J Herbmed Pharmacol. 2025; 14(4): 396-404.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2025.52790

Journal of Herbmed Pharmacology

# Exploring the potential of *Citrus hystrix* DC. extract as a cosmeceutical agent

**Widyastuti Widyastuti*, Diza Sartika, Sandra Tri Juli Fendri**

Faculty of Pharmacy, Universitas Perintis Indonesia, Padang, West Sumatera, Indonesia

**ARTICLE INFO**

***Article Type:***
Original Article

***Article History:***
Received: 18 Oct. 2024
Revised: 16 Dec. 2024
Accepted: 12 Mar. 2025
epublished: 1 Oct. 2025

***Keywords:***
Sunscreen
Skin-lightening
Antioxidant
Anti-aging
Natural product

**ABSTRACT**

**Introduction:** *Citrus hystrix* DC. contains secondary metabolites, such as phenolic and flavonoid compounds, which may have photoprotective and antioxidant properties. These compounds make them feasible for use as sunscreens, skin-lightening agents, and anti-aging agents in cosmetic preparations. This study aimed to evaluate *C. hysteric* plant extract as an active ingredient in sunscreen, skin-lightening, and anti-aging cosmetics.

**Methods:** Extraction was done on leaves, fruit peels, and pulp to assess extract characteristics and phytochemical compositions. The extracts containing phenolic and flavonoid compounds were analyzed for total phenolic content (TPC) and total flavonoid content (TFC). Finally, in vitro sunscreen, antioxidant, and tyrosinase inhibition activities were evaluated using spectrophotometric methods to determine cosmeceutical potential.

**Results:** The research revealed that ethyl acetate extract of fruit peels (EAP) had higher TPC and TFC content, compared to other extracts ($P<0.05$). Additionally, EAP had the best sunscreen activity with a Sun Protection Factor (SPF) value of 23.644 ± 0.007 at 200 µg/mL concentration. EAP was reported as the best tyrosinase inhibitor with an $IC_{50}$ value of 46.265 µg/mL. In contrast, ethanol leaf extract (EL) demonstrated the highest antioxidant activity, with an $IC_{50}$ value of 544.174 µg/mL. Finally, the best inhibitions of collagenase, elastase, and hyaluronidase were EAP, EL, and ethanol extracts of the fruit peels (EP) with $IC_{50}$ values of 26.045, 58.521, and 55.947 µg/mL, respectively.

**Conclusion:** Ethyl acetate extract from fruit peel extract from *C. hystrix* has potential for cosmetic preparations targeting sunscreen, skin-lightening, and anti-aging sites but needs clinical confirmation.

*Implication for health policy/practice/research/medical education:*
The ethyl acetate extract of fruit peels showed the potential to be used as an active substance in sunscreen, skin lightening, and anti-aging cosmetics.

*Please cite this paper as:* Widyastuti W, Sartika D, Fendri STJ. Exploring the potential of *Citrus hystrix* DC. extract as a cosmeceutical agent. J Herbmed Pharmacol. 2025;14(4):396-404. doi: 10.34172/jhp.2025.52790.

## Introduction

In the past ten years, the cosmetic industry has experienced rapid growth, becoming one of the fastest-growing sectors. Social media and digital resources have played a significant role in raising awareness of the potential risks of synthetic chemicals in cosmetics and promoting the advantages of using natural products sourced from plants and other natural materials. As a result, herbal ingredients derived from plants have become increasingly popular in formulating cosmetic products, particularly those intended for facial skin care. Generally, facial skin products are valued for their ability to protect the skin from external disturbances such as ultraviolet (UV) radiation, which causes hyperpigmentation and premature aging of the skin (1). Indeed, the skin has a natural protection against the harmful effects of UV radiation called melanin. However, excessive production of melanin leads to skin hyperpigmentation. Depigmentation cosmetics containing melanogenesis inhibitor compounds are often used to control melanin formation, making facial skin lighter. Unfortunately, the depigmentation agents obtained from synthetic compounds have several drawbacks, including poor bioavailability, photosensitivity, cellular harm, and insolubility. As a result, natural melanogenesis

***Corresponding author**: Widyastuti Widyastuti,
Email: widyastuti@upertis.ac.id

inhibitors have gained attention as important alternatives to synthetic agents (2). The primary enzyme involved in the melanogenesis process is known as tyrosinase. Some natural compounds, such as kojic acid from certain organisms, arbutin (glycosylated hydroquinone) extricated from bearberry plants and vanillin (phenolic aldehyde) from vanilla beans have demonstrated anti-melanogenesis effects. Additionally, various chemical compounds from nature, especially the plants appear to have distinctive restorative impacts, such as tyrosinase inhibitors with different restraints consistent and $IC_{50}$ values (3).

In addition to melanin inhibition, antioxidants from plant-derived compounds offer significant protective benefits for the skin. The antioxidant activity of auxiliary metabolites found in plants can help combat reactive oxygen species (ROS) and reduce oxidative harm to the skin. This activity also protects the skin from the negative impacts of UV radiation, which complements the action of chemical and physical UV filters. Together, these effects can decrease DNA harm that causes skin aging and the risk of skin cancer (4). In addition, some herbal plants containing secondary metabolite compounds can overcome skin aging, including protection against UV rays, preventing wrinkles and dull spots, and providing moisture to support skin health. These herbs avoid the impacts of skin aging through various mechanisms, such as cancer prevention agents, photoprotective operators, collagen or elastin amalgamation modulation, and melanin synthesis inhibitors (5).

Beyond general antioxidant benefits, *Citrus* plants offer a rich source of bioactive compounds that are particularly advantageous for skin health. *Citrus* plants are rich in bioactive compounds, such as polyphenols, carotenoids, and vitamins, that have antimicrobial and antioxidant properties and support the immune system. Approximately 50% of the natural products remain inedible waste, incorporating peel, seeds, mash, and portion buildups (6). Differences in solvents in extraction and *Citrus* peel types affect the amount of phenolic and flavonoid contents of the extracts, affecting their antioxidant and antibacterial activities. Several studies have found a direct effect of total phenolic and flavonoid content on extract activity (7). *Citrus* natural products also contain bioactive compounds, especially phenols, flavonoids, limonoids, and carboxylic acids. Spatial metabolomic analysis of lemon, lime, and mandarin has shown that flavonoids are concentrated in the albedo and flavedo, which are key components in checking DPPH radicals. The combination of flavonoids and limonoids contributes to clarifying the antioxidant activity in *citrus* natural products (8).

Among the *Citrus* species, Kaffir lime (*Citrus hystrix*) stands out for its unique bioactive properties. The leaf part is commonly used as cooking spices. *C. hystrix* belongs to the Rutaceae family. known for its antimicrobial, anti-inflammatory, antioxidant, and antitumor activities (9). The extraction of *C. hystrix* leaves using ethanol, hexane, and ethyl acetate obtained the highest flavonoids, phenols, and tannins in the ethanol leaf extracts. Meanwhile, the hexane extract had the highest saponin content. Notably, the most excellent antioxidant activities were found in the ethanol extract of *C. hystrix* leaves with an $IC_{50}$ of about 21.81 µg/mL (10). Additionally, the methanol extract of *C. hystrix* leaves had antioxidant, sunscreen, and inhibitory activity against bacterial and fungal growth. It contained secondary flavonoid and phenolic metabolite compounds (11). Fresh *C. hystrix* leaf essential oil and dry lime leaf pulp extract showed antioxidant activity, with $IC_{50}$ values of 51.63 and 51.31 ppm, respectively, placing them within the solid antioxidant category. The dry lime leaf pulp extract contained alkaloid, flavonoid, and saponin compounds (12). The most common compounds in *C. hystrix* leaf essential oil include linalool and citronellal, while the most common substances of the natural product basic oil are L-β-pinene, D-limonene, and L-α-terpineol (13).

Studies on the activity of *C. hystrix* have been primarily focused on antioxidant and antibacterial properties in its leaf extract. Therefore, our study explores the *C. hystrix* extracts from leaves and fruit peels using the maceration method with solvents of varying polarities and fruit pulp extracts using the freeze-drying procedure. These extracts were then tested for novel applications in cosmeceuticals, specifically for sunscreen, skin-lightening, and anti-aging properties.

## Materials and Methods

### Materials

To carry out this study on the bioactive properties of *Citrus hystrix* for cosmeceutical applications, we first collected plant samples and sourced various reagents and chemicals essential for the analysis. The data collection began by gathering *C. hystrix* plants in Tanah Datar district, West Sumatra, Indonesia. The plants were authenticated at the Andalas University Herbarium under number 411/K-ID/ANDA/VII/2023, confirming them as *Citrus hystrix* DC. For extraction and testing, we sourced high-purity reagents from Merck, including methanol (106009), ethanol (100983), Folin-Ciocalteu's phenol reagent (109001), potassium dihydrogen phosphate anhydrate (104873), aluminum chloride (801081), sodium hydroxide (106498), and dimethyl sulfoxide (102952) from Merck. Additional materials included ethanol (AT96) from Medika, ethyl acetate (A049) and n-hexane (N015) from Bratachem, and specialized chemicals from Sigma Aldrich, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) (D9132), mushroom tyrosinase (T3824), L-ascorbic acid (A92902), kojic acid (K3125), gallic acid (842649), quercetin (Q4951), tricine (T0377), trizma (T1503), bovine serum albumin (BSA) (A3608),

L-3,4-dihydroxyphenylalanine (L-DOPA) (D9628), collagenase from *Clostridium histolyticum* (C0130), N-(3-[2-furyl]acryloyl)-leu-gly-pro-ala (FALGPA) (F5135), elastase from porcine pancreas (E1250), N-SucAla$_3$-p-nitroanilide (S4760), hyaluronidase (H3506), hyaluronic acid (H5388), retinol (R7632), and niacinamide (N5535).

### Extraction

To begin extraction, the leaves of *C. hystrix* were cleaned, chopped, and weighed, before being macerated with ethanol. The macerate obtained was then concentrated using a rotary evaporator (Buchi Rotavapor R-200) to obtain a thick extract. This process was repeated for the maceration of *C. hystrix* leaves using ethyl acetate and n-hexane solvents. For fruit extraction, the *C. hystrix* fruit was cleaned, with the peel separated to collect only the flavedo part. The flavedo was thinly sliced, weighed, and macerated using ethanol. The macerate was concentrated using a rotary evaporator, obtaining a thick extract. The same process was repeated for maceration of the peeled fruit of *C. hystrix* fruit using ethyl acetate and hexane solvents. Following that, the pulp from *C. hystrix* fruit was squeezed. The squeezed results were put into a freeze dryer (Yamato DC-801) to obtain a powder-form extract. The extract obtained was calculated for yield, pH (pH meter Janwey 3510), solubility in water and ethanol, loss on drying, residue on ignition (Furnest Wisethern FH-03), phytochemical screening of the extract, determination of total phenolic content (TPC) total flavonoid content (TFC).

### Determination of sunscreen activity

In this stage, each extract was made into an arrangement with concentrations of 50–800 µg/mL in ethanol. The Sun Protection Factor (SPF) esteem was ensured by measuring the absorbance of the test arrangement employing a UV-Vis spectrophotometer (PG instrument T70) at a wavelength of 290–320 nm with an interim of 5 nm. The SPF esteem was then calculated based on the range beneath the bend (AUC) on the wavelength and absorbance bend. The protection grade UVA (PA) esteem was ensured by measuring the absorbance of the test arrangement employing a UV-Vis Spectrophotometer at the wavelengths of 290–400 nm with an interim of 5 nm. The region beneath the bend (AUC) on the wavelength and absorbance bend was calculated based on the UVB run (290–320 nm) and UVA (320–400 nm). Finally, the UVA/UVB proportion was calculated (14).

### Tyrosinase inhibition assay

Each extract was made into a solution with 50 to 800 µg/mL concentrations. Kojic acid (KA) was made into a solution with 12.5–200 µg/mL concentrations. Using a 96-well plate (Biologix), each test solution, tyrosinase solution 200 IU/mL, and L-DOPA 10 mM solution in phosphate buffer 50 mM pH 6.5 was inserted into the well. The same process was repeated to control the test solution (without tyrosinase) and control solution (without extract). The plate was put into a dark room for 60 minutes, and the absorbance was measured using a Microplate reader (Bio-Rad xMark) at 475 nm. To calculate the percentage of inhibition of tyrosinase, a curve between the concentration and percentage of tyrosinase inhibition wad made. The $IC_{50}$ value of each extract was also determined (15).

### Antioxidant activity assay

In this stage, each extract was weighed and dissolved in methanol at 12.5 to 400 µg/mL concentrations. L-ascorbic acid (AA) was used as a control with 4 to 20 µg/mL concentrations. Using a 96-well plate, each test arrangement was embedded into the well additionally 0.077 mM DPPH and cleared out for 30 minutes in a dull put. The absorbance was measured at a wavelength of 517 nm. After that, the percentage of DPPH inhibition was calculated. Finally, the $IC_{50}$ value of each extract was determined (8).

### Collagenase inhibition assay

The next stage began by weighing and making each extract into a series of 12.5–400 µg/mL concentrations. Using a 96-well plate, each test solution was inserted into the well with collagenase 0.1 mg/mL and Tricine buffer 50 mM (pH 7.5). The solution was left for 20 minutes at 25 °C. It was added to the well FALGPA 1.0 mM and left for 20 minutes at 25 °C in a dark place. The same plate was tested on the blank solution (without enzyme and extract). Further, absorbance was measured at a wavelength of 345 nm. Finally, the percentage of collagenase inhibition was calculated, and the $IC_{50}$ value of each extract was determined (16).

### Elastase inhibition assay

Each extract was weighed and made into a concentration series of 12.5–400 µg/mL. Then, each test solution was put into the well plus elastase 0,05 U/mL and Trizma® base solution pH 8.0 using a 96-well plate. The solution was left for 15 minutes. N-SucAla$_3$-pNA 0.1 mM was put into the well and left for 15 minutes in a dark place at 25 °C. Another test using the same plate was carried out on the blank solution (without enzyme and extract). The absorbance was measured at 410 nm. Finally, the percentage of elastase inhibition and the $IC_{50}$ value of each extract were calculated (17).

### Hyaluronidase inhibition assay

Each extract was weighed and made into a concentration series of 12.5–400 µg/mL. After that, each test solution, hyaluronidase 6 U/mL, and phosphate buffer pH 5.35 were inserted into the well using a 96-well plate. The solution was left for 10 minutes at 37 °C. Then, hyaluronic acid

0.03% was added and left for 45 minutes at 37 °C in a dark place. Additionally, the albumin acid solution was added and left at room temperature for 10 minutes. Another test using the same plate was carried out on the blank solution (without enzyme and extract). The absorbance was measured at 600 nm. Likewise, the percentage of hyaluronidase inhibition and the $IC_{50}$ value of each extract were calculated (16).

## Results

### Extraction of *Citrus hystrix*

Among the extraction results, the ethyl acetate peel extract (EAP) obtained the highest yield, while the hexane peel extract (HP) yielded the lowest. Overall, all extracts had a low acidic pH. Most extracts were difficult to dissolve in water; however, the hexane extracts of the leaves and fruit peels were notably more resistant to dissolving in water. Likewise, the extract was difficult to dissolve in ethanol, except for the ethyl acetate leaf extract (EAL). EAL was somewhat difficult to dissolve in ethanol, and the hexane leaf extract (HL), HP, and pulp extract (PE) were difficult to dissolve in ethanol. Further, the loss on drying extract ranged from 6.46 ± 0.22% to 9.58 ± 0.23%. Residue on ignition extract ranged from 1.51 ± 0.25% to 5.27 ± 0.04%. More details are presented in Table 1.

In a phytochemical screening of the extracts, EL, EAL, EP, EAP, and PE contained flavonoid and phenolic compounds. In none of the extracts the saponin compound was identified. Further, terpenoid compounds were not found in EL and EAL. Steroid compounds were identified in EL, EAL, EP, EAP, and PE, while alkaloid compounds were only identified in HL and HP (Table 1). The highest TPC and TFC were found in EAP, about 22.855 ± 0.049 mg GAE/g extract and 240.326 ± 0.042 mg QE/g extract. Meanwhile, the lowest contents of TPC and TFC in PE were around 6.287 ± 0.019 and 4.168 ± 0.065. The complete results can be seen in Table 2.

### Sunscreen activity of *Citrus hystrix* extract

The test found that the EAP had an SPF value >15, which was 23.644 ± 0.007 at 200 µg/mL. It was an extract with higher protection activity against UVB rays than other extracts with the same concentrations. Meanwhile, the best protection grade UVA (PA) value was obtained from PE, where at 50 µg/mL, it had a PA value of 0.931 ± 0.001, with a maximum protection category (4 stars). The complete results are presented in Table 3.

### Skin lightening activity of *Citrus hystrix* extract

The best skin-lightening activity was obtained in the EAP compared to other extracts, with a linear regression equation of $y = 0.1963x + 40.9180$ with $r^2 = 0.9803$ and an $IC_{50}$ value of 46.266 µg/mL. As a comparison, KA had a linear regression equation of $y = 0.9720x + 21.6010$ with $r^2 = 0.9992$ and an $IC_{50}$ value of 29.217 µg/mL. The results are illustrated in Table 4.

### Antiaging activity of *Citrus hystrix* extract

Based on this test, the best antioxidant activity was in the EL, which had a linear regression equation of $y = 0.0575x + 18.7100$ with $r^2 = 0.9957$ and an $IC_{50}$ about of 544.174 µg/mL. AA was used for comparison and had a linear regression equation of $y = 2.1857x + 14.0810$ with $r^2 = 0.9947$, so an $IC_{50}$ value of 16.434 was obtained (Table 4). In the collagenase inhibition assay, the EAP performed the best with a linear regression equation of $y = 0.0622x + 48.3800$ with $r^2 = 0.9329$ and an $IC_{50}$ value of 26.045 µg/mL. Retinol (RT) was used as a control and showed a

**Table 1.** Characteristics of *Citrus hystrix* extracts

| Testing | *C. hystrix* extracts | | | | | | |
|---|---|---|---|---|---|---|---|
| | EL | EAL | HL | EP | EAP | HP | PE |
| Yield (5) | 10.257 | 5.545 | 2.003 | 11.161 | 8.460 | 1.038 | 8.277 |
| pH (1% w/v solution) | 4.64 ± 0.11 | 4.75 ± 0.02 | 5.76 ± 0.18 | 4.84 ± 0.12 | 4.73 ± 0.02 | 4.54 ± 0.02 | 5.38 ± 0.02 |
| Solubility in water | 1:530 | 1:450 | 1:4660 | 1:500 | 1:450 | 1:2750 | 1:330 |
| Solubility in ethanol | 1:330 | 1:90 | 1:2910 | 1:200 | 1:100 | 1:1480 | 1:4550 |
| Loss on drying (%) | 9.06 ± 0.13 | 9.58 ± 0.23 | 6.46 ± 0.22 | 9.22 ± 0.33 | 7.68 ± 0.10 | 8.03 ± 0.10 | 9.13 ± 0.18 |
| Residue on ignition (%) | 2.71 ± 0.13 | 2.42 ± 0.31 | 1.51 ± 0.25 | 3.49 ± 0.32 | 2.33 ± 0.22 | 2.69 ± 0.25 | 5.27 ± 0.04 |
| Flavonoid | + | + | - | + | + | - | + |
| Phenolic | + | + | - | + | + | - | + |
| Saponin | - | - | - | - | - | - | - |
| Terpenoid | - | - | + | + | + | + | + |
| Steroid | + | + | - | - | - | - | + |
| Alkaloid | - | - | + | - | - | + | - |

EL: ethanol leaf extract; EAL: ethyl acetate leaf extract; HL: hexane leaf extract; EP: ethanol peel extract; EAP: ethyl acetate peel extract; HP: hexane peel extract; PE: pulp extract; +: identified; -: not identified. Data are presented as mean ± standard deviation

**Table 2.** Total phenolic (TPC) and flavonoid (TFC) contents of *Citrus hystrix* extracts

| Extract | TPC (mg GAE / g extract) | TFC (mg QE / g extract) |
|---|---|---|
| EL | 15.522 ± 0.065 | 69.323 ± 0.301 |
| EAL | 17.668 ± 0.019 | 61.244 ± 0.213 |
| EP | 18.700 ± 0.029 | 42.643 ± 0.107 |
| EAP | 22.855 ± 0.049 | 240.326 ± 0.042 |
| PE | 6.287 ± 0.019 | 4.168 ± 0.065 |

GAE: gallic acid equivalent; QE: quercetin equivalent; EL: ethanol leaf extract; EAL: ethyl acetate leaf extract; EP: ethanol peel extract; EAP: ethyl acetate peel extract; PE: pulp extract. Data are presented as mean ± standard deviation.

linear regression equation of y = 0.1371x + 45.6580 with $r^2$ = 0.9033, with an $IC_{50}$ value of 31.671 µg/mL (Table 4.). The best extract was again observed in EL for the elastase inhibition assay, with a linear regression equation of y = 0.1271x + 42.5620 with $r^2$ = 0.9050 and an $IC_{50}$ value of 58.521 µg/mL. RT had a linear regression equation of y = 0.1368x + 45.3440 with $r^2$ = 0.9142 and an $IC_{50}$ value of 34.035 µg/mL (Table 4.). In the hyaluronidase inhibition test, the best extract was obtained in the EP, with a linear regression equation of y = 0.1320x + 42.6150 with $r^2$ = 0.9617 and an $IC_{50}$ value of 55.947 µg/mL. In this test, niacinamide (ND) was used as a reference and showed a linear regression equation of y = 0.1220x + 44.4180 with $r^2$ = 0.9263 and an $IC_{50}$ value of 45.754 µg/mL (Table 4).

## Discussion

UV radiation has both advantages and disadvantages for skin health. The use of broad-spectrum sunscreen, for instance, protects the skin from UV radiation as it has a high SPF value. This value is the best preventive measure to maintain homeostasis in the skin (18). However, the prolonged use of sunscreen cosmetics might have dangerous potential if they contain toxic substances or compounds. In particular, some cosmetics contain synthetic chemicals that are added during the preparation process. These chemicals make the cosmetics not completely safe, although they are still allowed to be used with certain amount limits. To measure the safety of the cosmetics, cosmetic preparations are evaluated in vitro and in vivo to identify the materials in the cosmetics. Typically, most ingredients from nature, especially from plants, either in the form of extracts or pure isolated compounds, have milder side effects than synthetic chemical compounds (19).

Given these concerns about synthetic chemicals in sunscreens, it is important to explore natural alternatives that can offer protection without harmful effects. Skin areas exposed to UV radiation show symptoms of damage from the photoaging phenomenon. This phenomenon leads to signs such as dryness, uneven pigmentation, lentigo, hyperpigmentation, wrinkle arrangement, and diminished skin versatility. Strikingly, polyphenol compounds contained in plants can overcome these problems. Integration of polyphenol compounds as sunscreen is an action that can provide beneficial effects on UV radiation photo-protection. In addition, polyphenol compounds in the scope of dermatopathology can also overcome the skin's reaction to UV radiation from the sun, which incorporates incendiary cascades, oxidative

**Table 3.** Sunscreen activity of *Citrus hystrix* extract

| Test | Extract | Concentration (µg/mL) | | | | |
|---|---|---|---|---|---|---|
| | | 50 | 100 | 200 | 400 | 800 |
| SPF | EL | 1.462 ± 0.001 | 2.098 ± 0.002 | 4.794 ± 0.008 | 19.977 ± 0.028 | 88.540 ± 0.043 |
| | EAL | 1.655 ± 0.009 | 2.631 ± 0.003 | 8.546 ± 0,002 | 41,965 ± 0,081 | 89.801 ± 0.313 |
| | HL | 1.372 ± 0.001 | 1.695 ± 0.002 | 3.059 ± 0.128 | 7.618 ± 2.819 | 41.596 ± 0.112 |
| | EP | 2.023 ± 0.001 | 3.965 ± 0.005 | 16.992 ± 0.065 | 62.730 ± 0.226 | 77.870 ± 0.620 |
| | EAP | 1.632 ± 0.001 | 5.722 ± 0.011 | 23.644 ± 0.007 | 72.436 ± 0.580 | 78.158 ± 0.332 |
| | HP | 2.120 ± 0.001 | 4.264 ± 0.004 | 14.172 ± 0.014 | 60.888 ± 0.373 | 75.187 ± 0.462 |
| | PE | 1.106 ± 0.001 | 1.161 ± 0.001 | 1.324 ± 0.001 | 1.654 ± 0.002 | 2.706 ± 0.006 |
| PA | EL | 0.882 ± 0.001 | 0.783 ± 0.001 | 0.598 ± 0.001 | 0.395 ± 0.006 | 0.400 ± 0.001 |
| | EAL | 0.856 ±0.005 | 0.738 ± 0.002 | 0.516 ± 0.001 | 0.361 ± 0.001 | 0.524 ± 0.001 |
| | HL | 0.800 ± 0.001 | 0.683 ± 0.006 | 0.456 ± 0.021 | 0.290 ± 0.137 | 0.089 ± 0.001 |
| | EP | 0.648 ± 0.002 | 0.432 ± 0.002 | 0.188 ± 0.001 | 0.129 ± 0.001 | 0.284 ± 0.003 |
| | EAP | 0.867 ± 0.121 | 0.456 ± 0.002 | 0.262 ± 0.002 | 0.283 ± 0.003 | 0.522 ± 0.005 |
| | HP | 0.560 ± 0.002 | 0.331 ± 0.002 | 0.133 ± 0.001 | 0.055 ± 0.001 | 0.071 ± 0.001 |
| | PE | 0.931 ± 0.001 | 0.902 ± 0.001 | 0.833 ± 0.001 | 0.719 ± 0.001 | 0.543 ± 0.037 |

Grade of Sun Protecting Factor (SPF): 15–24 (good protection), 25–39 (very good protection), and >40 (excellent protection) (26). Star category description of UVA ratio of Protection Grade UVA (PA); <0.2 (too low for UVA claim), 0.2 – < 0.4 (moderate), 0.4 – < 0.6 (+ + good), 0.6 – < 0.8 (+ + + superior), and ≥ 0.8 (+ + + + maximum) (26). EL: ethanol leaf extract; EAL: ethyl acetate leaf extract; HL: hexane leaf extract; EP: ethanol peel extract; EAP: ethyl acetate peel extract; HP: hexane peel extract; PE: pulp extract. Data are presented as mean ± standard deviation.

**Table 4**. Half-maximal inhibitory concentration ($IC_{50}$) value of *Citrus hystrix* extract

| Sample | $IC_{50}$ value of *C. hystrix* extract (µg/mL) | | | | |
|---|---|---|---|---|---|
| | Tyrosinase | DPPH | Collagenase | Elastase | Hyaluronidase |
| EL | 162.212 | 544.174 | 30.301 | 58.521 | 179.770 |
| EAL | 119.206 | 934.569 | 167.702 | 59.891 | 157.731 |
| HL | 223.403 | 2862.119 | 320.366 | 245.844 | 95.231 |
| EP | 96.417 | 729.684 | 267.430 | 131.660 | 55.947 |
| EAP | 46.265 | 588.971 | 26.045 | 97.409 | 97.900 |
| HP | 112.477 | 3024.123 | 40.692 | 170.897 | 114.301 |
| PE | 599.052 | 1752.639 | 120.580 | 191.030 | 451.090 |
| KA | 29.217 | - | - | - | - |
| AA | - | 16.434 | - | - | - |
| ND | - | - | - | - | 45.754 |
| RT | - | - | 31.671 | 34.035 | - |

EL: ethanol leaf extract; EAL: ethyl acetate leaf extract; HL: hexane leaf extract; EP: ethanol peel extract; EAP: ethyl acetate peel extract; HP: hexane peel extract; PE: pulp extract; KA: Kojic acid; AA: Ascorbic acid; ND: Niacinamide; RT: Retinol; -: determination not done.

clutters, and DNA harm that cause flecks on the skin and wrinkles (20).

Among many potential plants, *C. hystrix* DC emerges as a promising candidate due to its rich phytochemical profile and therapeutic properties. Pharmacologically, *C. hystrix* has shown therapeutic potential as an antimicrobial, anti-mosquito, antioxidant, anti-cancer, anti-inflammatory, and neuroprotective agent (21). The study showed that the EP had the highest yield of 11.161%. This allows the EP to contain many polar compounds of flavonoids, phenolics, and terpenoids, as illustrated in Table 1. However, after TPC and TFC tests, the highest levels were obtained in the EAP. Hence, it is likely that the phenolic and flavonoid compounds contained in the EAP are semipolar (Table 2). The yield obtained in the present study was greater than the results obtained by Anggraeny et al. (2024), where the *C. hystrix* leaf extract using solvent by ethanol, ethyl acetate, and n-hexane solvents had yields of 5.28, 1.72 and 0.94%, respectively.

Research by Moolsup et al reported that the water extract of *C. hystrix* fruit peel contained higher levels of TPC, TFC, and total anthocyanidin compared to the ethanol extract of *C. hystrix* fruit peel (22). In particular, the methanol extract of dry *C. hystrix* leaves contained TPC and TFC values of approximately 0.64 ± 0.04 mg GAE/g dry leaf weight and 5.03 ± 0.21 mg QE/g dry leaf weight, respectively (11). Additionally, the ethanol extract of dry *C. hystrix* fruit peel was detected to contain the phenolic compounds gallic acid, catechin, caffeic acid, ferulic acid, and rutin alongside flavonoids such as hesperidin, naringenin, hesperetin, and nobiletin (23). Desmiaty et al further identified that the ethanol extract of *C. hystrix* fruit peel contains an extensive array of flavonoids, phenolics, saponins, triterpenoids, and coumarin compounds. Flavonoid compounds found within the ethanol extract of *C. hystrix* fruit peel contained hesperidin, neohesperidin, narirutin, naringin, nobiletin, tangeretin, apigenin, kaempferol, quercetin, rutin, eriocitrin, and sinensetin (24). Furthermore, *C. hystrix* leaves processed via green synthesis methods revealed bioactive compounds such as alkaloids, terpenoids, polyphenolic compounds, amino acids, tannins, polysaccharides, saponins, and steroids (25).

After exploring C. *hystrix's* potential for photoprotection, sunscreen activity was assessed by calculating the SPF value of each extract. In the present study, EAP demonstrated the highest SPF value, indicating superior activity compared to other extracts. As shown in Table 2, EAP contained the highest TPC and TFC levels. Thus, it can be stated that the TPC and TFC content may affect the SPF value of *C. hystrix* extract. Sunscreen may be a corrective item to secure the skin from harm caused by daylight radiation. However, traditional topical sunscreens cannot completely protect sensitive organs, like the eyes and lips. The types or classifications of sunscreens are UVB and UVA radiations (26).

This assessment focused on the 290 and 400 nm, where the wavelength of 290-320 was a filter for UVB and 320-400 a filter for UVA. The filter was then classified as 'broad spectrum', which had a significant part of its absorbance in UVA. The SPF value for sunscreen is based on erythema as the conclusion point. Hence, dynamic fixings that work essentially as UVB blockers significantly increment the SPF of restorative planning. For restorative arrangements, clarifying the UVA security advertised in the expansion to SPF is vital. The protection grade of UVA (PA) was calculated as the ratio of the added UVA retention to UVB (26). The high SPF value of EAP is not followed by a PA value. PE at a concentration of 50 µg/mL had a maximum protection grade of UVA (PA value) but had no protection against UVB (SPF value) (Table 3).

Skin protection using sunscreen against UV radiation

can reduce the signs of skin aging, such as flecks, hyperpigmentation, and wrinkles. UV radiation stimulates melanogenesis, where melanin acts as a natural protector but can also lead to hyperpigmentation. Depigmenting makeup can be utilized to control this issue. However, these makeups, often synthetic, have a few drawbacks, such as low bioavailability, photosensitization, cellular toxicity, quality, and insolubility. Natural alternatives, such as melanogenic inhibitors, are increasingly favored (2). Tyrosinase is a major target enzyme in melanogenesis, which can inhibit pigmentation. In some studies, kojic acid as a tyrosinase inhibitor from fungal species, serves as a comparison in tyrosinase inhibitor research (3).

The research found that EAP had good tyrosinase inhibition activity, with the lowest $IC_{50}$ value compared to other extracts. However, the $IC_{50}$ value of EAP was greater than kojic acid. Similarly, the TPC and TFC levels of EAP were also high. Thus, large phenolic and flavonoid compounds may result in better tyrosinase inhibition activity. Phenolic compounds that have fragrant rings with one or more hydroxyl bunches can possibly be melanogenesis inhibitors. Flavonoid subsidiaries, found in plants are the finest tyrosinase inhibitors. Additionally, there is a considerable relationship between the proficiency of flavonoid hindrance of tyrosinase and melanin union in melanocytes in vitro. Flavonoids are mostly categorized into flavones, flavonols, isoflavones, flavanones, flavonols, anthocyanidins, dihydroflavones, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones, and aurones. Deglycosylation of a few flavonoid glycosides utilizing infrared light can increase tyrosinase inhibitory activity (3). Cosmetic preparations as skin lighteners containing natural ingredients and marketed in Thailand have tyrosinase inhibitory activity ranging from 2.58% to 97.94% (27).

In addition to UV protection, antioxidant activity offers another layer of defense by counteracting free radicals generated by UV exposure. Antioxidant activity testing showed that EL had a lower $IC_{50}$ value than other extracts, which was 544.174 µg/mL. Interestingly, this $IC_{50}$ value was much higher than the AA, which had an $IC_{50}$ of about 16.434 µg/mL. The results also showed lower antioxidant activity than those obtained by (10), where the antioxidant activity of the ethanol extract of *C. hystrix* leaves obtained an $IC_{50}$ of about 21.81 µg/mL. The *C. hystrix* extract obtained from the hydrodistillation process had an antioxidant activity with an $IC_{50}$ of about 0.761 ± 0.049 µg/mL using the DPPH assay (28). ZnO in nanoparticles mediated by *C. hystrix* leaf extract through the extract-assisted green synthesis process has shown antioxidant activity with an $IC_{50}$ of about 133.9 µg/mL (25).

Oxidative stress, an awkwardness between intracellular cancer prevention agents and over-the-top ROS generation within the skin, can quicken the process of aging. Aging is a normal process that shows the dynamic decline of cell, tissue, and organ function (29). Strong or chronic exposure to various stressors (e.g., UV radiation, xenobiotic specialists, free radicals, and others) can cause untimely skin maturing, resistant concealment, and carcinogenesis (30).

The extracellular matrix (ECM) that shapes the peripheral layer of the skin is composed of fibroblasts and proteins such as collagen and elastin. ECM corruption is straightforwardly related to skin aging and is dependable for the expanded movement of certain chemicals such as collagenase, elastase, and hyaluronidase included in skin maturing. In aging, there is a diminishing level of elastin, collagen, and hyaluronic corrosive, which cause misfortune in skin quality and flexibility, resulting in wrinkles. In expansion, expanded movement of collagenase, elastase, and hyaluronidase with tall levels of ROS due to intemperate presentation of the skin to UV radiation can moreover cause skin maturing. A few plants, either extracts or decontaminated compounds, can repress chemicals in the maturing handle of the skin. Extracts or compounds that have activity as free radical scavengers or antioxidants can be used to prevent skin aging (31).

The assay was to measure the inhibition of the extract against the enzymes such as collagenase, elastase, and hyaluronidase. The test obtained the best collagenase inhibition in EAP with an $IC_{50}$ value, even lower than RT. The best activity of elastase inhibition was given by EL, but the $IC_{50}$ value of EL was greater than RT. RT was not used as a comparison in the hyaluronidase inhibition assay because it has an $IC_{50}$ of about 88.986 µg/mL compared to ND, which had an $IC_{50}$ of 45.754. The best hyaluronidase inhibition was given by EP, which had an $IC_{50}$ of 55.947 µg/mL. Based on the data obtained, it can be seen that the weak antioxidant activity in the extract does not cause weak enzyme inhibition activity. Even, EAP has better activity than RT. Despite these promising findings, the research is still limited to *in vitro* testing. There is a need for ongoing *in vivo* research to prove the activity of *C. hystrix* extract as a sunscreen, skin-lightener, and anti-aging agent so that it can be added as a safe and effective active cosmetic ingredient.

## Conclusion

EAP had a higher TPC and TFC content compared to other extracts. The best sunscreen activity was seen from the largest SPF value with the smallest concentration, where EAP had the best sunscreen activity. The best skin-lightening activity is EAP because it has a smaller $IC_{50}$ value than other extracts. The best antioxidant activity is in EL. As an anti-aging method, testing is done by measuring the inhibition of extracts against enzymes involved in the aging process. EAP is an extract that has the best collagenase inhibition, EL is the best extract as an elastase inhibitor, and EP is the best hyaluronidase inhibitor. The finest sunscreen, skin helping, and anti-aging exercises are

given by the fruit peel and cleared out, as well as the finest extricate from the ethyl acetic acid derivation extricate of the natural product peel. Leaf and fruit peel extracts can be added as active ingredients in cosmetic formulations.

## Authors' contribution

**Conceptualization:** All authors.
**Data curation:** All authors.
**Formal analysis:** All authors.
**Funding acquisition:** Widyastuti Widyastuti.
**Investigation:** All authors.
**Methodology:** All authors.
**Project administration:** Diza Sartika.
**Resources:** All authors.
**Software:** Sandra Tri Juli Fendri.
**Supervision:** Widyastuti Widyastuti.
**Validation:** Sandra Tri Juli Fendri.
**Visualization:** Widyastuti Widyastuti, Sandra Tri Juli Fendri.
**Writing–original draft:** All authors.
**Writing–review & editing:** Widyastuti Widyastuti, Diza Sartika.

## Conflict of interests

The authors declare no conflict of interest

## Ethical considerations

This research does not involve animals and humans in the experiment.

## Funding/Support

This research is not funded by any institution. The authors paid for the expenses.

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