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Anti-acetylcholinesterase and antioxidant activities and HPLC–MS analysis of polyphenol from extracts of *Nelsonia canescens* (Lam.) Spreng.

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PEER REVIEW

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Comments

Authors did a great contribution to investigate AchE and oxidant inhibition potentialities of *N. canescens* and also to identify and quantify 8 phenolic compounds. This research paper gives interesting phytochemistry and biological activities data of the species.

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ABSTRACT

Objective: To investigate the anti-acetylcholinesterase and antioxidant activities and to evaluate the major polyphenolic compounds of *Nelsonia canescens* extracts.

Methods: The anti-acetylcholinesterase activity was assessed using a kinetic inhibition standard method. Two methods, ABTS and lipid peroxidation, were used to estimate the antioxidant capacity. Polyphenols profile of the plant extract has been determined with a HPLC–MS method.

Results: The results showed that butanol extract exhibited the best anti-acetylcholinesterase activity with inhibition percentage of (55.62±1.49)%. The best 3 ethylbenzothiazoline–6–sulphonate radical cation scavenging capacity was found for ethyl acetate extract with a value of (56.20±0.77) mg equivalent trolox/g while the crude extract showed the highest inhibition of the rat liver lipid peroxidation (52.57±1.20)%. Polyphenols profile revealed the presence of five phenol acids (p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid and gentisic acid) and three flavonoids (apigenin, luteolin, quercetin).

Conclusions: All the extracts of *Nelsonia canescens* exhibited antioxidant and AChE inhibition capacities. The active compounds identified and quantified in this species are mainly responsible for these *in vitro* biological activities and allow to justify its widely use in Burkina Faso traditional medicine.

KEYWORDS

Anti-acetylcholinesterase, Antioxidant, Polyphenols, HPLC–MS, *Nelsonia canescens*

1. Introduction

The neurodegenerative disorder characterized by the nerve cell dysfunctions and loss of neurons in the central nervous system was first discovered in 1907 by a German scientist, Alois Alzheimer, and was named as Alzheimer's disease (AD). Nowadays, Neurodegenerative diseases are estimated to surpass cancer as the second most common cause of death among elderly by the 2040s[1]. There are

considerable financial, social and emotional burdens associated with the caring for patients with this disease. A great reduction of acetylcholinesterase (AChE) enzyme in the cerebral cortex is a significant factor in AD.

AChE is the principal enzyme involved in the hydrolysis of acetylcholine (ACh). Approaches to enhance cholinergic function in AD have included stimulation of cholinergic receptors or prolonging the availability of ACh released into the neuronal synaptic cleft by use of agents which restore

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the level of acetylcholine through inhibition of both AChE and BChE.

Since ancient times phytochemicals have been used in traditional cultures to restore and declining cognitive functions lost with neurodegenerative diseases[2]. Currently, only four acetylcholinesterase inhibitors namely, tacrine, donepezil, rivastigmine and galantamine have been approved by the US Food and Drug Administration[3]. Thus, the development of effective new agents as acetylcholinesterase inhibitors is needed. The search for new inhibitors of AChE derived from natural sources, with fewer side effects is urgently required[4]. Pharmacognosy research has shown that plants contain active compounds that have become new sources to investigate for the pharmaceutical industry. In traditional practices, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuropharmacological disorders.

Many medicinal plants and their phytochemicals have been investigated for their capacity of inhibiting AChE. For example, studies concerning the AChE inhibitory activity and chemical composition of commercial essential oils performed by Dohi *et al.* demonstrated for the first time that eugenol was a potent AChE inhibitor[5]. In addition galantamine, originally isolated from plants of the Amaryllidaceae family, has become an important treatment of AD[6].

Currently, there is global interest in finding new and safe antioxidants from natural sources, to prevent oxidative deterioration of foods and to minimize oxidative injury of living cells. Antioxidants are substances which extinguish free radicals or delay oxidation chain reactions through the course of different mechanisms. The human body can be protected from free radicals and reactive oxygen species through antioxidants. Reactive oxygen species (ROS) is harmful not only to lipids and proteins but can also damage DNA in the cells, which results in cell death. The oxidative damage caused by the over activity of ROS is responsible for the aging of skin and several other diseases, associated with the excessive generation of free radicals. AD for example is associated with oxidative stress[7].

Plant phenolic compounds such as flavonoids and lignin precursors commonly accumulate in epidermal cells of plant organs such as flowers, leaves, stems, roots, seeds and fruits, being found in glycosidic forms (glycosides) and non-glycosidic forms (aglycones). Subcellular localization of the glycosides is mainly confined to hydrophilic regions such as vacuoles and apoplasts. Phenolic compounds are important constituents of the human diet and they have been recognized largely as beneficial antioxidants, antibacterial and enzyme inhibitors.

Nelsonia canescens (*N. canescens*), a small perennial herb with soft decumbent villous branches is traditionally used for malarial[8], cancer, gout, cardiovascular and inflammatory diseases treatments[9–11]. In Burkina Faso, the species is

widely used in traditional medicine for the treatment of neurological diseases.

Research has reported the *in vivo* analgesic and anti-inflammatory activities of ethanolic extract from leaves of *N. canescens*[8]. The methanolic extracts of this plant exhibited an *in vivo* hepatoprotection activity based on carbon tetrachloride induced hepatic damage in rat[12]. Phytochemical investigations on *N. canescens* have identified concerned only two iridoids: shanzhiside methyl ester and galiridoside.

Our previous work on *N. canescens* showed a high level of phenolic compounds in the polar extract. In continuation of this preliminary screening, the present study was conducted to check the enzyme inhibition, antioxidant ability and the phenolic compounds identification.

2. Materials and methods

2.1. Chemicals

To carry out this study, we used solvents, enzymes and various classic reagents. All reagents were of analytical grade. Sodium chloride, quercetin, hydrochloric acid, ethyl acetate, acetic acid, p-iodonitrotetrazolium chloride (INT), *n*-hexane, acetonitrile, magnesium chloride, bovine serum albumin, acetylcholinesterase (EC 232. 559. 3. 3) (AChE), acetylcholine iodide (ATCI), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and 2-thiobarbituric acid were purchased from Sigma Aldrich chemie (Steinheim, Germany); potassium persulfate, 2,2'-azinobis (3 ethylbenzothiazoline-6-sulphonate) (ABTS) and trichloroacetic acid were supplied by Flukachemie (Buchs, Switzerland); dichloromethane, ferric dichloride, ethanol, methanol were sourced from Prolabo (Paris, France); butanol was sourced from SDS (Peyin, France).

Reference compounds: caftaric acid from Dalton (USA), gentisic acid, ferulic acid, sinapic acid, patuletin, luteolin from Roth (Germany), caffeic acid, chlorogenic acid, p-coumaric acid, hyperoside, isoquercitrin, rutoside, myricetol, fisetin, quercitrin, quercetol, kaempferol and apigenin were from Sigma (Germany).

2.2. Plant material

N. canescens plant species was collected in August 2010 in Loumbila, 15 km north of Ouagadougou, capital of Burkina Faso. The plant specimen was identified by Prof. Millogo-Rasolodimby botanist at the Plant Biology Department of the University of Ouagadougou. A voucher specimen with accession number ID 10152 was deposited at the OUA herbarium of the CIB (Centre d'Information sur la Biodiversité), UFR/SVT, University of Ouagadougou.

2.3. Preparation of extracts

Whole plants of *N. canescens* were dried at room temperature and ground to fine powder. Twenty five grams of powdered plant material was extracted successively with 250 mL of hexane, of dichloromethane, of acetonitrile, of ethyl acetate and butanol with Soxhlet apparatus. The final marc was used for a decoction. Thereafter, extract solutions were concentrated under reduced pressure in a rotary evaporator (BÜCHI, Rotavapor R–200, Switzerland) and dried at ambient temperature. Aqueous and aqueous–acetone extracts were frozen and lyophilized using a lyophilizer (Telstar–Cryodos 50, Spain). The obtained extracts were weighed before packing in waterproof plastic flasks and stored at 4 °C until use.

2.4. HPLC–MS analysis

This analysis was conducted according to the protocol described by Meda *et al.*[13].

2.4.1. Preparation of references and samples solutions

References and samples solutions were prepared. Stock solutions (consisting of 0.1 g/mL in methanol) of the following standard compounds were prepared: caftaric acid, gentisic acid, ferulic acid, sinapic acid, patuletin, luteolin, caffeic acid, chlorogenic acid, p–coumaric acid, hyperoside, isoquercitrin, rutoside, myricetol, fisetin, quercitrin, quercetol, kaempferol and apigenin. All stock solutions were stored in the dark at 4 °C, and an appropriate dilution of each of them was performed with double distilled water before analysis. The methanol extract of plant was used to prepare sample stock solution of 10 mg/mL in 50% methanol. The non hydrolysed samples were diluted at a concentration of 5 mg/mL in 50% methanol before injection. The hydrolyzed samples were prepared and adjusted to 10 mL with 50% methanol to get the same concentration as the non–hydrolyzed extract, before injection.

2.4.2. The equipment and the chromatographic conditions

The experiment was carried out using an Agilent 1100 HPLC system (Agilent, USA) equipped with degasser, binary gradient pump–, column thermostat–, autosampler– and UV detector. The HPLC system was coupled with an Agilent 1100 Mass–spectrometer (LC/MSD Ion Trap VL). For the separation, a reverse–phase analytical column was employed (Zorbax SB–C18 100×3.0 mm *i.d.*, 3.5 µm particle) and the work temperature was 48 °C. The UV detector was set at 330 nm for 17.5 min for phenolic acids (caftaric acid, gentisic acid, caffeic acid, chlorogenic acid, p–coumaric acid, ferulic acid, sinapic acid), then at 370 nm for flavonoids (hyperoside, isoquercitrin, rutoside, myricetol, fisetin, quercitrin, quercetol, patuletin, luteolin, kaempferol, apigenin). The

MS system operated using an electrospray ion source, in negative mode. The chromatographic data were processed using ChemStation and Data Analysis software from Agilent (USA).

The mobile phase was a binary gradient prepared from methanol and a solution of acetic acid 0.1% (v/v). The elution started with a linear gradient, beginning with 5% methanol and ending at 42% methanol, for 35 min; following with isocratic elution for the next 3 min with 42% methanol. The flow rate was 1 mL/min and the injection volume was 5 µL.

2.4.3. Identification and quantitative determinations of polyphenols

The MS signal was used only for qualitative analysis based on specific mass spectra of each polyphenol. The MS spectra obtained from a standard solution of polyphenols were integrated in a mass spectra library. Later, the MS traces/spectra of the analyzed samples were compared to spectra from library, which allows positive identification of compounds, based on spectral match. The UV trace was used for quantification of identified compounds from MS detection. Using the chromatographic conditions described above, the polyphenols eluted in less than 35 min. It was not possible to quantify four polyphenols in current chromatographic conditions due overlapping (caftaric acid with gentisic acid and caffeic acid with chlorogenic acid). However, all the four compounds can be selectively identified in MS detection (qualitative analysis) based on differences between their molecular mass and MS spectra. The detection limits were calculated as minimal concentration producing a reproductive peak with a signal–to–noise ratio greater than three. Quantitative determinations were performed using an external standard method. Calibration curves in the 0.5–50 µg/mL range with good linearity ($R^2 > 0.999$) for a five point plot were used to determine the concentration of polyphenols in plant samples.

2.5. Antioxidant tests

2.5.1. Trolox equivalent antioxidant capacity

The ABTS radical cation decolorization assay according to the procedure of Guenné *et al.*[14] with some modifications was used to determine the antioxidant capacity of extracts. ABTS radical cation (ABTS^{•+}) was produced by reacting aqueous ABTS stock solution (7 mmol/L) with 2.45 mmol/L potassium persulfate (final concentration). The mixture was put down in the dark at room temperature for 16 h before use. This mixture was diluted with ethanol to give an absorbance of (0.70±0.02) units at 734 nm using microtitre plates UV/visible light spectrophotometer (Epoch 251465, Biotek Instruments, USA) reader. A volume of 50 µL of the diluted sample (1 g/mL in methanol) was added with 200 µL of fresh ABTS· solution and the absorbance was taken 15 min exactly after initial mixing. Trolox was used to produce the calibration

curve ($R^2=0.99$) and the antioxidant capacity of extracts were expressed as mg trolox equivalent per gram of extract.

2.5.2. Rat liver–lipid peroxidation inhibition

The inhibitory activity of lipid peroxidation of extracts was determined according to the 2–thiobarbituric acid method with some modifications^[15]. Ferrous chloride (FeCl_2) with H_2O_2 was used to induce the liver homogenate peroxidation. In this method 0.2 mL of extracts (1.5 mg/mL) was mixed with 1.0 mL of 1% liver homogenate in Tris–HCl buffer, then 50 μL of FeCl_2 (0.5 mmol/L) and 50 μL of H_2O_2 (0.5 mmol/L) were added. The mixture was incubated at 37 °C for 60 min, then 1.0 mL of trichloroacetic acid (15%) and 1.0 mL of 2–thiobarbituric acid (0.67%) were added and the mixture was heated up in boiled water for 15 min. The absorbance was recorded at 532 nm using a spectrophotometer. Quercetin was used as the positive control. The percentage of inhibition effect was calculated according the following equation: Inhibition rate (%) = $1 - (A_1 - A_2) / A_0 \times 100$; where A_0 is the absorbance of the assay without the extracts, A_1 is the absorbance of the assay with the extracts and A_2 is the absorbance of the assay without homogenate.

2.6. AChE inhibitory activity

AChE inhibitory assay, with inhibition kinetics analysis, was conducted according to the protocol described by Kiendrebeogo *et al.* with some modifications^[16]. A volume of 50 μL of Tris–HCl buffer (50 mmol/L, pH 8, 0.1% BSA) was added with 25 μL of extract (final concentration of 100 $\mu\text{g}/\text{mL}$ dissolved in MeOH buffer 10%) and 25 μL of AChE (0.22 U/mL). The mixture was incubated at room temperature for 2 min before the addition of 125 μL of DTNB (3 mmol/L) and 25 μL of substrate (ATCI 15 mmol/L). The developing yellow color was measured at 405 nm after 4 min with a spectrophotometer. Galanthamine, a reference compound, used for Alzheimer cure, was used as a positive control at a final concentration of 2 $\mu\text{g}/\text{mL}$ in the assay mixture. The activity for acetylcholinesterase inhibition was expressed as inhibition percentage of AChE, calculated as $(1 - B/A) \times 100$, where A is a change in absorbance of the assay without the plant extract (Δ absorbance with enzyme– Δ absorbance without enzyme) and B is the change in absorbance of the assay with the plant extract (Δ absorbance with enzyme– Δ absorbance without enzyme).

2.7. Statistical analysis

The data were expressed as Mean \pm Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $P < 0.05$ and linear regression) was carried out with XLSTAT 7.1

3. Results

3.1. AChE inhibition activity of *N. canescens* extracts

The results of the AChE inhibitory activity of extracts

(0.1 mg/mL) were summarized in Table 1. Our finding was that the inhibitions decreased in the following order: butanol fraction > ethyl acetate fraction > acetonitrile fraction > dichloromethane fraction > hexane fraction > crude extract > water fraction. These results are lowest compared to the galanthamine inhibitory activity [$72.69 \pm 0.88\%$] at 0.02 mg/mL.

3.2. Radical scavenging and anti–lipid oxidation activity of *N. canescens* extracts

The results of antioxidant and lipid oxidation inhibitory activities from *N. canescens* extracts were indicated in Table 1.

In ABTS antioxidant assay, the antioxidant activity of the extracts at the concentration of 1 mg/mL ranged from (10.68 \pm 0.87) to (56.20 \pm 0.77) mg equivalent trolox/g of extract. Ethyl acetate extract [(56.20 \pm 0.77) mg equivalent trolox/g of extract] showed the best ABTS radical cation scavenging followed by butanol extract [(32.20 \pm 1.51) mg equivalent trolox/g of extract]. The water and hexane extracts were presented the lowest activities with values of (11.44 \pm 0.32) mg equivalent trolox/g of extract and (10.68 \pm 0.87) mg equivalent trolox/g of extract, respectively. The standards tested for their caffeic acid, luteolin, rutin and quercetin have shown the most potent ABTS radical cation scavengers (about 67 mg equivalent trolox/g). While the ethyl acetate extract showed the strongest and significantly antioxidant than the others extracts, this activity was still low compared to most of standards.

Table 1

Antioxidant and AChE inhibitory activities.

Samples or standards	AChE inhibition (%)	ABTS (mg ET/g)	LPO inhibition (%)
Crude extract	19.22 \pm 0.93 ^e	24.00 \pm 0.69 ^d	52.57 \pm 1.20 ^a
HE	30.30 \pm 0.99 ^d	10.68 \pm 0.87 ^g	46.63 \pm 1.75 ^b
DCME	36.49 \pm 1.75 ^c	14.41 \pm 0.79 ^e	36.44 \pm 1.17 ^e
ANE	37.19 \pm 0.17 ^c	26.15 \pm 0.65 ^d	40.29 \pm 0.90 ^{cd}
EAE	38.99 \pm 1.23 ^c	56.20 \pm 0.77 ^b	43.21 \pm 1.85 ^c
BE	55.62 \pm 1.49 ^b	32.20 \pm 1.51 ^c	31.49 \pm 0.20 ^f
WE	17.24 \pm 0.98 ^e	11.44 \pm 0.32 ^{fg}	25.83 \pm 1.59 ^g
Galanthamine	72.69 \pm 0.88 ^a	nd	nd
Cinnamic acid	4.43 \pm 0.87 ^f	13.96 \pm 0.00 ^{ef}	nd
Caffeic acid	11.05 \pm 1.03 ^f	67.71 \pm 0.06 ^a	nd
P–coumaric acid	not active	14.60 \pm 1.00 ^e	nd
Apigenin	4.40 \pm 0.08 ^f	14.55 \pm 0.72 ^e	nd
Luteolin	not active	67.05 \pm 0.89 ^a	nd
Rutin	not active	67.18 \pm 0.50 ^a	nd
Quercetin	not active	67.11 \pm 1.67 ^a	38.39 \pm 1.39 ^{de}

Values are Mean \pm SD ($n=3$). ET: Equivalent trolox; LPO: Lipid peroxidation. HE: Hexane extract, DCME: Dichloromethane extract, ANE: Acetonitrile extract, EAE: Ethyl acetate extract, BE: Butanol extract, WE: Water extract. nd: Not determined. Results within each column with different letters (a–g) differ significantly ($P < 0.05$) for our different fractions and standards.

Concerning the rat liver lipid peroxidation inhibition by *N. canescens* extracts, the crude extract demonstrated the best inhibition percentage (52.57 \pm 1.20)% followed by hexane extract (46.63 \pm 1.75)% and ethyl acetate extract (43.21 \pm 1.85)% at the concentration of 1.5 mg/mL (Table 1).

Excepted the dichloromethane, butanol and water extracts, the plant extracts showed greater anti-lipid peroxidative effect compared to quercetin (38.39 ± 1.39) % at the same concentration (1.5 mg/mL).

3.3 HPLC–MS analysis

In this study, 18 phenolic compounds have been investigated in *N. canescens* methanolic extract and the chromatographic profiles of phenolic acids and flavonoids were presented in Figure 1. After analysis, two flavones (apigenin and luteolin), one flavonol (quercetol), four cinnamic acid derivatives (p-coumaric acid, caffeic acid, chlorogenic acid and ferulic acid) and one benzoic acid (gentisic acid) derivative were found in the methanol extract (Table 2).

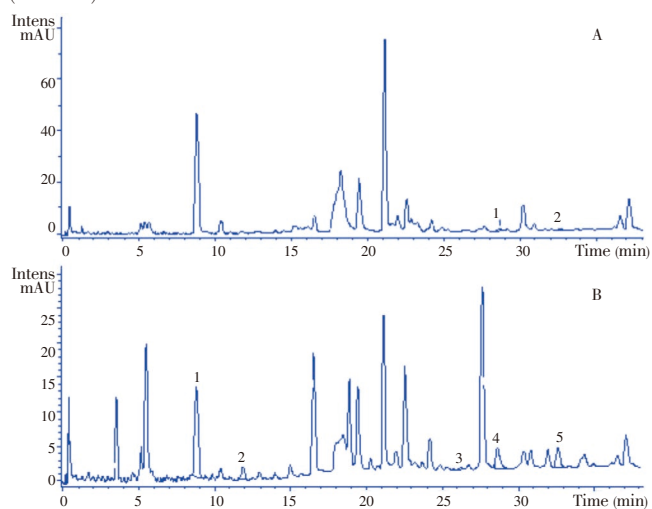


Figure 1. HPLC–MS chromatograms of *N. canescens* methanolic extract.

A: Non-hydrolyzed Sample. 1: Luteolin; 2: Apigenin.

B: Hydrolyzed Sample. 1: p-coumaric acid; 2: Ferulic acid; 3: Quercetol; 4: Luteolin; 5: Apigenin.

Table 2

Phenolic contents of *N. canescens* methanolic extract ($\mu\text{g/g}$ extract).

Polyphenols	Non-hydrolysed sample	Hydrolysed sample
Gentisic acid	+	+
Caffeic acid	+	+
Chlorogenic acid	+	+
P-coumaric acid	+	25 438
Ferulic acid	+	2 630
Quercetol	–	568
Luteolin	1 902	8 126
Apigenin	2 334	9 784

–: not found; +: low quantity.

Among the two flavones identified in non hydrolysed sample, apigenin was the most abundant flavonoid followed by luteolin with the concentrations of $2334 \mu\text{g/g}$ and $1902 \mu\text{g/g}$, respectively. These compounds could also be in their glycoside forms due to the increase of their concentrations in the hydrolysed sample with $9784 \mu\text{g/g}$ for apigenin and $8126 \mu\text{g/g}$ for luteolin. The quercetol absent in non-hydrolysis

extract was found after the hydrolysis at a concentration of $568 \mu\text{g/g}$.

It was found that p-coumaric acid ($25438 \mu\text{g/g}$) was the most abundant hydroxycinnamic acid followed by ferulic acid ($2630 \mu\text{g/g}$) after hydrolysis of *N. canescens* methanol extract. Due to overlapping, gentisic acid, caffeic acid and chlorogenic acid have only been identified selectively in MS detection in the non-hydrolyzed and the hydrolyzed samples.

Furthermore, considering the 18 standard compounds used in this study, several other peaks were not identified (data not shown). Among these compounds, the two major peaks were found in the non-hydrolysed extract of *N. canescens*. The retention time of the first compound was between 8 and 10 min and the second compound was between 20 and 21 min (Figure 1).

4. Discussion

ACh is one of the most important neurotransmitters in animal systems liberated at the synaptic gap. The most important changes observed in the brain are a decrease in cortical levels of this neurotransmitter. Increasing the levels of acetylcholine by inhibition of acetylcholinesterase (AChE, the enzyme responsible for its hydrolysis at the cholinergic synapses, serves as a strategy for the treatment of Alzheimer's disease (AD). The use of acetylcholinesterase inhibitors is the only therapy that has shown consistent positive results in the treatment of Alzheimer's disease^[17]. In this study the best anti-acetylcholinesterase activity obtained with butanol extract (55.62 ± 1.49)% at the concentration of 0.1 mg/mL is greater compared to those obtained by Kulišić-Bilušić *et al.*^[18] with aqueous infusions tea from *Juglansregia* L. and *Fragariaananassa* L. which show respectively 45% and 42.5% inhibition of AChE at the concentration of 1.36 mg/mL in there acting system. It's also known that oxidative stress is associated with age-related neurodegenerative diseases. In fact, ROS oxidize and damage nucleic acids, lipids and proteins contribute to brain aging and age-associated neurodegenerative diseases such as AD. Recently, new therapeutic perspectives point out the prevention of AD in the general population or those perceived as patients with an increased risk for this disease. This may be accomplished with drugs that can neutralize ROS involved as mediator agents in the inflammatory process that ends in neuronal death^[19]. Thus, according to numerous researches, the consumption of antioxidants is highly correlated with lower incidences of AD and the use of natural compounds with high levels of antioxidants has been proposed as an effective therapeutic approach for this disease. This suggests that treatment of AD should involve acetylcholinesterase inhibitors and antioxidants that can scavenge the excess

free radicals and antagonize the consequences of oxidative stress^[20]. Therefore, all the natural phenolic compounds identified in *N. canescens* extract may act as potent preventive agent in AD because they are especially known for their antioxidant effects. Indeed, previous studies are demonstrated that p-coumaric acid decreases low density lipoprotein peroxidation and possesses a potential protection on cardiac oxidative damage induced by doxorubicin, an anticancer antibiotic^[21]. Also, ferulic acid possesses a high antioxidant potential due to its resonance-stabilized phenoxy radical structure. This molecule is an effective scavenger of free radicals and has been approved in certain countries as food additive to prevent lipid peroxidation^[22]. It is demonstrated that pretreatment with ferulic acid protects the cell from γ -radiation-induced damage by inhibiting peroxidation of membrane lipids and free radicals induced DNA strand break formation which can be useful for cancer patients to prevent normal cell damage^[23]. Quercetol prevents oxidant injury and cell death by protecting against lipid peroxidation. Antioxidant capacities of apigenin in free radical scavenging and of luteolin against ABTS radicals have been shown^[24]. Caffeic acid is known for its *in vitro* antioxidant activity and multiple pharmacological properties including anti-inflammatory and antitumorigenic effects. For chlorogenic acid, its protective effects on oxidative stress *in vivo* have been reported^[25]. Ferulic acid is known for its high antioxidant and anti-inflammatory activities. It's showed that gentisic acid, an aspirin metabolite, inhibits potently low density lipoprotein oxidation *in vitro*. Quercetol prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals and chelating metal ions.

In addition to its potent antioxidant, chlorogenic acid is well known for its potent neuroprotective effects by the AChE inhibition and thus presents a double benefit^[26].

In this study, our finding was that acetylcholinesterase is inhibited at more than 50% by butanol extract at a final concentration of 100 $\mu\text{g/mL}$. Moreover, all the extracts of *N. canescens* exhibited an interesting antioxidant capacity using ABTS and lipoxygenase inhibition methods. Particularly, ethyl acetate extract exhibited a remarkable ABTS radical cation scavenging capacity while the crude extract showed the best result through the rat liver lipid peroxidation inhibition. In addition, two flavones (apigenin and luteolin), one flavonol (quercetol), four cinnamic acid derivatives (p-coumaric acid, caffeic acid, chlorogenic acid and ferulic acid) and one benzoic acid (gentisic acid) derivative were identified and quantified in this species after the HPLC-MS analysis and are mainly responsible for these biological activities *in vitro*. These extracts and their active components could emerge as natural antioxidants, alternative anticholinesterase drugs or serve as starting points for synthesizing more effective AChE inhibitors. These scientific data allow to justify the widely use of *N. canescens* in traditional medicine in Burkina Faso.

Further molecular investigations are necessary in order to isolate the bioactive structures identified by HPLC-MS for biological tests.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Alzheimer's disease is a slowly progressive neurodegenerative disease of the brain characterized by impairment of memory and eventually disturbances in reasoning, planning, language, and perception. Oxidative stress was very link with this disease. Therefore, the research is oriented for new AChE and oxidants inhibitors derived from natural sources.

Research frontiers

N. canescens is widely used traditionally in the treatment of degenerative diseases in Burkina Faso. A high level of phenolic in polar extract of the plant has been shown in preliminary study and there has been no study of the extracts inhibition on AChE and lipid peroxidation and there has been no identification of phenolic compounds.

Related reports

The obtained results are a substantial research contribution. Related reports here concern phytochemical profile data on phenolic. In this study more details obtained are compared to the results of Dasgupta *et al.* (2011 & 2012) for their phytochemical screening and those of Sawadogo *et al.* (2006) for total phenolic quantification.

Innovations & breakthroughs

In this paper, *N. canescens* is confirmed as a potential medicinal plant through the data obtained. And this paper will enhance the knowledge very well of this plant for its potent biological properties studied by authors and for the 8 phenolic compounds identified and quantified by HPLC-MS.

Applications

This study will be very important for traditional medicine practitioners and scientists throughout the world for the existence of scientific data and the plant using will be encouraged for treatment of neurodegenerative disorders in Burkina Faso.

Peer review

Authors of this paper did a great contribution to investigate AChE and oxidant inhibition potentialities of *N. canescens* and also to identify and quantify 8 phenolic compounds. This research paper gives interesting phytochemistry and biological activities data of the species.

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