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AN EVALUATION OF ANTIOXIDANT, ANTICHOLINESTERASE AND ANTIMICROBIAL ACTIVITIES OF LIQUIDAMBAR STYRACIFLUA L. LEAVES

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ABSTRACT

The sweet gum tree, *Liquidambar stryraciflua*, is traditionally used as medication. The aim of the present study was to evaluate the antioxidant, antimicrobial activities and acetylcholinesterase inhibitory capacity of 80% methanol extract of *Liquidambar styraciflua* leaves. Antioxidant activity was measured using the $ABTS^{\bullet+}$ and DPPH radical scavenging assays. Antimicrobial activity was tested using the disc diffusion assay, while acetylcholinesterase inhibition was determined using the Eldeen colorimetric method. Phytochemical analysis showed the presence of triterpenes and/or sterols, flavonoids, tannins and carbohydrates. Extracts of *Liquidambar styraciflua* leaves did not show any significant bioactivity with regards to acetylcholinesterase inhibition (IC_{50} > 100 µg/ml) or inhibition of microbial growth (no zone of inhibition), though moderate antioxidant activity was present (IC_{50} of 4.33 and 26.70 µg/ml in the $ABTS^{\bullet+}$ and DPPH assays, respectively). This study does not support the potential for the leaves of *L. styraciflua* to be used as a replacement for the roots in the treatment of wounds.

INTRODUCTION

Alzheimer's disease (AD) is one of the most widespread neurodegenerative diseases that involve dementia and mainly afflicts people over 65 years of age [1]. The therapy of early and moderate stages of AD is mainly based on acetylcholine esterase inhibitors such as galanthamine [1]**.** Furthermore, the increase in multiple-drug resistant microbes has indicated the need for the development and discovery of new antimicrobial drugs [2]. Plants are considered as natural antioxidant, antimicrobial and acetylcholinesterase

inhibitory activity resources. *Liquidambar styraciflua* L. (Hamamelidaceae) is commonly known as the sweet gum tree [3]. It is native to southeastern, east-central, and south-central United States, southern Mexico, and central America [3]. The bark is grayish brown, deeply furrowed into narrow, somewhat rounded ridges. The leaves are alternate, simple, dark green and lustrous above. Flowers are monoecious, female, on a slender stalk. The fruit is a dangling brown, woody spiny tipped “gum ball” with seeds brownish and winged [4, 5]. Traditionally the roots of the plant are used to treat wounds, skin sores, diarrhoea and dysentery, while the bark is used as a sedative for nervous patients or those presenting with fever [6, 7]. Harvesting of roots and bark from trees may incur plant damage or death, which in turn results in ecological damage. It is thus suggested that potential for substitution of plant parts be investigated to allow for less damage to be done. The aim of this study was to evaluate the antioxidant, antimicrobial and acetylcholinesterase inhibitory activity of methanol 80% extract of *Liquidambar styraciflua* leaves to assess its potential as possible drug lead.

MATERIALS AND METHODS

Reagents

Acetylthiocholine iodide, acetylcholinesterase (type VI-S from electric eel), 2,2'-azinobis-(3-ethyl benzothiazoline 6-sulfonic acid) (ABTS), bovine serum albumin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 5,5-dithiobis-2-nitrobenzoic acid, galanthamine, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), magnesium chloride, potassium peroxidisulfate and sodium chloride was procured from Sigma-Aldrich (St. Louis, USA). Methanol solvent was purchased from El-Nasr Company, Cairo, Egypt.

Plant collection and extract preparation

L. styraciflua leaves were collected from Al-Zohiriya Garden, Giza, Egypt in May 2011. The plant identity was verified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman Botanical Garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

Extract preparation

Air-dried powder (350 g) of *L. styraciflua* leaves were extracted by maceration method with methanol 80% six times at room temperature. The extract was concentrated under reduced pressure to yield 36 g of crude extract. Methanol 80% extract was phytochemically screened to identify the presence or absence of sterols and/or triterpenes [8], carbohydrates and saponins [9], flavonoids and alkaloids [10], coumarins [11] and tannins [12].

Antioxidant assays

2, 2'-azinobis-(3-ethyl benzothiazoline 6-sulfonic acid) radical ($ABTS^{•+}$) scavenging assay

The $ABTS^{•+}$ scavenging activity of the crude extracts was determined according to the method of Re et al. [13]. $ABTS^{•+}$ (7.46 mM) was prepared in distilled water and oxidized using 2.5 mM potassium peroxidisulfate at 4°C for 16 h. The oxidized $ABTS^{•+}$ solution was diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm (Lambda UV/VIS Spectrophotometer, Perkin Elmer). Into a 96-well plate was pipetted: 20 µl distilled water (negative control), Trolox (6.25, 12.5, 25, 50, 75 and 100 µg/ml; positive control) or crude extracts (half-log dilutions of 1 mg/ml) followed by 180 µl $ABTS^{•+}$ solution. Absorbance was measured at 405 nm after 15 min (Synergy 2, Bio-Tek Instruments, Inc.). The percentage inhibition of $ABTS^{•+}$ was determined relative to the negative control.

DPPH scavenging assay

The DPPH scavenging activity of the crude extracts was determined according to the method of Gyamfi *et al.* [14]. Into a 96-well plate was pipetted: 20 µl distilled water (negative control), Trolox (0.0313, 0.0625, 0.125, 0.25, 0.5 and 1 mg/ml) or crude extracts (half-log dilutions of 1 mg/ml) followed by 180 µl DPPH solution (240 µM). Absorbance was measured after 15 min at 570 nm. The percentage inhibition of DPPH was determined relative to the negative control.

Antimicrobial assay

Microorganisms

Candida albicans (ATCC 90028), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 9027) were purchased from The American Tissue Culture Collection (ATCC). A clinical isolate of each strain was obtained from the Department of Microbiology (National Health Laboratory Services, Pretoria, South Africa). Strains were maintained on either Mueller Hinton (fungus) or MacConkey agar (bacteria) at 4°C. Inocula were prepared from 24 h subcultures.

Disc diffusion assay

The disc diffusion assay was carried out as described by Bauer et al. [15] was used to identify extracts with antifungal activity. Sterile filter paper discs (Whatman No 1, 10 mm) were impregnated with 200 µl or 300 µl of the 10 mg/ml crude plant extract and the discs dried to drive off the solvent. Spread plates were prepared by placing 100 µl of the appropriate inoculum (5 x 105 CFU/ml) on the agar plates. The extract impregnated filter paper discs were placed on the inoculated plates and incubated at 37°C for 24 h. Amphotericin B was included as positive control for C. albicans and ampicillin for S. aureus and P. aeruginosa (Mast Diagnostics). The negative control was prepared by using the solvent. Antimicrobial activity was expressed as the mean diameter of the zone of inhibition (mm) around the disc.

Acetylcholinesterase inhibitory assay

The acetylcholinesterase inhibitory activity was determined according to the method of Eldeen *et al*. [16]. Three (A-C) 50 mM Tris-hydrochloride buffers (pH 8) were prepared, with buffer B containing an additional 0.1% bovine serum albumin, and buffer C containing an additional 0.1 M sodium chloride and 0.02 M magnesium chloride. All samples were diluted in buffer A. Into a 96-well plate was pipetted: 25 µl acetylthiocholine iodide (in distilled water), 125 µl 5,5-dithiobis-2-nitrobenzoic acid (3 mM in buffer C), 50 µl buffer B and 25 µl buffer A (negative control), galanthamine (half-log dilutions of 320 µg/ml) or crude extracts (half-log dilutions of 1 mg/ml). Absorbance was measured at 405 nm (four times) to account for spontaneous conversion of the substrate. A further 25 µl acetylcholinesterase (0.2 U/ml in buffer A) was pipetted and the absorbance measured at 405 nm every 45 s (fifteen times). Percentage inhibition of acetylcholinesterase was determined through the rate of the reaction (correcting for spontaneous colour changes) relative to the negative control.

Statistics

Results are expressed as the mean ± standard error of the mean (SEM). All experiments were performed in triplicate on three separate occasions. The concentration able to inhibit 50% of activity (IC_{50}) was determined using non-linear regression (variable, normalized slope). All statistical analysis was done using GraphPad Prism 5.0.

RESULTS AND DISCUSSION

Phytochemical analysis indicated the presence of flavonoids, tannins in high content, triterpenes and/or sterols, and carbohydrates in leaf extract of *L. styraciflua* (Table 1). Antioxidant activity was determined using the $ABTS^{•+}$ and DPPH assays, where the positive control (Trolox) was the most potent sample in terms of the IC_{50} ($ABTS^{•+}$ = 2.06 µg/ml; DPPH = 2.62 µg/ml) (Table 2).

Table 1. Phytochemicals tested for *Liquidambar styraciflua* methanol leaf extract.

(a) Chemical Constituents	(b) 80% methanol extract
Carbohydrates and/or glycosides	+
Tannins	
a. Condensed tannins	+
b. Hydrolysable tannins	+
Alkaloids and/or nitrogenous bases	-
Flavonoids	+
Sterols and/or triterpenes	+
Saponins	-
Coumarins	-
(+) denotes the presence of the constituents, (-) denotes the absence of the constituents	

Table 2: Antioxidant and acetylcholinesterase activity of *L. styraciflua* methanol leaf extract.

Plant	**IC_{50} (µg/ml) ± SEM**		
	Antioxidant activity		**Acetylcholinesterase inhibitory activity**
	$ABTS^{\bullet+}$	DPPH	
L. styraciflua	4.33 ± 1.07	26.70 ± 1.04	>100
Trolox	2.06 ± 1.03	2.62 ± 1.04	N/A
Galanthamine	N/A	N/A	1.02 ± 1.09

The extracts displayed a 6-fold greater affinity for the $ABTS^{\bullet+}$ radical (4.33 µg/ml) than that of DPPH (26.70 µg/ml), however, it did not perform as well as Trolox ($ABTS^{\bullet+}$ = 0.48-fold; DPPH = 0.10-fold). This activity does allow for further studies with regards to its ability to scavenge free radicals, and could deliver a possible antioxidant source. The presence of flavonoids could be responsible for the activity seen. The highest acetylcholinesterase inhibitory activity was observed for the positive control (galanthamine), with an IC_{50} of 1.02 µg/ml (Table 2). The extract showed slight acetylcholinesterase inhibitory activity with an inhibition of 41.07% at 100 µg/ml (IC_{50} >100 µg/ml). As the extract displayed weak inhibitory activity further development as an acetylcholinesterase inhibitor is not advisable. The extract did not show any antimicrobial activity against the ATTC or clinical strains of any of the tested microorganisms. Literature concerning the bioactivity of *L. styraciflua* is scarce, and the most prominent activity appears to relate to its potent anticancer activity displayed in extracts of the cones containing triterpenoids (Fukuda *et al*., 2005).

CONCLUSION

The results of this work indicated that methanol 80% extract of *L. styraciflua* leaves does not present with possibilities for development as an anticholinesterase or antimicrobial compounds. Its antioxidant activity however could be further investigated. As the leaves do not appear frequently used, it is thus suggested that further studies be carried out on other plant parts, which may yield more bioactive constituents with the potential to be used for substitution.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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