

# EVALUATION OF RICE BRAN OIL FROM RICEBERRY RICE (ORYZA SATIVA L.) AS ACTIVE INGREDIENT IN SKINCARE APPLICATION

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## Abstract

Rice bran oil (RBO) from riceberry rice is process from rice bran and contain health promoting benefits due to their biological activities. This study was aimed to determine phenolic, flavonoid and tannin contents of RBO and evaluate biological activities including in vitro antioxidant, anti-inflammation, anti-microbials, anti-tyrosinase, anti-elastase, wound-healing promotion and cytotoxicity of RBO against human skin fibroblast cells. Cold (mechanical) pressing was commercialized RBO extraction. Total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) of RBO were determined by colorimetric absorbance measurements of reaction mixture. Antioxidant property of RBO was evaluated by DPPH, NO radical scavenging and lipid peroxidation inhibition. In vitro anti-inflammation of RBO was evaluated by inhibition of NO production of LPS-induced macrophages. Anti-tyrosinase and anti-elastase of RBO were also evaluated according colorimetric methods. RBO cytotoxicity and wound healing was evaluated against human skin fibroblast cells by sulforhodamine B staining with survival cells and closed gap of cell cluster scratching, respectively. Anti-microbials of RBO was determined against four skin pathogens by disc diffusion method. TPC, TFC and TTC contained were  $1.41 \pm 0.11$  mg of GAE/g,  $6.61 \pm 0.11$  mg of QE/g and  $47.89 \pm 0.75$  mg of TE/g, respectively. RBO was scavenged DPPH and NO radicals ( $IC_{50} = 2.42 \pm 0.11$  and  $69.9 \pm 5.9$  mg/ml). RBO (1.0 mg/ml) was exhibited in vitro anti-inflammation and almost similar to positive control. RBO was slightly activated cell proliferation at 0.0001 to 0.1 mg/ml and had cytotoxicity at 1.0 mg/ml. However, there was lack of anti-tyrosinase, anti-elastase anti-microbial and wound healing activities.

**Keywords:** anti-inflammation, antioxidant, cell proliferation, cytotoxicity, *Oryza sativa* L., riceberry rice.

## INTRODUCTION

Asian rice (*Oryza sativa* L.) is one of most domestic specie, which is growing in worldwide with long history. Various of rice varieties and different of nutritive value are depending on genetic adaptation, geographical distribution, soil components, climatic condition and agronomic practices. Whole rice grain is composed of several components including edible part (70%), hull (20%), bran (7-8.5%) and rudiment (2-3%) [1, 2]. Rice bran oil (RBO) is process from rice bran and contain health promoting benefits. RBO is favored cooking oil in Asian countries, which made from mechanical pressing [3].

RBO is a healthy oil from recommendations of American Heart Association (AHA) and World Health Organization (WHO), due to its balancing of fatty acid ratio in the saponifiable fraction [saturated fatty acid, SFA (0.6): monounsaturated fatty acid, MUFA (1.1): polyunsaturated fatty acid, PUFA (1.0)] and other bioactive compounds contained in unsaponifiable fraction [4, 5].  $\gamma$ -oryzanol is most characterized phytochemical compound contained in RBO.

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Others bioactive compounds are  $\alpha$ -tocopherol,  $\gamma$ -tocopherol,  $\gamma$ -tocotrienol, squalene and phytosterols (including  $\beta$ -sitosterol, campesterol, stigmasterol and isofucosterol) contained in RBO [6]. In addition, rice brans from pigmented rice cultivars are contains anthocyanins and phenolic compounds [7, 8], which are possesses antioxidant property [9, 10]. Pigmented rice consumption can provide health benefits due to their biological activities i.e., anti-allergic, anti-mutagenic and anti-carcinogenic properties [11, 12]. In case of fine particles are obtained from the physical refining of organic cold pressed riceberry rice bran oils, which can be utilized as cosmetic ingredient, such as body mask exhibited antioxidant property [13]. Hence, pigmented rice is trend to become potential source for nutraceutical and cosmetic products. Since, animal testing in cosmetics is trend to abrogated as worldwide agreement, in silico and in vitro are become important in cosmetic products as on biochemical and cellular levels [14, 15, 16]. This work was aimed to determine phenolic, flavonoid and tannin contents of RBO as cosmetic ingredient screening and evaluate biological activities, which were related on skincare application, including in vitro antioxidant, anti-inflammation, anti-microbials, anti-tyrosinase (anti-melasma), anti-elastase (anti-wrinkle), and wound-healing promotion. Cytotoxicity of RBO was also evaluated against human skin fibroblast cells. This data can be supporting of RBO, which is a source of high value-added ingredient with skincare properties.

## MATERIALS AND METHODS

### Rice and RBO Preparation

Riceberry cultivar, Thai non-glutinous black rice was collected from agricultural community enterprise, Sing Buri province, Central of Thailand. The paddy was harvested and dehumidified by hot air (40 °C) until moisture content was reduced (< 14%). Then, paddy was removed husk and milled by local milling machine. Paddy milling was yielded 10% of rice bran, which was filtered prior to RBO extraction. Cold (mechanical) pressing was commercialized RBO extraction, which used to prepare RBO within this study [17]. Experimental studies on RBO had exempted by Suan Sunandha Rajabhat University Ethic Committees, Thailand (COE.1-098/2022).

### Determination of Total Phenolic, Flavonoid and Tannin Contents

Total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) of RBO were determined by chemical reactions based on colorimetric methods. Absorbance of reaction mixture for TPC, TFC and TTC measurements were done at 760, 420 and 725 nm, respectively. Gallic acid, quercetin and tannin was used to as standard curve; and TPC, TFC and TTC were reported as mg of gallic acid equivalent (GAE), quercetin equivalent (QE) and tannin equivalent (TE) per g, respectively [18, 19, 20].

### Determination of Antioxidant Activity

DPPH radical scavenging activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in present of RBO (0.01-1 mg/ml) was reduced DPPH absorbance. DPPH radical ( $6 \times 10^{-5}$  M) and ascorbic acid were used as negative and positive controls, respectively [21]. Lipid peroxidation inhibition (LPI) activity: lipid peroxidation in present of RBO (0.001-10 mg/ml) was reduced absorbance of ferric iron-tyrosinate complex.  $\alpha$ -tocopherol was used as positive control [22]. NO radical scavenging activity: nitric oxide (NO) radical in present of RBO (0.01-1 mg/ml) was reduced absorbance of Griess reagent reaction. Ascorbic acid was used as positive control [23]. Absorbance reaction mixture of all assays were monitored by micro-titer plate reader at maximum absorbance wavelength ( $\lambda_{max}$ ). Results was calculated from triplicate measurements and interpreted as 50% inhibitory concentration (IC<sub>50</sub>) of RBO.

### In Vitro Anti-Inflammatory Test

Monitoring of NO inhibition produced from lipopolysaccharide (LPS)-induced macrophage cells was used to evaluate anti-inflammatory activity of RBO. Briefly, mouse macrophage cell (RAW264.7) was obtained from Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. Cells were seeded in high-glucose Dulbecco's modified Eagle's medium (containing 10% fetal bovine serum, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin) and incubated at 37 °C (containing 5% CO<sub>2</sub>) in humidified incubator. After overnight culture in a 24-well plate ( $1 \times 10^5$  cells/well, 500  $\mu$ L medium/well), the cells were pre-treated with herbal mixture for 1 h and lipopolysaccharide (LPS) for an additional 24 h, the culture supernatant from each well was collected and used to measure NO production. 50  $\mu$ L of culture medium and 100  $\mu$ L of Griess reagents were added and incubated in a 96-well plate at room temperature (10 min). Absorbance of reaction mixture was then measured using a microplate reader. Triamcinolone acetonide (0.1 mg/ml) was used as positive control [24, 25]. Results was calculated from triplicate measurements and interpreted as 50% inhibitory concentration.

### In Vitro Enzymatic Inhibitory Tests

#### Anti-Tyrosinase Enzyme

Mushroom tyrosinase enzyme, tyrosine and kojic acid were purchased from Sigma-Aldrich (Sigma-Aldrich, Thailand). RBO was diluted with 10% (v/v) DMSO and tested concentrations were 0.001, 0.01, 0.1, 1 and 10 mg/ml. Each dilution was evaluated anti-tyrosinase activity by dopachrome method. Inhibition of tyrosinase enzyme was monitored the reduction of dopachrome by microplate reader. Activity was represented as 50% inhibitory concentration (IC<sub>50</sub>) of extract against tyrosinase enzyme and kojic acid was used for standard [26, 27].

#### Anti-Elastase Enzyme

Briefly, RBO was diluted with 10% (v/v) DMSO and tested concentrations were 0.001, 0.01, 0.1, 1 and 10 mg/ml. The

standard control, epigallocatechin gallate (EGCG) was diluted with Tris-HCl buffer and concentrations were 0.0001, 0.01, 0.1 and 10 mg/ml. Then, inhibitory of elastase enzyme by test samples and controls were monitored decrement of p-nitroanilide, which was hydrolyzed from elastase enzyme. Inhibitory activity was represented as 50% inhibitory concentration (IC<sub>50</sub>) of extract against elastase enzyme and EGCG was used for standard control [27].

### Evaluation of Cytotoxicity and Wound Healing

**Test of skin cell cytotoxicity:** Extract was dissolved in Dulbecco's modified eagle medium (DMEM) contained with 10% DMSO, 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Sample solution was sterile by 0.2 µM membrane filter and diluted to 0.0001, 0.001, 0.01, 0.1 and 1.0 mg/ml by sterile culture medium as previous described within micro-titer plate. Each diluted 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 survival cells. Cell viability of human skin fibroblast cells against extract was represented as percentage of cell survival, which was calculated from four-time repeated experiments. Sodium lauryl sulfate was used as positive control on cytotoxicity [28].

**Test of wound healing activation:** This assay was done after skin cell cytotoxicity test and maximum concentration of extract without cytotoxicity was used on activation of wound healing [28]. Human skin fibroblast cells were treated with extract and scratching of cell cluster was performed gap and can monitored and measured by high resolution of microscope (×40) within 0, 6, 24 and 24 h. DMEM and DMEM contained with 10% DMSO (10% DMSO) were used as negative controls. Ascorbic acid (1 mg/ml) was used as positive control on wound healing activation [29].

### Antimicrobial Activity

Skin pathogenic bacteria including *Staphylococcus aureus*, and *Cutibacterium acnes*; and skin pathogenic yeast including *Candida albicans* and *Malassezia furfur* were provided from Faculty of Medicine, Chiang Mai University and Institute of Scientific and Technological Research (TISTR), Thailand, respectively. Each bacterium and yeast were inoculated in brain heart infusion (BHI) and potato dextrose agar (PDA) plates, respectively. RBO was dissolved with 95% ethanol and applied to 6 mm-filter paper discs at 0.05, 0.5 and 5.0 mg, respectively. Disc diffusion

method was performed by applying of tested discs and control discs within same plate, and incubated at 37±1 °C for 24-48 h. Positive control discs were antibiotic discs including erythromycin (0.015 mg), clindamycin (0.002 mg), fluconazole (0.025 mg) and ketoconazole (0.2 mg) for *S. aureus*, *C. acnes*, *C. albicans* and *M. furfur*, respectively. Negative control disc was contained 95% ethanol. Diameter of inhibition zone (mm) of tested disc was measured and compared with controls [30].

## STATISTICAL ANALYSIS

Bioactive contents antioxidant, anti-inflammation, enzymatic inhibitions, skin cell cytotoxicity and proliferation, and anti-microbial activities of RBO were calculated and reported by descriptive analysis. All biological activities of RBO were reported and compared with controls.

## RESULTS

RBO characteristic was dark-brown, sticky-liquid with special odor. TPC, TFC and TTC contained in RBO were 1.41±0.11 mg of GAE/g, 6.61±0.11 mg of QE/g and 47.89±0.75 mg of TE/g, respectively. RBO was scavenged DPPH and NO radicals (IC<sub>50</sub> = 2.42 ±0.11 and 69.9±5.9 mg/ml), therefore, there was poorly inhibited lipid peroxidation (Table 1). RBO (1.0 mg/ml) was exhibited in vitro anti-inflammation by decreased NO production from LPS-induced macrophage cells (RAW 264.7), and this activity was almost similar to positive control. However, there was unable to inhibit key cosmetic enzymes including tyrosinase (as anti-melasma) and elastase (as anti-wrinkle) enzymes (Table 2). RBO was also lack of anti-microbial activity against skin pathogens, including *S. aureus*, *C. acnes*, *C. albicans* and *M. furfur* according by disc diffusion method (data not shown). RBO (1.0 mg/ml) had cytotoxicity effect on human skin fibroblasts (Fig. 1), which was slightly activated cell proliferation up to 0.1 mg/ml as maximum concentration (Table 3). No wound healing was occurred from RBO treated cells when compared with controls including vitamin C (positive control); DMEM and 10% DMSO within DMEM (negative controls). Gap of fibroblast cells incubated with RBO (48 h) was still similar to negative controls, while there was closed when treated with vitamin C (Fig. 2)

**Table 1:** Phytochemical and antioxidant screenings of rice bran oil (RBO) from riceberry rice

Sample / Test	TPC <sup>a</sup>	TFC <sup>a</sup>	TTC <sup>a</sup>	DPPH <sup>b</sup>	NO <sup>b</sup>	LPI <sup>b</sup>
RBO	1.41±0.11	6.61±0.11	47.89±0.75	2.42 ±0.11	69.9±5.9	> 1,000
Ascorbic acid	ND	ND	ND	0.02±0.00	0.07±0.00	ND
α-tocopherol	ND	ND	ND	ND	ND	0.02±0.00

<sup>a</sup>Total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) were reported as mg of gallic acid equivalent (GAE)/g, mg of quercetin equivalent (QE)/g, and tannin equivalent (TE)/g, respectively.

<sup>b</sup> 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging, nitric oxide (NO) and inhibition of lipid peroxidation were represented as 50% of inhibitory concentration (IC<sub>50</sub>)

ND = Not determined

**Table 2:** In vitro anti-inflammation and enzymatic inhibitions of RBO from riceberry rice

Sample / Test <sup>a</sup>	Anti-inflammation <sup>b</sup>	Anti-tyrosinase <sup>c</sup>	Anti-elastase <sup>c</sup>
RBO	34.73±4.50	NA	NA
Triamcinolone acetonide	36.11±3.00	ND	ND
Kojic acid	ND	0.007±0.00	ND
Epigallocatechin gallate (EGCG)	ND	ND	0.26±0.01

<sup>a</sup> Concentrations of each sample were ranged from 0.001 to 10.0 mg/ml

<sup>b</sup> Anti-inflammation of sample was monitored inhibition of NO production (%) from lipopolysaccharide (LPS)-induced macrophage

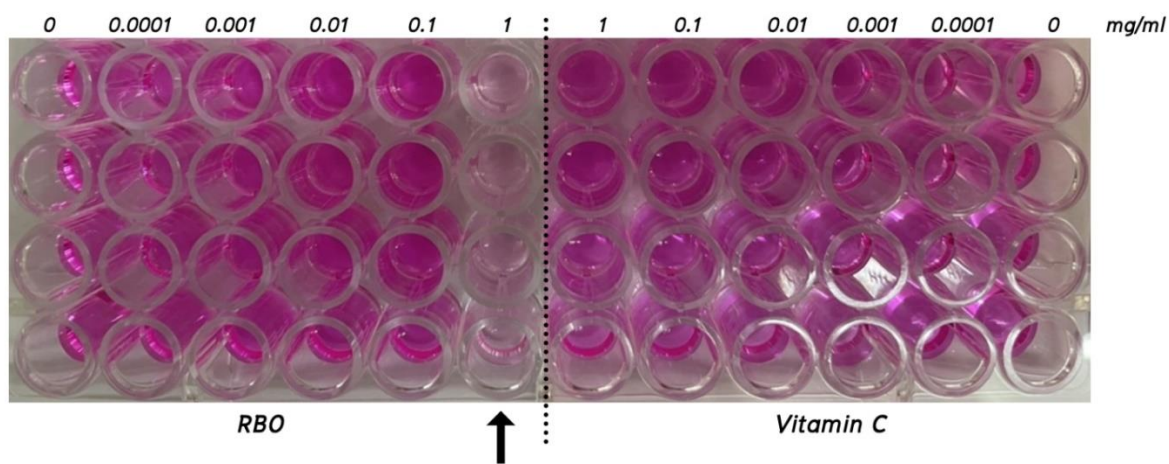
<sup>c</sup> Anti-tyrosinase and elastase of sample was represented as 50% of inhibitory concentration (IC<sub>50</sub>)

NA = No activity; ND = Not determined

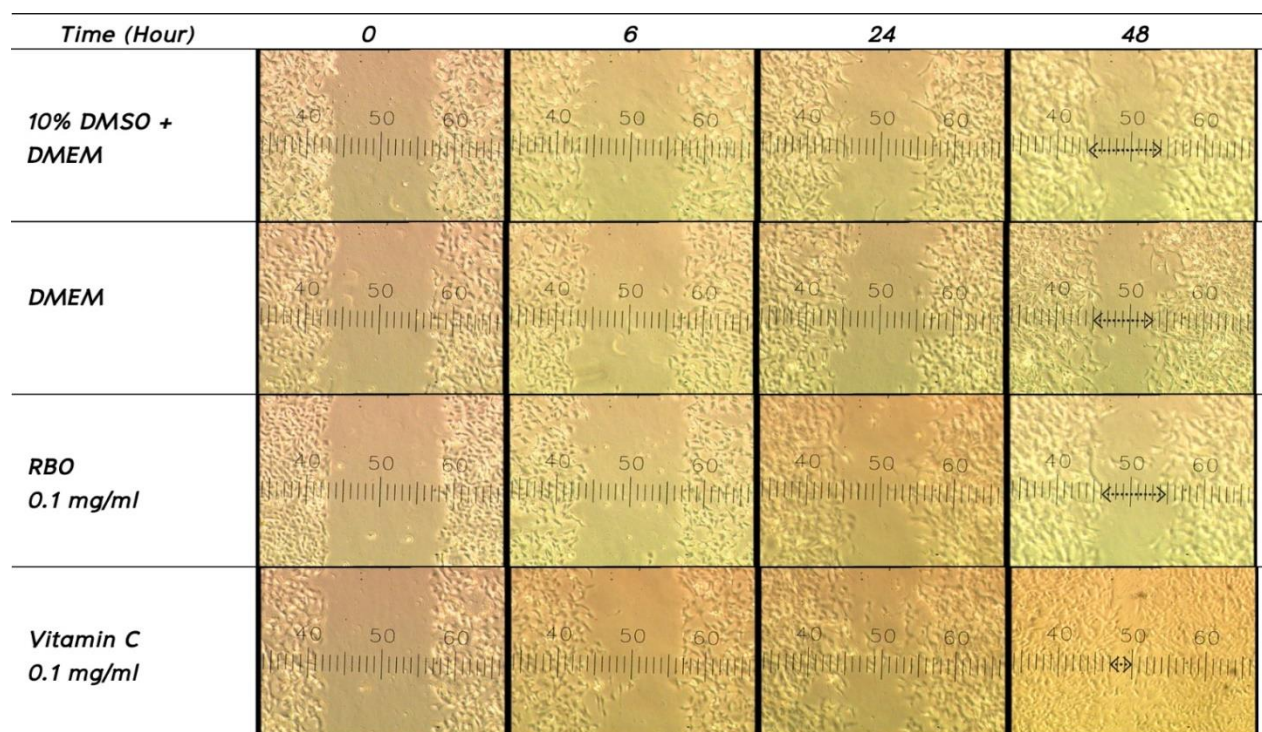
**Table 3:** Cell viability of human skin fibroblasts after incubated with RBO (0.0001-1.0 mg/ml) <sup>a</sup>

Sample / Conc. (mg/ml)	Cell viability of human skin fibroblasts (%)				
	0.0001	0.001	0.01	0.1	1.0
RBO	109.19±2.1	115.98±1.1	110.46±3.2	108.86±3.5	27.62±2.2
Vitamin C	97.59±3.5	98.74±2.7	95.26±1.8	91.12±1.7	84.65±3.7

<sup>a</sup> Result was represented as percentage (mean ±SD) calculated from four-time repeated experiments



**Fig. 1** Cytotoxicity of RBO at 1.0 mg/ml on human skin fibroblasts, which were unable to staining with cell vital color, sulforhodamine B (arrow pointed)



**Fig. 2** Wound healing test was represented scratching gap of cell cluster ( $\times 40$ ) after incubated with RBO and controls (48 h). RBO was lack of wound healing activity by unable of gap closing, which was similar to negative controls. While, cells and their clusters were grown and gap had closed after incubated with vitamin C.

## DISCUSSION

RBO bioactive components are namely  $\gamma$ -oryzanol and tocotrienol, which can inhibit cancer cell growth and induce apoptosis pathway via their antioxidant and anti-inflammatory properties [31, 32]. Reactive oxygen species (ROS) are reactive agents or radicals, which have producing from mitochondria dysfunction. They can biochemical oxidations, including protein, lipid and DNA, which are initiating several chronic diseases. RBO also can inhibit inflammatory pathway by inhibiting of the nuclear transcription factor kappa B (NF- $\kappa$ B), which is played role on inflammatory diseases; and slowdown action of tumor necrotic factor-alpha (TNF- $\alpha$ ), which is protect cell homeostasis [33]. Oxidative damage of skin is cause ultraviolet (UV) radiation exposure from sunlight especially without protective equipment or agent.  $\gamma$ -oryzanol extracted from RBO can be skin antioxidant, anti-aging and sunlight protectant [34, 35].

Riceberry rice is Thai black purple rice, which is cross breeding between Khao Hom Nin rice and Thai Jasmine (Khao Dawk Mali) rice [36]. RBO from riceberry rice is reduce oxidative stress and inflammation and also can improve DM and hyperlipidemia conditions in animal model. There is contain additional active compounds i.e., anthocyanins (cyanidin 3-glucoside and peonidin-3-glucoside) along with major active compounds in RBO including,  $\gamma$ -oryzanol and tocotrienols [37]. RBO from riceberry rice can called anthocyanin-rich RBO, which is

exhibited antioxidant, anti-inflammation and inducing apoptosis in gentamycin induced-hepatotoxicity rats. Thus, there are also possessed hepatoprotective effect [38]. Fine particles from riceberry bran are by-product from RBO processing by refining cold pressed-machine. There can utilized as ingredient of body mask and dietary supplement due to antioxidant and high dietary fiber rather than by-product from other vegetable oil processes [39]. We were found that tannin content was most active constituents in RBO and there was possible related to anthocyanins as polymeric anthocyanins (proanthocyanins and condensed tannins). Proanthocyanins are commonly in plant bark, seeds, flowers fruits and nuts. Several reports on human health benefits providing, such as antioxidation, anti-inflammation, immunomodulation, DNA repair, and antitumor activity [40]. In this study, RBO can inhibited DPPH and NO radicals, therefore, it was unable to inhibit lipid peroxidation. This finding may due to solubility of RBO in assay environment. RBO also inhibited NO radical produced from inflammatory cells and acted as anti-inflammatory agent. This result was corresponded to previous studies [33-35, 37, 38]. However, no key cosmetic enzyme inhibition. Low concentrations of RBO (0.0001-0.1 mg/ml) were slightly activated skin cell proliferation with no toxicity, whereas, 1 mg/ml of RBO was cytotoxicity to human skin fibroblast cells. There was implied that dose of RBO is most important on formulation of cosmetic products. Hence, we are suggested that further studies will be conduct on health promoting or cosmetic products, which is use of RBO as bioactive ingredient.

## CONCLUSION

RBO was contained tannins as most of bioactive compounds. RBO was exhibited antioxidant activity by scavenging of DPPH and NO radicals; and anti-inflammation activity by reduced NO radical production from LPS-induced macrophage cells. RBO was slightly activated skin cell proliferation with no toxicity at 0.0001-0.1 mg/ml, whereas, 1 mg/ml of RBO was cytotoxicity. RBO was unable to inhibited tyrosinase and elastase enzymes. No wound healing was occurred after incubated RBO with scratched-human skin fibroblast cells compared to controls.

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## CONFLICT OF INTEREST

There are no conflict of interest in this study

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