Research Article

Anti-Inflammatory, Anticholinesterase, and Antioxidant Potential of Scopoletin Isolated from Canarium patentinervium Miq. (Burseraceae Kunth)

R. Mogana, K. Teng-Jin, and C. Wiart

Center for Natural and Medicinal Products Research, School of Pharmacy, Faculty of Science, University of Nottingham (Malaysia Campus), Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia

Correspondence should be addressed to R. Mogana; khx1msa@nottingham.edu.my

Received 13 March 2013; Accepted 14 June 2013

Academic Editor: Mohd Roslan Sulaiman

Copyright © 2013 R. Mogana et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Bioassay guided fractionation of an ethanol extract of leaves of Canarium patentinervium Miq. (Burseraceae Kunth.) led to the isolation of scopoletin. The structure of this coumarin was elucidated based on spectroscopic methods including nuclear magnetic resonance (NMR-1D and 2D) and mass spectrometry. Scopoletin inhibited the enzymatic activity of 5-lipoxygenase and acetyl cholinesterase with an IC$_{50}$ equal to 1.76±0.01 μM and 0.27±0.02 μM, respectively, and confronted oxidation in the ABTS, DPPH, FRAP, and β-carotene bleaching assay with EC$_{50}$ values equal to 5.62±0.03 μM, 0.19±0.01 mM, 0.25±0.03 mM and 0.65±0.07 mM, respectively. Given the aforementioned evidence, it is tempting to speculate that scopoletin represents an exciting scaffold from which to develop leads for treatment of neurodegenerative diseases.

1. Introduction

The pathophysiology of Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis involves death of neurons upon exposure to excessive amounts of reactive oxygen species (ROS) [1]. Indeed, when ROS overwhelms enzymatic and nonenzymatic antioxidant mechanisms, DNA, lipid, and proteins are denatured compelling thus neuroapoptosis [2]. This offset of antioxidant and oxidant balance in the body is called oxidative stress. Oxidative stress results in the damage of biopolymers including nucleic acids, proteins, polyunsaturated fatty acids, and carbohydrates. Oxidative stress causes serious cell damage leading to a variety of human diseases like Alzheimer’s disease, Parkinson’s disease, atherosclerosis, cancer, liver disease, diabetes, AIDS, arthritis, immunological incompetence, neurodegenerative disorders, inflammation, and so forth [3, 4].

Inflammation in injured cells are both initiated and maintained by the overproduction of prostaglandins and leukotrienes, which are produced by separate enzymatic pathways, namely, the cyclooxygenase (COX) and lipooxygenase (LOX) pathways, respectively. 5-lipoxygenase, catalysing the oxidation of arachidonic acid, produces 5(S)-hydroxyperoxyeicosatetraenoic acid (5-HETE) which undergoes dehydration, resulting in the formation of leukotriene A$_4$ (LTA$_4$). Enzymatic hydrolysis of LTA$_4$, as well as conjugation with other substances, leads to the formation of inflammatory mediators [5]. Leukotrienes have been identified as mediators of a number of inflammatory and allergic reactions including rheumatoid arthritis, inflammatory bowel disease, atopic dermatitis, psoriasis, chronic urticaria, asthma [6, 7], and allergic rhinitis [8].

Furthermore, oxidative and inflammatory processes are among the pathological features associated with the central nervous system in Alzheimer’s disease (AD) [9]. The brain of patients suffering from AD is said to be under oxidative stress as a result of perturbed ionic calcium balances within their neurons and mitochondria [10, 11]. Moreover, there is
evidence that acetyl cholinesterase (AChE) inhibitors have an anti-inflammatory role through action against free radicals and amyloid toxicity, as well as through decreasing release of cytokines from activated microglia in the brain and blood [11]. There is an established link between the cholinergic system and inflammation as acetylcholine, the principle neurotransmitter, is reported to attenuate the release of cytokines in the parasympathetic anti-inflammatory pathway by which the brain modulates systemic inflammatory responses to endotoxin [12].

Canarium patentinervium Miq. is a rare plant from the family of Burseraceae and genus Canarium found in Asia Pacific region previously recorded for its usage in wound healing by the indigenous people of Malaysia [13, 14]. Wound healing involves manifold inflammatory processes of which notably the massive release of leukotrienes from arachidonic acid via the 5-lipoxygenase pathway (5-LOX) and the generation of nitric oxide (NO) from inducible nitric oxide synthase (iNOS) [15, 16]. Of note, nitric oxide (NO) is a free radical, and the generation of cytokines involves a reactive oxygen species (ROS) outburst [17]. Therefore, agents able to block the enzymatic activity of 5-LOX and to scavenge free radical are of immense interest against inflammatory conditions which englobe not only epidermal insults but also neurodegeneration and obesity [18, 19]. Cells produce superoxide anion ($O_2^-$), peroxide anion ($HO_2^-$), and hydroxyl ion ($HO^-$) as part of the physiological aerobic metabolism which are quickly scavenged by catalytic antioxidant defense system [17]. However, in the event of ageing or pathologies, the antioxidant defense system is overwhelmed, and cells suffer massive oxidative stress leading eventually to carcinogenesis or apoptosis [2]. In fact, oxidative stress is the main causative factor for cholinergic and dopaminergic neurons apoptosis hence AD and Parkinson's disease (PD) [2, 10]. In addition, there is a growing body of evidence that point to the fact that 5-LOX inhibitors are of immense therapeutic values [6, 7, 20–22].

In continuation of our earlier studies on the pharmacological properties of Canarium patentinervium Miq. [13, 23, 24], this study investigates the inhibition of 5-lipoxygenase, acetylcholinesterase, and antioxidant capacity of an isolated coumarin, scopoletin. To the best of our knowledge, this is the first comprehensive study on scopoletin isolated from Canarium patentinervium Miq. investigating the antioxidant capacity, anti-inflammatory, and anti-acetylcholinesterase activities.

2. Materials and Methods

2.1. Plant Material. The leaves and barks of Canarium patentinervium Miq. were collected from one individual tree from Bukit Putih, Selangor, Malaysia (3°5′24″N 101°46′0″E). The plant was identified by Mr. Kamaruddin (Forest Research Institute of Malaysia). A herbarium sample (PID 251210-12) has been deposited in the Forest Research Institute of Malaysia. The leaves were air dried and grinded into small particles using an industrial grinder.

2.2. Chemical and Reagents. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2'-Azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid), 2,4,6-tripyridyl-s-triazine (TPTZ), quercetin, β-carotene, 5,5′-Dithio-bis(2-nitrobenzoic) acid (DTNB), galanthamine, nordihydroguaiaretic acid (NDGA), and electric eel acetylcholinesterase (Ache) (Type-VI-S, EC 3.1.1.7) were purchased from Sigma Aldrich. Sodium chloride, ascorbic acid, ferric chloride, and glacial acetic acid were purchased from Systerm. Hexane and chloroform were purchased from Friendemann Schmidt Chemicals. Methanol and ethanol 95%, potassium persulfate powder, ferric chloride, ferrous sulphate, tween 20, and potassium phosphate were purchased from Kollin Chemicals. DMSO was from R&M Marketing, Essex UK. Linoleic acid, acetylthiocholine, and 5-lipoxygenase enzyme (human recombinant) were purchased from Calbiochem.

2.3. Extraction and Isolation. Dried and grinded sample of leaves (2.8 kg) were soaked in hexane with the ratio of 1:3 parts of sample to solvent for 2 h in a 60° C water bath then filtered and concentrated with a rotary evaporator (Buchi, R-200 Switzerland). This was repeated 3 times. Thereafter the leaves and barks were left to air dry completely for 3 days before repeating the whole process with chloroform and then ethanol, respectively. The yield for the hexane, chloroform, and ethanol extract of leaves were 1.25%, 1.11%, and 6.45%, respectively. The ethanol extract of the leaves (80 g) was then partitioned with petroleum ether, chloroform, and water to yield the respective solvent extracts. The chloroform extract (5 g) was further purified by silica gel chromatography (4 cm × 90 cm, 0.063–0.200 mesh) and eluted with a chloroform/methanol gradient elution (the ratio from 100:0 to 8:100). Thirteen column fractions were collected and analyzed by TLC (chloroform/methanol). Fractions with similar TLC pattern were combined to total of four fractions. Fraction 2 that was yielded from chloroform/methanol ratio 100:4 was rechromatographed on a preparative TLC (2 mm thickness) with solvent system chloroform/methanol (ratio of 1000:15) yielding total 7 bands. Band three was collected and rechromatographed on preparative TLC (0.5 mm thickness) with solvent system chloroform/methanol (ratio of 89:11) to yield four bands, with band two yielding 7-hydroxy-6 methoxycoumarin (49 mg) also known as scopoletin (Figure 1).

Scopoletin. Pale yellow powder; IH-NMR (400 MHz, CD$_3$Cl) δ; 3.98 (6-OCH$_3$, s, 3H), 6.30 (H-3, d, $J = 9.5$ Hz, 1H), 6.87 (H-5, s, 1H), 6.95 (H-8, s, 1H), 7.63 (H-4, d, $J = 9.5$ Hz, 1H); 13C-NMR (125 MHz, CD$_3$Cl) δ; 56.4 (6-OCH$_3$), 103.2 (C-5), 107.4 (C-8), 111.6 (C-3), 113.5 (C-10), 143.3 (C-4), 144.0 (C-6), 149.7 (C-9), 150.2 (C-7), 161.6 (C-2); ESI-MS: m/z (relative intensity): 192 (M+, 100), 177 (70), 164 (28) 149 (59).

2.4. Antioxidant Capacity Tests. Scopoletin was dissolved in dimethyl sulfoxide (DMSO, R&M) prior to assay at a stock concentration of 5 mM, and serial dilution was done accord-
ABTS remains stable for 2–3 days in the dark. The concentrated solution was then stored in the dark and 2.4mM potassium persulfate solution. The working control. The stock solution included 7mM ABTS solution (l-ascorbic acid), and quercetin were used as positive control antioxidants. The antioxidant activity scopoletin was determined using the colorimetric FRAP assay, as described by Benzie and Strain [29] with slight modifications. Aliquots of scopoletin were plated out in triplicate in a 96-well microtiter plate at different concentrations. The working FRAP reagent was prepared just before assay by mixing 300 mM of acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM of FeCl₃·6H₂O in ratio of 10:1:1. Briefly 180 μL of the FRAP reagent was mixed with 20 μL of the test sample so that the final dilution of the test sample in the reaction mixture was 1/10. After 30 minutes, the absorbance of the coloured product (ferrous triphyridyltriazine complex) was recorded. Fe(II) concentrations in the range of 1 μM–100 μM (FeSO₄·7H₂O) was used as standard for calibration curve, and equation of linearity is determined (y = ax + b). From the linearity equation, concentration of sample that produced same absorbance as 1 mM of Fe(II) was determined (y of sample filled in equation to obtain x). The antioxidant activity was calculated as Ferrous Equivalents, the concentration of samples which produced an absorbance value equal to that of 1 mM FeSO₄.

2.4.3. Ferric Reducing Ability of Plasma (FRAP) Assay. The antioxidant activity scopoletin was determined using the colorimetric FRAP assay, as described by Benzie and Strain [29] with slight modifications. Aliquots of scopoletin were plated out in triplicate in a 96-well microtiter plate at different concentrations. The working FRAP reagent was prepared just before assay by mixing 300 mM of acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM of FeCl₃·6H₂O in ratio of 10:1:1. Briefly 180 μL of the FRAP reagent was mixed with 20 μL of the test sample so that the final dilution of the test sample in the reaction mixture was 1/10. After 30 minutes, the absorbance of the coloured product (ferrous triphyridyltriazine complex) was recorded. Fe(II) concentrations in the range of 1 μM–100 μM (FeSO₄·7H₂O) was used as standard for calibration curve, and equation of linearity is determined (y = ax + b). From the linearity equation, concentration of sample that produced same absorbance as 1 mM of Fe(II) was determined (y of sample filled in equation to obtain x). The antioxidant activity was calculated as Ferrous Equivalents, the concentration of samples which produced an absorbance value equal to that of 1 mM FeSO₄.

2.4.2. 2,2′-Azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) Assay. The ABTS assay as described by Miller et al. [26], Rice-Evans [27], and Roberta et al. [28] was employed to determine the radical scavenging activity of the plant extracts. Aliquots of scopoletin were plated out in triplicate in a 96-well microtiter plate at different concentrations. Trolox, vitamin C (l-ascorbic acid), and quercetin were used as positive control antioxidants. The antioxidant activity scopoletin was determined using the colorimetric FRAP assay, as described by Benzie and Strain [29] with slight modifications. Aliquots of scopoletin were plated out in triplicate in a 96-well microtiter plate at different concentrations. The working FRAP reagent was prepared just before assay by mixing 300 mM of acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM of FeCl₃·6H₂O in ratio of 10:1:1. Briefly 180 μL of the FRAP reagent was mixed with 20 μL of the test sample so that the final dilution of the test sample in the reaction mixture was 1/10. After 30 minutes, the absorbance of the coloured product (ferrous triphyridyltriazine complex) was recorded. Fe(II) concentrations in the range of 1 μM–100 μM (FeSO₄·7H₂O) was used as standard for calibration curve, and equation of linearity is determined (y = ax + b). From the linearity equation, concentration of sample that produced same absorbance as 1 mM of Fe(II) was determined (y of sample filled in equation to obtain x). The antioxidant activity was calculated as Ferrous Equivalents, the concentration of samples which produced an absorbance value equal to that of 1 mM FeSO₄.

2.4.3. Ferric Reducing Ability of Plasma (FRAP) Assay. The antioxidant activity scopoletin was determined using the colorimetric FRAP assay, as described by Benzie and Strain [29] with slight modifications. Aliquots of scopoletin were plated out in triplicate in a 96-well microtiter plate at different concentrations. The working FRAP reagent was prepared just before assay by mixing 300 mM of acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM of FeCl₃·6H₂O in ratio of 10:1:1. Briefly 180 μL of the FRAP reagent was mixed with 20 μL of the test sample so that the final dilution of the test sample in the reaction mixture was 1/10. After 30 minutes, the absorbance of the coloured product (ferrous triphyridyltriazine complex) was recorded. Fe(II) concentrations in the range of 1 μM–100 μM (FeSO₄·7H₂O) was used as standard for calibration curve, and equation of linearity is determined (y = ax + b). From the linearity equation, concentration of sample that produced same absorbance as 1 mM of Fe(II) was determined (y of sample filled in equation to obtain x). The antioxidant activity was calculated as Ferrous Equivalents, the concentration of samples which produced an absorbance value equal to that of 1 mM FeSO₄.

2.4.4. β-Carotene Bleaching Assay. The β-carotene bleaching assay was conducted according to the method described by Habtemariam and Jackson [30] with some modifications. Aliquots of scopoletin were plated out in triplicate in a 96-well microtiter plate at different concentrations. Briefly, 1 mL of a β-carotene solution in chloroform (2 mg in 10 mL) was pipetted into a round bottom flask containing 40 μL of linoleic acid and 500 μL of Tween 20. After the removal of chloroform using a rotary vacuum evaporator at 45°C, 100 mL of deionised water were added with vigorous agitation. One hundred eighty microlitres of the emulsion was added to 20 μL of test samples at varying concentrations in 96-well microtiter plate. The absorbance was measured at 470 nm immediately against a blank consisting of the emulsion without β-carotene and after 3 h of incubation at 50°C using a spectrophotometer. The antioxidant activity of test agents was evaluated in terms of bleaching of β-carotene using the following formula: antioxidant activity AA (%) = [1 − (A₀ − Aₘ)/(A₀ − Aₜ)] × 100, where A₀ and Aₜ are absorbances measured at zero time of incubation for the test sample and control, respectively; Aₘ and Aₜ are the absorbances measured at 37°C, in a 3 cm cuvette. The total scavenging capacity of the extracts was quantified through the addition of 100 μL ABTS⁺ to 100 μL of test sample. The solutions were heated to 37°C for 7 min, after which the absorbance was read at 734 nm. The percentage decolourisation was calculated using equation below, and the extent of inhibition of the absorbance of the ABTS⁺ was plotted as a function of the concentration. This activity is given as percent ABTS radical scavenging, which is calculated with the following equation: ABTS radical scavenging capacity (%) = [(Abs control − Abs sample)/(Abs control)] × 100, where Abs control is the absorbance of ABTS radical + ethanol; Abs sample is the absorbance of ABTS radical + sample extract/standard.
measured in the test sample and control, respectively, after incubation for 3 hr.

2.5. The 5-Lipoxygenase Inhibition Assay. The 5-lipoxygenase assay was conducted according to the method described by Baylac and Racine [31] with some modifications. Ice-cold buffer (potassium phosphate) at 4°C was mixed with 100 U of the thawed 5-lipoxygenase enzyme. Test sample scopoletin and nordihydroguaiaretic acid (NDGA) which was used as positive control was dissolved in dimethyl sulfoxide (DMSO, R&M) prior to assay at a stock concentration of 5 mM, and serial dilution was done according to the assay to obtain a good EC50 curve. Twenty microliters of scopoletin was plated out in triplicate in a 96-well microtiter plate at different concentrations, followed by 160 μL of 0.1 M potassium phosphate buffer (pH 6.3) maintained at 25°C and 20 μL of enzyme solution. Mixture was agitated, and 10 μL of linoleic acid was added and incubated at 10 mins at 25°C. Absorbance was recorded at 234 nm using Thermo Scientific Varioskan Flash microtiter plate reader, linked to a computer equipped with (SkansIt Software 2.4.3). Percentage inhibition of enzyme was determined by comparison of rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH=8) using the formula (E−S)/E×100, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate.

2.6. Anti-Acetylcholinesterase Assay. Acetylcholinesterase (AChE) inhibitory activity was measured by slightly modifying the spectrophotometric method developed by Ellman et al. [32]. 5,5'-Dithio-bis(2-nitrobenzoic) acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of anti-AChE activity. All the other reagents and conditions were same as described previously [9]. Test sample scopoletin and galanthamine which was used as positive control was dissolved in dimethyl sulfoxide (DMSO, R&M) prior to assay at a stock concentration of 5 mM, and serial dilution was done accordingly to obtain a good EC50 curve. In brief, 130 μL of 0.1 mM sodium phosphate buffer (pH 8.0), 20 μL of DTNB, 20 μL of test solution, and 20 μL of AChE solution were added by multichannel automatic pipette (Eppendorf, Germany) in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of 10 μL of acetylthiocholine iodide. The hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate Thermo Scientific Varioskan Flash microtiter plate reader, linked to a computer equipped with (SkansIt Software 2.4.3). Percentage inhibition of AChE was determined by comparison of rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH = 8) using the formula (E−S)/E×100, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Galanthamine was purchased from Sigma (St. Louis, MO, USA) and used as reference.

2.7. Statistical Analysis. Concentration-response curves were calculated using the Prism software package 5.00 for Windows, GraphPad Software, San Diego, California, USA, http://www.graphpad.com/ (GraphPad, San Diego, USA), and data were obtained from three independent experiments, each performed in triplicates (n = 9) and represented as mean ± SD. Nonlinear best fit was plotted with mean ± SD. One way Anova was performed followed by Tukey’s multiple comparison tests. Throughout the analysis, P < 0.05 was considered significant.

3. Results and Discussions

3.1. Antioxidant Capacity. In vitro antioxidant capacity can be determined by hydrogen atom transfer (HAT) method and single electron transfer (SET) method [4]. HAT based methods measure the ability of an antioxidant to scavenge free radical by hydrogen donation to form a stable compound. SET based methods detect the ability of the antioxidant to transfer one electron to reduce compound including metals, carbonyls and radicals [33, 34]. β-carotene bleaching assay involves HAT method, FRAP assay involves SET method, while DPPH and ABTS assay involves both method predominantly via SET method [3, 35].

FRAP is the ferric reducing power of antioxidants by the reduction of the ferric ions to the ferrous ions, which form a blue coloured ferrous-tripyridyltriazine complex (ferric TPTZ) which is detected at 593 nm. Deeper blue colour indicates higher antioxidant potential [36]. The compounds absorbance equivalent to 1 mM FeSO4 was calculated from equation of linearity of ascorbic acid (y = 0.0301x + 0.0831, r2 = 0.9799), trolox (y = 0.1193x + 0.2815, r2 = 0.9904), quercetin (y = 0.3053x + 0.4857, r2 = 0.9646), and scopoletin (y = 0.0203x + 0.5259, r2 = 0.9837). Total FRAP value was determined from the absorbance value above using the standard Fe(II) calibration curve equation (y = 0.0105x + 0.0136, r2 = 0.9817) (figure not shown). Scopoletin displayed significant (P < 0.05) FRAP value (254.99 ± 0.64 μM) compared to ascorbic acid (1970.00 ± 0.23 μM).

In the β-carotene/linoleic model, linoleic acid reacts with ROS and O2 to form an unstable peroxy radical. β-carotene being an antioxidant will react with this radical to form stable epoxide causing the bleaching of yellow solution. Competition reaction occurs with the presence of another antioxidant (sample) to react with the peroxy radical resulting in slower bleaching of solution detected at 470 nm spectrophotometrically [37]. Scopoletin displayed a moderate antioxidant activity with an EC50 647.89 ± 0.07 μM.

DPPH assay is based in the ability of antioxidant to reduce stable DPPH radical to form yellow coloured α, α-diphenyl-β-picryl hydrizine thus decolourising the deep purple DPPH methanol solution. Greater discolourisation results in lower absorbance at 550 nm indicating higher antioxidant capacity [25, 36]. Scopoletin exhibited moderate antioxidant activity with an EC50 = 191.51 ± 0.01 μM. ABTS assay involves the reduction of the blue-green 2,2'-azino-bis(3-thylbenzothiazoline-6-sulfonate) radical cation (ABTS+) by antioxidants to its original colourless ABTS−.
form. Greater discolorisation results in lower absorbance at 734 nm indicating higher antioxidant capacity [26, 27]. Scopoletin displayed significant \( P < 0.05 \) EC\(_{50}\) (5.62 ± 0.03 \( \mu \)M) as opposed to ascorbic acid (8.74 ± 0.06 \( \mu \)M). The DPPH and ABTS assays have the same mechanism of action, but, in most cases, the results obtained from the ABTS assay are higher than those from DPPH assay. It has been documented that results reported for the ABTS assay do not only take into account the activity of the parent compound, but also the contribution of reaction products and other individual compounds on the activity, which is not the case in the DPPH assay [38, 39].

Isolated compound scopoletin showed significantly lower FRAP value (254.99 ± 0.64 \( \mu \)M) compared to quercetin and ascorbic acid and lower ABTS value (5.62 ± 0.03 \( \mu \)M) compared to ascorbic acid (Table 1). It can be concluded that scopoletin exhibits its antioxidant activity predominantly via the SET method.

### 3.2. Anti-Inflammatory Activity

This assay measures the inhibitory activity against 5-LOX enzyme, which is the key enzyme in the metabolism of arachidonic acid that is responsible for the formation of leukotrienes which play a pivotal role in the pathophysiology of chronic inflammatory and allergic diseases. 5-Lipoxygenase is known to catalyse oxidation of unsaturated fatty acids containing 1-4-diene and the modification of linoleic acid (1-4-diene into 1-3-diene) can be detected at 234 nm.

In the 5-LOX assay, scopoletin displayed potent enzyme inhibition (IC\(_{50}\) = 1.76 ± 0.01 \( \mu \)M) which was fiftyfold more than nordihydroguaiaretic acid (IC\(_{50}\) = 85.23 ± 0.02 \( \mu \)M) (Table 2). According to the 5-LOX enzyme inhibition activity measurement [39], scopoletin displays good enzyme inhibition activity with IC\(_{50}\) of 0.34 ± 0.01 \( \mu \)g/mL. (IC\(_{50}\) < 30 \( \mu \)g/mL: good activity; 30 < IC\(_{50}\) < 80 \( \mu \)g/mL: moderate activity; IC\(_{50}\) > 80 \( \mu \)g/mL: poor activity). A combination of anti-inflammatory and antioxidant assays constitutes a good indication on potential anti-inflammatory activity of a drug [40, 41], as inhibition of the lipoxygenases is due to reaction of the inhibitor with free radicals generated at the active site of the enzyme [42].

### 3.3. Anti-AChE Activity

This assay measures the inhibition activity against AChE, which is the key enzyme in the hydrolysis of acetylcholine that is responsible for muscle and organ relaxations. Acetylcholinesterase inhibitors are therefore used medicinally to treat myasthenia gravis to increase neuromuscular transmission and to treat Alzheimer’s disease (deficiency in the production of acetylcholine).

In the anti-AChE assay, scopoletin reported a moderate activity (IC\(_{50}\) = 270.00 ± 0.02 \( \mu \)M) compared to galantamine (IC\(_{50}\) = 2.57 ± 0.06 \( \mu \)M) as in Table 2.

### 4. Conclusion

Coumarins (known as 1,2-benzopyrones) consisting of fused benzene and pyrone ring, are an important group of low-molecular weight phenolics and have been widely used for prevention and treatment of various diseases. Hydroxycoumarins have attracted intense interest in recent years because of their diverse pharmacological properties. In particular, natural source of 7-hydroxycoumarins have been studied to have good pharmacokinetics because of their diverse pharmacological properties. In particular, natural source of 7-hydroxycoumarins have been studied for their antioxidant and anti-inflammatory actions [43]. Drug discovery from natural sources is an area pertinent to complementary and alternative medicine (CAM) [44]. Scopoletin has been studied to have good pharmacokinetics data with good absorption in the stomach and colon of rats [45]. Reported physical properties of scopoletin which passes the Lipinsky rule (mass < 500, log \( P \) < 5, donor count < 5, and acceptor count < 10), for possible lead compound in drug discovery [46] and, in agreement with its potent antioxidant power, good anti-inflammatory and moderate anti-acetylcholinesterase activity demonstrated in this study might be of value for the treatment of various diseases emerging from oxidative stress and thus warrants further in vivo studies. Given the aforementioned evidence it is tempting to speculate that scopoletin represents an exciting scaffold from which to develop leads for treatment of neurodegenerative diseases.

---

**Table 1:** Antioxidant capacity of scopoletin isolated from Canarium patentinervium Miq.

| Extracts | ABTS assay, EC\(_{50}\) (\( \mu \)M) | DPPH assay, EC\(_{50}\) (\( \mu \)M) | FRAP assay, FRAP value (\( \mu \)M) | \( \beta \)-carotene bleaching assay, EC\(_{50}\) (\( \mu \)M) |
|---------|-----------------|-----------------|-----------------|-----------------|
| SC      | 5.62 ± 0.03     | 191.51 ± 0.01   | 254.99 ± 0.64   | 64789.0 ± 0.07  |
| AA      | 8.74 ± 0.06     | 10.68 ± 0.01    | 1970.00 ± 0.23  | NA              |
| QC      | 2.91 ± 0.03     | 9.52 ± 0.02     | 284.00 ± 0.24   | 5.42 ± 0.04*    |
| TRO     | 2.72 ± 0.02     | 19.06 ± 0.04    | 131.85 ± 0.54   | 6.59 ± 0.03*    |

SC: isolated compound scopoletin, NDGA: nordihydroguaiaretic acid, NA: not applicable.

Data were obtained from three independent experiments, each performed in triplicates. Values with the same letter are not significantly different (\( P < 0.05 \)) according to Tukey multiple comparison test.

**Table 2:** Anti-inflammatory and antiacetylcholinesterase values of scopoletin isolated from Canarium patentinervium Miq.

| Compound  | Anti-inflammatory assay 5-LOX, IC\(_{50}\) (\( \mu \)M) | Antiacetylcholinesterase assay, IC\(_{50}\) (\( \mu \)M) |
|-----------|-----------------------------------------------|-----------------------------------------------|
| SC        | 1.76 ± 0.01                                  | 270.00 ± 0.02                                  |
| NDGA      | 85.23 ± 0.02                                 | NA                                            |
| Galantamine| NA                                             | 2.57 ± 0.06                                   |

SC: isolated compound scopoletin, NDGA: nordihydroguaiaretic acid, NA: not applicable.

Data were obtained from three independent experiments, each performed in triplicates. Values with the same letter are not significantly different (\( n = 9 \)) and represented as mean ± SD.

IC\(_{50}\) < 30 \( \mu \)g/mL: good activity; 30 < IC\(_{50}\) < 80 \( \mu \)g/mL: moderate activity; IC\(_{50}\) > 80 \( \mu \)g/mL: poor activity.
Conflict of Interests
The authors declare that they have no conflict of interests.

Acknowledgment
The authors wish to thank Professor Moody and team from University of Nottingham, UK for their assistance in NMR services.

References
[1] N. J. Larkins, “Free radical biology and pathology,” Journal of Equine Veterinary Science, vol. 19, no. 2, pp. 84–89, 1999.
[2] R. A. Roberts, D. L. Laskin, C. V. Smith et al., “Nitritative and oxidative stress in toxicology and disease,” Toxicological Sciences, vol. 112, no. 1, pp. 4–16, 2009.
[3] A. V. Badarinath, K. Mallikarjuna Rao, C. Madhu Sudhana Chetty, S. Ramkanth, T. V. S. Rajan, and K. Gnanaprakash, “A review on in vitro antioxidant methods: comparisons, correlations and considerations,” International Journal of PharmTech Research, vol. 2, no. 2, pp. 1276–1285, 2010.
[4] K. W. Joon and T. Shibamoto, “Antioxidant assays for plant and food components,” Journal of Agricultural and Food Chemistry, vol. 57, no. 5, pp. 1655–1666, 2009.
[5] A. W. Ford-Hutchinson, M. Gresser, and R. N. Young, “5-Lipoxygenase,” Annual Review of Biochemistry, vol. 63, pp. 383–417, 1994.
[6] H. E. Claesson and S. E. Dahlén, “Asthma and leukotrienes: antileukotriene drugs as novel anti-asthmatic drugs,” Journal of Internal Medicine, vol. 245, no. 3, pp. 205–227, 1999.
[7] B. Samuelsson, S.-E. Dahlén, and J. A. Lindgren, “Leukotrienes and lipoxins: structures, biosynthesis, and biological effects,” Science, vol. 237, no. 4819, pp. 1171–1176, 1987.
[8] R. A. Lewis, K. F. Austen, and R. J. Soberman, “Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases,” New England Journal of Medicine, vol. 323, no. 10, pp. 645–655, 1990.
[9] P. J. Houghton, M.-J. Howes, C. C. Lee, and G. Steventon, “Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant,” Journal of Ethnopharmacology, vol. 110, no. 3, pp. 391–400, 2007.
[10] G. Emilien, K. Beyreuther, C. L. Masters, and J. Maloteaux, “Prospects for pharmacological intervention in Alzheimer disease,” Archives of Neurology, vol. 57, no. 4, pp. 454–459, 2000.
[11] N. Tabet, “Acetylcholinesterase inhibitors for Alzheimer’s disease: anti-inflammatories in acetylcholine clothing!,” Age and Ageing, vol. 35, no. 4, pp. 336–338, 2006.
[12] L. V. Borovikova, S. Ivanova, M. Zhang et al., “Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin,” Nature, vol. 405, no. 6785, pp. 458–462, 2000.
[13] R. Mogana, T. D. Bradshaw, K. T. Jin, and C. Wiart, “In vitro antitumor potential of Canarium patensinervium Miq,” Biotechnology Research International, vol. 2011, Article ID 768673, 5 pages, 2011.
[14] I. H. Burkhill, A Dictionary of the Economic Products of the Malay Peninsula, Governments of Malaysia and Singapore, Ministry of Agriculture and co-operatives, Kuala Lumpur, Malaysia, 1966.
[15] F. D. Russell, T. Windegger, K. D. Hamilton, and N. W. H. Cheetham, “Effect of the novel wound healing agent, OPAL A on leukotriene B4 production in human neutrophils and 5-lipoxygenase activity,” Informit, vol. 19, no. 4, pp. 13–16, 2011.
[16] C. K. Sen, S. Khanna, G. Gordillo, D. Bagchi, M. Bagchi, and S. Roy, “Oxygen, oxidants, and antioxidants in wound healing: an emerging paradigm,” Annals of the New York Academy of Sciences, vol. 957, pp. 239–249, 2002.
[17] A. Soneja, M. Drews, and T. Malinski, “Role of nitric oxide, nitroxidative and oxidative stress in wound healing,” Pharmacological Reports, vol. 57, pp. 108–119, 2005.
[18] W. H. Suh, K. S. Suslick, and Y. Suh, “Therapeutic agents for Alzheimer’s disease,” Current Medicinal Chemistry, vol. 5, no. 4, pp. 259–269, 2005.
[19] J. B. Calixto, M. F. Otuki, and A. R. S. Santos, “Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor κ B (NF-κB),” Planta Medica, vol. 69, no. 11, pp. 973–983, 2003.
[20] S. Tavolari, M. Bonafé, M. Marini et al., “Licochelenone, a dual COX-5-LOX inhibitor, induces apoptosis in HCA-7 colon cancer cells through the mitochondrial pathway independently from its ability to affect the arachidonic acid cascade,” Carcinogenesis, vol. 29, no. 2, pp. 371–380, 2008.
[21] B. Zhang, C.-L. Wang, W.-H. Zhao et al., “Effect of 5-LOX/COX-2 common inhibitor DHDFM30 on pancreatic cancer cell Capan2,” World Journal of Gastroenterology, vol. 14, no. 16, pp. 2494–2500, 2008.
[22] O. Werz and D. Steinhilber, “Therapeutic options for 5-lipoxygenase inhibitors,” Pharmacology and Therapeutics, vol. 112, no. 3, pp. 701–718, 2006.
[23] C. Wiart, S. Mogana, S. Khalifah et al., “Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia,” Fitoterapia, vol. 75, no. 1, pp. 68–73, 2004.
[24] R. Mogana, T. D. Bradshaw, K. T. Jin, and C. Wiart, “In vitro antitumor potential of Canarium patensinervium Miq,” Academic Journal of Cancer Research, vol. 4, no. 1, pp. 1–4, 2011.
[25] M. Juan-Badaturuge, S. Habtemariam, and M. J. K. Thomas, “Antioxidant compounds from a South Asian beverage and medicinal plant, Cassia auriculata,” Food Chemistry, vol. 125, no. 1, pp. 221–225, 2011.
[26] N. J. Miller, C. Rice-Evans, M. J. Davies, V. Gopinathan, and A. Milner, “A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates,” Clinical Science, vol. 84, no. 4, pp. 407–412, 1993.
[27] C. A. Rice-Evans, “Chapter 5 Formation of free radicals and mechanisms of action in normal biochemical processes and pathological states,” New Comprehensive Biochemistry C, vol. 28, pp. 131–153, 1994.
[28] R. A. Roberta, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, “Antioxidant activity applying an improved ABTS radical cation decolorization assay,” Free Radical Biology and Medicine, vol. 26, no. 9-10, pp. 1231–1237, 1999.
[29] I. F. Benzie and J. I. Strain, “The ferric reducing ability of plasma (FRAP) as a measure of ‘antioxidant power’: the FRAP assay,” Analytical Biochemistry, vol. 239, no. 1, pp. 70–76, 1996.
[30] S. Habtemariam and C. Jackson, “Antioxidant and cytoprotective activity of leaves of Peltiphyllum peltatum (Torr.) Engl.,” Pharmacological Reports, vol. 57, pp. 109–119, 2005.
[32] G. L. Ellman, D. Courtney, V. J. Andres, and R. M. Featherstone, “A new and rapid colorimetric determination of acetylcholinesterase activity,” *Biochemical Pharmacology*, vol. 7, pp. 88–95, 1961.

[33] R. L. Prior, X. Wu, and K. Schaich, “Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements,” *Journal of Agricultural and Food Chemistry*, vol. 53, no. 10, pp. 4290–4302, 2005.

[34] D. Huang, O. U. Boxin, and R. L. Prior, “The chemistry behind antioxidant capacity assays,” *Journal of Agricultural and Food Chemistry*, vol. 53, no. 6, pp. 1841–1856, 2005.

[35] A. Karadag, B. Ozcelik, and S. Saner, “Review of methods to determine antioxidant capacities,” *Food Analytical Methods*, vol. 2, no. 1, pp. 41–60, 2009.

[36] A. Dapkevicius, R. Venskutonis, T. A. Van Beek, and J. P. H. Linssen, “Antioxidative activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania,” *Journal of Science of Food and Agriculture*, vol. 77, no. 1, pp. 140–146, 1998.

[37] P. Dhar, A. B. Tayade, P. K. Bajpai et al., “Antioxidant capacities and total polyphenol contents of hydro-ethanolic extract of phytococktail from trans-himalaya,” *Journal of Food Science*, vol. 77, no. 2, pp. C156–C161, 2012.

[38] M. J. T. J. Arts, J. S. Dallinga, H. Voss, G. R. M. M. Haenen, and A. Bast, “A critical appraisal of the use of the antioxidant capacity (TEAC) assay in defining optimal antioxidant structures,” *Food Chemistry*, vol. 80, no. 3, pp. 409–414, 2003.

[39] M. P. Paraskeva, S. F. van Vuuren, R. L. van Zyl, H. Davids, and A. M. Viljoen, “The *in vitro* biological activity of selected South African Commiphora species,” *Journal of Ethnopharmacology*, vol. 119, no. 3, pp. 673–679, 2008.

[40] C. W. Choi, S. C. Kim, S. S. Hwang et al., “Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison,” *Plant Science*, vol. 163, no. 6, pp. 1161–1168, 2002.

[41] G. A. Alitonou, F. Avlessi, D. K. Sohounhloue, H. Agnaniet, J.-M. Bessiere, and C. Menut, “Investigations on the essential oil of Cymbopogon giganteus from Benin for its potential use as an anti-inflammatory agent,” *International Journal of Aromatherapy*, vol. 16, no. 1, pp. 37–41, 2006.

[42] U. Takahama, “Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: mechanism of antioxidative function,” *Phytochemistry*, vol. 24, no. 7, pp. 1443–1446, 1985.

[43] I. Kostova, S. Bhatia, P. Grigorov et al., “Coumarins as antioxidants,” *Current Medicinal Chemistry*, vol. 18, no. 25, pp. 3929–3951, 2011.

[44] E. L. Cooper, “Drug discovery, CAM and natural products,” *Evidence-Based Complementary and Alternative Medicine*, vol. 1, no. 3, pp. 215–217, 2004.

[45] R. J. Yin, X. F. Xiao, Y. Y. Xu et al., “Research information and review on the leaves of Diospyros kaki L. II . Pharmacokinetics of major active compounds of Diospyros kaki L,” *Asian Journal of Pharmacodynamics and Pharmacokinetics*, vol. 10, no. 4, pp. 271–285, 2010.

[46] S. D. Shruthi and Y. L. Ramachandra, “RBP-J as a therapeutic target to rheumatoid arthritis- an in silico study,” *International Journal of Preclinical and Pharmacetical Research*, vol. 2, no. 1, pp. 38–44, 2011.