BioRisk 17: 309–315 (2022)
doi: 10.3897/biorisk.17.77052
https://biorisk.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
BioRisk

Plant products with acetylcholinesterase inhibitory activity for insect control

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Academic editor: Kalina Danova | Received 26 October 2021 | Accepted 13 January 2022 | Published 21 April 2022

Citation: Georgiev B, Nikolova M, Aneva I, Dzhurmanski A, Sidjimova B, Berkov S (2022) Plant products with acetylcholinesterase inhibitory activity for insect control. In: Chankova S, Peneva V, Metcheva R, Beltcheva M, Vassilev K, Radeva G, Danova K (Eds) Current trends of ecology. BioRisk 17: 309–315. https://doi.org/10.3897/biorisk.17.77052

Abstract

Acetylcholinesterase (AChE) inhibitors are widely used in Alzheimer's treatment, but they are also crucial for their action on organophosphorus insecticides. The latter exert their toxicity by inhibiting the AChE enzyme in insects, leading to their death. Amaryllidaceae alkaloids have been proven to be potent AChE inhibitors. In the present study methanolic extracts and essential oils being obtained from species of Asteraceae, Lamiaceae, Brassicaceae and Amaryllidaceae were evaluated in vitro for AChE inhibitory activity. Ellman's colourimetric method, with modifications, was used for AChE activity evaluation. According to the activity level, the tested plant products were divided into three categories. First: plant products with strong activity comparable to that of galanthamine; second: plant products with medium activity, with IC_{50} value about 1 mg/ml and the last group with low activity, with IC_{50} value greater than 1 mg/ml. Essential oils of *Origanum vulgare* subsp. *hirtum* Ietswaart., *Satureja pilosa* Vel., *Monarda fistulosa* L., *Thymus longedentatus* (Degen & Urum.) Ronniger and the methanolic extract of *Leucojum aestivum* L. showed the most potent activity and were referred to as the first group. Carvacrol was identified as the main component of the most active essential oils. In *L. aestivum* extract, galanthamine was found as the main alkaloid. The obtained results indicate that essential oils and alkaloid-rich plant extracts possess the strongest AChE inhibitory activity. This gives us a reason to recommend these plant products to be tested for insecticidal activity in the future.

Keywords

Acetylcholinesterase, alkaloids, carvacrol, essential oils, galanthamine

Introduction

The use of natural products as an alternative to synthetic insecticides is a priority for modern agriculture. At the root of the mechanism of action of organophosphorus insecticides is acetylcholinesterase inhibition (López et al. 2002; Lopez and Pascual-Villalobos 2010; Pang 2014; Mladenović et al. 2018). Amaryllidaceae alkaloids and terpenoids are secondary metabolites with proven high AChE inhibitory activity (López et al. 2002; Elgorashi et al. 2004; Wojtunik-Kulesza et al. 2017). Plant species of Amaryllidaceae, Lamiaceae and Asteraceae are rich in compounds from these chemical groups. Mono-, sesqui- and diterpenes, together with phenols, esters, oxides, ketones, alcohols and aldehydes are the main components of essential oils. Extensive studies on essential oils reveal that they possess various biological activities of great importance, such as toxic and repellent (insecticidal) activity (Isman 2000; Pascual-Villalobos and Ballesta-Acosta 2003; García et al. 2005).

In the present study, methanolic extracts and essential oils from species of Amaryllidaceae, Asteraceae, Brassicaceae and Lamiaceae were evaluated in vitro for AChE inhibitory activity.

Materials and methods

Plant material

Plant material was collected from natural localities of the studied species (*Artemisia santhonicum* L., *Artemisia lerchiana* L., *Micromeria dalmatica* Benth., *Thymus longedentatus* (Degen & Urum.) Ronniger, *Aurinia uechtritziana* (Bornm.) Cullen & T.R. Dudley, *Tanacetum parthenium* L., *Salvia forskaohlei* L., *Salvia sclarea* L., *Salvia aethiopis* L., *Thymus yankae* L., *Centaurea arenaria* M.Bieb. ex Willd., *Nepeta caria* L. and *Eupatorium perfoliatum* L.) or *ex situ* collections from the Institute of Biodiversity and Ecosystem Research (*Leucojum aestivum* L.) and the Institute of Roses and Aromatic Plants (*Origanum vulgare* subsp. *hirtum* Ietswaart., *Monarda fistulosa* L. and *Satureja pilosa* Vel.).

Extraction of plant material

Methanolic extracts. Air-dried powdered plant material (1 g) was extracted with methanol for 24 hours at room temperature. After filtration, the organic solution was evaporated and the dry extract stored at 4 °C until analysis.

Essential oils. The essential oil was extracted on a Clevenger apparatus by water distillation from 50 g of dry plant material in a flask with 500 ml water for 2 hours.

Acetylcholinesterase (AChE) inhibition assay

Acetylcholinesterase inhibitory activity of all samples was determined using Ellman's colorimetric method, as modified by López et al. (2002). The assay was performed in 96-

well microplates. Acetylthiocholine iodide (ATCI) in a solution with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) was used as a substrate for AChE from *Electrophorus electricus* (Sigma-Aldrich, Germany). Methanolic extracts and essential oils from the studied plants with concentrations between 0.001 and 1000 μg/ml were tested.

AChE (50 μl) at a concentration of 0.25 U/ml was dissolved in phosphate buffer (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4, 0.15 M NaCl, pH 7.5) and 50 μl of the sample dissolved in the same buffer were added to the wells. The plates were incubated for 30 minutes at room temperature before the addition of 100 μl of the substrate solution (0.04 M Na_2HPO_4, 0.2 mM DTNB, 0.24 mM ATCI, pH 7.5). The absorbance values were read on a microplate reader (BIOBASE, ELISA-EL10A, China) at 405 nm after 3 minutes. Enzyme activity was calculated as an inhibition percentage compared to an assay using a buffer without any inhibitor. Galanthamine was used as a positive control. The AChE inhibitory data were analysed in Microsoft Excel and the software package Prism 9 (Graph Pad Inc., San Diego, USA). The IC_{50} values were measured in triplicate and the results are presented as means.

GC/MS analysis

The most active samples were analysed for their bioactive components by GC/MS. The spectra were recorded on a Thermo GC, equipped with a Focus DSQ II mass detector, coupled to a HP-5MS capillary column (30 m length × 0.25 mm inner diameter × 0.25 μm film thickness). The chromatographic conditions for methanolic extracts and essential oils are described by Berkov et al. (2021) and by Traykova et al. (2019), respectively. The components were identified by comparing their mass spectra and retention indices (RI) with those of authentic standards and the National Institute of Standards and Technology (NIST) spectra library.

Results

Eighteen samples – 4 essential oils and 14 methanolic extracts of 17 plant species - were examined for AChE inhibitory activity by Ellman's colorimetric method, with modifications by López et al. (2002). The results are presented in Table 1.

According to the level of activity, the tested plant samples were divided into three groups. First group: plant samples with strong activity comparable to that of galanthamine (positive control: IC_{50} 4.03 μM = 0.0011 mg/ml), including the methanolic extract of *L. aestivum* and essential oils of *M. fistulosa*, *S. pilosa*, *O. vulgare* ssp *hirtum* and *T. longedentatus*; second group: plant products with moderate activity with IC_{50} value about 1 mg/ml, including the methanolic extracts of *S. aethiopsis*, *A. lerchiana*, *A. santhonicum*, *E.cannabinum*, *N. caria*, *M. dalmatica* and *O. vulgare* ssp *hirtum* and the last group with low activity with a IC_{50} value above 1 mg/ml; the third group comprises the rest of the samples which showed low activity. For *O. vulgare* ssp *hirtum*, it was shown that the essential oil is a stronger inhibitor of AChE than the methanolic extract.

Table 1. Acetylcholinesterase inhibitory activity of essential oils and methanolic extracts.

Plant species	Extract/EO	AChE activity IC_{50} [mg/mL]
Amaryllidaceae		
Leucojum aestivum	MeOH	0.20
Asteraceae		
Artemisia lerchiana	MeOH	1.08
Artemisia santhonicum	MeOH	0.94
Centaurea arenaria	MeOH	> 1
Eupatorium cannabinum	MeOH	1.07
Tanacetum parthenium	MeOH	> 1
Brassicaceae		
Aurinia uechtritziana	MeOH	> 1
Lamiaceae		
Micromeria dalmatica	MeOH	1.16
Nepeta caria	MeOH	1.12
Origanum vulgare ssp *hirtum*	MeOH	0.73
Salvia aethiopis	MeOH	1.23
Salvia forskaohlei	MeOH	> 1
Salvia sclarea	MeOH	> 1
Thymus yankae	MeOH	> 1
Monarda fistulosa	EO	0.0042
Origanum vulgare ssp *hirtum*	EO	0.30
Satureja pilosa	EO	0.0069
Thymus longidentatus	EO	0.72
Galanthamine	Positive control	0.0011

The most active samples were analysed for their bioactive components by GC/MS. The essential oil profiles of the studied samples are presented in Table 2. Monoterpenoid phenols – isomers carvacrol and thymol - were identified as main components of the essential oils of *S. pilosa*, *O. vulgare* ssp. *hirtum* and *M. fistulosa*. *p*-Cymene (16.26%) and *γ*-terpinene (16.07%) were found as the next most abundant constituents of *O. vulgare* ssp. *hirtum*. In the essential oil profile of *M. fistulosa*, thymoquinone (25.41%) and *p*-cymene (21.82%) were present in significant amounts. Neral and geranial were identified as major components of the essential oil of *T. longedentatus* with 24.9% and 27.95%, respectively.

In *L. aestivum* methanolic extract, galanthamine was found as the main alkaloid. In the methanolic extract of *O. vulgare* ssp., *hirtum* carvacrol (15.67%) was also detected, but in a much smaller amount compared to the essential oil. Rosmarinic acid (6.06%), flavonoid glycosides (1.49%), malic acid (1.09%) and catechin (0.23%) were also identified as bioactive compounds.

Discussion

Four essential oils and 14 methanolic extracts were studied for AChE inhibitory activity. All studied essential oils showed significant activity and their profiles were determined by GC/MS. Isomers – carvacrol and thymol - were identified as the main

Table 2. Main compounds identified in the essential oils of studied species (*Mf*: *M. fistulosa*; *Ovh*: *O. vulgare* ssp *hirtum*; *Sp*: *S. pilosa*; *Tl*: T. *longedentatus*); Area (%).

Compounds	RI	Studied essential oils			
		Mf	***Ovh***	***Sp***	***Tl***
α-Thujene	930	4.72	4.66	–	–
α-Pinene	932	1.29	1.43	–	–
Sabinene	971	–	–	–	0.43
ß-Myrcene	988	–	–	4	–
p-Cymene	1025	21.82	16.26	2.97	–
trans-ß-Ocimene	1044	–	–	–	1.15
γ-Terpinene	1059	–	16.07	1.04	–
Camphor	1141	–	–	–	1.34
Terpinen-4-ol	1175	0.98	–	–	0.74
Neral	1227	–	–	–	24.9
Carvacrol methyl ether	1245	1.98	2.54	1.73	–
Thymoquinone	1250	25.41	–	–	0.22
Geranial	1264	–	–	–	27.96
Thymol	1290	19.75	–	30.58	–
Carvacrol	1299	12.24	51.18	50.54	0.19
Neryl acetate	1359	–	–	–	12.79
Caryophyllene	1466	0.69	1.43	–	–
Caryophyllene oxide	1590	–	–	1.18	–

components of the essential oils of *S. pilosa*, *M. fistulosa* and *O. vulgare* ssp. *hirtum.* Carvacrol is a compound with a previously demonstrated strong AChE inhibitory activity (Jukic et al. 2007) and probably determines the activity of the essential oils. The established profile of *O. vulgare* ssp. *hirtum* is in accordance with data reported in literature for the natural populations of the species in Bulgaria from East Rhodopes, Strumska Valley and cultivated areas (Konakchiev et al. 2004; Alekseeva et al. 2021; Baycheva and Dobreva 2021). The composition of *S. pilosa* and *M. fistulosa* corresponds to that reported by Semerdjieva et al. (2020) and Ghosh et al. (2020), respectively. Citral isomers neral and geranial were determined as main components of the essential oil profile of *T. longedentatus* (Aneva et al. 2019).

Essential oils of many plant species have been examined as an alternative to synthetic insecticides (Isman 2000; Pascual-Villalobos and Ballesta-Acosta 2003; García et al. 2005).

To the best of our knowledge, we report for the first time AChE activity of the essential oils of *O. vulgare* ssp. *hirtum*, *T. longedentatus S. pilosa* and *M. fistulosa*. The established strong inhibitory activity of the tested essential oils is a prerequisite for the presence of insecticidal activity. For *O. vulgare* ssp *hirtum*, it was shown that the essential oil is a stronger inhibitor of AChE than the methanolic extract. Assuming the activity is dependent on the presence of carvacrol, the difference in its content between the essential oil and the methanolic extract may also determine the difference in AChE activity.

As galanthamine is a classic example for a substance with potent AChE inhibitory activity (Sramek et al. 2000), it undoubtedly determines the activity of the methanolic extract of *L. aestivum*.

Conclusion

The obtained results indicate that the essential oils of *Monarda fistulosa*, *Satureja pilosa*, *Origanum vulgare* subsp. *hirtum* and *Thymus longedentatus* and the methanolic extract of *Leucojum aestivum* possess the strongest AChE inhibitory activity. GC/MS analysis proved the presence of bioactive compounds in these plant products. Thus, we recommend them to be tested for insecticidal activity in the future.

Acknowledgements

This research was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science (Grant DN 16/2, 11.12.2017).

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