Value-added from microwave-assisted extraction of Musa sapientum fruit extract as an alternative safe and effective agent for the treatment of hyperpigmentation

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Abstract

Annually, a great number of bananas are produced, and those that do not satisfy commercialization criteria are discarded, resulting in a considerable amount of agricultural waste. This work aimed at valorizing rejected unripe *Musa sapientum* fruit by microwave-assisted extraction using glycerol-ethanol mixtures for development of cosmeceuticals. The fruit extract exhibited potent radical scavenging activity towards 2,2-diphenylpicrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assays in a concentration-dependent manner. Ferric reducing power was 199.13 μM FeSO₄/mg extract. Total phenolic content was 39 mg gallic acid equivalent/100 g extract and total flavonoid content was 26 mg quercetin equivalent/100 g extract. Vitamin C and E contents of the extract were 10.47 and 4.77 mg/100 g, respectively. The extract exhibited potent antibacterial activity against wide range of bacterial pathogens. The extract significantly reduced nitric oxide production in RAW 264.7 cells (p < 0.05). Biocompatibility assessment of the extract with L929 fibroblast cells demonstrated no toxic effects. *M. sapientum*-loaded serum significantly inhibited DPPH scavenging activity with a value of 50.62% when compared with commercially-available product (p < 0.05). The serum significantly suppressed tyrosinase activity about 37.46% (p < 0.05). This finding implies that *M. sapientum* has a high potential to be used as a depigmenting agent.

Statement Of Novelty

Our results demonstrated that *Musa sapientum*-loaded serum exerts skin whitening and antioxidant effects to prevent undesirable skin hyperpigmentation. The novelty of this study is the environment-friendly valorization of unripe *M. sapientum* fruit in cosmeceutical products utilizing microwave-assisted extraction incorporating glycerol-ethanol mixtures as solvents. Since glycerol is a non-toxic solvent with humectant properties, the obtained extracts may be employed without the need to remove the solvent for preparation of cosmetic formulations. This study entirely reported biologically active compounds of the extract including vitamin C, vitamin E, phenolic, and flavonoid components with potent antioxidant, antityrosinase, antibacterial, and anti-inflammatory properties with biocompatible. Successful adoption of these strategies will address present waste management issues, reduce landfill environmental consequences, and resolve farm owners’ sustainability challenges.

Introduction

Bananas are a prevalent fruit in the global market and are consumed as a staple food or as a processed product. According to the Food and Agriculture Organization in 2020, the total global production of bananas is 119 million tonnes [1]. Due to high commercialization expectations for high-quality bananas, the bananas are occasionally rejected at the processing plants. Furthermore, as a waste-agricultural source, a significant percentage of disfigured bananas are discharged into the living environment. These banana wastes pollute the environment by releasing poisonous gases like hydrogen sulfide and methane [2, 3], which causes huge losses for farmers and processors. *Musa sapientum* ABB group ‘Kluai Namwa’, belonging to the family Musaceae, is the most popular fruit in Thailand, grown in many regions for local
supply. Several nutrients are present in bananas, including dietary fiber, protein, carbohydrate [4], vitamin C, vitamin A, and mineral compositions [5]. Owing to the presence of secondary metabolites such as phenolic, flavonoid, and other polyphenolic compounds, the banana peel and pulp contain numerous antioxidants [6]. Procyanidin, coumarin, ferulic acid, and gallic acid, among other phenolic compounds found in banana extracts, have been shown to have substantial antioxidant capabilities [7]. Banana peel extract has been reported to have β-carotene, which absorbs ultraviolet B and acts as a reactive oxygen species scavenger system to reduce the inflammatory cytokines production [8]. In addition, glycosides are found in banana peel extract and have a moisturizing effect [9]. Due to alkaloids, flavonoids, tannins, and polyphenols, banana pulp and peel extracts possess tyrosinase enzyme inhibitory properties [10].

Previous work has documented that banana extract inhibited activity against a wide range of pathogenic bacteria [11]. Therefore, the banana extracts might be fascinating natural source with a wide range of functional properties that are desirable in cosmetic products.

Melasma is a skin disorder marked by hyperpigmented macular lesions in sun-exposed regions. In the biosynthesis of melanin, free radicals play a significant role. The catalytic reaction of tyrosinase produces dopaquinone, which requires free radicals and can undergo a variety of oxidation events, resulting in increased melanin biosynthesis during melanogenesis [12]. Bioactive compounds such as hydroquinone, kojic acid, and azelaic acid are commonly used as skin-lightening agents. However, most of these compounds have been related to several adverse reactions. For instance, hydroquinone has been linked to a treatment-related adverse skin reaction [13] and kojic acid may cause redness, burning sensations, and itching [14].

To overcome these problems, cosmetic industries are seeking an alternative using safer and natural ingredients to develop skin depigmenting agents. Further, recycling waste materials from industrial plants would significantly reduce the environmental concerns of industrial wastes and lead to value-added consumption. Therefore, this study aims to develop a skin depigmenting agent using valorized M. sapientum that inhibits the action of free radicals and the tyrosinase enzyme. In addition, microwave-assisted extraction of M. sapientum extract using glycerol-ethanol mixtures may be a value-added natural ingredient for cosmetic products.

Materials And Methods

Materials

Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Trolox were purchased from Gibco, (Paisley, UK). 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) was purchased from Sigma-Aldrich (Steinheim, North Rheine-Westphalia, Germany). Dimethyl sulfoxide (DMSO) and glycerol were obtained from Fisher Scientific (Loughborough, UK), Mueller Hinton broth (MHB), tryptic soy agar (TSA), and tryptic soy broth (TSB) were purchased from Difco (Port de Claix, France). Ethylene Tetrazolium Bromide (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H, MTT), and trypsin were purchased from Merck. Dulbecco's modified Eagle medium (DMEM) and foetal bovine serum were purchased from Gibco,
UK. Penicillin-streptomycin (5000 U/mL) was procured from Sigma-Aldrich (Steinheim, North Rheine-Westphalia, Germany).

**Bacterial Strains**

Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus* NPRCoE 160801, and *S. epidermidis* ATCC 35984), and Gram-negative bacteria (*Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853) were used for the antibacterial assay. The microorganisms were cultured in Mueller Hinton broth (MHB) and incubated at 37 °C for subsequent experiments.

**Preparation of *M. sapientum* Extracts**

The fruit of unripe *M. sapientum* was collected from a local market in Hat Yai, Songkhla, Thailand. The whole fruit, peel, and pulp were separately proceeded for preparing three different extracts. Each part was cut into small pieces, dried in a hot air oven at 50 °C and crushed into fine powder. Three hundred grams of the powders of each part were macerated in 1.5 L glycerol-ethanol mixture at the ratio of 1:1 by volume. The supernatant was evaporated under microwave-assisted extraction for 15 min with a 1 min cycle of on/off intervals, and microwave power of 900 watt. The obtained extract was then filtered using Whatman® No.4 filter paper and kept at 25 °C until further use.

**Antioxidant Assays**

**2,2-diphenylpicrylhydrazyl (DPPH) Radical Scavenging Assay**

DPPH radical is a widely used method for determining the ability of natural substances to scavenge free radicals. The DPPH radical scavenging activity of the extract was evaluated in a 96-well microtiter plate according to the method previously described [15]. Briefly, 20 μL of the extracts (31.25 – 1000 mg/mL) was added to 180 μL of 0.1 mM DPPH radical solution and incubated at room temperature in the dark for 30 min. Trolox was used as a standard control. Following incubation, the optical density of the mixing solution was measured at 517 nm using a microplate reader (Enspire® microplate readers, PerkinElmer, Waltham, MA, USA). Methanol was used as a blank. The percentage of DPPH scavenging was calculated as follows:

$$\text{Scavenging ability} (\%) = \left[ \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right] \times 100$$

**2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) Radical Scavenging Assay**

Free radical scavenging activity of the fruit, peel, and pulp extracts was further determined by ABTS radical cation decolorization assay [16]. Briefly, 7.4 mM ABTS⁺ (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) solution and 2.6 mM potassium persulfate solution were prepared as stock solutions. Then, the working solution was prepared by mixing equal volumes of the stock solutions and allowed to react for 12-16 h in the dark at room temperature. Twenty microliters of different concentrations of the
extracts (31.25 – 1000 mg/mL) and Trolox, a standard control, were mixed with 180 μL of ABTS•+ radical solution. After 6 min incubation, the absorbance of the mixing solution was measured at 734 nm. Percent inhibition of ABTS radical by the extracts was calculated using the formula:

\[
\text{Scavenging ability (\%)} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right) \times 100
\]

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP was determined using the method developed by Benzie and Strain with slight modifications [17]. Twenty microliters of different concentrations (31.25 – 1000 mg/mL) of fruit, peel, and pulp extracts were added to 96-well plates containing 180 μL of FRAP reagent. The mixture was incubated at 40 °C for 30 min in the dark and measured spectrophotometrically at 593 nm. Based on the standard curve, the FRAP value was recorded as μM FeSO₄ equivalent per 100 mg of extract.

Determination of Total Phenolic Contents

Total phenolic content in the *M. sapientum* fruit extract was determined by the modified Folin-Ciocalteu method [18]. Gallic acid was used as a standard drug. An aliquot of appropriately diluted extracts or gallic acid standard solutions (20 μL) were mixed with Folin-Ciocalteu’s reagent (100 μL) and sodium carbonate (80 μL) in a 96-well microtiter plate. The plate was mixed well and allowed to stand for 30 min to develop color. Absorbance values of the sample were measured at 765 nm. Total phenolic content was calculated using gallic acid equivalent.

Determination of Total Flavonoid Contents

The total flavonoid content in the *M. sapientum* fruit extract was determined by the previously described method [19]. Briefly, the mixture of 20 μL of *M. sapientum* fruit extract and 180 μL of 2% w/v aluminium chloride was incubated for 10 min. The absorbance was obtained at 415 nm against a blank. Each solution was analyzed in triplicate to obtain the mean absorbance values. Standard quercetin was serially diluted and treated with the same procedure to create a standard curve. Total flavonoid content was calculated using the quercetin calibration curve.

Quantification of Vitamins C and E in Fruit Extract of *M. sapientum* by High-Performance Liquid Chromatography (HPLC)

The analytical system consisted of an Agilent 1100 Series HPLC system equipped with a UV (DAD) detector (Waldbonn, Germany). The column was a Hypersil C-18 reverse phase, and the mobile phase consisted of 3.00 mM potassium dihydrogen phosphate in 0.35% (v/v) ortho-phosphoric acid. The mobile phase was pumped isocratically at a 0.8 mL/min flow rate. An analytical column temperature was kept at 50 °C. The injection volume of the sample was 20 μL. The working standard solutions were always analyzed together with the samples. The detection wavelength of vitamin C (ascorbic acid) and E (α-tocopherol) were 245 and 392 nm, respectively. The total run time was required less than 7.0 min. Chromatographic peaks were identified by comparing the retention times of samples with standard
compounds. According to the studies, the quantities of vitamin C and E were carried out by external standardization [20, 21].

**Determination of the Minimum Inhibitory Concentration (MIC) of the *M. sapientum* Fruit Extract Against Gram-positive and Gram-Negative Pathogenic Bacteria**

Antibacterial activity of *M. sapientum* fruit extract was investigated against Gram-positive and Gram-negative pathogenic bacteria, as mentioned in section bacterial strains. According to Clinical and Laboratory Standard Institute (CLSI) recommendations, the assay was performed in 96-well microtiter plates using two-fold broth microdilution method [22]. Briefly, the adjusted culture was diluted to obtain $10^6$ CFU/mL. One hundred microliters of the bacterial suspension were added to 96-well plates containing *M. sapientum* fruit extract at a final concentration ranging from 31.25 – 1,000 mg/mL. The plates were incubated at 37 °C for 18 h. The MIC value was obtained as the lowest concentration of the fruit extract that inhibits bacterial growth.

**Cytotoxicity Assay**

L929 fibroblast and RAW 264.7 murine macrophage cells were used to determine the cytotoxicity of *M. sapientum* fruit extract. The cell lines were cultured in high glucose Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Approximately $3 \times 10^4$ cells/well were seeded in 96-well plates and incubated at 37 °C for 24 h with 5% CO$_2$. After incubation, various concentrations of *M. sapientum* fruit extract (31.25 – 1000 mg/mL) were added to each well. The plates were further incubated at 37 °C for 24 h with 5% CO$_2$. Following incubation, the culture media containing *M. sapientum* fruit extract was removed. Cell viability after treatment was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Absorbance values were measured at 595 nm. Percentage of cell viability was calculated according to the equation:

$$Cell\ viability\ (%) = \left( \frac{Absorbance\ of\ test}{Absorbance\ of\ control} \right) \times 100$$

**Assessing the Anti-Inflammatory Potential of *M. sapientum* Fruit Extract by Nitric Oxide (NO) Quantification Assay**

The amount of NO production by RAW 264.7 cells after treatment with different concentrations of *M. sapientum* fruit extract was determined by measuring the accumulation of nitrite in the cell culture medium using the Griess reagent. Approximately $5 \times 10^4$ cells/well were seeded in 96-well plates in a complete medium and plates were incubated at 37 °C for 24 h with 5% CO$_2$. Subsequently, cells were stimulated with 1 µg/mL of LPS and treated with the extract at various concentrations for 24 h. Griess reagent was mixed with the cell culture supernatant at a 1:1 ratio and the plate was incubated at room temperature for 10 min. The NO level was determined at 540 nm using a microplate reader. The presence of NO production was calculated as follows:
NO production (%) = (Absorbance of test / Absorbance of control) × 100

Preparation of *M. sapientum*-Loaded Serum

Two-fifty μL of *M. sapientum* fruit extract was loaded into 4.75 mL of serum base. Then, the mixture was stirred and kept at room temperature. The obtained *M. sapientum*-loaded serum was used for further experiments. The serum base and the commercially-available product were used as controls.

**Antioxidant Potential of *M. sapientum*-Loaded Serum**

DPPH radical scavenging assay to determine the antioxidant potential of *M. sapientum*-loaded serum was carried out using the method described in section DPPH radical scavenging assay.

**Skin-Lightening Property of *M. sapientum*-Loaded Serum**

According to previous study, a skin-lightening property of *M. sapientum*-loaded serum was performed using L-3,4-dihydroxyphenyl alanine (L-DOPA) as substrate [23]. The assay was carried out in a 96-well microtiter plate. In each well, 40 μL of *M. sapientum*-loaded serum, 80 μL of phosphate buffer (0.1 M, pH 6.8), 40 μL of tyrosinase enzyme (60 units/mL), and 40 μL of L-DOPA (3.5 mM) were added. The mixture was incubated at 37 °C for 10 min and the absorbance was measured at 475 nm. The percentage of tyrosinase inhibition was calculated as follows:

Tyrosinase inhibition (%) = (Absorbance of test / Absorbance of control) × 100

**Statistical Analysis**

The results were obtained from three independent experiments carried out in triplicate. The mean and standard deviation were used to express the data. A paired Student's t-test was used to compare the tests and controls. Values of p < 0.05 were considered statistically significant.

**Results And Discussion**

**Antioxidant Activity of *M. sapientum* Extracts**

Antioxidants are used as preventative and therapeutic agents in the pharmaceutical and cosmetic industries. Plant antioxidants are becoming more popular and they may eventually replace synthetic antioxidants. A single assay is not adequate to assess the antioxidant potential of plant extracts. In this study, the antioxidant activity of *M. sapientum* was evaluated using three different standard assays such as DPPH, ABTS, and FRAP. As shown in Fig. 1a, the increasing concentrations of extract significantly enhanced the DPPH radical scavenging activity in a dose dependent manner (p < 0.05). At 100 mg/mL concentration, the fruit extract of *M. sapientum* had the maximum DPPH radical scavenging activity to the level of 89%, followed by peel and pulp extracts, which had the scavenging activity to the level of 76 and 72%, respectively. Similarly, the extract substantially inhibited the ABTS radicals in a concentration dependent manner. At 25 mg/mL concentration, the fruit extract of *M. sapientum* destroyed 99.91% of
ABTS radical cation. It was followed by peel (97.87%) and pulp (92.44%) extracts (Fig. 1b). With a FRAP value of 199.13 µM/mg, the fruit extract displayed the highest ferric reduction antioxidant capacity. The peel and pulp extracts have FRAP values of 70.55 and 45.39 µM/mg, respectively (Fig. 1c). Previously, it has been reported that an increase in microwave power enhanced the antioxidant potential of unripe *M. acuminata* peel waste [23]. Therefore, high microwave power (900 watt) used in this study was responsible for potent antioxidant activity of *M. sapientum*. It might be due to the direct impacts of microwave energy on biomolecules through dipole rotation and ionic conduction in the extraction system. Overall, the fruit extract of *M. sapientum* exhibited more potent antioxidant activity than the peel and pulp extracts in all the performed standard radical scavenging assays. Therefore, the fruit extract of *M. sapientum* alone was selected for further investigations.

**Total Phenolic and Flavonoid Contents in M. sapientum Fruit Extract**

*Musa* spp. are rich sources of phenolic and flavonoid contents with potent antioxidant activities. The total phenolic and flavonoid contents of the fruit extract are shown in Table 1. Total phenolic content was 39 ± 0.30 mg gallic acid equivalent/100 g extract and total flavonoid content was 26 ± 0.22 mg quercetin equivalent/100 g extract. The obtained result in this study goes well with another report, who have reported that microwave-assisted extraction effectively recovers phenolic antioxidants from banana peels [25]. The increased phenolic recovery through microwave-assisted extraction may be attributed to inner superheating, which may induce liquid vaporization inside the cells and causing the cells to rupture [26].

**Vitamins C and E in M. sapientum Fruit Extract**

Vitamin C, water-soluble organic molecule, plays a significant role in various biological functions. It acts as an antioxidant to safeguard cells from oxidative stress-induced damages [27]. Its antioxidant potential makes it essential for skin health. Vitamin E is a lipid-soluble organic molecule needed for the body's defense against the formation of hydroperoxyl radicals in the lipid milieu. It is a phenolic antioxidant and protects membrane lipids from oxidative damage. The antioxidant capabilities of vitamin E play a significant role in protecting erythrocyte membranes and neural tissues from damages [28]. In this study, high performance liquid chromatography was done to quantify the vitamin C and E contents in the fruit extract of *M. sapientum*. The obtained results revealed that 100 g of *M. sapientum* fruit extract contained 10.47 and 4.77 mg of vitamins C and E, respectively (Table 1). The total vitamin C content of *M. sapientum* obtained in this study using microwave-assisted extraction was higher than the conventional extraction [29].

**Antibacterial Activity of M. sapientum Fruit Extract**

The antibacterial activity of *M. sapientum* fruit extract was determined against Gram-positive and Gram-negative pathogenic bacteria. The MICs of *M. sapientum* fruit extract against the tested bacteria ranged from 250–1000 mg/mL (Table 2). Since potential was shown against both Gram-positive and Gram-negative pathogenic bacteria, the results obtained from this assay indicate that *M. sapientum* fruit extract might be a great source of broad-spectrum antibacterial agents. The antibacterial action of *M. sapientum*
extract might be ascribed to its high phenolic and flavonoid contents, which are associated with bacterial cell metabolism suppression. The presence of antibacterial phenolic compounds in *M. sapientum* acts as preservatives during storage [30, 31].

**Cytotoxicity of M. sapientum Fruit Extract**

Cytotoxicity assays underpin the principle that early screening of biological materials for toxicity can determine their therapeutic and biological usefulness. In this study, the cytotoxicity of the extract was investigated on L929 fibroblast cells and murine macrophage (RAW 264.7) cell lines using an MTT assay. The results demonstrated that the extract at concentrations ranging from 31.25–1,000 mg/mL had no impacts on the viability of both cell lines following treatment (Figs. 2 and 3a). As a result, the extracts obtained in this study may be utilized directly to develop cosmetic or therapeutic formulations without removing the solvent. *M. sapientum* fruit extract may be used as a commercial product without any risk of toxic effects.

**Anti-inflammatory Effects of M. sapientum Fruit Extract**

Skin inflammation may cause redness, swelling, and disruption in skin physiological activities [32], which significantly affects natural skin health and appearance. Therefore, herbal cosmetics with anti-inflammatory activity are one of the most important aspects of controlling skin inflammation. The anti-inflammatory potential of *M. sapientum* fruit extract was assessed by inhibiting nitric oxide (NO) production in macrophages activated with lipopolysaccharide (LPS). Nitric oxide is a chemical mediator that affects several physiological processes, including inflammation. NO inhibition may diminish the triggering of pro-inflammatory substances and immunological responses, potentially leading to anti-inflammatory treatment. The nitrite generated by the spontaneous oxidation of NO’ was evaluated using Griess reagent to measure NO production. The results revealed that the extract significantly inhibited LPS-induced NO in a concentration dependent manner (p < 0.05) (Fig. 3). The obtained result in this study corroborates well with another report, who have reported that *M. sapientum* water extract of fresh ripe peel and 95% ethanol extract of dried unripe peel showed significant NO inhibitory action with IC$_{50}$ values of 6.68 ± 0.34 and 36.62 ± 3.68 µg/mL, respectively [33]. Additionally, they disclosed that antioxidant activity of dried unripe extract was dependent on phenolic contents.

**Antioxidant and Skin-Lightening Properties of M. sapientum-Loaded Serum**

Harmful effects of oxidative stress are minimized by antioxidants. Therefore, antioxidant potential of *M. sapientum*-loaded serum was evaluated using the DPPH radical scavenging method. As shown in Table 3, *M. sapientum*-loaded serum significantly inhibited DPPH radical scavenging activity with value of 50.62 ± 0.42%. Further, the *M. sapientum*-loaded serum exhibited potent radical scavenging action than the commercially-available product (10.25 ± 2.36%). Antioxidants also protect pharmaceutical and cosmetic products against oxidation during storage and usage.
The overexpression of tyrosinase enzyme leads to a significant level of melanin production in human skin and triggers hyperpigmentation. Tyrosinase inhibitory effect of *M. sapientum*-loaded serum was determined by mushroom tyrosinase assay. *M. sapientum*-loaded serum considerably suppressed tyrosinase activity to the level of 37.46 ± 1.11% (Table 4). *M. sapientum* contains vast quantities of phenolic, alkaloids, flavonoids, β-carotene, tannin, riboflavin, and ascorbic acid, all of which have potent antioxidant and tyrosinase inhibitory properties [10, 11, 34]. Further, phenolic compounds present in bananas have been reported to suppress melanogenesis in B16F10 mouse melanoma cells by downregulating the synthesis of the tyrosinase enzyme and inhibiting melanogenesis via p38 signaling pathway. The vitamin C derivative magnesium phosphate ascorbyl has been found to suppress melanin production in three-dimensional cultured human skin models and cultured human melanoma cells [35]. Another study revealed that using a lotion containing sunscreen, buffered glycolic acid, hydroquinone, vitamins C and E instead of sunscreen alone resulted in a significant decrease in pigmentation [36]. This impact is hypothesized to be caused by ability of vitamin C to interfere with the function of the rate-limiting enzyme tyrosinase. As earlier mentioned, our results reveal a high content of phenolic compounds and vitamin C in banana fruit extract. Therefore, it may be one of the factors for tyrosinase inhibitory potential of *M. sapientum*-loaded serum.

**Conclusions**

This study reveals the possible usage of banana waste as a promising material for the development of cosmeceuticals to treat hyperpigmentation. Owing to its high contents of total phenols, flavonoids, vitamin C, and E, it also exhibits excellent radical scavenging, antibacterial, and anti-inflammatory properties with biocompatible. Taken together, this finding suggests that *M. sapientum* might be a valuable source of skin-lightening agents for the treatment of hyperpigmentation.

**Declarations**

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**Authors’ Contributions** Conceptualization: Siwaporn Bihlman, Supakit Paosen, Supayang Piyawan Voravuthikunchai; Methodology: Siwaporn Bihlman, Suttiwan Wunnoo, Krittima Dumjun, Tassanai Wengnoen; Formal analysis and investigation: Siwaporn Bihlman, Supakit Paosen, Srinivasan Ramanathan, Suttiwan Wunnoo; Writing—original draft preparation: Siwaporn Bihlman, Supakit Paosen, Srinivasan Ramanathan, Suttiwan Wunnoo, Sakkarin Lethongkam; Writing—review and editing: Siwaporn Bihlman, Supakit Paosen, Srinivasan Ramanathan, Sakkarin Lethongkam, Nattha Kaewnopparat, Supayang Piyawan Voravuthikunchai; Funding acquisition: Siwaporn Bihlman, Supayang Piyawan Voravuthikunchai; Resources: Supayang Piyawan Voravuthikunchai; Supervision: Supakit Paosen, Supayang Piyawan Voravuthikunchai.
Data Availability  The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest  The authors have not disclosed any conflict of interest.

Author identification


References


Tables

Tables 1-4 are available in the Supplementary Files section.

Figures
Antioxidant activity of *Musa sapientum* extracts determined by **a** 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH), **b** 2,2’-azino-bis(3-ethylbenzothiazoline6-sulphonic acid) diammonium salt (ABTS), **c** Ferric reducing antioxidant power. The values indicate the means ± SD from three independent experiments performed in triplicate. * Significant difference between fruit and peel, fruit and pulp extract (p < 0.05)
Figure 2

Cytotoxic effects of *Musa sapientum* fruit extract on L929 fibroblast cells. Cell viability was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide metabolic assay following exposure to different concentrations (31.25 – 1000 mg/mL) of the *Musa sapientum* fruit extract for 24 h. The cell viability was quantified and expressed in percentages. The values indicate the means ± SD for three independent experiments performed in triplicate.
Figure 3

Effects of *Musa sapientum* fruit extract on the viability of RAW 264.7 cells stimulated by lipopolysaccharides (LPS). **a** RAW 264.7 cells were treated with different concentrations (31.25 – 1000 mg/mL) of *Musa sapientum* fruit extract and LPS for 24 h at 37 °C in a humidified atmosphere containing 5% CO₂. The cell viability was quantified and expressed in percentage, **b** the anti-inflammatory potential of *Musa sapientum* fruit extract was assessed by quantifying nitric oxide production in RAW
264.7 cells upon treatment with different concentrations (31.25 – 1,000 mg/mL) of *Musa sapientum* fruit extract and LPS. The values indicate the means ± SD from three independent experiments performed in triplicate. * Significant difference in the treatments compared with LPS-stimulated control (p < 0.05)

**Supplementary Files**

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