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Phenolic composition, antioxidant, antimicrobial and antiproliferative activities of water caltrop pericarps extract

Haining Yu [a], Shengrong Shen [b, *]

[a] College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou, 310014, PR China
[b] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058, PR China

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ABSTRACT

Pericarps from three different Chinese water caltrop cultivars (*T. acornis* Nakano., *T. bispinosa* Roxb., and *T. quadrispinosa* Roxb.) were collected and extracted using the system of methanol: water (7:3, mL:mL) and hot water. Phenolic compositions analysis indicated that gallic acid and ferulic acid contributed 74.82–99.44 mg phenolic acids/100 mg phenolic acids determined in the extraction. All water caltrop pericarps extracts (WCPE) exhibited high antioxidant capacity. WCPE showed strong *in vitro* inhibitory action against gram positive bacteria, gram negative bacteria, yeast and fungi. The lowest Minimum inhibitory concentration (MIC) to gram positive bacteria was 0.5 mg/mL. MTT assay and cell cycle analysis revealed that WCPE inhibited PC-3 cancer cell growth by inducing G0/G1 phase arrest without any action on RWPE-1 normal cells compared with 5-fluorouracil that showed relatively higher cytotoxicity to RWPE-1 than PC-3.

1. Introduction

Trapa sp. is an aquatic free-floating plant that belongs to Angiospermae, Dicotyledoneae, Myrtales and Trapaceae. Water caltrop, a popular traditional edible vegetable in China, is the fruit of trapa. The mature water caltrop generally has 1–4 spines at the shoulder of its outer pericarp. In China, various cultivars of trapa are classified according to the spines of water caltrop, such as 'Wujiaoling' without spine (*T. acornis* Nakano.), 'Erjiaoling' with two spines (*T. bispinosa* Roxb.) and 'Sijiaoling' with four spines (*T. quadrispinosa* Roxb.), etc. During the processing of water caltrop, pericarps are discarded as agriculture waste without taking into consideration their possible biological activity.

Pericarps, skins or shells of most fruits and vegetables are generally not consumed and are discarded as waste. Recent studies reported that pericarps of many fruits such as lychee, longan, wampee and mangosteen contain large amounts of bioactive compounds and exhibit antioxidant and anticancer activities both *in vitro* and *in vivo* (He, Wang, Yang, Lu, & Sun, 2009; Prasad, Xie, Hao, Yang, & Qiu, 2010; Wang, Yuan, Wang, Lin, & Liu, 2006; Yu, Zhao, Yang, & Bai, 2009). Water caltrop pericarp is considered as traditional medicine in most Asian countries, including China, India and Bangladesh (Wang et al., 2011). In some parts of China, dried water caltrop pericarps are boiled in water and drunk like tea to treat cancer and clean up heat detoxification (Lin, 1996; Niu, Li, Dong, Liu, & Cheng, 2001). In recent years, it is reported that pericarps extracts of *Trapa taiwanensis* (Chiang & Ciou, 2010; Wang et al., 2011), *Trapa manshurica* (Niu et al., 2001; Shang, Xuan, Zhu, & Cui, 2007) and *Trapa natans* (Ning et al., 2011) possess antioxidant and anticancer actions, which have been attributed to their bioactive components, such as polyphenols, flavonoids and alkanoids.

It has been reported that phenolic acids generally accumulate in the outer parts of plants such as shells, skins, pericarps, etc. (Bravo, 1998). To the best of our knowledge, phenolic composition of water caltrop pericarps has not been reported. Furthermore, in view of the safety of synthetic microbial agents v (Fang, Bo, Bao, Jin & Gui. 2009; Mohammed, Mahmood, Salmah, Zahra & Suhailah. 2012), it is crucial to explore natural sources of antimicrobial compounds for their possible use in food preservation. The objective of this study was to analyze the contents of phenolic compounds in the pericarps of 3 well-known and widely consumed trapa cultivars in China as well as their potential uses as antioxidant and antimicrobial agents.

2. Materials and methods

2.1. Reagents and standards

Gallic acid, Protocatechuic acid, Catechuic acid, Chlorogenic acid, Caffeic acid, Isovanillic acid, *p*-Coumaric acid, Ferulic acid, 2,4,6-

* Corresponding author. Tel./fax: +86 571 88982539.
E-mail address: shrshen@zju.edu.cn (S. Shen).

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tris(2-pyridyl)-s-triazine(TPTZ), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonicacid) (ABTS), propidium iodide (PI), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT), 5-fluorouracil were purchased from Sigma (St. Louis, MO, USA). RPMI-1640 medium and F-12 medium, fetal bovine serum and penicillin-streptomycin were obtained from GIBCO (Grand Island, NY, USA).

2.2. Plant materials and sample extraction

The pericarps of three water caltrop cultivars (*T. acornis* Nakano., *T. bispinosa* Roxb., and *T. quadrispinosa* Roxb.) were collected from Nanhu Lake in Jiaxing City, Zhejiang Province, China. The fresh pericarps were washed, dried in sunshine, ground into powder and stored at −20 °C till use.

Methanol extraction: 2 g of water caltrop pericarp powders were extracted twice with the mixture of 35 mL methanol and 15 mL water at 70 °C for 10 min. The extracts were centrifuged at 3600 g for 30 min. The supernatant was collected and made up to a final volume of 100 mL.

Water extraction: 2 g of water caltrop pericarp powders were extracted twice with 50 mL of distilled water at 100 °C for 1 h and 30 min. The extracts were centrifuged at 3600 g for 30 min. The supernatant was collected and made up to a final volume of 100 mL.

2.3. Analysis of phenolic compounds in the extract

Phenolic acids were prepared according to Nardini with some modifications (Nardini, Cirillo, Natella, Mencarelli & Comisso. 2002). An aliquot of water caltrop pericarps extracts (WCPE) were mixed with 4 mol/L NaOH (containing 1 mL ascorbic acid/100 mL NaOH and 10 mmol/L EDTA) (v:v, 1:1) for alkaline hydrolysis at room temperature for 4 h. After acidification to pH 2.0 by 6 mol/L HCl, the mixture was centrifuged at 2200 g for 8 min. The supernatants were extracted 3 times with ethyl acetate (v:v, 1:1). The ethyl acetate extracts were dried by evaporation under vacuum. The dry residues were dissolved in methanol and used for further analysis.

HPLC analysis was conducted on an Alliance 2695 HPLC system (Waters, Milford, USA) with Xterra C18 column, 4.6 × 250 mm, 5 μm particle size linked to a PDA 2996 (Waters, Milford, USA). The mobile phase consisted of 0.1 mL formic acid/L filtered deionized waters as solvent A and 0.1 mL formic acid/L acetonitrile as solvent B, with a flow rate of 1.0 mL/min, and the column temperature was maintained at 30 °C. The gradient elution increased solvent B linearly from 0% to 15% from 0 to 15 min, then increased to 30% at 20 min, 60% at 30 min, 90% at 40 min, 90% at 45 min and finally returned to 0% at 50 min to allow stabilization and return to initial concentration. Injection volume was 20 μL. Peak areas were quantified at 254 nm.

2.4. Antioxidant capacity evaluation

2.4.1. 2,2-Azino-bis-3-enthylbenzothiazoline-6-sulfonic acid (ABTS) radical-scavenging assay

This assay was based on the method developed by Ozgen, Reese, Tulio, Scheerens, and Miller (2006) with some modifications. ABTS radical cation was produced by reacting ABTS stock solution (7 mmol/L) with potassium persulphate (2.45 mmol/L) and allowing the mixture to stand in the dark at room temperature for 16 h. The ABTS solution was diluted to get an absorbance of 0.70 ± 0.02. ABTS solution 3.9 mL was mixed with WCPE (water caltrop pericarps extracts) 0.1 mL and kept in the dark for 6 min. The decrease in absorbance was measured at 734 nm. Ascorbic acid was used as standard. Results were expressed as mg/g AEAC (Acid equivalent antioxidant capacity).

2.4.2. Ferric reducing antioxidant power (FRAP) assay

This assay was carried out according to the modified method described by Benzie and Strain (Benzie & Strain. 1996). FRAP reagent containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) and 20 mmol/L ferric chloride (v: v: v, 10:1:1) was prepared. 0.1 mL of WCPE was added to 1.9 mL FRAP reagent, and the absorbance was measured at 593 nm after 10 min. Ascorbic acid was used as standard. Results were expressed as mg/g AEAC.

2.5. Antimicrobial activity

Baclillus cereus, *Bacillus subtilis*, *Staphyloccocus aureus*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Penicillium glaucum* organisms were obtained from the Microbiology Laboratory, Department of Food and Nutrition, Zhejiang University (Hangzhou, China) to test the ability of the water caltrop pericarps extract on their growth. .

2.5.1. Inhibition zone assay

Agar-hole diffusion method described by Mohammed et al. (Mohammed et al., 2012) with minor modifications was used to determine the inhibition zone of microoganisms of WCPE. Solvent used for WCPE extraction was removed under vacuum using a rotary evaporator. The dry extracts were dissolved in water to 30 mg/mL 100 μL of 10^4-10^5 cfu/mL microorganisms were spread onto an agar plate. Punch 5 mm diameter holes in the plate and then 30 μL of WCPE extract was employed in each hole. Water was used as negative control, and potassium sorbate and sodium benzoate were used as positive controls. The diameter of the zone of inhibition was measured after incubation of bacteria plates at 37 °C for 24 h and fungi plates at 28 °C for 48 h.

2.5.2. Minimum inhibitory concentration (MIC) assay

The dry extracts were dissolved in water and diluted to form final concentrations at 1, 3, 5, 10, 20, 50, 60, and 100 mg/mL. These solutions (1.0 mL) were poured into plates with agar media (9.0 mL) at about 55 °C. 30 μL of microorganisms with concentration adjusted to 10^4–10^5 cfu/mL was spread onto the agar plates. The plates with bacteria were incubated at 37 °C for 24 h, and those with fungi were incubated at 28 °C for 48 h. The MIC was the concentration of the extract at which no growth of the microorganism was observed.

2.6. In vitro antiproliferative activity against cancer cells

2.6.1. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The antiproliferative activities of extracts using methonal:water (7:3, mL:mL) and water of water caltrop pericarps from three cultivars on human prostate cancer cell line PC-3 and human prostate normal endothelial cell line RWPE-1 were measured using MTT assay. 5-fluorouracil, a widely used anticancer drug, was used as the positive control. The cell lines were obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. PC-3 cell and RWPE-1 cell were grown in F-12 medium and RPMI-1640 medium separately, supplemented with 100 mL fetal bovine serum/L and 100 U/mL penicillin-streptomycin in a humidified 37 °C, 5% CO_2 incubator. Solvent of WCPE was removed under vacuum using a rotary evaporator. The dry extracts were dissolved in DMSO and diluted with medium to give final concentrations at 500, 400, 300, 200, 100 μg/mL. Cells were harvested and seeded in 96-well plates