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RESPONSIBLE EDITOR Prof. Ugo Oliviero, Department of Translational Medical Sciences, Federico II University, Via pansini 5, Napels, Campania, 80131 Italy, e-mail: ugo.oliviero@unina.it

PUBLISHER Walter de Gruyter GmbH, Berlin/Boston, Genthiner Straße 13, 10785 Berlin, Germany

JOURNAL MANAGER Katharina Appelt, De Gruyter, Genthiner Str. 13, 10785 Berlin, Germany, Tel.: +49 (0)30 260 05-325, e-mail: jbcpp.editorial@degruyter.com

RESPONSIBLE FOR ADVERTISEMENTS Kevin Göthling, De Gruyter, Genthiner Straße 13, 10785 Berlin, Germany, Tel.: +49 (0)30 260 05-170, e-mail: anzeigen@degruyter.com

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Frontmatter

Page range: i-ii

Original Articles

Unlicensed | June 25, 2021

Cost of illness of diabetes mellitus in Indonesia: a systematic review

Yohana Febriani Putri Peu Patty, Mufarrihah, Yunita Nita

Page range: 285-295

Abstract

Objectives Diabetes Mellitus (DM) is a group of insulin metabolism disorder that affects the socio-economic conditions of the community. The cost of treating diabetes in 2019 was USD 760 billion and by 2045 there are predicted to be 700 million people living with diabetes. The purpose of this systematic review was to provide an overview of the economic burden caused by Diabetes Mellitus for the government, health care providers, and for the community. Methods This systematic review was carried out by considering the related studies about the cost of illness, evaluation of disease costs, or therapeutic costs for various types of diabetes mellitus that were published in both English and Indonesian. The search engines PUBMED, DOAJ, SCOPUS, SCIENCE DIRECT, and GOOGLE SCHOLAR were used without date published restrictions. Results A systematic search identifies 18 eligible studies conducted in various regions in Indonesia. The study was retrospective with variation in their perspectives and methods to estimate the diabetes cost. Drug cost was the major contributor to direct medical cost followed by complications cost while other cost was affected by transportation cost, productivity losses, and time spent by family accompanying patients. Conclusions Diabetes mellitus

creates a significant financial burden and affects the health care system as well as the individual and society as a whole. Research about the cost of diabetes in the future should be carried out on a large scale in order to get a more specific cost estimation.

Unlicensed | June 25, 2021

Social media health interventions to improve diabetes mellitus patient outcome: a systematic review

Riza Alfian, Umi Athiyah, Yunita Nita

Page range: 297-304

Abstract

Objectives The use of modern technology and social media has revolutionized the way health information is distributed to diabetes mellitus patients. Social media can be used as a medium of providing health interventions to improve patient health outcomes. Social media is able to provide a more intensive communication facility between healthcare professionals and patients. We aim to systematically review and describe the effect of social media interventions on health outcomes of patients with diabetes mellitus. Methods A systematic review was carried out from three electronic databases (Pubmed, Scopus, and Medline). Eligible publications are studies that describe the application of social media interventions on the health outcomes of patients with diabetes mellitus. Results Fourteen studies were selected for this systematic review, 10 studies with a randomized controlled trial design, and 4 studies with a nonrandomized controlled trial design. Six studies only used interventions using social media, A blend of face-to-face social media intervention was used in 6 studies, 2 studies used a combination of telephone and social media intervention. One study had treatment behavior outcomes with improvement in treatment behavior, 6 studies had clinical outcomes (an improvement in HbA1c values in the four studies), 6 studies had treatment behavior outcomes and clinical outcomes (1 study had improved treatment behavior and clinical outcomes, 3 studies had improved treatment behavior outcome only), and 1 study had medication adherence outcome (no improvement in medication adherence). Conclusions These findings indicate that the intervention using social media can improve the health outcomes of diabetes mellitus patients.

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Developing pharmacokinetics–pharmacodynamics model of valproic acid syrup based on prediction of population pharmacokinetics parameter and seizure frequency in Indonesian pediatric epilepsy outpatients

I Komang Prawira Nata Nugraha, Anita Purnamayanti, I Gusti Ngurah Made Suwarba, Nani Parfati

Page range: 305-311

Abstract

Objectives Valproic acid (VPA) is a broad-spectrum antiepileptic drug with known efficacy profile in pediatric patients, despite of its narrow therapeutic index. There is lack of VPA's pharmacokinetics profile in Indonesian pediatric subjects, partly due to limited pediatric blood volume taken for conducting therapeutic drug monitoring. This study aimed to determine the correlation between VPA pharmacokinetics parameters based on population data and seizure frequency in pediatric epilepsy outpatients. Methods This observational study was conducted at Sanglah General Hospital during June–December 2019. The subjects of this research were 38 pediatric epilepsy patients who adhered to VPA syrup monotherapy for at least 3 weeks. Five subjects randomly selected for blood sample collection. Thus, VPA concentration level in the blood being analysed as a comparison to its concentration predicted from Yukawa's steady state equation. Monolix2019R2 ® software was used to identify VPA population pharmacokinetics–pharmacodynamics (PK–PD) parameters at steady state level. Results Population PK–PD of VPA syrup at steady state level were ka_pop = 6.25/h, Vd_pop = 3.36 L, Cl_pop = 3.17·e −11 mL/min, IC 50 _pop = 1.85·e −6 , correlation of Vd_pop and Cl_pop = 0.966. Kendall Tau Correlation of predicted VPA steady state concentration and frequency of seizure was −0.66. Mean prediction error

between predicted steady state concentration of five subjects and their related blood levels was ≤25% and considered as within clinically acceptable limit. Conclusions It needs further study to develop best matched PK–PD model of VPA syrup at steady state condition in pediatric epilepsy.

Unlicensed | June 25, 2021

Acetylcholinesterase inhibitory activity of extract and fractions from the root of *Rauvolfia serpentina*(L.) Bth.ex Kurz

Suciati, Debora Poerwantoro, Aty Widyawaruyanti, Kornkanok Ingkaninan

Page range: 313-317

Abstract

Objectives Alzheimer's disease (AD) is a degenerative brain disease characterized by confusion, behavior changes, decline in memory and cognitive skills. One of the strategies in the treatment of AD is to use acetylcholinesterase (AChE) inhibitors. The current study aims to determine the AChE inhibitory activities of the extract and fractions of the root of Rauvolfia serpentina . Methods Extraction was carried out by maceration method using ethanol, followed by liquid–liquid partition using n -hexane, ethyl acetate and n -butanol. Further fractionation was conducted by using vacuum liquid chromatography (VLC). The AChE inhibitory assays were performed by using Ellmann's method. Phytochemical screening was carried out by TLC method. Results The ethanolic extract of R. serpentina showed inhibition against AChE enzyme with an IC 50 value of 7.46 µg/mL. The extract and fractions showed higher inhibition against butyrylcholinesterase (BChE) compared to AChE. Amongst three fractions obtained, the n -butanol fraction showed the strongest inhibition with an IC 50 value of 5.99 µg/mL against AChE. VLC fractionation of the n -butanol fraction yielded 13 subfractions (VLC 1–VLC 13). Four out of 13 subfractions gave more than 80% inhibition against AChE, namely subfractions 4–7, with IC 50 values ranging from 4.87 to 47.22 µg/mL. The phytochemical screening of these subfractions suggested the presence of alkaloids. Conclusions The ethanolic extract, as well as fractions of R. serpentina root, are potential for AChE inhibitor. The alkaloid compound may be responsible for this activity.

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Green tea and its active compound epigallocathechin-3-gallate (EGCG) inhibit neuronal apoptosis in a middle cerebral artery occlusion (MCAO) model

Abdulloh Machin, Imam Susilo, Djoko A. Purwanto

Page range: 319-325

Abstract

Objectives To determine the effect of green tea with the active ingredient epigallocathechin-3-gallate (EGCG) on the inhibition of apoptosis in the middle cerebral artery occlusion (MCAO) model. Methods Four month old male Rattus norvegicus rats with a body weight of 200–275 g was used for the MCAO model and divided into five groups, and the treatment was carried out for 7 days. Before being sacrificed, the subject had 1 cc of blood drawn for high mobility group box 1 (HMGB-1) examination using enzyme-linked immunosorbent assay (ELISA), and after being sacrificed, the brain tissue specimen was taken to examine caspase-3 and B-cell lymphoma 3 (BCL-3) using immunohistochemistry methods. Results There was no significant difference in HMGB-1 results for the treatment group compared to the control group (P1: 384.20 ± 231.72 [p = 0.553]; P2: 379.11 ± 268.4 [p = 0.526]; P3: 284, 87 ± 276.19 [p = 0.140]; P4: 435.32 ± 279.95 [p = 0.912]). There is a significant increase in BCL-2 expression between the treatment group compared to the control group (P1: 2.58 ± 0.51 [p = 0.04]; P2: 3.36 ± 0.50 [p<0.001]; P3: 4.00 ± 0.42 [p<0.001]; P4: 3.60 ± 0.52 [p<0.001]). There was a significant difference in caspase-3 expression compared to the control group in the P3 group (P1: 4.33 ± 0.49 [p = 0.652]; P2: 4.09 ± 0.30 [p = 0.136]; P3: 3.58 ± 0.51 [p = 0.01]; P4: 3.89 ± 0.42 [p = 0.063]). There is no correlation between HMGB-1 and caspase-3 (r = −0.063; p = 0.613) or BCL-2 (r = −0.106; p = 0.396). There is significant negative correlation between caspase-3 and

Suciati*, Debora Poerwantoro, Aty Widyawaruyanti and Kornkanok Ingkaninan

Acetylcholinesterase inhibitory activity of extract and fractions from the root of *Rauvolfia serpentina*(L.) Bth.ex Kurz

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Abstract

Objectives: Alzheimer's disease (AD) is a degenerative brain disease characterized by confusion, behavior changes, decline in memory and cognitive skills. One of the strategies in the treatment of AD is to use acetylcholinesterase (AChE) inhibitors. The current study aims to determine the AChE inhibitory activities of the extract and fractions of the root of *Rauvolfia serpentina*.

Methods: Extraction was carried out by maceration method using ethanol, followed by liquid–liquid partition using *n*-hexane, ethyl acetate and *n*-butanol. Further fractionation was conducted by using vacuum liquid chromatography (VLC). The AChE inhibitory assays were performed by using Ellmann's method. Phytochemical screening was carried out by TLC method.

Results: The ethanolic extract of *R. serpentina* showed inhibition against AChE enzyme with an IC_{50} value of 7.46 μg/mL. The extract and fractions showed higher inhibition against butyrylcholinesterase (BChE) compared to AChE. Amongst three fractions obtained, the *n*-butanol fraction showed the strongest inhibition with an IC_{50} value of 5.99 μg/mL against AChE. VLC fractionation of the *n*-butanol fraction yielded 13 subfractions (VLC 1–VLC 13). Four out of 13 subfractions gave more than 80% inhibition against AChE, namely subfractions 4–7, with IC_{50} values ranging from 4.87 to 47.22 μg/mL. The phytochemical screening of these subfractions suggested the presence of alkaloids.

Conclusions: The ethanolic extract, as well as fractions of *R. serpentina* root, are potential for AChE inhibitor. The alkaloid compound may be responsible for this activity.

Keywords: acetylcholinesterase inhibitor; Alzheimer's disease; *Rauvolfia serpentina*.

Introduction

Alzheimer's disease (AD) is a degenerative brain disease characterized by confusion, behavior changes, decline in memory and cognitive skills. This disease has been recorded as the most common cause of dementia in the elderly population [1, 2]. AD often begin with minor symptoms and grow into severe brain damage or even death. The onset of AD usually occurs in population at the age of 65 years or above [3]. The increase in life expectancy means that the number of people suffering from AD is anticipated to increase each year if there is no effective medication found. The pathophysiology of AD is credited to several factors including the acetylcholine (ACh) deficiency due to neuronal loss in the brain. ACh is a neurotransmitter, produced in the nerve ending of the presynaptic nerve, which is associated with memory and cognitive functions. Therefore one of the target treatment of AD is the use of acetylcholinesterase (AChE) inhibitors, which can prevent the hydrolysis of ACh to choline and ethanoic acid [4]. There are three AChE inhibitors approved to be used by FDA for AD, namely galantamine, rivastigmine and donepezil. Donepezil is of a synthetic origin, while rivastigmine is a derivative developed from the natural compound physostigmine [5]. Galantamine is an alkaloid isolated from natural sources, *Galanthus nivalis* (Amaryllidaceae) [6]. The AChE inhibitors are beneficial to improve cognitive, functional and behavioral effects on AD patients. However, studies have shown the presence of several side effects and limited effectiveness of these medications [7, 8]. A sesquiterpenoid alkaloid Huperzine A, isolated from *Huperzia* spp., has also been reported as a potential

***Corresponding author: Suciati**, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia; and Natural Product Medicine Research and Development, Institute of Tropical Diseases, Universitas Airlangga, Surabaya, Indonesia, E-mail: suciati@ff.unair.ac.id
Debora Poerwantoro, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
Aty Widyawaruyanti, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia; and Natural Product Medicine Research and Development, Institute of Tropical Diseases, Universitas Airlangga, Surabaya, Indonesia
Kornkanok Ingkaninan, Bioscreening Unit, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok, Thailand

therapy for AD. The product containing extract of *Huperzia* spp. has been commercialized as a food supplement for memory improvement in China [1].

Plants containing alkaloids have been the target of screening for AChE inhibitors. Recently, we have reported the potency of several *Cassia* spp. as well as several marine sponges as AChE inhibitor [9, 10]. In the current study, investigation on the AChE inhibitory activities of extract and fractions from the root of *Rauvolfia serpentina* was conducted. The root of *R. serpentina* possesses high therapeutic properties. It is traditionally used as a tranquillizer for nervous and mental disorders [11]. The root also provides high protein, starch and micronutrient which is useful for treating malnutrition [12–14]. More than 60 indole alkaloids have been reported from the root of *R. serpentina* [11, 15]. These alkaloids, especially reserpine and rescinnamine have an important role in the well-known hypotensive activity of this plant [11]. The presence of alkaloid compounds in this plant made this worth to be investigated for AChE inhibitory assay.

Materials and methods

Reagents

AChE from electric eel (AChE typeVI-S), horse-serum butyrylcholinesterase (BChE), acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI), 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), and bovine serum albumin (BSA), tris buffer and galantamine were purchased from Sigma–Aldrich.

Plant collection

The dried root of *R. serpentina* was obtained from the local market in Surabaya, East Java, Indonesia. The sample specimen (voucher number: PSR 06) was kept at the Institute of Tropical Diseases Universitas Airlangga. The identification of the plant material was conducted by Purwodadi Botanic Garden (Identification letter number: 814/IPH.06/HM/VIII/2019).

Extraction and fractionation

The dried root was grinded to obtain powdered material. One kilogram of the powdered root was extracted with ethanol 96% by using the maceration method. The sample was submerged in the solvent (2 L) for 24 h, followed by filtration. The residue was re-extracted with ethanol using the same procedure three times. The filtrate was then evaporated in the rotary evaporator to obtain a crude ethanolic extract (18.23 g). A portion of the ethanolic extract (18.0 g) was dissolved with a mixture of ethanol and H_2O (1:1) (220 mL), then further separated by a liquid–liquid partition with *n*-hexane (250 mL), ethyl acetate (250 mL), and followed by *n*-butanol (50 mL). Fractionation with each solvent was conducted three times. Each of the fractions obtained was evaporated *in vacuo*, and yielded *n*-hexane (3.61 g), ethyl acetate (6.80 g), and *n*-butanol (2.63 g) fractions. Further fractionation of the *n*-butanol fraction was conducted by using vacuum liquid chromatography (VLC). Sample (2.6 g) was mixed with silica gel (2.6 g) to obtain a dried powdered sample, which was then applied to the VLC. The sample was eluted with a combination of dichloromethane and methanol in order of increasing polarity, and yielded 13 subfractions namely VLC 1–VLC 13.

Phytochemical screening

Samples were dissolved in ethanol. The solutions were applied on the TLC plate (Silical gel F_{254}), which was then developed with a combination of dichloromethane:methanol (1:3). The plate was visualized under UV 254 and 366 nm, followed by Dragendorff spray to indicate the presence of alkaloid.

Cholinesterase inhibitory assay

The assay was performed according to the modified Ellman's method [9, 10, 16]. The solution of plant extract and fractions were made in methanol at a concentration of 10 mg/mL, which was then diluted to 1 mg/mL with 50 mM Tris buffer. The final test concentration is 100 µg/mL after the dilution of samples in the microplate well. Sample solutions were added to a 96-well microplate, followed by the addition of 1.5 mM ATCI or 1.5 mM BTCI (25 µL), 3 mM DTNB (125 µL), and Tris buffer (50 µL). The substrate was then hydrolyzed by the addition of 25 µL of 0.22 U/mL of either *Ee*AChE or BChE.The solutions were shaken for 30 s in a microplate reader (Bio-Tek Instrument, USA) before measurement. The product, 5-thio-2-nitrobenzoate, indicated by a yellow color, was measured at 405 nm every 5 s for 2 min. Every experiment was carried out in triplicates. Galantamine was used as a positive control, and 10% methanol was used as a negative control. For the $IC_{50,}$ measurement serial concentrations of the samples were prepared ranging from 0.2–200 µg/mL. The enzyme activity was calculated as a percentage of the velocity of the test sample, compared with that of the nontreated control. The inhibitory activity was calculated as:

$$\%\text{Inhibition} = \frac{(\text{mean velocity of control} - \text{mean velocity of sample})}{\text{mean velocity of control}} \times 100$$

Data analysis

The 50% inhibitory concentration (IC_{50}) was determined using GraphPad Prism 7.04 software by plotting log concentrations as axis and % inhibition as ordinate. The inhibition data of the samples against AChE and BChE were analyzed using unpaired *t*-tests in GraphPad Prism 7.04.

Results

The ethanolic extract of the root of *R. serpentina* showed inhibition against the AChE enzyme with an IC_{50} value of 7.46 µg/mL. To investigate the selectivity against cholinesterase enzyme, the sample was also tested against both

AChE and BChE at a concentration of 100 µg/mL. The results shown in Figure 1 indicated that the extract inhibited both enzymes; however, the inhibition was higher against BChE compared to AChE. Likewise, the *n*-hexane, ethyl acetate, and *n*-butanol fractions also gave higher inhibition against BChE than against AChE enzyme (Figure 1). Amongst the three fractions, the *n*-butanol fraction gave the strongest inhibition against AChE with an IC_{50} value of 5.99 µg/mL (Table 1). The determination of the IC_{50} value was not conducted on the *n*-hexane fraction since it only gave low inhibition against the AChE enzyme.

Subfractionation of the *n*-butanol fraction by using VLC yielded 13 subfractions. All subfractions were subjected to AChE inhibitory assay. The results presented in Figure 2 showed that four subfractions, namely subfractions 4–7 gave more than 80% inhibition against the tested enzyme. The IC_{50} values of these subfractions were also determined. The results (Table 2) showed that subfraction 5 gave the strongest inhibition against the AChE with IC_{50} value of 4.87 µg/mL. Subfractions 4–7 were subjected to phytochemical screening by using TLC method. The results showed that after spraying with Dragendorff dye, dark orange color spots on a yellowish-orange background were observed. This suggested that subfractions 4–7 of *R. serpentina* roots contain alkaloid.

Discussion

ACh is a neurotransmitter, produced in the nerve ending of the presynaptic nerve, that has been discovered to be involved in the pathogenesis of AD. This neurotransmitter plays an important role in memory and learning function [17]. In AD patients, the ACh that is released has a very short half-life due to the presence of large amounts of the cholinesterase enzymes, namely AChE and BChE [4, 18].

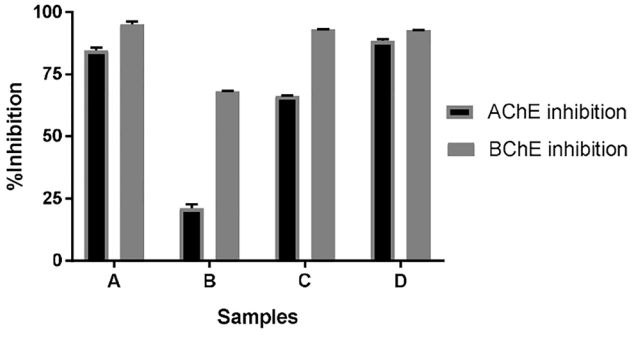

Figure 1: Inhibition of ethanolic extract (A), *n*-hexane (B), ethyl acetate (C), and *n*-butanol (D) fractions of *R. serpentina* root against AChE and BChE at 100 µg/mL.

Table 1: IC_{50} values of extract and fractions of the root of *R. serpentina* against AChE.

Samples	IC_{50}, µg/mL
Ethanol extract	7.46 ± 0.28
Ethyl acetate fraction	23.62 ± 2.97
n-Butanol fraction	5.99 ± 0.86
Galantamine	0.63 ± 0.05

Data presented as mean ± standard deviation of three independent experiments, each done in triplicate.

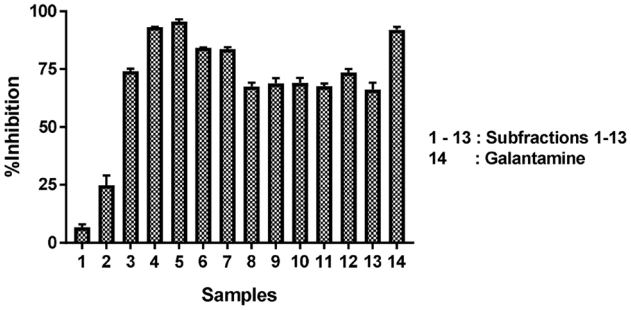

Figure 2: Inhibition of subfractions 1–13 of *R. serpentina* (100 µg/mL) and galantamine (100 µM) against AChE.

Table 2: IC_{50} values of selected VLC subfractions of the root of *R. serpentina* against AChE.

Samples	IC_{50}, µg/mL
Subfraction VLC 4	7.08 ± 0.53
Subfraction VLC 5	4.87 ± 0.45
Subfraction VLC 6	19.88 ± 1.58
Subfraction VLC 7	47.22 ± 1.08

Data presented as mean ± standard deviation of three independent experiments, each done in triplicate.

These enzymes hydrolyze the ACh molecule into choline and ethanoic acid. In the healthy brain, AChE predominates, while BChE plays a minor role. However, in AD patients, the activity of BChE is increased progressively, while the activity of AChE remains unchanged [19]. The cholinergic strategy in the therapy for AD patients included stimulation of cholinergic receptors or increasing the availability of ACh by inhibiting AChE and BChE enzymes [8].

The current AChE inhibitors approved by FDA as medication for AD patients are donepezil, rivastigmine, and galantamine. Considering that all the three drugs are alkaloids that are synthetic or natural origins, searching on the new AChE inhibitor from natural sources has focused on the alkaloids, although other class of compounds, such terpenes, sterols, and flavonoids have also shown potency as AChE inhibitor [20].

Plant from the genus *Rauvolfia* has been known to contain alkaloids. The well-known compound reserpine and rescinnamine have been used as antihypertensive agents. In the present studies, we evaluated the anti-AD activity of the ethanolic extract as well as fractions of *R. serpentina* by measuring the inhibition against AChE and BChE. The extract as well as several fractions have shown strong inhibitions against both enzymes. The *n*-hexane fraction gave the lowest inhibition compared to the ethanolic extract, the ethyl acetate and the *n*-butanol fractions. These results indicated that the active compounds are semipolar to polar compounds. At the concentration of 100 μg/mL, the samples tested demonstrated higher inhibition against BChE compared to AChE enzymes. Analysis by using unpaired t-test, suggested that there were significant differences observed between the inhibition of extracts as well as *n*-hexane, ethyl acetate, and *n*-butanol fractions against AChE and BChE enzymes with p-value < 0.001 for *n*-hexane and ethyl acetate fractions and p-value = 0.003 and 0.004 for the ethanolic extract and *n*-butanol fraction, respectively.

Since the highest enzyme inhibition was given by the *n*-butanol fraction, further fractionation was conducted on this sample by using VLC. Thirteen subfractions were yielded and subjected to the AChE inhibitory assay. The results showed that subfractions 4–7 gave higher potency compared to other subfractions. These subfractions were eluted in a solvent combination of dichloromethane:methanol (85:15) to dichloromethane:methanol (60:40), which suggested that the active compounds are semipolar.

The phytochemical screening of subfractions 4–7 indicated the presence of alkaloid. *R. serpentina* has been known to contain more than 60 alkaloids in the root [11, 15]. Another plant from the same genus, *Rauvolfia reflexa*, has been reported to show inhibition against AChE enzyme. The methanol extract as well as the isolated indole alkaloids from *R. reflexa* demonstrated inhibition against both AChE and BChE enzymes [21]. Several indole alkaloids were found to be more selective against BChE compared to AChE [21, 22]. This suggested that indole alkaloids present in the root of *R. serpentina* may responsible for the cholinesterase inhibitory activity.

Conclusions

The ethanolic extract as well as fractions from the root of *R. serpentina* inhibited cholinesterase enzymes. The alkaloid compounds in the extract and fractions may contributed to the AChE and BChE inhibition.

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Top of page

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36

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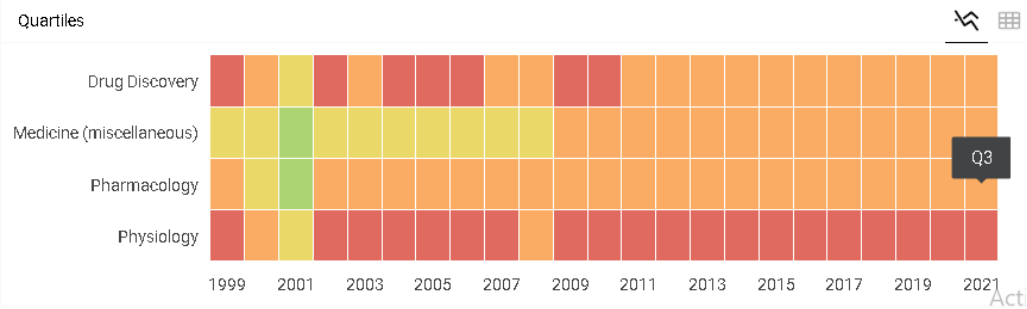

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