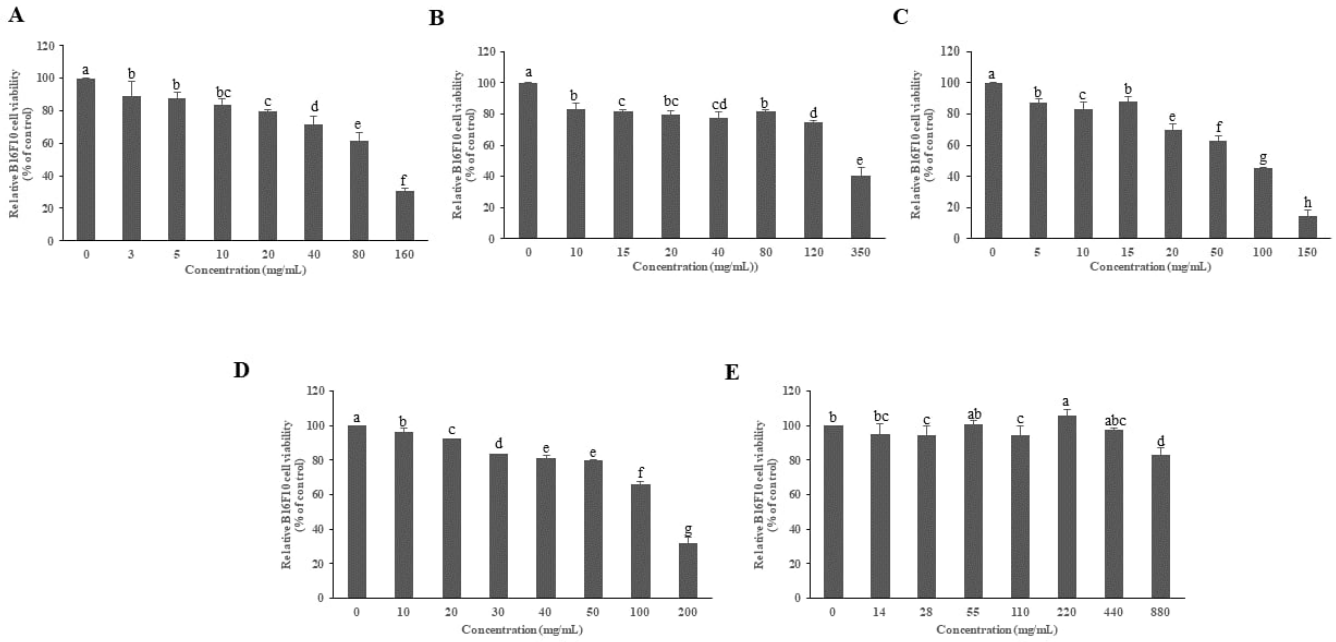

Figure 2. The effect of Trang peppercorn extract on cell viability in B16F10 melanoma cells.: A: black peppercorn (BP), B: red peppercorn (RP), C: white peppercorn (WP), D: floating peppercorn (FP) and E: kojic acid (positive control). $^{a-h}$Data arepresented as mean ± SD and the same letters indicate non-significant differences within the same sample.

that piperine had little change throughout the simulation, as seen by the analysis of root mean square deviation (RMSD) and root mean square fluctuation (RMSF) (Safithri et al., 2023). Furthermore, research has examined the efficacy of other Piper species, such as *P. erecticaule*, *P. retrofractum*, *P. abbreviatum*, *P. magnibaccum*, *P. caninum*, *P. betle,* and *P. stylosum*, in reducing the activity of the tyrosinase enzyme (Hashim et al., 2019; Safithri et al., 2023; Salleh et al., 2014). The inhibition of hyaluronidase activity causes the skin to create a tissue matrix that influences tissue development, growth, and damage. The inhibition of elastase activity makes the skin flexible, which helps prevent premature aging (Goodman et al., 2019). Hyaluronidase and elastase are most efficiently inhibited by compounds belonging to the polyphenols, phenolic, and flavonoids groups. This aligns with the research on RP, which showed that significant quantities of TPC and TFC exhibit the most efficacy in suppressing both kinds of enzymes. Scientists have shown that compounds such as polyphenols, phenolics, and flavonoids interact with the carbonyl and hydroxyl groups of hyaluronidase and elastase to produce metalloenzymes (Pientaweeratch et al., 2016; Wahab et al., 2014). Therefore, the enzyme is unable to facilitate interactions. Moreover, phenolic compounds with a hydroxyl group may establish hydrogen bonds within the elastase structure, there by rendering the enzyme incapable of catalyzing chemical processes (Masuda et al., 2009).

Acne is caused by gram-positive bacteria, including *P. acnes* and *S. epidermidis*, in both females and males, with the stimulation of the sebaceous glands by sex hormones resulting in increased sebum production, leading to clogged pores (Bunrathep et al., 2020). Several studies have shown evidence of the effectiveness of different flavonoids and phenolic compounds in preventing the growth of *P. acnes* and *S. epidermidis*. The chemicals could be found in Trang peppercorn, as shown in Table 1. RP is characterized by its significant phenolic content and the highest concentration of total flavonoids. Researchers investigated the impact of flavonoids on the strength of cell walls and the creation of septa during cell division. Weng et al. (2024) showed that flavonoids can inhibit medication resistance in both gram-positive and gram-negative bacteria. In addition, low-polarity chemicals like essential oils, found in Trang peppercorn extract, may interact with the lipid components of bacterial cell walls. This interaction disrupts the permeability of the cell walls, causing the release of diverse cellular contents (Weng et al., 2022). In addition, a mechanism for inhibiting acne-causing bacteria was found through the use of substances with the properties of a photosensitizing agent sensitive to light, which may be present in Trang peppercorn extract. The mechanism is triggered when the photosensitizing agent is exposed to light, producing reactive oxygen species (ROS), which kill or reduce the number of acne-causing bacteria, *P. acnes* and *S. epidermidis*. Additionally, an effect on the destruction of blood vessels supplying fat, leading to reduced oil production from the sebaceous glands and subsequent improvement in acne, was observed (Lee et al., 2024). However, the mechanism may require further studies on pure substances, along with research in living organisms to assess treatment efficacy, as well as studies on the quantity and toxicity of the extracts to cells and skin. In order to assess the effect of Trang peppercorn extract on the

survival of cells, B16F10 melanoma cells were used as an in vitro model setting. Kojic acid was used as a control in the experiment. The results of this research align with a previous study that found piperine does not exhibit toxicity against B16F10 melanoma cells (Sadangri et al., 2019).

5. CONCLUSION

This study presents an investigation of the phytochemical composition and cosmetic properties of Trang peppercorn extract. Initially, an exhaustive examination of metabolites was conducted using TPC, TFC, and LC-HRMS/MS, which revealed substantial amounts of biologically active piperamides. RP contain rich tyrosinase, elastase, and hyaluronidase inhibitors, and are also non-toxic to B16F10 cells. All samples exhibited a significant and efficient protective effect, suggesting the possible application of these extracts to protect from UV radiation. Then, the samples were evaluated to determine their efficacy against bacteria that contributes to the formation of acne. The following specific Trang peppercorn not only shows a moderate level of effectiveness against *P. acnes* and *S. epidermidis*, but it also offers valuable information about the phytochemicals found in this species. This shows its potential for usage in the cosmetics industry. Additional research is required to determine the chemical components of this plant, their modes of operation, and their interaction between them.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in this study.

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ORCID

Thanakorn Damsud	0009-0005-4201-2127
Chutinut Sujarit	0009-0005-1511-6369
Preecha Phuwapraisirisan	0000-0001-6481-7712

AUTHOR CONTRIBUTIONS

TD- Research concept and design; CS, TD - Collection and/or assembly of data; CS, PP, TD - Data analysis and interpretation; TD - Writing the article; CS, PP, TD - Critical revision of the article, TD - Final approval of the article.

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