

# PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITY OF FINGER-ROOT (*BOESENBERGIA ROTUNDA* L.) RHIZOME ON SKINCARE APPLICATION

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## Abstract

Traditional plants are most attractive ingredient of skincare products. The research purposes were to screen phytochemical constituents contained in ethanol extract of fingerroot (*Boesenbergia rotunda* L.) rhizome; and to evaluate in vitro biological activities on skincare, including antioxidant, anti-inflammation, anti-melasma, anti-wrinkle and its cytotoxicity against human skin fibroblast cells. Ethanol extract of fingerroot rhizome (FRE) was positive for triterpenes determined by qualitative phytochemical screening. Total phenolic content (TPC) and total flavonoid content (TFC) were 91.51±0.76 milligram of gallic acid equivalent per gram and 21.05±1.53 milligram of quercetin equivalent per gram, respectively. FRE was scavenged only DPPH radicals (IC<sub>50</sub> = 3.80 ±0.04 mg/ml). FRE (1 mg) was demonstrated in vitro anti-inflammation property by reduced nitric oxide (NO) production (24.5±4.07%) from LPS-induced macrophage cells. FRE was also inhibited tyrosinase (IC<sub>50</sub> = 0.11 ±0.03 mg/ml) and can compared with kojic acid. Therefore, FRE was lack of anti-elastase activity. FRE (0.0001-1.0 mg/ml) was non cytotoxic against human skin fibroblasts. In this study, we were reported in vitro anti-melasma activity of FRE as anti-tyrosinase inhibitor, therefore it was lack of anti-wrinkle activity due to unable to inhibit elastase enzyme. The finding was supporting that FRE can be ingredient of skincare products as antioxidant, anti-inflammation, anti-tyrosinase inhibitor and skin disorder relive without harmful effect.

**Keywords:** *Boesenbergia rotunda* L., fingerroot, anti-tyrosinase, anti-inflammation.

## INTRODUCTION

The skin is rapid growing organ, which is controls various physiological functions, such as environmental protection and adaptation, responding of stimuli, water control, vitamin D synthesis and body barrier [1]. Skin characteristic is also important on social interface among personals [2]. Skin aging is affected from multi-factors including genetic variation, sunlight contact, mechanical stress and physiological alteration. Chronic exposure of UV radiation, especially UV-B range is cause photoaging, which is commonly cause of skin [3, 4]. UV is responsible for free radical generation, which is cause lipid peroxidation, biomolecular damage and inflammatory responses as premature skin aging and cancer [4, 5].

Signs of skin deterioration in photoaging are including wrinkle, dryness, roughness, shallowness, dyspigmentation and histological change [6, 7]. In skincare products, antioxidants are excellent ingredients, which are prevent the effects of free radical damage [8].

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Lightening agents are control melanin production that activated from sunlight and environments. There are act as inhibitors on tyrosinase, melanin pigments and melanocyte maturation [9]. Inflammation is one of the most significant factors in the pathogenesis of acne. Anti-inflammatory agents from different plant species are used to control pain, swelling and redness of skin inflammation. Reduction of inflammatory molecules and free radicals from plant extract is also prevent mitochondrial dysfunctions and cellular aging through stimulate antioxidant system [10, 11]. Traditional plants are most attractive ingredient of skincare products, which is due to low possibility of adverse effects and lead to consumer acceptances. Natural skincare products are also largest section of cosmetic markets followed by hair care, make-up and oral products [12]. Due to low toxicity, natural plant origins are providing antioxidant, anti-melasma and anti-inflammation properties, which can be formulate as ingredients [13, 14].

Fingerroot (*Boesenbergia rotunda* L.) in Thai namely “krachai”, is a perennial herb that grows mainly in certain tropical countries. Fresh rhizomes have been used as a food spice and as a local medicine for the treatment of colic, dry cough rheumatism, and muscular pains [15]. Phytochemical properties of *B. rotunda* are related with skincare including anti allergic, antibacterial, anti inflammatory, antioxidant and wound healing [16]. *B. rotunda* extract is also exhibited strong protection against UV irradiation in human skin fibroblast cells [17] and in mice [18]. As previous studies, *B. rotunda* extract is able to natural ingredient of skincare products. Thus, this study was purposed to screen phenolic compounds and flavonoids contained in ethanol extract of *B. rotunda*; and to evaluate in vitro biological activities on skincare, including antioxidant, anti-inflammation, anti-melasma, anti-wrinkle and its cytotoxicity against human skin fibroblast cells. The finding will support *B. rotunda* extract as new natural ingredient in cosmetic formulae.

## MATERIALS AND METHODS

### Sample Authentication and Extraction

*B. rotunda* is a perennial with a short stem that is replaced by pseudostems, formed by leaf sheaths growing up to 50 cm tall. There are 3-4 leaves which are 7-11 cm in width and 25-50 cm in length, which are not divided, oval or elongate shape. Rhizomes are finger appearance and light brown color. Inside of rhizomes are ovoid-globose, yellow color and strongly aromatic [19]. Rhizomes of fingerroot were purchased from local markets in Bangkok, Thailand. Botanical identification and sample voucher were done by senior physiochemist. Air-dried fingerroot rhizomes ( $\square$  45°C) were ground and extracted with 95% ethanol by maceration during 72 h. Ethanol extract (yield  $\square$  10-12%) was filtered and evaporated by vacuum-rotary evaporator. All laboratories were done as in vitro, which was exempted on approval of Ethical Committees, Suan Sunandha

Rajabhat University, Bangkok, Thailand (COE. 2-392/2022).

### Phytochemical Screening

#### Qualitative Phytochemical Screening

Bioactive compounds of fingerroot rhizome extract (FRE) including alkaloids, anthraquinones, carotenoids, flavonoids, glycosides, tannins, xanthenes, triterpenes and steroids were determined by colorimetric methods based on chemical reactions [20-24].

#### Determination of total phenolic and flavonoid contents

Total phenolic content (TPC) and total flavonoid content (TFC) of FRE were evaluated according by colorimetric methods. TPC was monitored chemical reaction of phenolic compounds and Folin-Ciocalteu reagent and absorbance of mixture measured at 760 nm. Gallic acid was used for standard curve and result was reported as milligram of gallic acid equivalent (GAE) per gram. TFC was monitored chemical reaction of flavonoids and aluminium chloride reagent and absorbance of mixture measured at 420 nm. Quercetin was used for standard curve and result was reported as milligram of quercetin equivalent (QE) per gram [25-28].

#### In Vitro Antioxidant Assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: ability of FRE on DPPH radical scavenging was measured by monitoring of DPPH absorbance reduction. Negative and positive controls were initial concentration of DPPH radicals ( $6 \times 10^{-5}$  M) and ascorbic acid, respectively [25]. Lipid peroxidation inhibition (LPI) assay: ability of FRE on inhibition of lipid peroxidation was measured by monitoring of ferric iron-thiocyanate complex absorbance. Positive control was  $\alpha$ -tocopherol [26]. Nitric oxide (NO) radical scavenging assay: ability of FRE on NO radical scavenging was measured by monitoring of Griess reagent reaction absorbance. Positive control was ascorbic acid [27]. Micro-titer plate reader was used to determined absorbance of mixture at maximum absorbance wavelength ( $\square$ max), which was appropriated to each assay. Results from all assays were calculated from reference formulae with triplicate absorbance measurements, which were reported as 50% inhibitory concentration (IC50) of FRE.

#### Assay for Anti-Inflammation

Anti-inflammation activity of FRE was evaluated by reduction of NO produced from lipopolysaccharide (LPS)-induced macrophage cells. Mouse macrophage cell (RAW264.7) was obtained from Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand and cultured in Dulbecco's modified Eagle's medium, containing fetal bovine serum (10%), penicillin (100 U/mL), and streptomycin (100  $\mu$ g/mL) by humidified incubator (5% CO<sub>2</sub>) at 37 °C for 24 h. Cell culture was transferred to a 24-well plate and suspended to  $1 \times 10^5$  cells with 500  $\mu$ L of medium/well. Each cell culture well was treated with different concentration of FRE (or

controls) for 1 h and with LPS for an additional 24 h. After that supernatant of culture medium from each well was determined for NO production. Culture medium (50 µL) and of Griess reagent (100 µL) was mixed in a 96-well plate and stand for 10 min at room temperature. Absorbance of reaction mixture was monitored by micro-titer plate reader [28, 29]. Positive control was triamcinolone acetonide (0.1 mg/ml). Results was calculated from reference formula with triplicate absorbance measurements, which was reported as 50% inhibitory concentration (IC50) of FRE.

#### Assays for Enzymatic Inhibition

##### Anti-Tyrosinase Enzyme

Mushroom tyrosinase enzyme, tyrosine and kojic acid were purchased from Sigma-Aldrich, Thailand. Briefly, FRE concentration was adjusted with 10% (v/v) DMSO, which was 0.001, 0.01, 0.1, 1 and 10 mg/ml. Anti-tyrosinase activity of FRE sample was determined by dopachrome method. Enzymatic inhibition was monitored absorbance of dopachrome reduction by micro-titer plate reader. Results was calculated from reference formula with triplicate absorbance measurements, which was reported as 50% inhibitory concentration (IC50) of FRE against tyrosinase enzyme. Positive control was kojic acid [30].

##### Anti-Elastase Enzyme

Elastase enzyme, N-succinyl-Ala-Ala-Ala-p-nitroanilide and epigallocatechin gallate (EGCG) were purchased from Sigma-Aldrich, Thailand. Briefly, FRE concentration was adjusted with 10% (v/v) DMSO, which was 0.001, 0.01, 0.1, 1 and 10 mg/ml. While, EGCG was adjusted with Tris-HCl buffer in same concentration range. Enzymatic inhibition of elastase was monitored absorbance reduction of p-nitroanilide by micro-titer plate reader. p-nitroanilide was product from N-succinyl-Ala-Ala-Ala-p-nitroanilide hydrolyzed by elastase enzyme. Results was calculated from reference formula with triplicate absorbance measurements, which was reported as 50% inhibitory concentration (IC50) of FRE against elastase enzyme. Positive control was EGCG [31].

##### Assay for Cytotoxicity

FRE was dissolved in Dulbecco's modified Eagle's medium, containing DMSO (10%), 10 fetal bovine serum (10%), penicillin/streptomycin (1%), filtered with 0.2 µm membrane and the adjusted concentration within each well of micro-titer plate by sterile culture medium (0.0001, 0.001, 0.01, 0.1 and 1.0 mg/ml). Each sample or control well was added human skin fibroblast cells (2.2-3.3 x 10<sup>4</sup> cells/ml) and continued incubation (48 h). Cytotoxicity was evaluated by sulforhodamine B staining with vital cells. Cell viability of human skin fibroblast cells against FRE was represented as percentage of cell vitality, which was calculated from four-time repeated experiments. Cytotoxicity positive control was sodium lauryl sulfate and

negative control was Dulbecco's modified Eagle's medium [32].

#### STATISTICAL ANALYSIS

Screening of bioactive compounds were reported as qualitative data. Antioxidant, anti-inflammation, enzymatic inhibitions, and cytotoxicity of FRE were demonstrated by descriptive analysis and compared with controls.

## RESULTS

FRE was semi-solid appearance, orange-brown color and strongly aromatic odor. In qualitative of phytochemical screening in FRE, only triterpene was determined positive. However, TPC and TFC contained in FRE were 91.51±0.76 mg of GAE/g and 21.05±1.53 mg of QE/g, respectively. FRE was scavenged only DPPH radical and (IC50 = 3.80 ±0.04 mg/ml) and can comparable with ascorbic acid. Therefore, FRE was poorly inhibited lipid peroxidation and lack of NO radical scavenging activity (Table 1). FRE was possessed anti-inflammation activity by NO production reduction produced from LPS-induced macrophage cells (24.5±4.07%). FRE (1 mg/ml) was 0.78 time of anti-inflammation activity when compared with triamcinolone acetonide (30.73±3.56%). Therefore, anti-inflammation activity of FRE at high concentration was undetermined due to color disturbance on absorbance determination (Table 2). FRE was inhibited only tyrosinase (IC50 = 0.11 ±0.03 mg/ml) and can compared with kojic acid, commercial anti-melasma agent. Therefore, FRE was lack of anti-elastase activity (Table 3). FRE was non cytotoxic against human skin fibroblasts at all range of FRE concentrations. There was implied that FRE was safe to skin when use FRE as ingredient of skincare products (Table 4).

Table 1: Active constituents and antioxidant activity of fingerroot rhizome extract (FRE) a

Sample / Assay	TPC <sup>b</sup>	TFC <sup>c</sup>	DPPH	NO	LPI
FRE	91.51±0.76	21.05±1.53	3.80 ±0.04	NA	>1,000
α-tocopherol	-	-	-	-	0.02±0.0
Ascorbic acid	-	-	0.04±0.01	0.07±0.0	-

a Antioxidant activity was demonstrated as IC50 (mg/ml); b Total phenolic content (TPC) was demonstrated as mg of GAE per g; c Total flavonoid content (TFC) was demonstrated as mg of QE per g; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; NO = nitric oxide radical scavenging activity; LPI = lipid peroxidation inhibition activity; NA = no activity

Table 2: Anti-inflammation activity of fingerroot rhizome extract (FRE)

Sample (mg/ml)	Inhibition of NO production (%) <sup>a</sup>					
	0.0001	0.001	0.01	0.1	1.0	10.0
FRE	-	15.02±3.15	21.78±2.85	23.71±4.12	24.03±4.07	9.55±3.39 <sup>b</sup>
Triamcinolone acetonide	18.45±4.29	24.12±3.62	25.38±4.75	27.90±4.86	30.73±3.56	-

a Inhibition of NO production from lipopolysaccharide (LPS)-induced macrophage demonstrated as percentage;

b Color of FRE was disturbed NO measurement

Table 3: Anti-tyrosinase and anti-elastase activities of fingerroot rhizome extract (FRE)

Sample / Assay	Anti-tyrosinase <sup>a</sup>	Anti-elastase <sup>a</sup>
FRE	0.11±0.03	NA
Kojic acid	0.01±0.00	-

epigallocatechin gallate	-	0.29±0.08
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a Enzymatic inhibition was represented as 50% of inhibitory concentration, IC50 (mg/ml)

NA = no activity

Table 4: Cytotoxicity of fingerroot rhizome extract (FRE) against human skin fibroblast

Sample (mg/ml)	Cell survival rate (%)				
	0.0001	0.001	0.01	0.1	1.0
FRE	111.51±4.86	110.86±3.35	106.18±3.02	105.57±2.54	93.62±2.93
Sodium lauryl sulfate	99.52±3.42	96.64±2.81	96.29±6.06	94.42±1.05	9.02±0.66

## DISCUSSION

Many antioxidants are mainly divided to water-soluble and lipid-soluble antioxidants. Ascorbic acid, glutathione, coffeeberry, resveratrol, and green tea are common water-soluble antioxidants. While, retinol,  $\alpha$ -tocopherol and coenzyme Q10 are lipid-soluble antioxidants [33]. This antioxidant activity of FRE was lower than methanol extract of fingerroot rhizome, which had reported by previous study. However, FRE in this study was use ethanol extraction, which were safer and more preferable on application as skincare rather than methanol extract [34]. Several different plant species have represented anti-inflammatory activity, which are including turmeric, licorice root, oats, lavender, calendula, chamomile, witch hazel, yarrow and oak bark plants include turmeric, licorice root, oats, feverfew, willow bark, lavender, calendula, chamomile, witch hazel, yarrow and oak bark [10]. Our finding on anti-inflammation of FRE was corresponded to previous studies and there was also related to anti-allergic activities. Thus, fingerroot rhizome can use in relieve of skin disorders including dermatitis, rash and swelling. These biological activities may be affected from chalcones and other flavonoids contained in fingerroot rhizome [35, 36]. In addition, fingerroot rhizome is contains aromatic oils, which can inhibit some of pathogenic bacteria, which can relieve skin infection [37]. Ethanol extract of fingerroot rhizome is also activate wound healing in rat, which is implied that there can increase curable rate on skin injury or damage [38]. In this study, we were reported in vitro anti-melasma activity of fingerroot rhizome extract as anti-tyrosinase inhibitor, therefore it was lack of anti-wrinkle activity due to unable to inhibit elastase enzyme. Hence, our finding was supporting that ethanol extract of fingerroot rhizome can be ingredient of skincare products as antioxidant, anti-inflammation, anti-tyrosinase inhibitor and skin disorder

relieve without harmful effect.

## CONCLUSION

Ethanol extract of fingerroot rhizome (FRE) was inhibited DPPH radical, and possessed anti-inflammation and anti-tyrosinase activities. FRE was non-cytotoxicity against human skin fibroblasts.

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## CONFLICT OF INTEREST

Non conflict of interest was found in this study.

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