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Chemical Composition, Tyrosinase Inhibitory Activity and Antibacterial Activity of Coconut Coir Dust Extract

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ABSTRACT

Coconut industrial waste, particularly coconut coir dust, is an abundant and underutilized agricultural biomass. A large amount of coconut coir dust is generated during the extraction of coir fiber from coconut husk and accumulates as a waste product. The phenolic phytochemicals in coconut coir dust are considered a rich source with potential applications in a wide range of industries. Therefore, this study aims to assess the extract from coconut coir dust, a plentiful agricultural by-product of the coconut industry, as a potential source of natural components for skincare and cosmetic products. The bioactive compounds from coconut coir dust were extracted by ultrasound-assisted solvent extraction. The chemical composition of the coconut coir dust extract was determined by liquid chromatography-electrospray ionisation-mass spectrometer (LC-ESI-MS) and five bioactive compounds were identified. The extract was also evaluated for tyrosinase inhibitory activity by the mushroom tyrosinase inhibitory assay. Antibacterial activity was tested against the three human pathogenic strains including *Staphylococcus aureus*, *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus* (MRSA). Results revealed that the extract exhibited tyrosinase inhibitory activity with an IC_{50} value of 874.17 µg/mL and showed antibacterial activities against all the tested bacterial strains. Minimal inhibitory concentration (MIC) values ranged from 0.80 - 25.60 mg/mL and minimal bactericidal concentration (MBC) values ranged from 3.20 - 120.40 mg/mL. In the light of this, coconut coir dust extract has the potential to replace artificial chemicals in skincare and cosmetic applications.

Keywords: Antibacterial activity, Chemical composition, Coconut coir dust, Tyrosinase inhibitory activity.

Introduction

Coconut (*Cocos nucifera* L.) is an important fruit crop widely distributed in tropical regions including Thailand. According to Thailand's Office of Agricultural Economics, there were 137,510 hectares of coconut palm plantations in 2020, and the nation produced 591 million whole coconuts. Mature coconut dry matter is composed of 25% water, 28% white meat, 12% shell (endocarp) and 35% husk (mesocarp).[1] After processing, large amounts of coconut shell and husk are generated as the major underutilised by-products. Coconut husk consists of 75% fibre and 25% fine material. Extraction of coir fibre from the husk generates coconut coir dust.[2] Coir fibre is used to produce mats or ropes, while the dust accumulates as a waste product. Poor management of agricultural wastes such as open burning and improper disposal generates environmental pollution with negative impacts on the health of people living nearby.

Polyphenols are organic compounds that contain one or more hydroxyl groups attached to an aromatic ring. They play important roles in plant defence mechanisms against infectious diseases caused by microorganisms such as bacteria, viruses and fungi.[3]

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Natural phenolic extracts from plants are now widely used as active ingredients in many health care products such as food supplements, medicines and cosmetics, with broad-ranging beneficial effects on human health such as antiproliferative activity on leukemic cells and normal blood lymphocytes,[4] antibacterial activity, antiviral activity and free radical scavenging activities.[5,6]

Valadez-Carmona *et al.*[1] and Dey *et al.*[7] reported that the phenolic compositions of coconut husk extract were a good source of phytochemicals containing catechin, epicatechin, 4-hydroxybenzoic acid, ferulic acid and condensed tannin, while Chakraborty & Mitra[8] found that caffeoyl derivatives exhibiting anti-HIV and antiviral activities were major components in a methanolic extract of coconut husk.[9,10] It was also discovered that extracts of coconut shell and coconut coir dust contained high total phenolic contents that exhibited potent antioxidant activities.[11]

Therefore, isolation of bioactive polyphenols from coconut wastes and exploring their properties may generate further applications in various industrial applications.

This research identified the chemical compositions of bioactive compounds extracted from coconut coir dust using ultrasound-assisted solvent extraction and liquid chromatography-electrospray ionisation-mass spectrometry (LC-ESI-MS). Tyrosinase inhibitory activity and the antibacterial activities of the extract were also investigated against three human pathogenic bacterial strains.

Materials and Methods

Raw material

Coir dust of *Cocos nucifera* L. was collected in August 2021 at a coconut processing plant in Samut Songkhram Province, Thailand. The sample was dried in an oven at 50°C to constant weight, then ground and passed through a screen sieve mesh to obtain a particle size of less than 240 µm and stored in an airtight container for further analysis.

Extraction of bioactive compounds from coconut coir dust by ultrasound-assisted solvent extraction (UAE)
The coconut coir dust sample was extracted with 50% aqueous ethanol (v/v) using a 100:1 (v/w) ratio of solvent to sample. The extraction was performed in an ultrasonic cleaning bath (Bandelin Sonorex Digitec, DT 510 H, 35 kHz, 16 W) for 120 min at 30°C according to the method described by Siramon *et al.*[11]

Chemical composition analysis of coconut coir dust extract by LC-ESI-MS
The coconut coir dust extract was analysed by LC-ESI-MS (Agilent Technologies 6420 Triple Quad) according to the method of Siramon & Wongsheree.[12] A ZORBAX Eclipse Plus C18 analytical column (4.6×100 mm, 3.5µm; Agilent) was used for LC separation. The solvents used were deionised water containing 0.1% glacial acetic acid (solvent A) and acetonitrile containing 0.1% glacial acetic acid (solvent B). The solvent flow rate was 1 mL/min, wavelength of the photodiode array (PDA) was 280 nm and sample injection volume was 20 µL. The linear gradient of the HPLC solvent was as follows: B was increased from 8 to 10% for 2 min, then from 10 to 30% for 25 min, from 30 to 90% for 23 min, from 90 to 100% for 10 min and kept at 100% for 5 min before returning to the initiation state. Full mass spectra were recorded across a range of 100-1500 *m*/*z*. The ESI interface was operated in the positive/negative ionisation modes.

Tyrosinase inhibitory activity determination
The tyrosinase inhibition assay was modified from the methods of Kubo *et al.* and Saewan *et al*.[13,14] The reaction mixture contained 1.8 mL of phosphate buffer (0.1 M, pH 6.8), 100 µL of mushroom tyrosinase solution (138 units), 100 µL of extract solution and 1 mL of 2.5 mM L-DOPA. The solution was incubated at 25°C for 10 min and absorbance was measured by a UV-Vis spectrophotometer at 475 nm. Kojic acid, a common tyrosinase inhibitor, was used as the positive control. All experiments were performed in triplicate and percentage inhibition of tyrosinase was calculated. A concentration giving 50% inhibition of tyrosinase activity was determined by interpolation of concentration-response curves.

Antibacterial activity evaluation
Preparation of extract solution
A stock solution of coconut coir dust extract in dimethyl sulfoxide (DMSO) at 204.80mg/mL was prepared and the extract solution was sterilized by passing through a 0.45 µm membrane filter.

Microbial strains
The three bacterial strains used in this study include *Staphylococcus aureus* DMST 8840, *Staphylococcus epidermidis* DMST 15505 and methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20651 were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. All bacterial strains were grown and maintained on a nutrient agar slant. Inoculum size of each test strain was 10^8 bacteria/mL.

Determination of MIC and MBC values
Minimum inhibitory concentration (MIC) of the extract solutions was determined by the two-fold serial microdilution method, as described by Rahman *et al.*[15] The extract solutions were added to sterile Mueller Hinton broth and spread on microtiter plates before adding the diluted bacterial suspension. Each extract solution was measured in triplicate. The bacterial suspensions were used as the positive control and extracts in broth were used as the negative control. The minimum bactericidal concentration (MBC) of the extract solutions was determined according to Basri and Fan[16] by a subculture of the well showing no apparent growth in a sterile agar plate. The lowest concentration showing no visible growth on the agar subculture was taken as the MBC value. Antibiotic erythromycin was used as the standard.

Statistical Analysis
Statistical analysis was performed with the SPSS Version 22 statistical software package. The data was presented as the means ± standard deviation (SD).

Results and Discussion

Chemical composition identification
The total ion chromatogram of the coconut coir dust extract is shown in Figure 1, with the analyses of mass spectra in positive/negative ionisation modes shown in Figure 2 (a-e). Five chemicals were identified as follows:
Peak 1 (RT 8.905-9.252; Figure 2a) was identified as a coumaroycaffeoylquinic acid isomer (molecular weight 499.8); showing $[M - H]^-$ ion of *m*/*z* 498.8 ($C_{25}H_{24}O_{11}$). The product ion of coumaroylquinic acid at *m*/*z* 337.1 was found (after the loss of a caffeoyl unit: 162 amu).[17,18]
Peak 2 (RT 33.484-33.986; Figure 2b) was identified as kaempferol ($C_{15}H_{10}O_6$, molecular weight 286.3); showing $[M - H]^+$ ion of *m*/*z* 287.3.[19,1]
Peak 3 (RT 35.304-36.161; Figure 2c) was identified as isorhamnetin ($C_{16}H_{12}O_7$, molecular weight 316.0) showing $[M - H]^+$ at *m*/*z* 317.0. The product ion of quercetin at *m*/*z* 303.3 (molecular weight 302.3) was found.[20,21]
Peak 4 (RT 44.524; Figure 2d) was identified as a hydroxycinnamic acid derivative (molecular weight 422.1) showing $[M - H]^+$ at *m*/*z* 423.1. This yielded a fragment at *m*/*z* 321.0 of hydroxycinnamic acid (molecular weight 320.0). [19,22]
Peak 5 (RT 45.398-48.334; Figure 2e) was identified as quercetin ($C_{15}H_{10}O_7$, molecular weight 302.1) showing $[M - H]^+$ at *m*/*z* 303.1.[20,21]

Tyrosinase inhibitory activity
Inhibition of tyrosinase activity by the coconut coir dust extract solution and kojic acid (positive control) was measured and the IC_{50} values were calculated (data are shown in Table 1). The extract solution and kojic acid gave IC_{50} values of 874.17 and 221.36 µg/mL respectively. The extract solution showed good inhibitory activity against tyrosinase but lower activity than the positive control. These test results were consistent with other studies that reported the potential of polyphenol and flavonoid derivatives as tyrosinase inhibitors.[23,24] Recently, demand for natural tyrosinase inhibitors has increased to replace synthetic chemicals in cosmetic products.[25,26] The findings of this study suggested that a naturally occurring tyrosinase inhibitor such as coconut coir dust extract showed promise as an alternative depigmenting agent in skincare products.

Determination of antibacterial activities
The antibacterial activities of coconut coir dust extract solution against *S. aureus*, *S. epidermidis* and MRSA are shown in Table 2. The extract solution exhibited antibacterial activities against all tested bacterial strains, with minimal inhibitory concentration (MIC) values in the range 0.80-25.60 mg/mL and minimal bactericidal concentration (MBC) values ranging 3.20-120.40 mg/mL.

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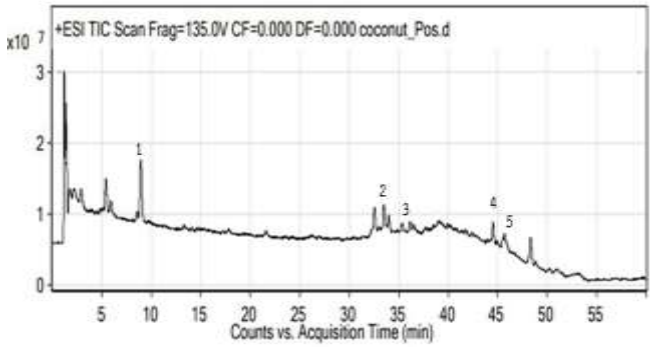

Figure 1: Total ion chromatogram of the coconut coir dust extract

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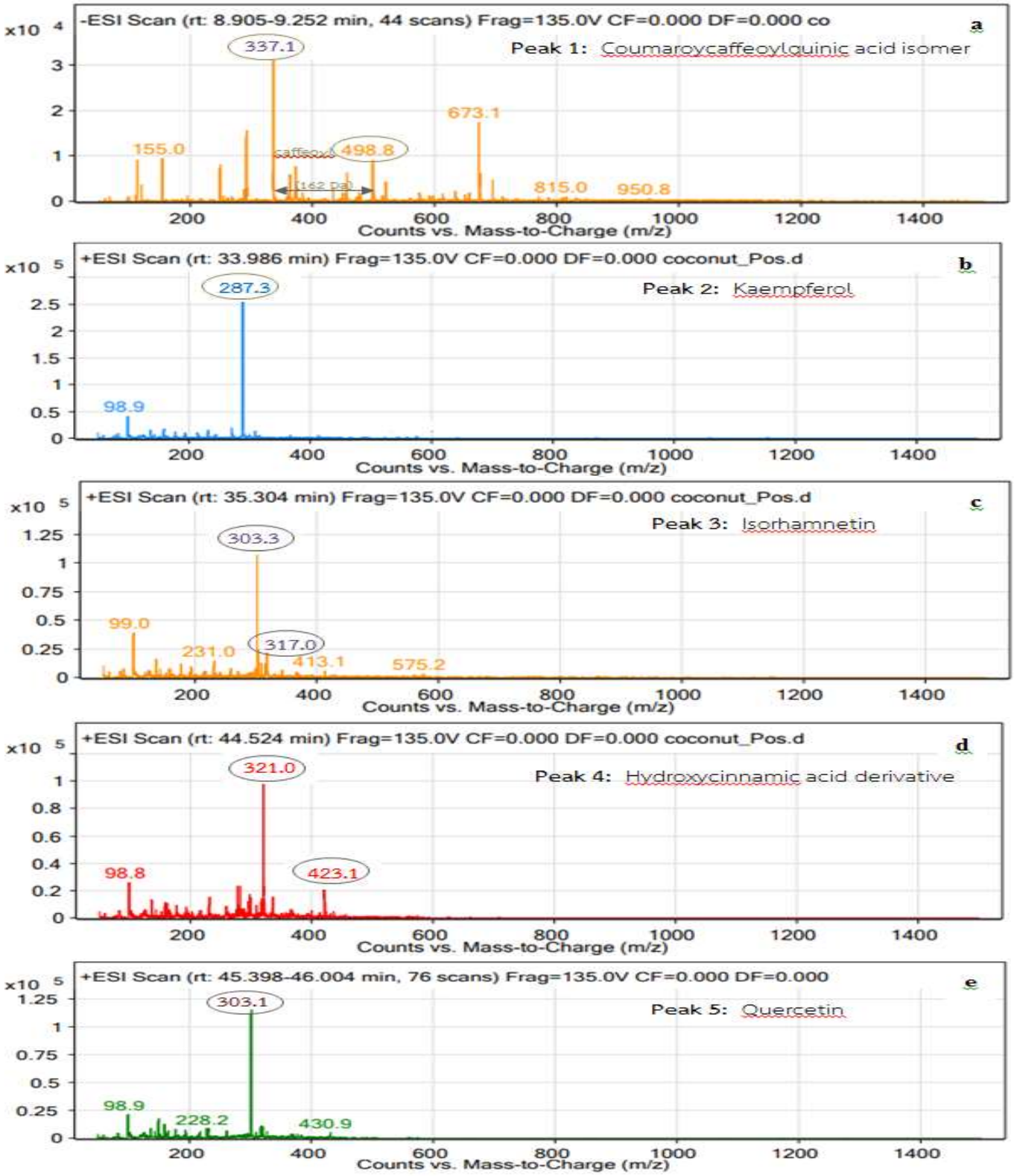

Figure 2: Mass spectra showing identified peaks in the chromatogram

Table 1: Inhibition of tyrosinase (IC_{50}) by coconut coir dust extract and standard kojic acid

Sample	IC_{50} (µg/mL)
Coconut coir dust extract	874.17±67.96
Kojic acid	221.36±0.64

Results are expressed as the mean (n = 3) ± standard deviations.

These activities were influenced by the chemicals present in the extract as (1) flavonoids and their derivatives: kaempferol, quercetin, isorhamnetin, and (2) phenolic acids - hydroxycinnamic acid derivatives: coumaroycaffeoylquinic acid, hydroxycinnamic acid. The antimicrobial activities of plant phenolics representing phenolic acids and flavonoids are well known. These compounds have wide-ranging pharmacological effects including strong inhibition of various microbial growths and synergistic effects between individual

compounds.[27-29] The antibacterial mechanisms of polyphenols permeate bacterial membranes and inhibit crucial factors such as enzymes or toxins, while also suppressing bacterial biofilm formation.[30-32]

Table 2: Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for coconut coir dust extract and the erythromycin standard

Bacterial strains	***S. aureus***		***S. aureus* (MRSA)**		***S. epidermidis***	
Concentration (mg/mL)	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Crude extract	0.80	3.20	1.60	6.40	25.60	102.40
Erythromycin	0.80	0.80	0.10	0.10	3.20	3.20

Conclusion

Five bioactive compounds were identified from the coconut coir dust extract including coumaroycaffeoylquinic acid isomer, kaempferol, isorhamnetin, hydroxycinnamic acid derivatives and quercetin. The extract exhibited tyrosinase inhibitory activity with an IC_{50} value of 874.17 µg/mL and also exhibited antibacterial activities against all tested bacterial strains with minimal inhibitory concentration (MIC) values in the range 0.80-25.60 mg/mL and minimal bactericidal concentration (MBC) values ranging 3.20-120.40 mg/mL. Based on the test results, coconut coir dust extract with tyrosinase inhibition and antibacterial actions showed promise as an alternative natural ingredient to synthetic chemicals, with applications in skincare formulations and cosmetics.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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