ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS AND DETERMINE QUERCETIN A BIOMARKER USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF BUTEA MONOSPERMA (LINN.)

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ABSTRACT

A study on the development of innovation and process of facial skin care products from flower of *Butea monosperma* (Linn.) (BM) Taub. Flowers extract to promote green travel, Udon Thani Province to create readiness for product and service standards to increase competitiveness in a sustainable way. The objective is to develop a facial skin care set consisting of cleansing gel, essence, facial cream, serum from BM flowers extract. to test its pharmacological activity and the safety of the facial skin care set from BM flowers extract to develop a facial skin care set from guava flower extract to get a product registration number Testing the efficacy and safety of facial care sets from BM. Quantitative determination of quercetin by HPLC method. After the analysis, it was found that Example of BM flowers extract at a concentration of 10 mg/mL, the quercetin peak was found in the chromatogram of the above sample at 6.166 ± 0.068 min and the quercetin content was 0.0110 ± 0.0003. mg per 100 mg of sample weight. This study is only the development of facial skincare products from BM.

Keywords: *Butea monosperma* (Linn.), BM flowers extract, facial care, moisture, elasticity

INTRODUCTION

BM is one of the indigenous plants of Thailand, as it originated from the hot regions in Southeast Asia such as Thailand, Myanmar, Cambodia, Laos, Malaysia, Sri Lanka, Nepal, Pakistan, and Bangladesh. In Thailand, it can be found in every region, mostly in lowlands and in the forests of deciduous trees at an altitude of 80-300 meters above sea level, but it is more commonly found in the northern and northeastern regions. (Provincial Development Plan for Udon Thani Province, 2561-2565)

BM or Golden Shower Tree is considered a native plant of Thailand and other regions in Southeast Asia, with its origins in the hot areas of the eastern and southern regions of the continent. It can be found throughout Thailand in lowland areas and in the Mixed Forest, which is located at an altitude of 80-300 meters above sea level. However, it is more commonly found in the northern and northeastern regions of Thailand than in other areas.

Various parts of the BM tree have been used in many ways. For example, the bark can be used to make rope and paper, while the branches and wood can be used to make household tools, as well as for building houses and boats (in shaded areas), and for making charcoal. Today,

BM are popularly planted in residential and public areas, as well as along roadsides, as they provide good shade and have beautiful and eye-catching flowers.

According to Thai medicinal texts, the BM also has medicinal properties. Its roots have a warm and spicy taste and can be used to nourish the body's elements and nerves, relieve stomachache and diarrhea, drive out pathogens, relieve muscle pain, relieve urinary problems, and help treat eye inflammation and redness. Its leaves can be used to treat skin problems, such as itching, redness, and rashes, while its seeds can help expel intestinal worms, drive out pathogens, and relieve skin inflammation and irritation. Finally, the sap of the BM can be used externally to treat wounds and internally to help relieve diarrhea and stomach problems. (Goodbody, 2561)

The presence of phenolic acids and flavonoids in BM (golden shower) extract was examined using a semi-experimental HPLC method with a PDA detector. BM is known to contain components that exhibit inhibitory and antioxidant properties against enzymes (Baessa, M., 2018). The root extract of BM demonstrated significant antibacterial activity against Staphylococcus faecalis, *S. falecalis*, *S. cohni*, *Escherichia coli* (E. coli), and *Serratia ficaria* (S. ficaria) using a well diffusion method. The extract exhibited good inhibitory effects against the tested bacteria (Tiwari, P, 2012). The hot water and ethanol extracts of BM leaves showed significant antibacterial properties and can be used in the treatment of infections caused by tested bacteria as complementary and alternative medicine (Sahu, M. C. and R. N. Padhy, 2013). The anti-inflammatory properties of the extract were evaluated by measuring the levels of cytokines IL-1β, IL-6, IL-8, prostaglandin E_2, and metalloproteinases MMP-1, -2, -9, and -10 in cell supernatant. It was found that the extract reduced the secretion of IL-1β, IL-6, and IL-8 pro-inflammatory cytokines by -32%, -33%, and -18%, respectively (Krolikiewicz-Renimel, I. 2013). The extract from BM flowers exhibited anti-inflammatory activity against carrageenin-induced edema in rats. The reduction in paw edema was significant when using carrageenin BM at doses of 600 mg/kg and 800 mg/kg. BM also inhibited granuloma tissue formation significantly and reduced the levels of lysosomal enzymes (SGOT, SGPT, and ALP) and lipid peroxides compared to the control group (Shahavi, V. M. and S. K. Desai, 2008). The major compounds separated from BM flowers include dihydromonospermoside chalcones, butein, monospermoside, and isoliquiritigenin, such as flavone, 7,3',4'-trihydroxyflavone (1), (-)-Butran (3a), (+)-isomonospermoside (5b), and (-)-liquiritigenin (9a), as well as isoflavones such as formononetin, afrormosin, and formononetin-7-O-β-D-glucopyranoside (Chokchaisiri, R, 2009). In toxicity testing, the methanolic extract of BM showed hepatoprotective effects and inhibited the promotion stage by inhibiting oxidative stress and pathogenic pathways (Sehrawat, A, 2006). Research confirms the safety of BM extract, which has the potential for the development of antidiabetic pharmaceuticals (Khan, W, 2017). The evaluation of the toxicity of golden shower seed powder revealed a tendency to induce toxic effects when used in powder form (Donga, S, 2011).

Regarding BM the tincture allowed for a moderate inhibition of AChE, the decoction was able to inhibit α-glucosidase and no activity was observed towards BuChE, α-amylase or lipase. All extracts had a low or moderate inhibition towards tyrosinase, and significant RSA and metal chelating potential. (Baessa, M, 2019).

The ethanolic extract and the acetone fraction of BM stem bark showed the significant wound healing activity which was evident by the increased rate of wound contraction, reduction in the period of epithelialization and increase in collagen deposition (Muralidhar, 2011). The alcoholic bark extract of BM accelerated wound healing action by increasing cellular proliferation and collagen synthesis at the wound site, as evidenced by increase in DNA, total protein and total collagen content of granulation tissues (Sumitra, M, 2005).

The wound healing activity was assessed by the breaking strength in case of incision wounds, epithelialization, and wound contraction in case of excision wound and granulation tissue dry weight, breaking strength and hydroxyproline content in case of dead space wound. The ethanolic extract and the acetone fraction showed the significant wound healing activity on all three wound models. The phytochemical investigations revealed the presence of alkaloids, tannins, flavonoids, phenolic compounds and steroids. The increased rate of wound contraction and hydroxyproline content in the ethanolic extract and the acetone fraction treated animals provides a scientific base to the ethnomedicinal use of BM which is largely attributable to the additive or synergistic effect of their constituents. (Muralidhar, 2011).

Based on in vitro lipoxygenase assay BBM was selected for in vitro compound 48/80 induced mast cell degranulation assay and in vivo lipopolysaccharide induced inflammation in rats. BBM 50, 100 and 200 µg/ml inhibited compound 48/80 induced mast cell degranulation. BBM exhibited dose dependent (50, 100 and 200 mg/kg i.p.) inhibition of lipopolysaccharide-induced increased in total cell count, differential leukocyte count, nitrate-nitrate, total protein and albumin levels in bronchoalveolar fluid (BALF) and myeloperoxidase (MPO) levels in lungs homogenate. (Shirole, 2013).

In the present study, safety of the topical gels and creams containing the flower and leaves extract of BM was examined by means of skin irritation tests on rabbit skin (N = 5 for each type of formulation) and healthy volunteers (N=17). Safety was evaluated based on the Primary Irritation Index (PII) by Draize test. The Draize results showed that the gels and creams were safe in rabbit as PII was observed 0.07 to 0.13 which falls under category of negligible irritant reactions. All reactions disappeared at 72-hr observations. Study on human revealed that none of the volunteers shows the any skin reaction after application of highest concentration of gel (1.5%) and cream as well. Since the cream and gel containing the extract of BM exhibited a somewhat negligible irritant property thus BM formulations can be used safely as topical preparation to treat various skin diseases or as topical cosmetics. (More, B., 2013).

In the province of Udon Thani and surrounding areas, there are many medicinal herbs that can be developed into skincare products. One such plant is the tamarind tree, which is a herbal plant and also a symbol of Udon Thani province. Tamarind flower extract has been reported to contain amino acids and polyphenols that help stimulate the immune system, fight breast cancer, and have skin nourishing properties. It can help moisturize the skin, reduce wrinkles, inhibit collagen destruction, and reduce dark spots and hyperpigmentation.

Currently, products that reduce wrinkles and promote clear, glowing skin are very popular. Natural products are particularly sought after because they are safer and less likely to cause allergic reactions than synthetic chemicals. Therefore, plants are important in the cosmetics industry, not only for their established medicinal properties but also for their potential to be further developed into useful skincare ingredients.

Based on the information above, the researcher is interested in developing BM into a unique line of facial skincare products that promote tourism. BM extract has been shown to

contain amino acids and polyphenols that can stimulate the immune system and potentially prevent breast cancer. In addition, it has skin-nourishing properties that can help to moisturize, reduce fine lines, inhibit collagen breakdown, and reduce pigmentation and freckles. By utilizing this local Thai plant in their skincare products, the researcher aims to create a unique and locally inspired line of products that can attract tourists interested in exploring Thailand's natural resources and beauty traditions.

OBJECTIVE

1. Determine antioxidant activity with DPPH Assay of BM
2. Determine total phenolic content of plant extracts was determined using the FolinCiocalteu colorimetric method of BM
3. Determine quercetin a biomarker using high-performance liquid chromatography of BM

MATERIALS AND METHODS

The process involves sourcing and cleaning the herbs from natural sources, then chopping them into small pieces and drying them in an oven at 50°C. Weighing the herbs according to the specified amount, they are then roughly ground using a disk mill, and macerated with 95% ethanol for 3 days. The mixture is filtered through filter paper, and the residue is re-macerated and filtered twice more. The resulting extracts are combined and concentrated using a rotary evaporator. The percentage yield (%Yield) is calculated, and the antioxidant activity is evaluated using the DPPH assay, which measures the ability to scavenge free radicals. The total phenolic content is also determined, freeze-thaw stability testing,

The formula for calculating the % yield of plant extract (Phrompittayarat et al., 2007) is to compare the amount of extract obtained with the initial amount of plant material used, in order to determine the production cost for each extraction.

$$\% \text{ yield} = \text{weight of plant extract} / \text{dry weight of plant material} \times 100.$$

Antioxidant activity with DPPH Assay (Yuttana Sudjaroen, 2012)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a method used to analyze the ability of a test substance to scavenge free radicals. DPPH• is a stable nitrogen-centered free radical with a violet color in solution. The analysis involves measuring the decrease in absorbance at 517 nm by a spectrophotometer because of adding a free radical scavenger. The DPPH radical is used to test the scavenging activity of the test substance. The DPPH• solution has a violet color in ethanol, and upon receiving an H, it changes to a yellow-colored solution, according to the following equation

$$\text{DPPH}\bullet + \text{H} \square \text{DPPH-H}$$

The measured value will indicate the ability of the test substance to scavenge free radicals in the % inhibition value, calculated using the following equation:

$$\% \text{ inhibition} = [(\text{A Control} - \text{A Test sample}) / \text{A Control})] \text{ x } 100$$

where:

A test sample = Absorbance value of the DPPH solution with the test sample

A control = Absorbance value of the DPPH solution without the test sample

The antioxidant activity of the mango seed kernel extract was evaluated by DPPH assay. The extract was dissolved in distilled water to obtain concentrations of 0.001, 0.01, 0.1, 1, and 10 mg/mL, and tested for radical scavenging activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical in a microplate assay. The SC_{50} value, which is the concentration of the extract required to scavenge 50% of the DPPH radicals, was calculated (Manosroi, et al., 2010).

The total phenolic content of plant extracts was determined using the Folin-Ciocalteu colorimetric method (Maliński MP, 2021)

Analysis of total phenolic compounds content using Folin-Ciocalteu (FC reagent) method involves determining the amount of total phenolic compounds present in the sample extract. The concentration of the total phenolic compounds is determined by measuring the absorbance of the sample at a specific wavelength and comparing it to a standard curve. The standard curve is obtained by measuring the absorbance of different concentrations of a standard solution of gallic acid.

To determine the total phenolic compounds content, the following formula is used:

$$A = [C \times V \times F] / [W] \times [1000\ mL]$$

where A is the amount of total phenolic compounds content (mg/g of sample), C is the concentration of total phenolic compounds from the standard curve (mg/L), V is the volume of sample extract (mL), F is the dilution factor, and W is the weight of the sample (g).

The concentration of the sample extract (C) obtained from the standard curve is substituted into the formula to determine the total phenolic compounds content of the sample.

RESULTS

Percentage of yield of extract

Table showing percentage yield of extract

Percentage of yield of substance	Weight before extraction (g)	Extract weight (g)	yield percentage (% Yield)
BM	1000	132.31	13.231

The result of the antioxidant activity test using the DPPH assay

Table 1. IC_{50} values of test samples (BM extract) compared to standard substances.

	BM	BHT
IC_{50}	49.43±3.4	12.688±2.4

From the DPPH assay testing table, the IC_{50} value is reported to indicate the strength of the sample's antioxidant activity in inhibiting the DPPH radical by 50%. It was found that the BM extract has antioxidant activity with an IC_{50} value of 49.43 ± 3.4 mg/ml.

The results of determination of the total phenolic content of the BM extract

Table 2. The results of the total phenolic content of the BM extract.

crude extract	Total phenolic content ± SD (milligrams of gallic acid per 100 grams of dry weight)
BM	86.01 ± 0.03

From the table, it was found that the crude extract of BM contains a total phenolic content of 86.01 ± 0.03 milligrams per 100 grams of dry weight.

Apparatus and chromatographic conditions (Mehta, J. P., et al. 2014).

The analytical method for determination of markers in SHT extract was carried out by using a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan). The HPLC by using a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) system composinged of aquaternary pump (LC20AD), an autosampler (SIL 20ACHT), a diode array detector (SPD M20A). All markers were separated along a C18 HPLC column (5-micron, 4.6 mm x 250 mm; Phenomenex, USA) protecting by a C18 guard cartridge (5-micron, 4.6 mm x 3 mm; Phenomenex, USA).

The mobile phase composed nsisted of acetronitrile: and 0.1% v/v phophoric acid in water using gradient eluting as follow: 0-20 min 5:95; 21-49 min 50:50; 50-54 min 95:5; 55-60 min 1005:095. The flow rate was set at 1.0 ml/min. The eluted markers were detected at wavelength of 256 nm. The injection volume was 10 µl. The operating temperature was maintained at room temperature.

Result of quercetin content using HPLC in a sample of BM extract

From the chromatogram of the quercetin standard solution with a concentration of 0.1 mg/mL, the retention time was found to be 6.497 minutes (Figure 1). The calibration curve of the quercetin standard solution at different concentrations is shown in Figure 2. Upon analysis, it was found that in the sample of BM with a concentration of 10 mg/mL, the quercetin peak was found in the chromatogram of the sample at 6.166 ± 0.068 minutes (Figure 3), and the amount of quercetin was calculated to be 0.0110 ± 0.0003 mg per 100 mg of sample, as shown in the table.

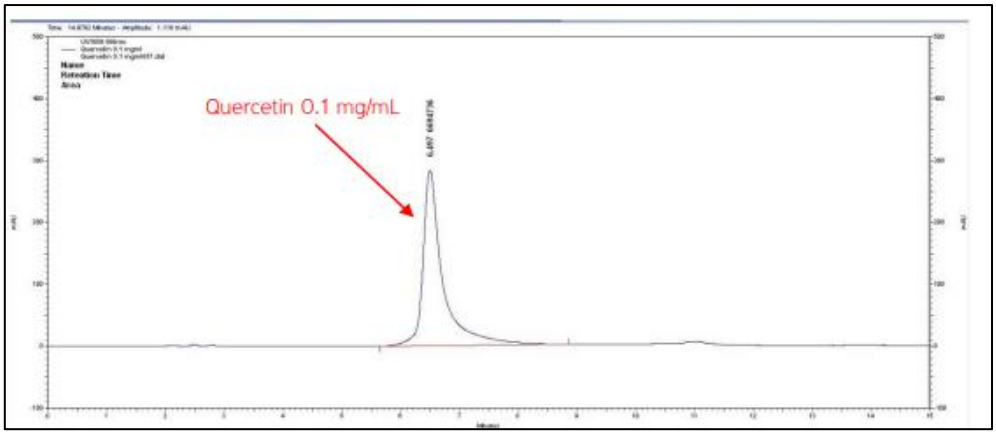

Figure 1 the HPLC chromatogram of quercetin standard at a concentration of 0.1 mg/ml.

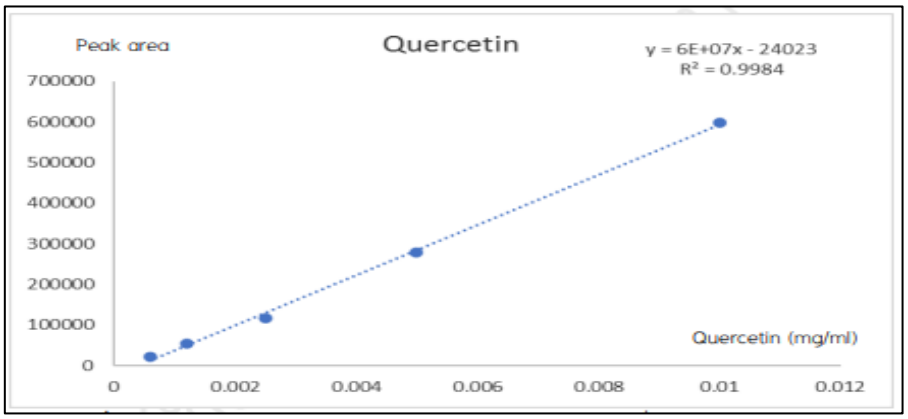

Figure 2 the calibration curve of the quercetin standard at different concentrations.

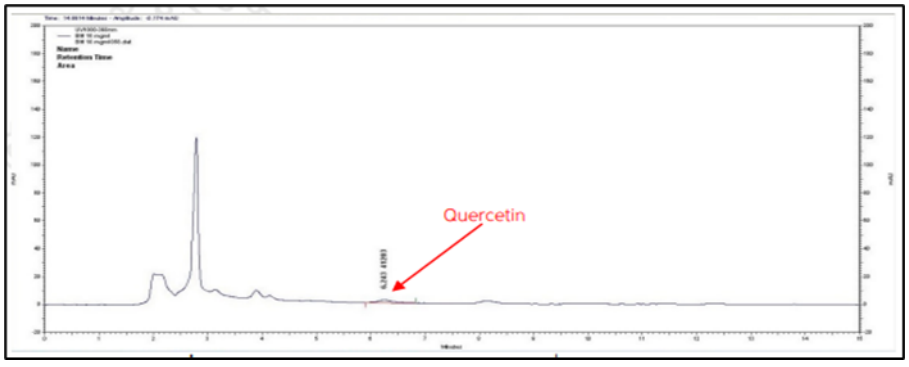

Figure 3 the HPLC chromatogram of the sample extract of from BM at a concentration of 10 mg/ml.

Table showing the details of retention time and quercetin content of the sample of the from BM extract.

crude extract	Quercetin	
	Retention time (min)	Quantity (mg per 100 mg sample, $\bar{x}$±SD)
BM	6.166 ± 0.068	0.0110 ± 0.0003

When analyzing the BM sample using the HPLC technique, it was found that the amount of quercetin was 0.0110 ± 0.0003 mg per 100 mg of the sample.

CONCLUSION

The study successfully demonstrated the antioxidant activity of BM extract through the DPPH assay, showing an IC50 value of 49.43 ± 3.4 mg/ml. The total phenolic content of the BM extract was also determined to be 86.01 ± 0.03 milligrams of gallic acid per 100 grams of dry weight, showcasing its antioxidant potential. Using high- performance liquid chromatography (HPLC), Mehta, J. P., et al. (2014). the research identified quercetin as a biomarker in the BM extract, with a calculated amount of 0.0110 ± 0.0003 mg per 100 mg of sample. This provides valuable insights into the composition of BM extract and its potential skincare applications.

Recommendation

The findings support the idea of developing BM into a unique line of facial skincare products that can help moisturize skin, reduce fine lines, inhibit collagen breakdown, and address pigmentation issues. However BM flower bloom once a year, during December - February. We should plan the harvest of raw materials to be consistent with production.

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