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Short communication

Chemical composition, antioxidant and antimicrobial activity of Chinese tallow tree leaves

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ABSTRACT

In the present study, the chemical compositions, antioxidant and antimicrobial activities of fractions of extracts of Chinese tallow tree (CTT, *Sapium sebiferum* L.) leaves were evaluated. The ethanol extract of CTT leaves was fractionated, then obtained ethyl acetate fraction (EAF), n-butyl alcohol fraction (nBF) and water residue fraction (WF). UPLC-PDA method was adopted for chemical composition analysis; Four chemical methods, including DPPH and ABTS radicals scavenging, reducing power and ORAC assays were adopted for the determination of antioxidant activities; MIC method was employed to test inhibitory effects on *Staphylococcus aureus* growth. Eight phenolic compounds were identified, including gallic acid, rutin, ellagic acid, hyperin, isoquercitrin, astragalin, quercetin and keampferol. EAF contained higher contents of all identified components except rutin. Meantime, WF contained more un-retained, high polar compounds. All fractions exhibited antioxidant activities. EAF exhibited strongest antioxidant activity, nBF taken second place, while antioxidant of WF was weakest. The anti-bacterial activity was also found in order of EAF > nBF > WF. Results of bioactivity tests were in accordance to the relative content of most identified phenolic compounds, which indicate that identified compounds may be responsible for the bioactivity of CTT leaves. This study provided evidence for the selection of EAF of CTT leaves as a bioactivity resource, which might improve the development and application of CTT leaves.

1. Introduction

Chinese tallow tree (CTT, *Sapium sebiferum* L., Euphorbiaceae) is originated in China and is cultivated as an ornamental plant. It grows primarily in the subtropical regions around the world and has been considered to be an invasive species in America. CTT has high economic value as it is a wood energy plant, meantime, which also has medicinal value. Its leaf, root or bark can be used as medicine in Traditional Chinese Medicine (TCM). Leaves of CTT have the potential to be a better choice due to its resource advantage, which is helpful for the treatment of eczema, edema, shingles, swelling, scabs, ascites, and snakebites. Some published works have indicated that the leaves of CTT have anti-microbial, anti-inflammatory, antihypertensive, and analgesic activities (Binxue et al., 2004; Chaudhary et al., 2011; Hsu et al., 1994; Pankaj et al., 2011; Zhou, 2007). The chemical composition of CTT leaves has been also researched, and some compounds have been identified, including gallic acid, β-sitosterol glycoside, quercetin, kaempferol, astragalin, isoquercetin, ellagic acid, methyl gallate, 6-*O*-galloyl-D-glucose, and methyl-3,4,5-trihydroxybenzoate. Most publications about bioactivity have been focused on different crude solvent extracts, or ethyl acetate fraction of ethanol extract. Only one paper has been done to evaluate the difference of antimicrobial activity of various fractions of ethanol extract of CTT leaves (Zhou, 2007). The author studied the antimicrobial activity of different fractions by determination of the inhibition zone and exhibited that ethyl acetate fraction exerted the strongest inhibitory effect on the organism. In the present study, we analyzed the chemical composition of three fractions of ethanol extract of CTT leaves and evaluated the antioxidant and antimicrobial activities.

2. Material and methods

2.1. Reagents

Vitamin C (Vc), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)(ABTS), fluorescein and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO,

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USA). Herbal reference standards including gallic acid, rutin, ellagic acid, hyperin, isoquiercitrin, astragalin, quercetin and kaempferol were obtained from Chengdu Herbpurify Co., Ltd. (Chengdu, China). Methanol, acetonitrile, formic acid and ammonium formate were HPLC grade for UPLC analysis. All other chemicals and reagents were analytical grade.

2.2. Preparation of fractions of extract

CTT leaves were collected in April 2012 in Renshou County (Sichuan Province, China) and authenticated by A.P. Jie Bai, School of Life Sciences, Sichuan University. The voucher specimen (No. 00721412) was deposited in the Herbarium of Sichuan University.

The materials were dried and grounded. 10 g was weighed and extracted for two hours with 100 ml of 95% ethanol (v/v) in a Soxhlet apparatus, and repeat once. The two extracts were mixed and concentrated using a vacuum rotary evaporator. The extract was re suspended in water and partitioned with petroleum (Boiling range: 30–60 °C) (10 times), ethyl acetate (8 times) and *n*-butyl alcohol (6 times) using liquid–liquid partitioning. The extract fractions were concentrated and lyophilized. As petroleum fraction was too much pigment and impossible to get dehydrated, which was not included in our last test. These fractions were dissolved in methanol and 50% ethanol to perform UPLC analysis and antioxidant assays, respectively.

2.3. UPLC analysis

The analysis of three fractions of CTT leaves was performed on a Waters Acquity System (Waters Co., Milford, MA, USA). Sample separation was performed using an Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm; Waters Co., MA, USA), and the column temperature was set at 40 °C. The mobile phases were (A) acetonitrile and (B) 0.1% formic acid and 10 mM ammonium formate water solution. The gradient was as follows: 2% A for 0.5 min; 2–17% in 1 min; 17% A for 1 min; 17-20% A in 0.5 min; 20-30% A in 0.5 min; 30-50% A in 2 min; 50% A for 0.5 min, followed by re-equilibration of the column for 2.5 min with 2% A. The flow rate was 0.5 ml/min and injection volume was 2.0 μl. UV–vis absorption spectra was recorded on-line from 200 to 400 nm during the UPLC analysis.

2.4. Antioxidant activity

The DPPH radical scavenging activities were determined using the previous method (Fu et al., 2014a). The ABTS radical scavenging activities were determined using the published method (Fu et al., 2014b). The reducing power was measured according to the reported method (Fu et al., 2014b). The ORAC values were established using the method of (Fu et al., 2014a).

2.5. Anti-bacterial activity

Staphylococcus aureus (ATCC 29213) was used as test organism, which was activated by cultured at 37 °C for 24 h with fresh MHA. Inocula was prepared by transferring microorganism to sterile distilled water. The suspensions were adjusted to 0.5 McFarland standard. Working suspensions were obtained by further diluted in MHB (10 times). Fractions were dissolved in 100% dimethylsulphoxide (DMSO) (102.4 mg/ml) and further diluted in sterile MHB before experiment. The minimum inhibitory concentration (MIC) was determined using the micro-broth dilution method (Tekwu et al., 2012). The experiment was performed in 96-well sterile microplates. 100 μl of MHB with different concentrations of fractions (final concentration 16–2048 μg/ml) and 100 μl of the inoculum suspension was added and mixed. Three wells served drug-free controls. Another two-fold serial dilutions of ampicillin (final concentration 1–128 μg/ml) was used as a positive control. The highest concentration of DMSO was 2% followed by a two-fold dilution. Each microplate was covered and incubated at 37 °C for 18 h. The MIC was defined as the lowest concentration as the sample which prevents visible growth.

2.6. Statistical analysis

All experiments were conducted in triplicate and results are expressed as the mean ± SD. GraphPad Prism (GraphPad software Inc., San Diego, CA, USA) was adopted for the statistical analysis and determination of IC_{50} values. Data were analyzed using one-way analysis of variance (ANOVA) with multiple comparisons, followed by Dunnett's *t*-tests. $P < 0.05$ were considered as statistically significant.

3. Results and discussion

3.1. Chemical composition

The typical UPLC chromatograms detected at 260 nm of fractions of CTT leaves are shown in Fig. 1. In the present study, we have identified eight phenolic compounds, including gallic acid (1), rutin (2), ellagic acid (3), hyperin (4), isoquercitrin (5), astragalin (6), quercetin (7) and keampferol (8) by comparing the retention times and UV–vis spectra with those of herbal reference standards. As we can see from the result, EAF contains more moderate polar compounds, which can be retained in the chromatographic column. nBF exhibited less related compounds, but with more un-retained composition. As for WF, there were almost no identified compounds, which included more high polar compounds. These results were in accordance with our original intention and some published papers (Morales and Paredes, 2014; Yang et al., 2014), ethyl acetate fractions contained more flavonoids and phenolics. As the composition difference, we next evaluated the antioxidant and antimicrobial activities of three fractions and aimed to confirm if these identified compounds were responsible for the bioactivity of CTT.

3.2. Antioxidant activity

DPPH radical scavenging activities of EAF, nBF and WF are represented in Fig. 2(A). They all exhibited a dose-dependent manner. IC_{50} was established as concentration that causes 50% scavenging in DPPH radical concentration by GraphPad software, which as shown in Table 1. EAF exhibited a comparative DPPH radical scavenging activity contrast with Vc; however, nBF and WF were weaker than Vc. The radical scavenging activity was in the order of Vc > EAF > nBF > WF.

All of three fractions were found to exhibit ABTS scavenging activities, and their activities were in a dose-dependent manner (Fig. 2(B)). The IC_{50} values are represented in Table 1. Vc exhibited a lower IC_{50} than WF significantly ($P < 0.05$) but not EAF and nBF. EAF and nBF showed stronger radical scavenging activity than WF ($P < 0.05$). No significantly difference was detected between EAF and nBF. The ABTS radical scavenging capacity was found in the order of EAF > Vc > nBF > WF.

The reducing power of three fractions was illustrated in Fig. 2(C). Greater absorbance at 700 nm indicated stronger reducing power. The reducing powers exhibited dose-dependent manners. $A_{0.5}$ were also determined, which represent the concentration that exhibited reducing power of A700 is 0.5. The $A_{0.5}$ of three fractions are represented in Table 1. A lower $A_{0.5}$ means a higher reducing power. Vc exhibited the strongest reducing power. Three fractions also exhibited significant difference when compared with each other. The reducing power was found in the order of Vc > EAF > nBF > WF.