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Original Article

Cholinesterase inhibitory activity and chemical constituents of *Stenochlaena palustris* fronds at two different stages of maturity

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ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form
1 November 2015
Accepted 4 December 2015
Available online 4 March 2016

Keywords:
anticholinesterase
Stenochlaena palustris
Blechnaceae
phytochemical profile
frond maturity

ABSTRACT

Stenochlaena palustris fronds are popular as a vegetable in Southeast Asia. The objectives of this study were to evaluate the anticholinesterase properties and phytochemical profiles of the young and mature fronds of this plant. Both types of fronds were found to have selective inhibitory effect against butyrylcholinesterase compared with acetylcholinesterase. However, different sets of compounds were responsible for their activity. In young fronds, an antibutyrylcholinesterase effect was observed in the hexane extract, which was comprised of a variety of aliphatic hydrocarbons, fatty acids, and phytosterols. In the mature fronds, inhibitory activity was observed in the methanol extract, which contained a series of kaempferol glycosides. Our results provided novel information concerning the ability of *S. palustris* to inhibit cholinesterase and its phytochemical profile. Further research to investigate the potential use of this plant against Alzheimer's disease is warranted, however, young and mature fronds should be distinguished due to their phytochemical differences.

1. Introduction

The central cholinergic system plays a key role in the regulation of cognitive functions. Damage in such systems is thought to be responsible for cognitive decline, which is the key features of Alzheimer's disease (AD), dementia, and other neurodegenerative diseases [1]. Since cholinergic markers have been found to be greatly reduced in the postmortem brain samples of AD patients, and the decline in neurotransmitter acetylcholine can be correlated to the degree of cognitive impairment [2], inhibition of cholinesterases

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http://dx.doi.org/10.1016/j.jfda.2015.12.005

involved in the hydrolysis of acetylcholine is thought to be a plausible strategy for the treatment and control of memory loss associated with AD [3]. A number of foods from plants have been found to exhibit anticholinesterase activity. These include ginger [4], a blend of black chokeberry, and lemon juice [5], as well as green tea [6]. Other plant species containing alkaloids, xanthones, and polyphenols [7–9] have also been reported to exert inhibitory effects on cholinesterase and, thus, have potential applications in prevention of cognitive decline.

In this study, we evaluated the potential of a vegetable fern, *Stenochlaena palustris*, to inhibit cholinesterases and the phytochemicals involved. *S. palustris* is known as 'Paku Miding' in Malaysia, or 'Kalakai" in Borneo and Kalimantan. It is a creeping fern found widely across India, through Malaysia and Polynesia, and into Australia [10]. The *S. palustris* frond is dimorphic and can be classified as fertile or sterile based on morphology. Fertile fronds have thin, long pinnae that bear spores, and are seasonal and inedible. However, the sterile fronds that are edible have broad pinnae with sharply toothed margins and are available throughout the year [11]. The young, sterile fronds of the fern have a crispy texture and are usually cooked with shrimp paste into a vegetable dish. They are also used traditionally to treat fever, diarrhea, skin diseases, cutaneous disorders, and gastric ulcers [11–13]. According to the Malaysian Agricultural and Development Institute, *S. palustris* has great potential to be exported to foreign markets. Therefore, research has been carried out to improve postharvest handling and packaging conditions in order to extend the storage period of the plant [14]. Furthermore, effort has also been undertaken to evaluate its cultivation and economic potential [15]. Despite having great promise in the food market, information concerning its health functions and nutritive properties remained scarce.

There have been accounts concerning the antifungal activity of the methanolic leaf extract [16] and antibacterial properties of flavonol glycosides [17] from *S. palustris*. Additionally, other researchers, including our group, found strong antioxidant activities and high polyphenolic content in the fronds [18–20]. Given that natural antioxidants may have potential in treating AD due to their neuroprotective properties [21,22], we explored the neuroprotective potential of *S. palustris* by evaluating its cholinesterase inhibitory properties. This study was carried out on young, sterile fronds, which are commonly consumed as vegetables, as well as on the mature sterile fronds, which are not usually eaten, in order to verify differences between them.

2. Materials and methods

2.1. Plant material

S. palustris (Burm.). Bedd. (Blechnaceae) was collected from Sungai Petani, Kedah, Malaysia. The taxonomic identity of the plant was authenticated by Ms Maliga Gnasan, a botanist at the Penang Botanic Gardens. A voucher specimen (No. 1645) was then deposited at the premises.

2.2. Chemicals and reagents

Hexane, dichloromethane (DCM), and methanol (MeOH) used for the extraction of plant materials were of analytical grade (Merck, Darmstadt, Germany). Reagents and standards used for estimating the total phenolic content and total flavonoid assays were: Folin-ciocalteu and quercetin hydrate from Sigma-Aldrich (St. Louis, MO, USA); sodium carbonate, sodium hydroxide, and sodium nitrite from Classic Chemicals (Shah Alam, Malaysia); gallic acid and aluminum chloride from Merck. For the anticholinesterase assay, acetylthiocholine iodide and acetylcholinesterase from electric eel, bovine serum albumin, 5,5′-dithiobis (2-nitrobenzoic acid), and butyrylcholinesterase from equine serum, and S-butyrylthiocholine chloride and physostigmine were purchased from Sigma-Aldrich.

2.3. Extraction of plant material

Prior to carrying out the extraction, young and mature fronds were separated based on their physical appearance. The young fronds are tender, juicy, and have a reddish–orange hue, while mature fronds are stiff and pure light green in color (Fig. S1). Upon separation, the fronds were cleaned, freeze-dried, and subsequently pulverized with a mill grinder. The powdered materials were extracted sequentially with *n*-hexane, DCM, and MeOH, twice for each solvent and for 20 minutes for each extraction, in a B-5510 ultrasonic cleaning bath operating at 42 kHz and 135 kW (Branson Ultrasonics Corporation, Danbury, CT, USA). The extracts obtained for each solvent were combined, filtered, and evaporated to dryness under reduced pressure at temperature < 40°C. The percentage yield of each plant extract was calculated based on the weight of the dried extract obtained (g) for every g of dried plant material used.

2.4. Cholinesterase inhibitory assay

Cholinesterase inhibitory potential of the samples was determined using a spectrophotometric method that was modified from that described by Ellman et al [23]. For the acetylcholinesterase (AChE) inhibition assay, 140 μL 0.1M Na_2PO_4 buffer (pH 8) was added to a 96-well microplate, followed by the addition of 20 μL test sample and 20 μL acetylcholinesterase enzyme (0.09 U/mL). Ten microliters 10mM 5,5′-dithiobis (2-nitrobenzoic acid) was then added to each well, followed by addition of 10 μL acetylthiocholine iodide (14mM). The absorbance of the colored end-product was measured at 412 nm at designated intervals for 30 minutes after initiation of the enzymatic reaction by a Infinite 200 ProMicroplate Spectrometer (Tecan, Männedorf, Switzerland).

For the butyrylcholinesterase (BChE) inhibition assay, the same procedure as described for AChE was followed; however, the enzymes and substrates were substituted with butyrylcholinesterase from equine serum and S-butyrylthiocholine chloride, respectively.

A set of five concentrations of each plant extract or isolated compound was used to estimate the 50% inhibitory concentration (IC_{50}). Physostigmine, a cholinesterase inhibitor, was used as the reference standard. Absorbance of the test