



Flavonoid composition, antibacterial and antioxidant properties of tartary buckwheat bran extract

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ARTICLE INFO

Article history:

Received 24 February 2013

Received in revised form 26 April 2013

Accepted 26 April 2013

Keywords:

Anti-acne

Flavonoids

Tartary buckwheat bran

Antibacterial

Antioxidant

ABSTRACT

The tartary buckwheat [*Fagopyrum tataricum* (L.) Gaench] bran, which is an important by-product during the production of tartary buckwheat tea, is a good source of flavonoids but has not been made full use of. Some studies reveal its antioxidant activity. However no research is found for its antibacterial activities against *Propionibacterium* and *Staphylococci* species. The 60% (v/v) EtOH extract of the tartary buckwheat bran (TBBE) was prepared at room temperature and the flavonoids content was determined by HPLC. Rutin (541.3 ± 9.3 mg/g), isoquercetin (9.33 ± 0.16 mg/g) and quercetin (66.3 ± 1.14 mg/g) were detected in the TBBE. The inhibition zone of TBBE against four bacterial strains varied from 7.6 mm to 11.6 mm; minimum inhibition concentration (MIC) values were from 512 μ g/mL to 2048 μ g/mL. IC₅₀ of DPPH scavenging activity and relative ORAC values were 8.36 ± 0.27 μ g/mL and $11,090 \pm 1278$ μ mol TE/g, respectively. For the constituents of TBBE quercetin showed higher antioxidant and antibacterial properties than TBBE and its glycosides (isoquercetin and rutin). These results suggest that TBBE might be useful to develop new types of antibacterial substance and new skin care cosmetics to prevent or improve acne.

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1. Introduction

Presently, both in practice and in science, there is growing interest for new preparations of natural origin bioactive fractions, especially in the control and prevention of various human diseases (Kosanic et al., 2012). Acne is a follicular rash that can cause comedo, red papules, and pustule (Higaki, 2003). It affects 79–95% of adolescents (Costa et al., 2010). Many acne patients undergo spontaneous and complete resolution of their lesion, whereas others have permanent damage such as disfigurative scarring and keloids which can lead to psychological disorders (Falcocchio et al., 2006). *Propionibacterium* and *Staphylococci* species are two predominant bacterial groups in sebaceous sites (Grice et al., 2009). The excessive growth of the two major bacteria and inflammation which caused by the reactive oxygen species are the most important physiological factors in the pathogenesis of acne (Marcinkiewicz et al., 2006). Azelaic acid, retinoids and antibiotics are commonly used to treat acne (Degitz and Ochsendorf, 2008; Kanlayavattanukul and Lourith,

2011; Krautheim and Gollnick, 2004). However the chemical drugs for acne have a number of limitations such as the occurrence of widespread resistance *P. acnes* (Patel et al., 2010; Tzellos et al., 2011; Webster and Graber, 2008) and skin irritation (Smith et al., 2011; Zeichner, 2012). In contrast, plant extracts are likely to have a similar degree of efficacy without the troublesome side effects associated with chemical drug treatment. Presently, there is growing interest in phytochemical remedies for the treatment of acne because of their antibacterial and antioxidant abilities. It has been reported that the flavonoids extracted from many plants have antibacterial activity against *Propionibacterium* strains (Kim et al., 2010; Lim et al., 2007; Pothitirat et al., 2009).

Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaench], which is a very important edible and medicinal plant in China, has been received increasing attention for its antioxidant, hypocholesterolemic, and antidiabetic activities (Qin et al., 2010). Tartary buckwheat tea is one of the tartary buckwheat-based food products available in the market and has gained popularity among China, Japan, South Korea and European. But during the production of the tartary buckwheat tea the whole seeds subject to dehulling procedure after soaking, steaming and drying (Qin et al., 2013). The dehulling procedure separate the whole seeds into about 70% dehulled groats, which is used to produce tartary buckwheat tea, and approximately 30% bran yet most of which is discarded. Previous research has shown that the bran contained about 7.7% total

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flavonoids (Liu and Zhu, 2007), meaning it could be an important raw material for phytochemical remedies. However, no research was found for the antibacterial activity against *Propionibacterium* and *Staphylococci* species of tartary buckwheat bran extract to the best of our knowledge.

Therefore, the objective of this study was to analyze the flavonoids content by RP-HPLC and *in vitro* investigate the antibacterial and antioxidant properties of TBBE. The structure–activity relationship of its principal components is also discussed. This could provide information to find new antibacterial compounds against *Propionibacterium* and *Staphylococci* species and to develop new natural cosmetic products to prevent acne.

2. Experimental

2.1. Reagents and chemicals

Trifluoroacetic acid (TFA, >99%), Trolox, 1,1-Diphenyl-2-picrylhydrazyl (DDPH), 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein (3',6'-dihydroxyspiro [isobenzofuran-1-[³H], 9' [⁹H]-xanthen]-3-one, FL) were purchased from Sigma–Aldrich (Shanghai, China). Rutin (>99%) and quercetin (>99%) which were used as standards references purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Isoquercetin (>98%) was prepared by a high temperature and pressure method in our laboratory (Qin et al., 2013). Erythromycin, retinoic acid, azelaic acid were purchased from Beijing Mengyimei Biotechnology Co., Ltd. (Beijing, China). Agar, Actinomycete Broth Medium (GAM broth), Muller-Hinton Broth (MHB) medium and Muller-Hinton Agar (MHA) medium were purchased from Beijing Aoboxing Biotech Company Ltd. (Beijing, China).

2.2. Plant material

Tartary buckwheat bran used in this study was a by-product of tartary buckwheat tea processing, using Heifeng 1 a variety of tartary buckwheat that was planted in Yanmen of Shanxi Province. They were collected during summer 2009; after the collection the bran was stored at -20°C .

2.3. Tartary buckwheat bran extracts

Before the extraction, the bran was grounded and passed through 60 mesh screens. The extract of tartary buckwheat bran (TBBE) was prepared as follows: 100.00 g of the homogenized bran powder was extracted twice with 1 L 60% (v/v) EtOH for 14 h, at room temperature with a stirring rate of 13 revolutions per minute. The mixture was filtered through Whatman (no. 2) filter paper under vacuum; the clarified extract was collected and then evaporated to dryness using a rotary evaporator (Buchi, Switzerland) at 60°C . The extraction was done in triplicate and an average value of the yield was calculated, which was $6.25 \pm 0.53\%$ (w/w). The dried extracts (TBBE) were stored at -20°C for further use.

2.4. HPLC analysis

2.4.1. Chromatographic condition

The HPLC system was SHIMADZU Prominence LC-20A (Shimadzu Corporation, Kyoto, Japan) equipped with an YMC ODS-A column (4.6 mm $\times$ 250 mm, YMC Co., Ltd., Kyoto, Japan). The wavelength of the UV detector was set at 375 nm. Mobile phase A consisted of a water solution containing 0.05% (v/v) TFA, while mobile phase B was acetonitrile. A gradient program at a flow rate of 0.8 mL/min was as follows: 0–8 min: 18% B; 8–18 min: 18–28% B; 18–28 min: 28% B; 28–35 min: 28%–100% B; 35–40 min: 100% B;

40–45 min: 18% B. The injection volume was 20 μL . The identification of rutin, isoquercetin and quercetin (Fig. 1) in TBBE were evaluated by HPLC-PDA, observing the retention time (Rt) and UV–vis spectra.

2.4.2. Preparation of standard curves

Stock solutions were prepared by dissolving rutin (15.00 mg), quercetin (6.00 mg), and isoquercetin (2.00 mg) in methanol (50 mL), respectively. Then the solutions were diluted step by step with methanol to give six different concentrations of working standard solutions. The working standard solutions were analyzed by the established method in triplicate. Calibration curves were constructed by plotting the peak area (Y) versus the concentration of rutin, quercetin, and isoquercetin (X), respectively.

2.4.3. Recovery test

The recovery test was performed according to the method of Tomczyk et al. (2012) and Sun et al. (2012). Three different concentrations of rutin, quercetin, and isoquercetin were added to known amounts of the pre-analyzed TBBE sample solutions, respectively. The spiked samples were analyzed three times by the established HPLC method.

2.5. Antibacterial activity testing

Four bacterial species were used for the experiment: *S. aureus* (ATCC25923), *S. epidermidis* (ATCC12228), *P. acnes* (ATCC11827) and *P. acnes* (ATCC6919). *S. aureus* and *S. epidermidis* were cultured in MHB for 24 h, at 37°C and *P. acnes* was cultured in GAM broth at 37°C for 24 h in YQX-II anaerobic incubator (Shanghai, China) for further use. The anaerobic incubation atmosphere contained 5% (v/v) CO_2 , 10% (v/v) H_2 and 85% (v/v) N_2 . Cell suspensions were diluted in sterile MHB or GAM to provide initial cell counts of about 10^8 colony-forming unit per mL (CFU/mL). All antibacterial agents were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 200 mg/mL for the subsequent antibacterial experiments.

2.5.1. Determination of growth inhibition

The inhibition effect on the growth of *P. acnes*, *S. aureus* and *S. epidermidis* was determined by disk diffusion method (Lim et al., 2007). Briefly, the bacteria were adjusted to the McFarland 0.5 standard and used to inoculate MHA or GAM agar plates. Disks (6 mm diameter) containing 500 μg of test agents or 6.4 μg of erythromycin or DMSO only were placed on the plates. The *S. aureus* and *S. epidermidis* were incubated in an artificial climate chambers and the GAM agar plates were incubated in YQX-II anaerobic incubator at 37°C for 24 h (or 48 h). All determinations were performed in triplicate. The inhibition zone was determined by measuring the diameter.

2.5.2. Determination of MIC and MBC

MIC (minimum inhibition concentration) and MBC (minimum bactericidal concentration) values of TBBE, rutin, isoquercetin, quercetin, and three antibacterial agents were determined by micro-broth dilution method (Fu et al., 2009; Lim et al., 2007). The diluted suspensions of *S. aureus*, *S. epidermidis*, and *P. acnes* were inoculated into each well of a 96 well micro-plate, each containing a different concentration of the test samples. All the test broths and antibacterial agents-free broths contained 5% (v/v) DMSO. Agents-free broths were incubated as growth controls. The final range of test samples dilutions was 5000–0.0625 $\mu\text{g}/\text{mL}$ in the MHB and GAM broth. The final bacteria concentration in each dilution was 1×10^5 CFU/mL. The plates of *S. aureus*, and *S. epidermidis* were incubated in an artificial climate chambers well the *P. acnes* was in the anaerobic incubator. They were all incubated at 37°C for 24 h, but

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