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In vitro antioxidant, cholinesterase and tyrosinase inhibitory activities of *Calophyllum symingtonianum* and *Calophyllum depressinervosum* (Guttiferae)

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PEER REVIEW

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Comments

This is a very good piece of research work in which authors have demonstrated the DPPH radical scavenging, β-carotene bleaching, cholinesterase inhibitory and tyrosinase inhibitory activities of the extracts from leaves and heartwood of *C. symingtonianum* and stem bark of *C. depressinervosum*. These results showed that both *Calophyllum* species are potential sources of antioxidant and cholinesterase inhibitors.

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ABSTRACT

Objective: To screen the antioxidant, cholinesterase and tyrosinase enzymatic inhibition activities of the leaves and heartwood of *Calophyllum symingtonianum* (*C. symingtonianum*), and the bark of *Calophyllum depressinervosum* (*C. depressinervosum*).

Methods: Samples of leaves and heartwood of *C. symingtonianum* and bark of *C. depressinervosum* were tested for their total phenolic content and *in vitro* antioxidant assay by 2,2-diphenyl-1-picrylhydrazyl radical scavenging and β-carotene bleaching. Cholinesterase inhibition by Ellman's method and tyrosinase inhibition using L-3,4-dihydroxyphenylalanine as a substrate were also tested.

Results: All methanol extracts were found to exhibit strong 2,2-diphenyl-1-picrylhydrazyl radical scavenging effects. Extracts from the heartwood of *C. symingtonianum* gave a low IC_{50} (5.17±0.04) μg/mL followed by bark of *C. depressinervosum* [(7.30±0.14) μg/mL] and *C. symingtonianum* leaves [(15.70±1.43) μg/mL]. The methanol extract of *C. depressinervosum* bark showed 95.08% inhibition of β-carotene bleaching. All extracts showed moderate inhibition towards tyrosinase activity with an IC_{50} of more than 100 μg/mL. The methanol extract of *C. depressinervosum* stem bark showed the highest inhibition (78.46%) against butyrylcholinesterase.

Conclusions: These results showed that both *Calophyllum* species are potential sources of antioxidant and cholinesterase inhibitors. Further study is needed for the isolation and characterization of the active metabolites responsible for both activities.

KEYWORDS

Calophyllum symingtonianum, *Calophyllum depressinervosum*, Antioxidant, Cholinesterase, Tyrosinase, Guttiferae

1. Introduction

Currently, there is global attention focused on finding antioxidants from natural sources, due to common synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene (BHT) being reported to cause adverse effects including enzymatic and lipid alterations[1]. Antioxidants protect the body from free radicals and reactive oxygen species (ROS). ROS activity leads to skin ageing and generation of radicals in body apart from cancer. There is enough evidence in the literature to relate oxidative stress caused by ROS activity to age-related neurodegenerative diseases like Alzheimer's disease. The use of antioxidants has been investigated in an effort to slow down the progression of such diseases[2].

Alzheimer's disease is a progressive neurodegenerative disorder

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and a leading cause of dementia among older people. Alzheimer's disease becomes more prevalent as the aging population increases all over the globe. Decrement of acetylcholine levels due to hydrolysis of cholinesterase enzymes significantly causes impairment in cognitive function. One of the approaches used to prolong the availability of acetylcholine levels is by inhibiting acetylcholine hydrolysis through the inhibition of cholinesterase enzymatic activity[3]. Known cholinesterase inhibitors are recognized to have several limitations such as hepatotoxicity, short biological action duration and low bioavailability[4]. Therefore, the search for new inhibitors with low toxicity from natural plants continues.

Tyrosinase is a key enzyme known to be important for melanin biosynthesis. Melanin is the pigment responsible for the colour of human skin and takes place in a lineage of cells known as melanocytes. Excessive tyrosinase activity can cause dermatological disorders such as hyper-pigmentation of skin, age spots and freckles. Over-production of melanin can be prevented by avoiding excessive UV exposure and by consuming skin-lightening agents such as hydroquinone and kojic acid[5]. However, some commercially available skin products are chronically, cytotoxic and have mutagenic effects. Therefore, alternative natural tyrosinase inhibitors have become increasingly important for pharmaceutical industries.

Calophyllum belongs to the Guttiferae or Clusiaceae family, which is a pan-tropical genus comprising of approximately 200 species. Generally, these species are confined to the tropical rain forest and locally known in Malaysia as "bintangor". The plants are commonly employed in folk medicine to treat bronchitis, gastric and hepatic disturbances pain, inflammation, diabetes, hypertension, diarrhea, rheumatism varicose, hemorrhoids, chronic ulcers, and in the prevention of wound infection[6]. *Calophyllum* spp. has received considerable attention following the discovery of anti-HIV RT-1 compounds from *Calophyllum lanigerum* var. *austrocoriaceum* from Sarawak[7]. There has been a continual interest to further investigate the chemistry and phytochemical profiles of *Calophyllum* spp. Several studies on *Calophyllum* spp. reported that this genus is a rich source of biologically active secondary metabolites such as coumarins[8], xanthones[9], chromanone carboxylic acids[10], flavonoids[11], and terpenoids[12]. *Calophyllum symingtonianum* (*C. symingtonianum*) and *Calophyllum depressinervosum* (*C. depressinervosum*) are two *Calophyllum* species endemic to the Malaysian Peninsula. Previous studies on the antimicrobial and antioxidant activities of extracts from the barks of *C. symingtonianum* and *C. depressinervosum* have been reported[13,14]. Herein we report the antioxidant, cholinesterase and tyrosinase inhibitory activities of extracts from leaves and heartwood of *C. symingtonianum* and bark of *C. depressinervosum*.

2. Material and methods

2.1. Chemicals and reagents

β-Carotene was purchased from Fluka, ascorbic acid was purchased from GCE Laboratory Chemicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), linoleic acid and galanthamine hydrobromide were purchased from Calbiochem. Folin-Ciocalteu's phenol, gallic acid, butylated hydroxytoluene (BHT), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholinesterase (AChE) from *Electrophorus electricus* (electric eel) type VI-S lyophilized powder, acetylthiocholine iodide, butyrylcholinesterase (BChE) from equine serum lyophilized powder, butyrylthiocholine iodide, tyrosinase enzyme from mushroom, L-3,4-dihydroxyphenylalanine (L-DOPA) and kojic acid were all purchased from Sigma Aldrich.

2.2. Plant material

Samples of *C. symingtonianum* and *C. depressinervosum* were collected from Hutan Air Terjun Sungai Pandan, Kuantan, Pahang on 22 February 2013. The samples were identified by Dr. Shamsul Khamis from Universiti Putra Malaysia and the plant specimens were deposited (with voucher number of M.T.26 for *C. symingtonianum* and M.T.25 for *C. depressinervosum*) at the Herbarium, Kulliyyah of Pharmacy, International Islamic University Malaysia, Pahang.

The dried and ground leaves (2.02 kg), heartwood (2.34 kg) of *C. symingtonianum* and bark of *C. depressinervosum* (3.46 kg) were macerated sequentially with CH_2Cl_2 and MeOH at room temperature for 3 d each and the process was repeated three times. After concentration *in vacuo* by using rotary evaporator, all extracts were stored at 4 ℃ for further use.

2.3. Determination of total phenolic content

The total phenolic content of the extracts was determined by Folin-Ciocalteau's assay[15]. In brief, 40 μL of sample with concentrations from 1 000.00 to 7.81 μg/mL were mixed with 20 μL of Folin-Ciocalteau's reagent and incubated for 5 min at room temperature. 80 μL of sodium carbonate was added followed by 60 μL of distilled water. The mixture was kept in the dark for 90 min and the absorbances were recorded using an Epoch Biotek microplate spectrophotometer at 760 nm. A calibration graph of standard gallic acid was constructed. Total phenolic content of the extract was expressed as mg Gallic acid equivalent (GAE) per gram of extract.

2.4. Determination of antioxidant activity

2.4.1. DPPH radical scavenging activity

DPPH radical scavenging activity of all tested samples was carried out using 96-well microplate as described by Kassim *et al*[15]. Sample stock solution of concentration 1 000 μg/mL was prepared by dissolving the sample in MeOH. In brief, 30 μL of DPPH (300 μmol/L in MeOH) was added to 170 μL of samples ranging from dilutions of 500.00 to 7.81 μg/mL obtained from a twofold dilution method. The reaction mixture was allowed to incubate in dark conditions at room temperature for 30 min. The conversion of colour from purple to yellow indicates that the samples were active as antioxidant agents. The absorbance for DPPH radical inhibition was measured at 517 nm. The radical scavenging activity was examined and compared with ascorbic acid and butylated hydroxytoluene (BHT) as the references. The DPPH radical scavenging inhibition was calculated by using the following formula:

Scavenging concentration (%)=$[A_{DPPH\ blank}-(A_{sample}-A_{blank\ sample})]/A_{DPPH\ blank}\times 100\%$

Where, $A_{DPPH\ blank}$ is the absorbance of DPPH solution, A_{sample} is the absorbance of tested sample and DPPH solution and $A_{blank\ sample}$ is the absorbance of tested sample without DPPH solution.

2.4.2. β-Carotene bleaching assay

The evaluation of β-carotene bleaching activity was determined following the method used by Kassim *et al.* and Barros *et al.*[15,16] with minor modifications. In brief, 210 μL of β-carotene solution (1 mg/mL in chloroform) was mixed with 5 μL of linoleic acid and 42 μL of Tween 40. The chloroform was evaporated at 40 ℃ before addition of 10 mL of distilled water with vigorous shaking to form emulsion. Then, 50 μL of sample (1 mg/mL in MeOH) were added to 200 μL of emulsion in 96-well microplates. The absorbance of all samples was recorded at 470 nm during the initial time of incubation (t=0 h) and after 2 h incubation (t=2 h) at 50 ℃ in the dark. The antioxidant activity was calculated according to the following formula:

Antioxidant activity (%)=$(A_{t=2\,h}/A_{t=0\,h})\times100\%$

2.5. Cholinesterase inhibitory assay

The cholinesterase inhibitory activity of all samples was assessed by Ellman's method as reported by Yang *et al.*[17] with minor modifications. The selectivity of samples towards two types of cholinesterase enzymes known as AChE and BChE was compared. Twenty microlitre of sample (1 000 μg/mL) was added into 96 well microplate followed by the addition of 10 μL of 0.01 mol/L DTNB in sodium phosphate buffer (pH 7.0), 15 μL of 0.28 U/mL AChE/BChE in sodium phosphate buffer (pH 8.0) and 140 μL of 0.10 mol/L sodium phosphate buffer (pH 8.0) before being pre-incubated for 15 min. Ten microlitre of 0.075 mol/L acetylthiocholine iodide/butyrylthiocholine iodide in deionized water was added and further 30 min incubation was then allowed. Galanthamine hydrobromide was used as a positive control for this assay and the absorbances were measured at 412 nm. Enzyme activity was calculated by comparing the rate of reaction for the samples relative to the blank. The percentage of inhibitory activity was calculated according to the following formula, where A_{sample} is the absorbance of the sample and A_{blank} is the absorbance of the solution containing all the components except the enzyme.

Inhibition concentration (%IC)=$[(A_{blank}-A_{sample})/A_{blank}]\times100\%$

2.6. Tyrosinase inhibitory assay

Tyrosinase inhibitory activities were assessed as described by Abdillahi *et al*[18]. All samples were dissolved in dimethylsulfoxide with concentrations ranging from 100.00 to 6.25 μg/mL. A 40 μL aliquot of each sample was added to 80 μL of 0.1 mol/L phosphate buffer (pH 6.8) followed by 40 μL of tyrosinase enzyme (100 units/mL) and 40 μL of L-DOPA (2.5 mmol/L) as the substrate. The reaction was allowed to incubate for 30 min at room temperature under dark conditions. Kojic acid which is known to inhibit tyrosinase was used as positive control. Absorbance results were compared with absorbance of a blank consisting of all components except L-DOPA. The percentage of tyrosinase was calculated by using the following formula:

Inhibition concentration (%IC)=$[(A_{blank}-A_{sample})/A_{blank}]\times100\%$

2.7. Statistical analysis

Three replicates of each sample were used for statistical analysis with values reported as mean±SD. The Student's *t*-test was used for comparison between treatment of samples and positive controls. A value of $P<0.05$ was considered significantly different. Pearson's correlation and regression analysis were carried out using SPSS software (version 22) to study the relationship between the two different assays.

3. Results

The total phenolic content was expressed as mg GAE per gram of dry weight using the standard curve equation of Gallic acid (y= 0.0107x+2.6402, R^2=0.9978) as a standard compound. All methanol extracts from both *Calophyllum* species showed high phenolic content with the highest value from heartwood of *C. symingtonianum* (110.17 mg/g) followed by the bark of *C. depressinervosum* (92.18 mg/g) and leaves extract (74.72 mg/g).

The results of the antioxidant activities of *C. symingtonianum* and *C. depressinervosum* extracts were assessed based on the DPPH radical scavenging activity and β-carotene bleaching inhibition. The DPPH assay indicated that the methanol extracts possessed strong antioxidant activity with IC_{50} values comparable to ascorbic acid as shown in Table 1. The methanol extract from heartwood of *C. symingtonianum* gave the lowest IC_{50} [(5.17±0.04) μg/mL] followed by bark of *C. depressinervosum* [(7.30±0.14) μg/mL] and leaves of *C. symingtonianum* [(15.70±1.43)μg/mL]. In contrast, all dichloromethane extracts showed moderate activity with IC_{50} values of more than 250 μg/mL. All extracts showed more than 50% bleaching inhibition in the β-carotene bleaching assay. The inhibitory activity of methanol extract from bark of *C. depressinervosum* was (95.08±1.07)% which surpassed the BHT inhibition at 87.10%. All dichloromethane extracts also showed good β-carotene bleaching inhibition contradicting with DPPH radical scavenging activities. All extracts tested for DPPH and β-carotene activities had a statistically significant difference ($P<0.05$) from the positive controls.

Table 1
Antioxidant activity of crude extracts from *C. symingtonianum* and *C. depressinervosum* (mean±SD).

Calophyllum species	Plant parts	Crude extracts	Phenolic content (mg GAE/g dry weight)	DPPH IC_{50} (μg/mL)	β-carotene bleaching (IC% at 1000 μg/mL)
C. symingtonianum	Leaves	DCM	0.00	>1000*	62.39±6.84*
		MeOH	74.72±2.73	15.7±1.43*	60.10±4.25*
	Heartwoods	DCM	0.00	281.67±3.12*	61.69±3.58*
		MeOH	110.17±2.23	5.17±0.04*	75.85±5.84
C. depressinervosum	Barks	DCM	0.00	258.33±3.21*	52.12±3.80*
		MeOH	92.18±1.26	7.30±0.14*	95.08±1.07*
Ascorbic acid			ND	4.30±0.10	35.93±2.81
Butylated hydroxytoluene			ND	48.30±0.73	87.10±5.62

Values are means of three replicates determination; DCM: Dichloromethane; ND: Not determined; *: Values in a column are significantly different ($P<0.05$).

Pearson's correlation of total phenolic content and DPPH radical scavenging activity was evaluated to establish the relationship between both parameters using various concentrations (1000.00-7.81 μg/mL). The correlation coefficient and the significant values were determined and tabulated in Table 2. The Pearson's correlation coefficient (R^2) of all extracts showed strong positive correlation. All extracts showed significant correlation ($P<0.05$) between the total phenolic content with DPPH radical scavenging activity.

Table 2
Pearson's correlation coefficient between total phenolic content and DPPH radical scavenging activity.

Calophyllum species	Plant parts	Crude extracts	R^2	*P* value
C. symingtonianum	Leaves	DCM	0.872	0.005
		MeOH	0.872	0.005
	Heartwoods	DCM	0.851	0.007
		MeOH	0.895	0.003
C. depressinervosum	Barks	DCM	0.847	0.008
		MeOH	0.812	0.014

DCM: Dichloromethane; R^2: Pearson's correlation coefficient.

Table 3 displays the percentage of inhibition concentration of selectivity studies between AChE and BChE enzymatic activities. In general, all extracts showed statistically significant difference with positive control (P<0.05) and were more selective towards inhibition of BChE enzyme compared to AChE enzyme except for the methanol extract of *C. depressinervosum* bark and dichloromethane extract from leaves of *C. symingtonianum*. On the other hand, all dichloromethane extracts exhibited weaker activity compared to their respective methanol extracts except for extract of bark of *C. depressinervosum*. It showed the highest inhibition against BChE activity [(78.46±3.79)%] comparable with the positive control, galanthamine hydrobromide. Both extracts from *C. depressinervosum* were able to inhibit both AChE and BChE enzymatic activities by more than 60%. It differs with the activity of *C. symingtonianum* extracts, in which strong activities were more concentrated to its methanol extracts.

Table 3
Cholinesterase inhibition activity of crude extracts from *C. symingtonianum* and *C. depressinervosum* (mean±SD).

Calophyllum species	Plant parts	Crude Extracts	Inhibition concentration (IC% at 1000 μg/mL)	
			AChE	BChE
C. symingtonianum	Leaves	DCM	38.83±0.77*	31.38±0.39*
		MeOH	55.63±4.77*	68.20±2.44*
	Heartwoods	DCM	24.70±1.67*	55.39±3.39*
		MeOH	64.37±2.30*	67.00±0.63*
C. depressinervosum	Barks	DCM	62.94±3.15*	78.46±3.79
		MeOH	75.41±2.61*	59.60±0.33*
Galanthamine hydrobromide			88.70±0.20	80.14±0.66

Values are means of three replicates determination; DCM: Dichloromethane; *:Values in a column are significantly different (P<0.05).

Table 4
Tyrosinase inhibiton activity of crude extracts from *C. symingtonianum* and *C. depressinervosum* (mean±SD).

Calophyllum species	Plant parts	Crude extracts	IC_{50} (μg/mL)	Inhibition concentration (IC% at 100 μg/mL)
C. symingtonianum	Leaves	DCM	99.88±0.68*	47.94±0.68*
		MeOH	>100*	45.18±0.55*
	Heartwoods	DCM	65.08±0.79*	56.39±0.57*
		MeOH	96.31±0.24*	50.69±0.91*
C. depressinervosum	Stem barks	DCM	98.90±0.67*	48.86±0.18*
		MeOH	>100*	44.26±0.21*
Kojic acid			28.22±0.51	80.66±0.10

Values are means of three replicates determination; es determination; DCM: Dichloromethane; *:Values in a column are significantly different (P<0.05).

The ability of extracts from both *Calophyllum* species to inhibit tyrosinase enzymatic activity was also evaluated with L-DOPA as the substrate. Table 4 depicts the IC_{50} and percentage of inhibition at 100 μg/mL. The most active extract was dichloromethane extract of heartwood of *C. symingtonianum* which showed moderate inhibition at (65.08±0.79) μg/mL. Meanwhile, all dichloromethane extracts showed IC_{50} values of approximately 100 μg/mL and showing a significant difference with kojic acid (P< 0.05).

4. Discussion

The antioxidant activities of extracts can be determined by monitoring their reactions with a stable radical of DPPH in a methanol solution. The DPPH free radical gives strong absorption at 517 nm and can be indicated by a purple solution colour. The purple colour converts to yellow when the radical electron pairs with hydrogen from a free radical scavenging antioxidant to form reduced DPPH-H. The reduction of the DPPH radical causes a decrease in the absorbance at characteristic wavelength. The determination of antioxidant activity also was assessed by measuring the inhibition of β-carotene bleaching by linoleic acid. Linoleic acid will produce hydroperoxides and act as a radical generator during its incubation at 50 ℃. Oxidation of unsaturated β-carotene by hydroperoxide will bleach its yellow colour. The existence of antioxidants will inhibit discoloration by neutralizing the radical presence in the system. This method is more sensitive due to strong absorption of β-carotene [Ɛ=2 280 L/(mol^{-1}·cm^{-1})] whereas it is slower than the DPPH method[19].

Phenolic compounds are always associated with strong antioxidant properties. The measurement of total phenolic content in all methanol extracts exhibited high levels of total phenolic content with a strong correlation to DPPH radical scavenging activity. This suggests that the phenolic content is a contributor to antioxidant properties. High biflavonoid content and a large number of xanthones were reported to be isolated from *Calophyllum* genus[20,21]. In previous studies, DPPH activity of the methanol extract from bark of *C. symingtonianum* and *C. depressinervosum* were found to be at IC_{50} 100 μg/mL and 270 μg/mL[13,14], respectively. These reported values were higher compared to our current findings which gave a lower IC_{50} thus indicating that the current measuring method by using an ELISA microplate reader is more sensitive.

In contradiction, all dichloromethane extracts displayed β-carotene bleaching inhibition despite weak activity in the DPPH assay. These results suggest that the oxidative reactions of all dichloromethane extracts demonstrated the "polar paradox" phenomenon. The "polar paradox" is a complex interfacial phenomenon occur which influence the antioxidant behavior. Non-polar antioxidants will exhibit stronger oxidative properties in relatively more polar media such as oil-in-water emulsions since they concentrate at the lipid:air surface[22]. Meanwhile, polar antioxidants will be more diluted and remain in the aqueous phase, thus exhibit weaker oxidative properties as shown by ascorbic acid[23].

The role of oxidative stress in age-related neurodegenerative diseases such as Alzheimer's disease has received substantial attention. For that reason, cholinesterase activity was also evaluated since both *Calophyllum* species exhibited potentially antioxidative agents. The cholinesterase family comprises of AChE and BChE. AChE is an enzyme that is involved in the hydrolysis of acetylcholine at cholinergic synapses in the nervous systems[24], whereas BChE is an enzyme defined as a pseudocholinesterase or plasmatic cholinesterase since it is predominantly distributed in the liver and excreted into the plasma.

It is the second major form of cholinesterase after AChE[25]. Inhibition of cholinesterase enzyme was evaluated by colorimetric determination following the methodology used by Ellman. Acetylcholinesterase hydrolyzes the substrate acetylthiocholine to produce thiocholine which later reacts with Ellman's reagent, DTNB. This enzymatic reaction results in the formation of 5-thio-2-nitrobenzoate (yellow colour) and 2-nitrobenzoate-5-mercaptothiocholine as products which can be detected at wavelength 412 nm.

Shikimate-derived compounds especially from phenolic compounds with a skeleton derived from one phenylpropanoid unit, two units combined together or one or more phenylpropanoid units combined with fragments arising from other metabolic pathways have been reported to display a wide range of AChE inhibitory activities such as coumarins and xanthones[26]. Terpenoids also appeared as potent cholinesterase inhibitors. The synergistic effects of monoterpenes in essential oils as well as a test on triterpenes taraxerol and ursolic acid exhibited strong inhibition towards AChE[27,28]. A steady stream of reports on the isolation of pyranocoumarins, xanthones and triterpenoids from various parts of the *Calophyllum* species especially from methanol extracts ruled out that possible cholinesterase inhibitors can be isolated from both *C. symingtonianum* and *C. depressinervosum* crude extracts.

Tyrosinase is a multifunctional copper-containing enzyme involved in the biosynthesis of melanin. It catalyze the ortho-hydroxylation of tyrosine (monophenol) to 3,4-dihydroxyphenylalanine or DOPA (*o*-diphenol) and oxidation of DOPA to dopaquinone (*o*-diphenol). Dopaquinone can undergo a series of enzymatic and non-enzymatic reactions to form melanin pigments[29]. Many polyphenol derivatives of flavonoids show anti-melanogenic properties especially with 4-resorcinol and catechol moieties. 4-Resorcinol type inhibitors bind to the enzyme binuclear active site while catechol may chelate the copper ions present in the active site, suppressing the tyrosinase activity[30]. Based on the tyrosinase inhibition activity of both *Calophyllum* species, it was deduced that there are a lack of phytochemicals with 4-resorcinol or catechol moieties of flavonoids in the crude extracts since structure-activity relationship studies on tyrosinase activities reported that the phytochemicals with a 4-resorcinol group play important roles in the inhibition of tyrosinase activity.

Based on the current findings, both *Calophyllum* species showed strong antioxidant activity especially from methanolic extracts, suggesting potent antioxidant agents may be isolated. Strong inhibitive activity towards both AChE and BChE enzymes showed that both *Calophyllum* species may serve as possible sources for new cholinesterase inhibitors. The isolation of active phytochemicals is needed to provide comprehensive data for tested bioactivities.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Antioxidants protect the body from free radicals and ROS. ROS activity leads to skin ageing and generation of radicals in body apart from cancer. Alzheimer's disease, an oxidative stress to age-related neurodegenerative diseases is also caused by ROS activity. The use of antioxidants has required to investigate in an effort to slow down the progression of such diseases.

Research frontiers

The present research work deals with the antioxidant, cholinesterase and tyrosinase enzymatic inhibition screening activities of leaves and heartwood of *C. symingtonianum*, and the bark of *C. depressinervosum*.

Related reports

Previous studies on the antimicrobial and antioxidant activities of extracts from the bark of *C. symingtonianum* and *C. depressinervosum* have been reported.

Innovations and breakthroughs

Based on the results, both *Calophyllum* species exhibited strong antioxidant activity, suggesting potent antioxidant agents may be isolated.

Applications

The leaves and heartwood of *C. symingtonianum*, and the bark of *C. depressinervosum* showed strong antioxidant activity especially from methanolic extracts, suggesting the presence of potent antioxidant agents. Strong inhibitive activity towards both AChE and BChE enzymes showed that both *Calophyllum* species may serve as possible sources for new cholinesterase inhibitors.

Peer review

This is a very good piece of research work in which authors have demonstrated the DPPH radical scavenging, β-carotene bleaching, cholinesterase inhibitory and tyrosinase inhibitory activities of the extracts from leaves and heartwood of *C. symingtonianum* and bark of *C. depressinervosum*. These results showed that both *Calophyllum* species are potential sources of antioxidant and cholinesterase inhibitors.

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