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Chemical profiling, volatile oil analysis and anticholinesterase activity of *Hydrocotyle umbellata* L. aerial parts cultivated in Egypt

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ABSTRACT

Hydrocotyle umbellata L. (commonly known as Acaricoba) is an aquatic perennial plant belonging to family Araliaceae. The plant has been used in folk phytotherapy and in the Ayurvedic medicine to reduce anxiety and as a memory enhancer herbal remedy. This study was designated to explore the diversity of the accumulated bioactive metabolites that might account for the potential cholinesterase inhibition activity of *H. umbellata* L. aerial parts. UPLC/PDA/ESI-qTOF-MS secondary metabolites profiling revealed similar chemical composition of the leaves and flowers. The major identified secondary metabolites in both samples were caffeoylquinic acid isomers, quercetin derivatives, and fatty acids. Also, the volatile oil analysis of fresh leaves and flowers using GC/MS showed similar composition. The monoterpenes; α-pinene, β-pinene, and limonene represent the main components of the oil. Many reports were found dealing with the cholinesterase inhibition activity of the major identified caffeoylquinic acids, quercetin derivatives, as well as the identified volatile monoterpenes. Therefore, the anticholinesterase activity of the ethanolic extract of the aerial parts was evaluated using Ellman's spectrophotometric method. The ethanolic extract exhibited significant cholinesterase inhibitory activity with IC_{50} 1.88 mg/ml $\pm$ 0.19 compared to standard cholinesterase inhibitor eserine (IC_{50} 0.27 mg/ml $\pm$ 0.15). These findings suggest that the significant cholinesterase inhibition activity of the ethanolic extract is likely to be mediated by the major identified bioactive molecules and support the folkloric use of *Hydrocotyle umbellata* L. as a memory enhancer herbal medicine.

1. Introduction

Genus *Hydrocotyle* (Family Araliaceae) comprises about 130 species of creeping perennial plants (Mabberley, 1997) distributed in tropical and temperate regions (Karuppusamy et al., 2014). Plants of the genus have been used in Taiwan folk medicine for treatment of several inflammatory disorders, common cold, dysentery, zoster, eczema and period pain (Lin et al., 2003).

Hydrocotyle umbellata L. is a creeping water-loving specimen, widely grown in the Americas and mainly native to Brazil (Rocha et al., 2011). It is a long petiolated plant with peltate-shaped leaves, simple umbel inflorescence somewhat exceeding the length of the leaves, numerous small white flowers and flattened capsule-shaped fruits (Fig. 1) (Godfrey and Wooten, 1981; Florentino et al., 2013).

Traditionally, *H. umbellata* L. has been used as anti-inflammatory, memory stimulant and anxiolytic herbal medicine (Reis et al., 1992; Lin et al., 2003). The ethanolic extract of the underground parts of the plant was reported to possess analgesic, anti-inflammatory (Florentino et al., 2013) and anxiolytic activities (Rocha et al., 2011). The main secondary metabolites previously identified in the plant were volatile constituents (Rojas et al., 2009), flavonoids (Adams et al., 1989), sterols (Chavasiri et al., 2005), and saponins (Sosa et al., 2011).

Based on *H. umbellata* L. presumed therapeutic value and lack of reports concerning the biological and chemical investigation of *H. umbellata* L. aerial parts, this study was designed to evaluate the potential anticholinesterase activity of the ethanolic extract of *Hydrocotyle umbellata* L. aerial parts using Ellman's spectrophotometric method, and to correlate this activity with its secondary metabolites composition. Also, to provide a detailed map for various chemical classes (*i.e.* phenolic compounds, fatty acids and volatile oil constituents) distribution in leaves, as well as flowers using different chromatographic methods.

UPLC-PDA-ESI-qTOF-MS has been widely used in metabolomic studies and became a powerful tool for secondary metabolites profiling of complicated components in herbal medicines; as it provides rapid metabolites analysis and excellent chromatographic separation with high sensitivity, accuracy, and precision (Farag et al., 2013).

Alzheimer's disease (AD), the most common cause of dementia, is a neurodegenerative disorder that affects the elderly population characterized by progressive cognitive disability with declining in daily living activities and neuropsychiatric symptoms or behavioral changes (Lane et al., 2006).

Natural plants are considered a potential source of bioactive secondary metabolites that might serve as leads for the development of novel

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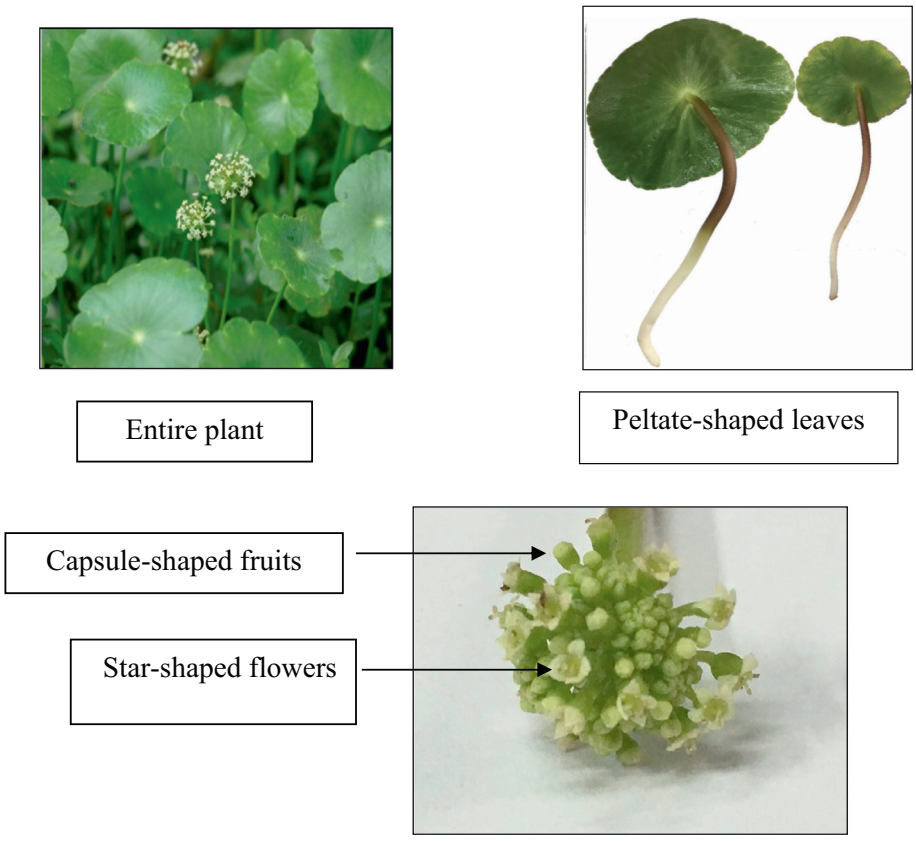

Fig. 1. Photographs of *H.umbellata* L. plant.

drugs with the ability to inhibit the acetylcholinesterase enzyme, which hydrolyzes the acetylcholine, increasing the acetylcholine available for transmission at the cholinergic synapse (Miyazawa and Yamafuji, 2005).

Based on the cholinergic theory, these natural cholinesterase inhibitors improve the cholinergic functions in patients suffering from Alzheimer's disease and mitigate the symptoms of this neurological disease (Perry and Howes, 2011).

2. Methodology

2.1. Plant material

The plant material was collected in March 2014 at the flowering stage from El-Orman Botanical Garden, Giza, Egypt and kindly identified by Eng. Threase Labib, consultant in Orman Garden and National Gene Bank, Ministry of Agriculture and confirmed by Dr. Mohammed El-Gebaly, the senior taxonomist at National Research Center. A voucher specimen (No. 17-8-2016) was kept at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt.

2.2. Preparation of ethanolic extract

The air-dried powdered aerial parts (2.3 kg) were extracted with 70% ethanol by cold maceration till exhaustion. The combined ethanolic extracts were evaporated under reduced pressure at 55 °C to dryness.

2.3. Chemicals and reagents

Acetylcholinesterase (Electric-eel EC 3.1.1.7), Acetylcholine iodide, 5,5-Dithiobis[2-nitrobenzoic acid] (DTNB), Buffers and all other chemicals of the highest purity and analytical grade were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Extraction and sample preparation for UHPLC–qTOF– PDA–MS analysis

Leaves and flowers were separately air-dried, reduced to fine powder (20 mg each) and extracted with 2.5 ml 70% methanol (MeOH) containing 5 μg/ml umbelliferone (as an internal standard for relative quantification) using a Turrax mixer (11,000 RPM) for five 20 s periods. To prevent heating, a period of 1 min separated each mixing period. Extracts were then vortexed vigorously and centrifuged at 3000*g* (gravity force) for 30 min to remove plant debris before UPLC-MS analyses, 500 μl was aliquoted and placed on a C_{18} cartridge (500 mg) preconditioned with methanol and water. Samples were then eluted using 3 ml 70% MeOH and 3 ml 100% MeOH, the eluent was evaporated under a nitrogen stream and the obtained dry residue was resuspended in 500 μl methanol. Three microliters, aliquots, were used for analysis. Samples were analyzed in negative ESI ionization mode.

2.5. High-resolution UPLC-MS analysis

Qualitative analysis of the secondary metabolites was performed by UPLC on an HSS T3 column (100 × 1.0 mm, packed with silica gel RP-C_{18}, particle size 1.8 μm; Waters) by applying the following binary gradient, at a flow rate of 150 μl min^{-1}: 0 to 1 min, isocratic 95% A (water/formic acid, 99.9/0.1 [v/v]), 5% B (acetonitrile/formic acid, 99.9/0.1 [v/v]); 1 to 16 min, linear from 5 to 95% B; 16 to 18 min, isocratic 95% B; 18 to 20 min, isocratic 5% B. The injection volume was 3.1 μl (full loop injection). Eluted compounds were detected from *m/z* 100 to 1000 in negative ion mode using the following instrument settings: nebulizer gas, nitrogen, 1.6 bar; dry gas, nitrogen, 6 l min^{-1}, 190 °C; capillary, −5500 V (+4000 V); end plate offset, −500 V; funnel 1 RF, 200 Vpp; funnel 2 RF, 200 Vpp; in-source CID energy, 0 V; hexapole RF, 100 Vpp; quadrapole ion energy, 5 eV; collision gas, argon; collision energy, 10 eV; collision RF 200/400 Vpp (timing 50/50); transfer time, 70 μs; pulser frequency, 10 kHz; spectra rate, 3 Hz. Internal mass calibration