Recovery of Natural Bioactive Extract with Antioxidants and Anti-Tyrosinase from Coffee Silver Skin (CSS)

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Abstract

Coffee silver skin (CSS) is a thin tegument of the outer layer of coffee bean, CSS is a by-product of the coffee, appearing during the roasting step. Although this is produced in large amounts as an agricultural waste, the chemical components and biological activity have never been explored. In this study, CSS was extracted using three different polarity solvents and three extracts were evaluated for antioxidant and anti-tyrosinase activity. The ethyl acetate extract showed the most potent inhibition against DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) with IC_{50} values of 0.31 ± 0.06 and 0.49 ± 0.03 (mg/mL), respectively. In addition, ethyl acetate extract also revealed the most potent inhibition (25%) against tyrosinase at the concentration of 0.25 mg/mL. This finding suggested that CSS would be developed as a prolific source of antiaging agents such as cosmetics.

Keywords: Coffee Silver skin / Total Phenolic / Anti-Tyrosinase / Antioxidants / Bioactive Extract

1. Introduction

One of the main problems that occur due to the production of goods and services are the offshoot of waste as a byproduct, most of these many different kinds of waste do not get disposed of properly, causing widespread damage that builds up to unimaginably high degrees over year and years of inaction. The focus of this research paper is to study one of these types of wastes, more specifically Coffee Silver skin which is a by-product of processing and roasting coffee. And one of the largest sources of discarded waste in the world [1].

Coffee is one of the world's most consumed drinks in the world, with each small cup of coffee requiring ~11 grams of coffee beans. In the perspective of the world with over millions of people consuming coffee at an average of over 2.25 billion cups of coffee consumed worldwide every day, the amount of waste is bound to be astronomical. In 2023 alone, global coffee production reach approximately over 10 billion kg [2]. Coffee is one of the worlds' global economic plants and the global coffee market was valued at approximately 223.78$ billion in 2023 with forecasts of even further growth in the coming years, [3] supporting the livelihoods of roughly 25 million small producers all over the globe, with >90% of coffee production in developing countries.

Despite Coffee Silver skin typically making up only 1-2% of the mass of roasted coffee beans, it accumulates to significant quantities annually due to the massive demand and consumption of coffee production. Data from industrial coffee roasters indicates approximately 1 ton of coffee silver skin by-product produced every 120 tons of roasted coffee which along with the process in which coffee is produced is extremely water intensive especially in wet processing methods has contributed and snowballed into many other worldwide problems such as deforestation and carbon emissions. [4, 6]

Though there are a lot of disposal methods used currently, they face a lot of challenges when imposed. In many regions of metropolitan areas that have high business surrounding coffee consumption, the disposal methods consist of sending coffee silver skin to composting facilities near the area. However, taking into account the logistics and management of the transportation of coffee silver skin, it would not doubt be very costly—studies have reported disposal costs of around 447.55 pounds per ton when utilizing composting facilities as a disposal method [5]. Another well used method is incineration and landfilling with these methods posing a lot of environmental challenges through the release of methane and carbon dioxide adding onto greenhouse gas emissions which may even top that of coal combustion. All of these methods put an economically impossible limit for small businesses to invest in other lose value processing due to high logistical and handling costs [7].

Recent research has explored various coffee silver skin methods of disposal by incorporating it into functional and economically feasible products such as bakery items, water adsorbents and most importantly cosmetics which had significantly reduced disposal costs. An instance of this was in Naples where they had replaced part of their wheat flour with small amounts of coffee silver skin in bakery products, managing to reduce disposal costs by 190.09 pounds per ton [4].

The main purpose of this research paper is to explore the bioactive components of Coffee Silver skin, most notably their antioxidants and anti-tyrosinase efficacy from its compounds. Using three different polarity extracts to separate specific compounds of the substances resting in Coffee Silver skin, this research aims to evaluate its phenolic activity for the purpose of potential utilization in economical and healthcare fields. The research could be used to maximize the efficiency of disposal of coffee silver skin through providing other means of use for companies to build off of their waste to decrease waste costs and increase profit.

2. Objective

1. To identify the potent bioactive extract inhibiting against free radical and tyrosinase.

3. Scope of the Research Study

1. Evaluation of CSS extracts against antioxidant and anti-tyrosinase together with determination of their total phenolic and flavonoid contents.

4. Experiment

4.1 Plant Material

The coffee silver skin (CSS) were obtained from Nestlé (Thai) Ltd in June 2024. A voucher specimen was deposited at the Natural Products Research Laboratory of Center of Excellence in Natural Products, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

4.2 Extraction and Isolation

The coffee silver skins (CSS) (1.5kg) were mashed and macerated with MeOH (3 L each, 2 times for 2 days) at room temperature. The MeOH extract was then partitioned with hexane and EtOAc. Removal of the solvent under reduced pressure yielded hexane EtOAc and MeOH extracts (Figure 1).

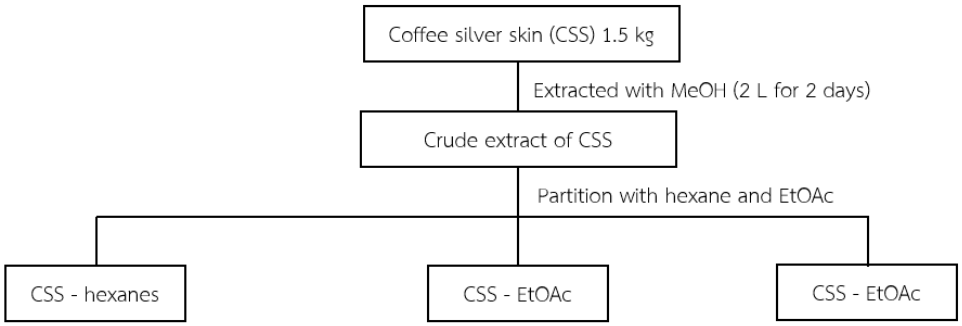

Figure 1 Chromatographic separation of coffee silver skin (CSS) extract.

4.3 Estimation of Total Phenolic Content (TPC)

Total phenolic content was evaluated using the Folin-Ciocalteu method [6, 8]. Briefly, each sample of coffee silver skin (CSS) (1 mg/mL) was diluted with DMSO to make five concentrations 2, 1, 0.75, 0.50, 0.25, 0.125 mg/mL. Then, the coffee silver skin (CSS) solution (25 µL) was mixed with 125 µl of 0.2 N Folin-Ciocalteu reagent for 5 min. Subsequently, 100 µl of 75% (w/v) sodium carbonate (Na_2CO_3) was added. The absorbance at a wavelength of 700 nm was measured using a microplate reader against DMSO as a blank. All reactions were performed in triplicate. The results were expressed as mg of gallic acid equivalents (GAE)/g of partitioned extract using a standard graph for gallic acid in the range of 0.02–0.1 mg/mL.

4.4 Estimation of Total Flavonoid Contents

Total flavonoids content was evaluated using the aluminium chloride ($AlCl_3$) colorimetric method in a 96 well microplate reader. Briefly, twenty-five microliters of the coffee silver skin (CSS) extract solution dissolved in DMSO was mixed with one hundred microliters of distilled water. Then, 7.5 µL of 5% (w/v) $NaNO_2$ solution was added and incubated at room temperature for 5 min, followed by the addition of 7.5 µL of 10% (w/v) $AlCl_3$. After being 6 min incubation at room temperature was added 50 µL of 1 M NaOH. The absorbance at wavelength of 510 nm was measured. The TFC was expressed as mg of quercetin equivalents (QE)/g of partitioned extract using a standard graph for quercetin (0.1–1 mg/mL).

4.5 Determination of the antioxidant capacity

The DPPH free radical scavenging activity assay

The potential of the crude extracts was determined as previously described [6, 8]. Five different concentrations of each sample were prepared in DMSO. The each well was mixed with 20 µL of the sample and 80 µL of 0.15 mM DPPH in MeOH for each concentration and incubated at room temperature for 30 min. The absorbance was measured at a wavelength of 517 nm (A_{517}) using a microplate reader. Ascorbic acid (vitamin C) was used as the positive control. Each assay was performed in triplicate. The free radical scavenging activity was calculated from Equation (1):

$$\% \text{ DPPH radical scavenging activity} = [(A-B)/(A)] \times 100, \quad (1)$$

where A is the $A_{517}$of the negative control and B is the A_{517} of the treatment. The % inhibition (Y axis) was plotted against the extract concentrations (X axis) and the effective concentration at 50% (EC_{50}) was obtained from the graph.

4.6 Determination of the Trolox equivalent antioxidant capacity (TEAC)

The Trolox equivalent antioxidant capacity (TEAC) assay followed the described method [6, 8]. The stock solution of $ABTS^{\bullet+}$ was prepared by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate solution in distilled water at a 1:1 (v/v) ratio in the dark at room temperature for 16 h before use. The stock solution (one mL)

mixed ethanol (Thirty-five mL) to obtained an absorbance at 734 nm (A734) of 0.700 ± 0.025. The 0.3 mL of each extract at different concentrations dissolved in DMSO was mixed with 2.7 mL of the prepared $ABTS^{\bullet+}$ solution, left for six min in the dark and then the A_{734} was read. The percentage of inhibition was calculated according to Equation. (2),

$$\% \text{ ABTS radical scavenging activity} = [(A-B)/(A)] \times 100, \quad (2)$$

where A is the A_{734} of the negative control and B is the A_{734} of the treatment. The % inhibition (Y axis) was plotted against the respective extract concentration (X axis) and the EC50 value was obtained from the graph. The results were then compared with the Trolox standard curve (0-0.2 mM) and the results are expressed as µmole Trolox equivalents (TE)/g partitioned extract.

4.7 *In vitro* tyrosinase inhibition

Anti-tyrosinase activity was conducted as described by [6, 7, 8] using L-DOPA as the substrate and kojic acid as a positive control. Briefly,120 µL of 2.5 mM L- DOPA in 80 mM phosphate buffer (pH 6.8), 30 µL of 80 mM phosphate buffer (pH 6.8), and 10 µL of partitioned extract or kojic acid (positive control) at different concentrations in DMSO. All sample solutions were incubated at 37°C for 10 min and 300 µL of L-DOPA was added. After mixing, the reaction was added 40 µL of 165 units (U)/mL mushroom enzyme in phosphate buffer was added and incubated at 25 ˚C for 5 min. The absorbance was measured using spectrophotometry at 475 nm was measured using a microplate reader. The tyrosinase activity was calculated as the IC_{50} value. Each assay was performed in triplicate and the percentage TYRI was calculated from Equation. (3);

$$\text{The inhibition Percentage of tyrosinase inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100, \quad (3)$$

where $A_{control}$ is the absorbance of the enzyme activity and A_{sample} is the absorbance of enzyme activity in the addition of the sample solution. The concentration at 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage and sample concentration.

5. Result and discussion

The partitioned extracts of coffee silver skin (CSS)

The methanolic extract was first prepared from the coffee sliver skin (CSS) by soaking in methanol. The methanol soluble portion was separated by liquid-liquid partition with hexane and EtOAc, respectively. Three partitioned extracts, namely hexane, EtOAc and methanol extracts, were obtained. The EtOAc partitioned extracts had the highest yield (above 40%). The MeOH partitioned extracts exhibited a sticky solid form, whereas the hexane partitioned extracts exhibited an oil form.

Determination of the total phenolic compound and total flavonoid compound

The TPC and TFC were determined from the calibration curves of gallic acid ($y = 7.5447x - 0.004$; $R^2 = 0.9975$) and quercetin ($y = 0.4651x + 0.0519$; $R^2 = 0.9781$). The biological activities of hexanes, ethyl acetate

and methanol extract from coffee silver skin (CSS) were summarized in (Tables1). The TPC of ethyl acetate (EtOAc) extract was the highest with value of 62.91 ± 1.46 mg GAE/g extract and the highest TFC was shown by ethyl acetate extract with value of 247.41 ± 6.28 mg QE/g extract.

Antioxidant activity of the fractionation

The results of partitioned extracts with antioxidant assays from the coffee silver skin are shown in (Table 2) along with that for ascorbic acid as the standard reference for the DPPH and Trolox as the standard reference for the ABTS assays. The ethyl acetate was the most potent inhibitor against DPPH with EC_{50} values of 0.31 ± 0.06 mg/mL. Although this was still almost 22-fold less effective than ascorbic acid (EC_{50} of 0.01 ± 8.9 mg/mL), while the hexanes extract had the weakest DPPH scavenging capacity. The ethyl acetate showed a significantly strong ABTS radical scavenging ability with (EC_{50} values of 0.49 ± 0.03 mg/mL), although this was still over four-fold less effective than Trolox EC_{50} of 0.12 ± 0.02 mg/mL.

Tyrosinase inhibition

All the partitioned extracts were initially screened for tyrosinase activity **[7]**. Generally, the ethyl acetate was the most potent inhibitor against tyrosinase activity with %inhibition (36.04%) shown in Figure 2. On the other hand, hexane showed weak or no inhibition, except for MeOH that show a significant suppression against tyrosinase with %inhibition (28.06%).

It should be noted that higher inhibition against radical and tyrosinase is corresponding to higher total phenolic and flavonoid. We therefore assume that the active components in EtOAc and methanol extracts are possibly phenolic and flavonoid type compounds.

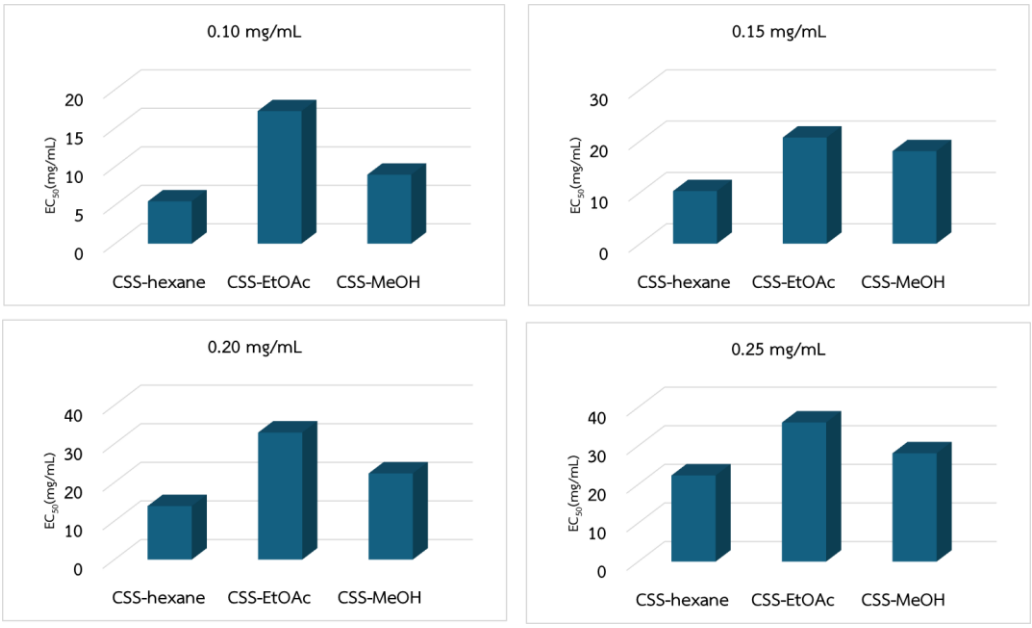

Figure 2 Tyrosinase inhibition of coffee silver skin (CSS).

Table1 The TPC and TFC of the coffee silver skin (CSS) extracts

Sample	Total phenolic (mg GAE/ g extract)	Total flavonoids (mg QE/ g extract)
CSS – hexanes	ND	ND
CSS - EtOAc	62.91 ± 1.46	247.41 ± 6.28
CSS - MeOH	58.59 ± 0.77	142.67 ± 6.18

Notes. Data are shown as the mean ± 1SD derived from three replicates. EC_{50} = concentration at 50% inhibition ND = Not detectable.

Table 2 Antioxidant activity of the spent coffee grounds extracts.

Sample	DPPH EC_{50} (mg/mL)	ABTS EC_{50} (mg/mL)
CSS – hexanes	NI	NI
CSS - EtOAc	0.31 ± 0.06	0.49±0.03
CSS - MeOH	0.54 ± 0.04	0.83±0.02
Ascorbic acid	0.01 ± 8.9	-
Trolox	-	0.12 ± 0.02

Notes. Data are shown as the mean ± 1SD derived from three replicates. EC_{50} = concentration at 50% inhibition NI = percentage inhibition less than 30% at highest concentration tested

6. Conclusion

The ethyl acetate had the highest antioxidant activities, which were correlated to the highest TPC and TFC. Their fractionation led to an enrichment of bioactivities, suggesting these might be the major active compounds in the coffee silver skin (CSS). The ethyl acetate was the most potent inhibitor against tyrosinase with highest %inhibition.

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