Pyrroloquinolone A, a new alkaloid and other phytochemicals from Atractylis cancellata L. with antioxidant and anticholinesterase activities

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Abstract

A new alkaloid pyrroloquinolone A (**1**), along with fifteen known compounds **2-16** were isolated from the petroleum ether, EtOAc and *n*-BuOH extracts of the whole plant *Atractylis cancellata* L. Their structures were elucidated on the basis of extensive spectroscopic analysis including 1D- and 2D-NMR and HR-ESI-MS techniques. This is the first report of alkaloids in the genus *Atractylis*. Some of the isolated compounds and extracts were evaluated for their antioxidant potential (scavenging activity of DPPH and ABTS radicals, and reducing Fe^{+3} and Cu^{+2} power assays) and acetylcholinesterase and butyrylcholinesterase inhibitory activities. Compounds **8** and **11** showed good antioxidant capacity compared to ascorbic acid, BHA, and BHT used as standards, whereas compounds **1** and **2** exhibited good anticholinesterase activities compared to galantamine used as standard.

1. Introduction

The Asteraceae family called also Compositae is the most diverse of all plant families, and it is growing in the entire globe except for Antarctica, with 24000-30000 species and 1600-1700 genera (Funk et al. 2005). The *Atractylis* genus belonging to this family is widespread in the Mediterranean area, including 16 species in North Africa due to its variable climate (arid, semi-arid, tropical and desert) (Chabani et al. 2013).

The *Atractylis* species are used in folk medicine against urinary retention, intestinal parasites and snakebite poisoning, and for their anti-inflammatory and antipyretic activities (Larrey and Pageaux, 1995, El Rhaffari and Zaid 2002, Daniele et al. 2005, Melakhessou et al. 2018). In addition, many studies have been reported for *Atractylis* plants, which allowed the identification of diterpenes, triterpenes, saponins and flavonoids (Sadek et al. 1998, Chabani et al. 2013, Chabani et al. 2016a, Chabani et al. 2016b). *Atractylis cancellata* L., used in folk medicine for the treatment of skin disorders (Bammou et al. 2015), is an herbaceous endemic plant; it grows in semi-arid zone of Mediterranean area (Quezel and Santa 1963).

In this paper, we report the isolation and structural elucidation of one new alkaloid type pyrroloquinolone A (**1**), together with fifteen known secondary compounds including one alkaloid **2**, seven flavonoids **3-9,** three phenolic acids **10-12**, and four triterpenes **13-16**. In addition, the antioxidant capacities of extracts (PE, EtOAc and *n*-BuOH) and some phenolic

compounds were measured using DPPH, ABTS, CUPRAC, and reducing power methods. Furthermore, the acetylcholinesterase and butyrylcholinesterase inhibitory activities of extracts and the alkaloids **1** and **2** were tested.

It is worth to note that this work represents the first phytochemical and biological investigations of *A. cancellata* and the first report of alkaloids in the genus *Atractylis.*

2. Results and discussion

2.1. Chemical constituents

The 70% ethanol extract of dried whole plant *A. cancellata* was partitioned by liquid/liquid chromatography into three extracts PE, EtOAc and *n*-BuOH. Purification of the PE, and EtOAc soluble parts gave the known compounds **13**-**15**, and **3-6** and **16**, respectively. While, one new alkaloid **1**, in addition of seven known compounds **2** and **7**-**12** were isolated from the *n*-BuOH soluble part (Figure 1). Their structures were elucidated on the basis of 1D- and 2D-NMR and HR-ESI-MS techniques, and comparison with data reported in the literature. The known compounds were elucidated as the alkaloid 4-methoxy-1-methyl-2-quinolone (**2**) (Nayar et al. 1971), the flavonoids chrysin (**3**) (Liu et al. 2010), apigenin (**4**) (Liu et al. 2010), tricine (**5**) (Ghasemi et al. 2018), quercetin (**6**) (Liu et al. 2010), quercetin 3-*O*-β-D-glucopyranoside (**7**) (Chang et al. 2009), isoorientin (**8**) (Chang et al. 2009), and diosmin (**9**) (Szeleszczuk et al. 2017), the phenolic acids 4-*O*-caffeoyl-2-*C*-methyl-D-threonic acid (**10**) (Chang et al. 2009), chlorogenic acid methyl ester (**11**) (Chang et al. 2009), and 5-*O*-caffeoylshikimic acid (**12**) (Khaligh et al. 2016). Finally, the triterpenes lupeol (**13**) (Silva et al. 2017), and oleanolic acid (**14**) (Seebacher et al. 2003), were identified with *β*-sitosterol (**15**) (Chaturvedula et al. 2012), and *β*-sitosterol-3-*O*-β-D-glucoside (**16**) (Peshin and Kar, 2017).

2.2. Structural elucidation of the new compound

Compound **1** was isolated as a white amorphous powder. The positive HR-ESI-MS showed a molecular ion peak at *m/z* 198.0791 [M]$^+$ (calcd. $C_{12}H_{10}N_2O$, 198.0793). The ^{1}H-NMR spectrum of **1** showed a methyl group signal at δ_H 4.02 (3H, s) and six aromatic proton signals at δ_H 7.12 (1H, d, *J*=3.1 Hz, H-3), 7.46 (1H, d, *J*=3.1 Hz, H-2), 7.61 (1H, t, *J*=7.6 Hz, H-8), 7.75 (1H, ddd, *J*=8.7, 7.6, 1.3 Hz, H-7), 8.01 (1H, d, *J*=8.7 Hz, H-6), and 8.24 (1H, brd, *J*=8.0 Hz, H-9). According to the correlation observed in the ^{1}H-^{1}H-COSY spectrum between protons H-3/H-2, H-8/H-7, H-8/H-9, and H-7/H-6, and their coupling constants, we assumed

that the protons H-6, H-7, H-8 and H-9 belong to the same aromatic ring which is di-substituted in *ortho* position; and the two protons H-2 and H-3 belong to an another aromatic ring. The ^{13}C-NMR spectrum showed signal at δ_C 152.4 corresponding to a quaternary carbon of an ester or an amid group. The other signals are in the aromatic area shifts (δ_C 103.9, 108.6, 115.1, 116.9, 121.5, 124.9, 125.1, 129.0, 134.8, and 135.1), in addition to a signal at δ_C 33.1 attributed to a methyl group. The chemical shifts of the carbon and the protons signals (δ_H 4.02/ δ_C 33.1) of this methyl group suggested it to be linked to a nitrogen atom. The HSQC spectrum allowed the assignment of the protons at δ_H 7.12, 7.46, 7.61, 7.75, 8.01, and 8.24 to their carbons at δ_C 103.9 (C-3), 125.1 (C-2), 124.9 (C-8), 129.0 (C-7), 116.9 (C-6), and 121.5 (C-9), respectively. The HMBC spectrum exhibited $^3J_{H/C}$ correlation between the protons of the *N*-methyl group (δ_H 4.02) and carbons C-6, C-6a (135.1 ppm), and C-4 (152.4 ppm) (Figure 2) indicating that the nitrogen is related to the aromatic ring by the bond N-(C-6a), and the function will be an amid. The two protons of the aromatic ring H-6 and H-8 showed $^3J_{H/C}$ correlations with the quaternary carbon at δ_C 115.1, ascribable to C-9a. The HMBC spectrum exhibited also the correlations H-6/C-8, H-7/C-6a, H-7/C-9 and H-9/C-6a, H-6/C-9b (134.8 ppm), and H-9/C-9b (Figure 2). According to the HMBC correlations H-2/C-9b and H-3/C-9b, the carbon chemical shifts of C-2 (125.1 ppm) and C-9b (121.5 ppm), and the molecular formula obtained by HR-ESI-MS spectrum ($C_{12}H_{10}N_2O$), the carbons C-2 and C-9b were proposed to be connected by a second nitrogen atom (C-9b-NH-C-2). The HMBC correlations H-3/C-3a (108.6 ppm), H-2/C-3a and H-2/C-4 confirmed that C-3 is connected to C-3a (Figure 2), and were included in a pyrrole ring.

According to these data, this compound is composed of benzene and pyrrole rings linked to a pyridine ring including the amide group, which lead us to the structure of pyrroloquinolone. Thus, compound **1** was elucidated as 5-methyl-4,5-dihydro-1*H*-pyrrolo[3,2-c]quinolin-4-one, previously synthetized by Tim and Keith, 1997, and named pyrroloquinolone A.
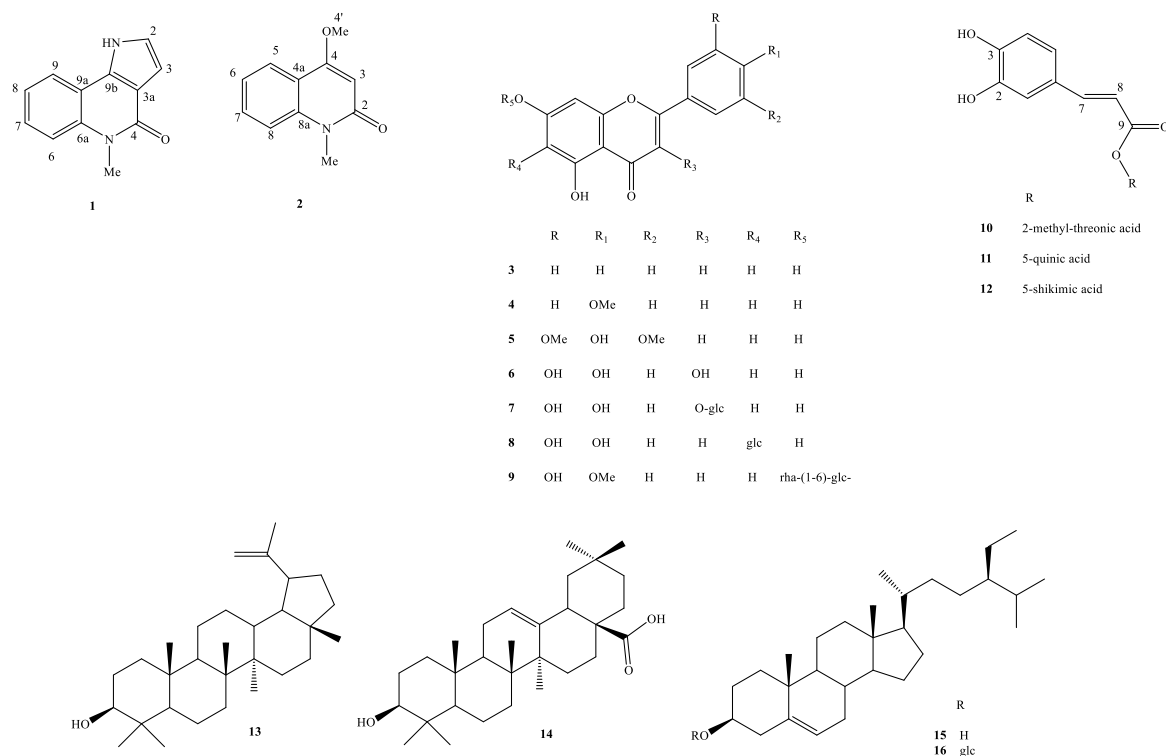
Figure 1. Chemical structures of compounds **1-16** isolated from *A. cancellata* L.

2.3. Spectral data

Pyrroloquinolone A (**1**): White amorphous powder; UV λ_{max} (MeOH): 249, 316, 328 nm; ^{1}H NMR (500 MHz, CD$_3$OD): δ_H 4.02 (3H, s, N-CH$_3$), 7.12 (1H, d, *J*=3.1, H-3), 7.46 (1H, d, *J*=3.1, H-2), 7.61 (1H, t, *J*=7.6 Hz, H-8), 7.75 (1H, ddd, *J*=8.7, 7.6, 1.3 Hz, H-7), 8.01 (1H, d, *J*=8.7 Hz, H-6), 8.24 (1H, brd, *J*=8.0 Hz, H-9); ^{13}C NMR (125 MHz, CD$_3$OD) δ_C 33.1 (*N*-CH$_3$), 103.9 (C-3), 108.6 (C-3a), 115.1 (C-9a), 116.9 (C-6), 121.5 (C-9), 124.9 (C-8), 125.1 (C-2), 129.0 (C-7), 134.8 (C-9b), 135.1 (C-6a), 152.4 (C-4); HR-ESI-MS *m/z* 198.0791 [M]$^+$ (calcd. C$_{12}$H$_{10}$N$_2$O, 198.0793).

2.4. Phenolic and flavonoid contents

The total phenolic and flavonoid contents (TPC, TFC) were determined in the PE, EtOAc and *n*-BuOH extracts. The results were expressed by gallic acid equivalent as (mg GAE/g Extract) for TPC, and by quercetin equivalent as (mg QE/g Extract) for TFC. The *n*-BuOH extract showed the higher content of phenols (53.20 ± 1.21 mg GAE/g) comparing to the EtOAc (4.55 ± 0.13 mg GAE/g) and the PE (28.44 ± 2.85 mg GAE/g). The same result was obtained

for content of flavonoids (*n*-BuOH 49.39 ± 0.98 mg QE/g EtOAc 8.72 ± 1.08, and PE 6.38 ± 1.01 mg QE/g) (Table S1).

2.5. Biological activity

The results of phenolic and flavonoid contents were confirmed by the phytochemical investigation. Indeed, many phenolic and flavonoid compounds were isolated from the EtOAc and *n*-BuOH extracts. The antioxidant activity of the PE, EtOAc and *n*-BuOH extracts and some isolated compounds **5**, **7-9** and **10-12** was evaluated by four methods DPPH, ABTS, CUPRAC, and reducing power assay; using ascorbic acid, BHA and BHT as positive control (Table S2).

The DPPH assay indicated a good activity for five tested compounds, in particular compounds **8** and **11** which afforded a potent activity (IC_{50} 8.16 ± 0.27 and 18.81 ± 0.17 µg/mL, respectively) compared to ascorbic acid (IC_{50} 13.94 ± 2.81), BHA (IC_{50} 5.73 ± 0.41 µg/mL) and BHT (IC_{50} 22.32 ± 1.19 µg/mL) as standards, while the IC_{50} for compounds **5** and **9** were not achieved at 100 µg/mL (Table S2). Compounds **8** and **11** showed also the highest activity in $ABTS^{+.}$ radical scavenging assay (IC_{50} 7.14 ± 0.13 and 5.98 ± 0.00 µg/mL, respectively) compared to ascorbic acid (IC_{50} 1.74 ± 0.10), BHA (IC_{50} 1.81 ± 0.10 µg/mL), and BHT (IC_{50} 1.29 ± 0.30 µg/mL), whereas good scavenging activity was observed for compounds **5**, **7**, **10** and **12** (IC_{50} 47.23 ± 2.44, 30.79 ± 1.42, 14.81 ± 0.47 and 17.09 ± 0.64 µg/mL, respectively). The CUPRAC assay was applied and all compounds revealing an excellent ability to reduce Cu^{+2} (IC_{50} < 0.78 µg/mL). In the reducing power assay, compounds **8** and **10**-**12** showed a good ability to reduce Fe^{+3} (IC_{50} 10.76 ± 0.80, 27.45 ± 1.16, 14.18 ± 0.25, and 19.83 ± 0.60 µg/mL, respectively), compared to ascorbic acid (IC_{50} 6.37 ± 0.42), BHA (IC_{50} 8.41 ± 0.67 µg/mL) and BHT (IC_{50} >100 µg/mL) (Table S2).

Cholinesterases (ChEs) play a vital role in regulating cholinergic transmission. Inhibition of ChEs is thought to bean emerging and useful therapeutic target for neurodegenerative disorders through restoration of acetylcholine levels in the brain (e.g. Alzheimer's disease) (Bensouici et al. 2016). Since derivatives of quinoline alkaloids such as tacrine have promising ChE and BChE inhibitory effects (Cabral et al. 2012, Konrath et al. 2013, Mermer et al. 2018, Mishra et al. 2019), the unexplored quinoline alkaloid derivatives **1** and **2** and extracts in which they were detected, were evaluated for their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity (S3). Galantamine was used as positif control Mishra et al 2019, (Table S3). The *n*-BuOH extract exhibited moderate ChE (IC_{50}

48.58 $\pm$ 0.26 µg/mL) and good BChE (IC$_{50}$ 22.85 $\pm$ 2.15 µg/mL) inhibitory activities compared to galantamine (IC$_{50}$ = 6.27 $\pm$ 1.15, AChE and 34.75 $\pm$ 1.99 µg/mL, BChE). Compound **1** exhibited a significant AChE inhibitory activity (IC$_{50}$ = 18.48 $\pm$ 0.33 µg/mL) and a potent BChE inhibitory activity (9.66 $\pm$ 0.16 µg/mL), whereas compound **2** exhibited a moderate BChE inhibitory activity (IC$_{50}$ 37.49 $\pm$ 1.61 µg/mL).

3. Conclusion

In summary, one new alkaloid, pyrroloquinolone A (**1**), along with fifteen known compounds **2-16** were isolated and identified from the whole plant *Atractylis cancellata* L. The antioxidant activity, evaluated by DPPH, ABTS, CUPRAC, and reducing power methods, showed that compounds **8** and **11** possesses good antioxidant activity. Furthermore, the *n*-BuOH extract, pyrroloquinolone A**,** and compound **2** showed good AChE and BChE inhibitory activities. This study reports for the first time the occurrence of alkaloids in the *Atractylis* genus.

Supplementary material

General experimental methods, UV, HR-ESI-MS, ^{1}H and ^{13}C NMR, COSY, HSQC and HMBC spectra of compound **1** are available online.

Disclosure statement

No potential conflict of interest was reported by the authors.

Statistical analysis

All data on both chemical analysis and bioassays activity tests were the average of triplicate analyses. The data were recorded as mean values $\pm$ standard deviation. The IC$_{50}$ values were calculated by linear regression analysis, and one-way analysis of variance ANOVA to detect significant differences (p < 0.05) by Tukey using XLSTAT.

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SUPPLEMENTARY DATA

Pyrroloquinolone A, a new alkaloid and other phytochemicals from *Atractylis cancellata* L. with antioxidant and anticholinesterase activities

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Abstract

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Keywords: Asteraceae, *Atractylis cancellata* L., Pyrroloquinolone A, NMR, Antioxidant activity, Anticholinesterase activity

Experimental

General experimental procedures

1D- and 2D-NMR spectra were carried out in $CDCl_3$, CH_3OH-d_4, or DMSO-d_6 on Bruker Avance DRX III 500 instrument using standard Bruker microprograms (Karlsruhe, Germany). ESI-MS experiments were performed using a Micromass Q-TOF micro-instrument (Manchester, UK). TLC were realized on pre-coated silica-gel 60 F_{254} Merck and were visualized under UV light at 254 and 366 nm and by spraying the dried plates with 50% H_2SO_4, followed by heating. Flash chromatography was performed on a Grace Reveleris system equipped with dual UV and ELSD detection using Grace cartridges (Silica gel or RP-C18), and the effluents were monitored at 205 and 254 nm. The Medium pressure liquid chromatography (MPLC) was employed using a Buchi pump system coupled (Bushi, France), with C_{18}-silica gel packed glass column (15×230 and 26×460 mm). Semi-prep. HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a diode array detector UVD 340S and a Chromeleon software (ThermoFisher Scientific, France). RP-C_{18} column (Phenomenex 250 -15 mm, Luna 5 m) was used for semi preparative HPLC with a binary gradient eluent (H_2O pH 2.4 with TFA; MeCN) and a flow rate of 5 mL/min (interchim, France); the chromatogram was monitored at 205, 254, 300, and 360 nm.

The bioactivity measurements were carried out at National Center of biotechnology Research (Algeria) on a 96-well microplate reader, Perkin Elmer Multimode Plate Reader EnSpire (Perkin Elmer, France). Folin-ciocalteu's reagent (FCR), 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxylanisole (BHA), butylated hydroxyltoluene (BHT), α-tocopherol, ascorbic acid, neocuproine, 2,2′-azinobis(3-ethyl-benzothiazoline-6-suphonic acid) diammonium salt (ABTS), trichloroacetic acid (TCA), potassium ferricyanide, ammonium acetate, trichloro- acetic acid, potassium acetate, aluminum nitrate and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), potassium ferricyanide, were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Stern-heim, Germany). Sodium carbonate, aluminium nitrate, iron (III) chloride, iron (II) chloride, copper (II) chloride, potassium persulfate, potassium acetate, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), acetylcholine iodide, butylcholine iodide were obtained from Biochem Chemopharma (Biochem Chemopharma, France). All other chemicals and solvents were of analytical grade.

Plant material

The whole plant *Atractylis cancellata* L. was collected in May 2015 in Djerma from semi-arid region of Batna (North eastern of Algeria) by Prof. Hamada Haba. It was authentified by Prof. Bachir Oudjehiche from the Agronomic Institute of the University of Batna-1 (Algeria). A voucher specimen (846/LCCE) has been deposited at the Faculty of Sciences of Matter, University of Batna-1, Algeria.

Preparation of plant extracts

The dry powdered whole plant of *A. cancellata* (1.2 kg) was macerated in EtOH 70% (2 × 12 L, 48h) at room temperature, and then concentrated under low pressure to remove EtOH. The aqueous residue was diluted with 500 mL of H_2O, and successively extracted with Petroleum Ether (3 × 300 mL), ethyl acetate (3 × 300 mL), and *n*-butanol (3 × 300 mL). The obtained organic phases were evaporated to give 13.4 g of PE, 10 g of EtOAc and 16.8 g of *n*-BuOH extracts. All the extracts were evaporated at 45 °C, under low pressure using rotary evaporator and the extracts and their fractions were stored in darkness at room temperature before fractionation and purification.

Chemical screening of plant extracts

Total phenolic content (TPC) was measured for the obtained extracts (PE, EtOAc and *n*-BuOH) using the method of Muller et al. (2010), for the calibration, the gallic acid solution at various concentrations was used and the result was given as gallic acid equivalents (µg GAE/mg extract).

The measurement of total flavonoids content (TFC) was carried out by the procedure of Topçu et al.(2007), the TFC of extracts was expressed as quercetin equivalents (µg QE/mg extract), with quercetin for calibration.

Isolation and purification

The PE extract (5 g) was fractionated by Vacuum Liquid Chromatography (VLC) over silica gel eluted with PE-EtOAc (1:0 - 0:1), to give 9 fractions (P1-P9). Fraction P3 (725 mg) was applied to silica Column Chromatography (CC) gel and eluted with PE-EtOAc (1:0 - 0:1) to afford compounds **15** (172 mg), **13** (13 mg) and **14** (7 mg).

The EtOAc extract (7 g) was subjected to silica gel VLC eluted with PE-EtOAc-MeOH (1:0:0 - 0:1:0 - 0:0:1) to result 13 fractions (Ac1-Ac13). Ac5 (549 mg) was separated over silica gel CC (PE-EtOAc 1:0 - 0:1) and 14 sub-fractions were collected (Ac5-A to Ac5-N). The sub-fraction Ac5-J was fractionated over polyamide CC (Toluene-MeOH 1:0 - 0:1) in order to

obtain compounds **4** (1.3 mg) and **6** (3 mg). The fractions Ac6-Ac8 were combined (2.7 g) and subjected to silica gel CC eluted with CH_2Cl_2-Acetone-MeOH (1:0:0 - 0:1:0 - 0:0:1) to give 8 sub-fractions (Ac6-A to Ac6-H). The sub-fraction Ac6-C contained the compound **3** (3 mg). Fraction Ac9 (1.1 g) was fractionated by silica gel CC (PE-EtOAc-MeOH 1:0:0 - 0:1:0 - 0:0:1) to afford 15 sub-fractions (Ac9-A to Ac9-O), then Ac9-G (85 mg) was purified with silica gel CC (CHCl$_3$-MeOH 1:0 - 90:10) to furnish compound **16** (5 mg). Fractions Ac10-Ac11 were combined (2.8 g) and subjected to silica gel CC (CHCl$_3$-MeOH 1:0 - 0:1) to give 15 sub-fractions (Ac10-A to Ac10-O). Ac10-H (285 mg) was purified with polyamide CC (Toluene-MeOH 1:0 - 0:1) to obtain compound **5** (7 mg).

The *n*-BuOH extract (13.4g) was subjected to VLC over RP-18 eluted with MeOH-H$_2$O (25:75 - 1:0) to afford 4 fractions (Bu1-Bu4). Bu2 (2.7 g) was purified by preparative HPLC using MeCN (isocratic elution 10% for 30 min, 10%→100% in 20 min) to yield 21 sub-fractions (Bu2-A to Bu2-U). Compound **8** (42 mg) was precipitated in MeCN of sub-fraction Bu2-Q. Sub-fraction Bu2-H (80 mg) was purified by semi-prep. HPLC (10% MeCN, isocratic elution) to give compound **11** (R_t 15.2 min, 24 mg). Sub-fraction Bu2-K (115 mg) was purified by semi-prep. HPLC (isocratic elution 10% MeCN) to afford compound **10** (R_t 25.5 min, 25 mg). Fraction Bu2-P (160 mg) was fractionated by flash chromatography over silica gel eluted with CH_2Cl_2-MeOH-H$_2$O (1:0:0 - 70:30:0 - 70:30:5) to give ten sub-fractions (Bu2-P1-Bu2-P10). Bu2-P4 (14 mg) was purified by semi-prep. HPLC (14→20% MeCN in 20 min) to yield compound **12** (R_t 11.0 min, 3 mg). The combined fractions Bu3 and Bu4 (2.2 g) were applied to flash chromatography over silica gel, eluted with CH_2Cl_2-MeOH-H$_2$O (90:10:0 - 70:30:0 - 70:30:5) affording 22 sub-fractions (Bu3-A-V). Compound **9** (3 mg) was precipitated in MeOH solution of sub-fraction Bu3-G (99 mg). Bu3-P (300 mg) was subjected to prep. HPLC (15% MeCN in 30 min, then 15% →100% MeCN in 20 min) to give 22 sub-fractions (Bu3-P1 to Bu3-P22). The fractions Bu3-P2 (9 mg), Bu3-P10 (14 mg) and Bu3-P15-19-t (33 mg) were purified separately by semi-prep. HPLC (10→20% MeCN in 20 min) to give compounds **2** (R_t 12.5, 2 mg), **7** (R_t 10.0 min, 3 mg), and **1** (R_t 7.7 min, 2 mg), respectively.

Biological studies

In order to evaluate the antioxidant activity, we have applied four different methods: DPPH free radical scavenging, ABTS, CUPRAC and reducing power assays. The ascorbic acid, BHA and BHT were used as antioxidant standards. The radical scavenging activity (%) or reducing capacity ratio (%) were is calculated by the following formula: $100 \times [A_0 - A/A_0]$,

where A_0 and A are the absorbances of the system in the absence and presence of sample, respectively. The IC_{50} values were interpolated from a linear regression plot of sample concentration against scavenging effect/or reducing capacity in percentage.

DPPH free radical scavenging assay

The free radical scavenging assay was evaluated by the DPPH assay described by Blois (1958) with a slight modification. Briefly, 180 µL of DPPH solution (0.4 mM) was added to 20 µL of samples dissolved in MeOH, at different concentrations; a control containing 40 µL of MeOH with 160 µL of DPPH solution was used, after 30 min in darkness the absorbance was measured at 517 nm.

ABTS cation radical decolorization assay

The method of Re et al. (1999) was used with slight modification to assess the capacity of extracts and isolated compounds to scavenge the $ABTS^{.+}$ radical. The $ABTS^{.+}$ radical-cation is regenerated by adding 2.45 mM of potassium persulfate to an aqueous solution of 7 mM ABTS which is stored in the dark at room temperature for 12 hours. To each well, in 96- well plate, 180 µL of $ABTS^{.+}$ solution was added to 20 µL of sample solution at different concentrations. After 10 min, the absorbance was measured at 734 nm.

Cupric reducing antioxidant capacity (CUPRAC) assay

The cupric-reducing antioxidant capacity was evaluated according to the method of Apak et al. (2004), with slight modifications. To each well, in a 96-well plate, 50 µL 10 mM copper (II) chloride, 50 µL 7.5 mM neocuproine and 60 µL ammonium acetate buffer (1 M, pH 7.0) solutions were added. 40 µL of the sample at different concentrations was added to the initial mixture. After 60 min, the absorbance was measured at 450 nm.

Reducing power assay

The reducing power of the tested compounds was determined according to the method of Bouratoua et al. (2017). In order to evaluate the reducing power activity, 10 µl of serial diluted sample was added into a 96 well round-bottomed plate. Following this, 40 µL of 0.2 M phosphate buffer (pH 6.6) and 50 µL of potassium ferricyanide (1%), were added to each well and the plate was incubated at 50 °C for 20 min. Finally, 50 µL of TCA (10%) and distilled water (40 µL) and 10 µL of ferric chloride (0.1%), were added into each well in order to measure the reducing power activity. Then, the absorbance was measured in a microplate reader at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

Anticholinesterase activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity was measured using the spectrophotometric method developed by Ellman et al. (1961). Briefly, 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution dissolved in methanol at different concentrations and 20 µL AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) solution were mixed and incubated for 15 min at 25 ℃; then 10 µL of 0.5 mM DTNB [5,5´-dithio-bis(2-nitrobenzoic) acid] was added. The reaction was then initiated by the addition of 10 µL of acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM). The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion, as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, at a wavelength of 412 nm, every 5 min for 15 min, using a 96-well microplate reader (Perkin Elmer Multimode Plate Reader EnSpire, USA) in triplicate experiments. Galantamine was used as reference compound. The inhibition of AChE or BChE (%) was calculated using the formula: $(E-S)/E \times 100$, where E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample. The IC_{50} values were interpolated from a linear regression plot of extracts and compounds concentration against inhibition effect in percentage.

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Table S1. Phenolic and flavonoid contents in *A. cancellata* extracts

Extracts	TPC (mg GAE[x]/g)	TFC (mg QE[y]/g)
PE	28.44 ± 2.85^a	6.38 ± 1.01^b
EtOAc	4.55 ± 0.13^b	8.72 ± 1.08^b
n-BuOH	53.20 ± 1.21^c	49.39 ± 0.98^a

[x] Gallic acid equivalent, [y] quercetin equivalent

Table S2. Antioxidant activity of *A. cancellata* extracts and some isolated compounds by the DPPH, ABTS, CUPRAC and reducing power assays

Extracts/Compounds	Antioxidant Activities			
	DPPH assay	ABTS assay	CUPRAC assay	Reducing Power assay
	IC_{50} (µg/mL)		IC_{50} (µg/mL)	
PE extract	>100	>100	194.17 ± 2.36^a	>100
EtOAc extract	>100	>100	148.56 ± 0.28^b	>100
n-BuOH extract	60.55 ± 2.18^a	36.58 ± 2.41^b	36.59 ± 0.76^c	49.25 ± 0.08^a
5	>100	47.23 ± 2.44^a	0.58 ± 0.23^g	>50
7	57.91 ± 1.83^a	30.79 ± 1.42^c	0.73 ± 0.48^g	>50
8	8.16 ± 0.27^e	7.14 ± 0.13^e	0.73 ± 0.35^g	10.76 ± 0.80^e
9	>100	NT	0.60 ± 0.39^g	>50
10	25.80 ± 0.39^b	14.81 ± 0.47^d	0.71 ± 0.60^g	27.45 ± 1.16^b
11	18.81 ± 0.17^c	5.98 ± 0.00^e	0.70 ± 0.20^g	14.18 ± 0.25^d
12	25.01 ± 0.55^b	17.09 ± 0.64^e	0.71 ± 0.57^g	19.83 ± 0.60^c
BHA	5.73 ± 0.41^e	1.81 ± 0.10^f	$3.64 \pm 0.19^{f,g}$	8.41 ± 0.67^e
BHT	$22.32 \pm 1.19^{b,c}$	1.29 ± 0.30^f	$9.62 \pm 0.87^{e,g}$	>100
Ascorbic acid	13.94 ± 2.81^d	1.74 ± 0.10^f	12.43 ± 0.09^d	6.37 ± 0.42^g

IC_{50} values are defined as the concentration of 50% inhibition percentages and calculated by linear regression analysis and expressed as Mean $\pm$ SD (n=3). The IC_{50} values with different superscripts (a, b, c,…) in the same column are significantly different ($p < 0.05$). BHA: Butylatedhydroxyanisole, BHT: Butylatedhydroxytoluene, NA: not tested.

Table S3. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of various extracts and isolated alkaloids of   *A. cancellata*

Extract/compound	Anticholinesterase activity	
	AChE	BChE
	IC_{50} (µg/mL)	
PE extract	>200	>200
EtOAc extract	>200	>200
***n*-BuOH extract**	48.58 ± 0.26[a]	22.85 ± 2.15[b]
1	18.48 ± 0.33[b]	9.66 ± 0.16[c]
2	>50	37.49 ± 1.61a
Galantamine	6.27 ± 1.15[c]	34.75 ± 1.99[a]

IC50 values is defined as the concentration of 50% inhibition percentages and calculated by linerar regression analysis and expessed as mean ± SD (n=3). The IC50 values with different superscripts (a, b, c …) in the same column are significantly different ($p < 0.05$).

Table S4. NMR spectroscopic data of compound **1** in MeOH-d_4 (in ppm and *J* in Hz)

position	1	
	δ_C	δ_H
2	125.1	7.46 (1H, d, 3.1 Hz)
3	103.9	7.12 (1H, d, 3.1 Hz)
3a	108.6	-
4	152.4	-
N-Me	33.1	4.02 (3H, s)
6a	135.1	-
6	116.9	8.01 (1H, d, 8.7 Hz)
7	129.0	7.75 (1H, ddd, 8.7, 7.6, 1.3 Hz)
8	124.9	7.61 (1H, t, 7.6 Hz)
9	121.5	8.24 (1H, brd, 8.0 Hz)
9a	115.1	-
9b	134.8	-