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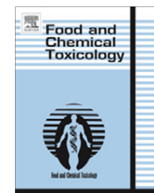

Eclipta prostrata L. phytochemicals: Isolation, structure elucidation, and their antitumor activity

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ABSTRACT

Eclipta prostrata L., (Asteraceae), is used in China for both food and medicine purposes. This research is concerned with the isolation and purification of phytochemical constituents from the aerial parts of *E. prostrata*, using gradient solvent fractionation, macroporous resin, silica gel, Sephadex LH-20 and ODS columns, and TLC analyses. Four fractions (water, 30% ethanol, 60% ethanol and 90% ethanol) were obtained. Four compounds, wedelolactone (I), eclalbasaponin I (II), luteolin (III) and luteolin-7-*O*-glucoside (IV) were purified and their structures were identified by the interpretation of spectroscopic analyses including MS, ^{1}H and ^{13}C NMR. Antitumor activities of extracts (total fraction), four fractions and the isolated compounds were assessed using hepatoma cell smmc-7721 as an *in vitro* assay system. The 30% ethanol fraction and eclalbasaponin I dose-dependently inhibited the proliferation of hepatoma cell smmc-7721 with IC_{50} values of 74.2399 and 111.1703 μg/ml, respectively, more strongly compared with 5-fluorouracil positive control group with the IC_{50} value of 195.3131 μg/ml. Antitumor activities of other fractions and compounds were lower than positive control. These results suggested that some specific compounds or extracts from *E. prostrata* are potential sources of natural anti-tumor materials and worthy of further study.

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1. Introduction

Eclipta prostrata L. belongs to therophyte herb of the family Asteraceae and is a widely distributed medicinal plant (Pithayanukul et al., 2007), principally in the provinces of Jiangsu, Jiangxi and Zhejiang in China (Li and Wang, 2010), Korea, India, Philippines and Japan. Its stem is cylindrical, with longitudinal ridges, 2–5 mm in diameter; externally greenish-brown or dark green (Wagner et al., 2011). *Eclipta prostrata* is used in the traditional medical systems of China as a health food or tonic to treat infectious hepatitis, jaundice, liver cirrhosis, aching and weakness of the knees and loins, spitting of blood, epistaxis, hematuria and diarrhea with bloody stools, and abnormal uterine bleeding (Ying, 2008).

The previous phytochemical studies on *E. prostrata* revealed the presence of thiophene-derivatives, steroids, triterpenes (Yahara et al., 1997), flavonoids, polyacetylenes, polypeptides, coumestons, etc. (Santhosh et al., 2006). *E. prostrata* has been extensively studied for its hepatoprotective activity. A number of herbal preparations comprising of *E. prostrata* are available for treatment of diverse symptoms e.g. hyperlipidemia, atherosclerosis and skin diseases (Prachayasittikul et al., 2010). This plant has also been reported to possess activities like immunomodulatory (Liu et al., 2000; Jayathirtha and Mishra, 2004), antioxidant (Kim et al., 2008), antimicrobial (Khanna and Kannabiran, 2008), anti-inflammatory (Arunachalam et al., 2009; Tewtrakul et al., 2011), hypolipidemic (Dhandapani, 2007), analgesic (Sawant et al., 2004), antivenom (Mors et al., 1989) and anti-aging (Thakur and Mengi, 2005). There was also report on the anticancer-cytotoxic activity of isolated saponins and dasyscyphin C from *E. prostrata*, which was tested under *in vitro* conditions on HeLa cells (Khanna and Kannabiran, 2009). The results showed the isolated saponins were not toxic to vero cells, and the saponins and dayscyphin C had significant anticancer-cytotoxic activity on HeLa cells under *in vitro* conditions.

E. prostrata, a widely applied traditional medicine and functional food, was extensively explored for its bioactivities (Zhong et al., 2009). However the studied bioactivities were only based on its crude extracts. The antitumor activity of single compounds extracted from *E. prostrata* has not been reported yet. Considering the rich natural resources of *E. prostrata*, the extraction researches of phytochemicals from *E. prostrata* will have a highly practical value. Therefore, in the present research, we tried to extract and separate active components from *E. prostrata*. Additionally, the inhibitory effects of various fractions and single compounds of *E.*

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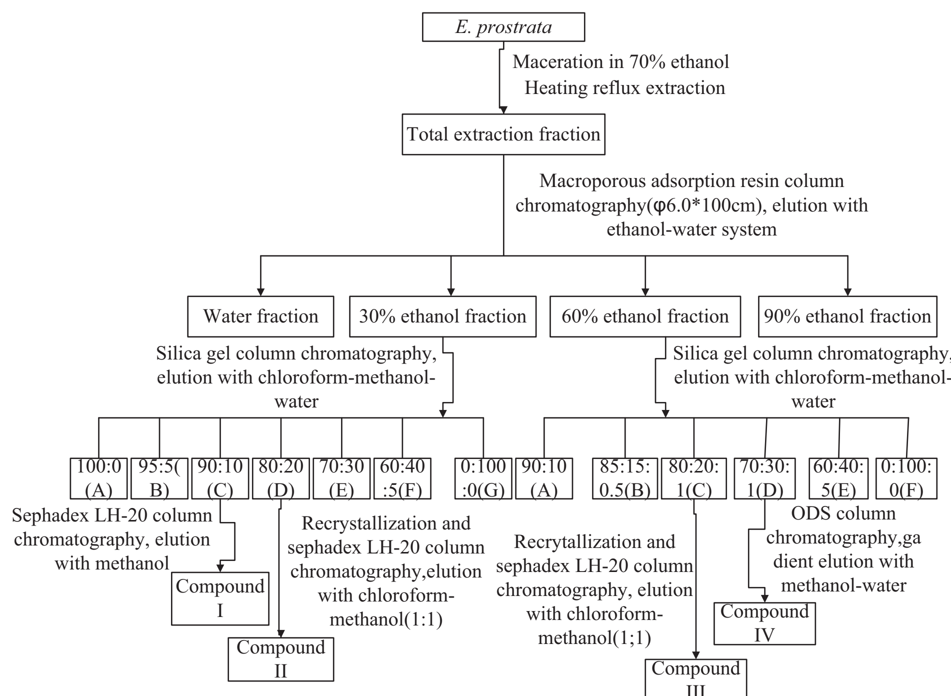

Fig. 1. The extraction and separation procedure of *E. prostrata*.

prostrata on hepatoma cell smmc-7721 were tested to evaluate their anti-tumor activity.

2. Materials and methods

2.1. Plant material

The dry *E. prostrata* (Voucher number: PTBG0000045130) was obtained from Qingping TCM market (Guangzhou, China) and identified by Prof. Ding-yong Wang (Dept. of Chemistry, Guangdong College of Pharmacy). Then it was ground in a cutting mill to pass through 50-mesh sieve to obtain fine powder, and stored in a well-closed container for further use.

2.2. Reagents

All chemicals used in the study, such as methanol, chloroform, petroleum ether, concentrated sulfuric acid, glacial acetic acid, vanillin, deuterium and reagents, ethyl acetate and *n*-butanol, were of analytical grade and ethanol absolute was of food grade and DMSO, BIBCO, MTT, Trypsin and Trypan blue, all which were purchased from east giant experiment instrument company (Guangzhou, China). 5-Fluorouracil was produced in Zhengzhou alpha chemical Co. Ltd. and hepatoma cell smmc-7721 was obtained from Shanghai cell bank of Chinese Academy of Sciences.

2.3. Chromatographic materials

Silica gel for column chromatography 200–300 and 100–200 mesh, Sephadex and silica gel GF-254 5 × 20 cm TLC plates were purchased from east giant experiment instrument company (Guangzhou, China).

2.4. Extraction, isolation and purification of bioactive constituents

The pre-prepared powder (5.0 kg) of *E. prostrata* was exhaustively extracted with 70% ethanol for 3 h and concentrated under reduced pressure at 70–80 °C three times. The crude extract was concentrated to dryness under vacuum at 40 °C to isolate the total extraction fraction (270.1 g). And then the total extraction fraction, dissolved in thermal distilled water, was successively partitioned with macroporous resin chromatography and eluted with ethanol/water of different ratios (0:100, 30:70, 60:40, 90:10, respectively). After the elution, concentration was conducted and four fractions were yielded, including the water fraction (140.0 g, yield coefficient 51.83%), 30% ethanol fraction (27.0 g, yield coefficient 10.00%), 60% ethanol fraction (53.6 g, yield coefficient 19.84%) and 90% ethanol fraction (5.29 g, yield coefficient 1.96%). In the next anti-tumor activity screening tests, 30% ethanol fraction and 60% ethanol fraction showed better anti-tumor activity among the four fractions. So these two fractions were chosen for further purification.

The 30% ethanol fraction (25.0 g) was subjected to a silica gel column chromatography with a gradient of chloroform–methanol–water (100% chloroform up to 100% methanol, mixture of increasing polarity), to yield 7 sub-fractions (subfr.). After TLC analysis, subfr. 3 (chloroform/methanol 90:10) was then chromatographed by column chromatography over Sephadex LH-20 and eluted with methanol, yielded compound I. Subfr. 4 (chloroform/methanol 80:20) was further subjected to Sephadex LH-20 chromatography and eluted with chloroform/methanol (1:1), to yield compound II. The extraction and separation procedures were demonstrated in Fig. 1.

The 60% ethanol fraction (31.4 g) was subjected to a silica gel column chromatography with a gradient of chloroform–methanol–water (100% chloroform up to 100% methanol, mixture of increasing polarity), to yield 6 subfrs. After TLC analysis, subfr. 3 (chloroform/methanol 1:1) was then chromatographed by column chromatography over Sephadex LH-20 and eluted with chloroform/methanol (1:1), yielded compound III. Subfr. 4 (chloroform/methanol/water 70:30:1) was further subjected to ODS chromatography and eluted with a gradient of methanol–water, to yield compound IV. The extraction and separation procedure was as Fig. 1.

2.5. Measurement of inhibition activity on hepatoma cell smmc-7721

The anti-tumor activities of 5-fluorouracil control (Diasio and Harris, 1989), total extraction fraction, water fraction, 30% ethanol fraction, 60% ethanol fraction, 90% ethanol fraction, and monomer compounds were respectively tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay (La et al., 2009; Tavakkol-Afshari et al., 2008) with hepatoma cell smmc-7721 *in vitro*.

2.5.1. Cell culture

Hepatoma cells smmc-7721 were obtained from Zhongshan University, Guangzhou city, China, and were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U ml^{-1}) and streptomycin (100 μg/ml) under an atmosphere of 5% CO_2 at 37 °C.

2.5.2. Cell proliferation assay

One hundred microliters of exponentially growing cells (8×10^4 cells ml^{-1}) were seeded in 96-well microculture plates and attached for 24 h, and then the supernatant was removed. Hundred microliters of samples (200, 100, 50, 25, 12.5 μg/ml, final concentrations of DMSO (0.2%) other than 30% fraction 100, 50, 25, 12.5, 6.26 μg/ml) were seeded into each well for 24 h at 37 °C. After incubation, the medium solution was removed and an aliquot of 100 μl of medium containing MTT (0.1%) was loaded to the plate. The cells were incubated for 4 h at 37 °C and then the medium solution was removed. An aliquot of 150 μl DMSO was added to the plate, which was shaken until the crystals dissolved. Controls and blanks

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