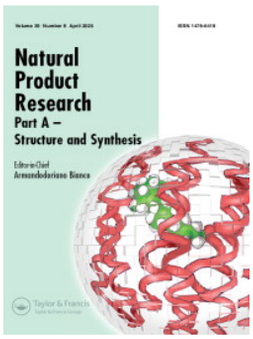

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Antiradical and anti-acethylcholinesterase constituents from the methylene chloride extract of *Ganoderma applanatum* (Pers.) Pat (Ganodermataceae) and molecular docking study

Roméo Toko Feunaing[a], Joël Abel Gbaweng Yaya[a,b], Jean Noël Nyemb[c], Noël Issa Bassigue[a], Hervé Landry Ketsemen[d], Céline Henoumont[e], Antoine Kavaye Kandeda[f], Alessandro Venditti[g], Sophie Laurent[d] and Emmanuel Talla[a]

[a]Department of Chemistry, Faculty of Science, University of Ngaoundere, Ngaoundere, Cameroon; [b]Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, Yaounde, Cameroon; [c]Department of Refining and Petrochemistry, National Advanced School of Mines and Petroleum Industries, The University of Maroua, Kaele, Cameroon; [d]Department of Organic Chemistry, Faculty of Science, The University of Yaounde 1, Yaounde, Cameroon; [e]Department of General Organic and Biomedical Chemistry, Faculty of Medicine and Pharmacy, University of Mons, Belgium, Avenue Maistriau, Mons, Belgium; [f]Department of Biology and Animal Physiology, Faculty of Science, The University of Yaounde 1, Yaounde, Cameroon; [g]Independent Researcher, Italy

ABSTRACT

Alzheimer's disease (AD) is a non-communicable disease with global impact. Inhibitors of acetylcholinesterase (AChE) are suitable therapies for AD. In this work, we report the isolation of antiacetylcholinesterase compounds from the methylene chloride (DCM) extract of the medical fungus *Ganoderma applanatum* (Pers.) Pat (Ganodermataceae). Chemical evaluation of this extract using chromatographic technics led to the isolation of a (1:1) mixture of ergosterol (**1**) and stellasterol (**2**), palmitic acid (**3**), ganodermanondiol (**4**), lucidumol B (**5**) and lupeol (**6**). Structures of these compounds were determined using spectroscopic analysis such as IR, MS, 1D & 2D NMR and literature. The acetylation reaction has been performed on the mixture (**1**+**2**) and compound **4**, leading to the obtention the mixture of 3-acetyl-ergosterol (**7**) and 3-acetylstellasterol (**8**) along with 24-acetyl-ganodermanondiol (**9**) respectively. Total phenolic content was determined for DCM, Ethyl acetate and *n*-butanol extracts. To assess their antiradical scavenging potential, DPPH was used as free radical. The Inhibition power of acetylcholinesterase was evaluated *in vitro* using the Ellman reagent. Amongst all tested extracts, the DCM extract showed the high amount of total phenolic compounds with a value of 133.9512 mg EAG/g EX. The same extract showed a very good antiradical scavenging potential with an IC_{50} of 0.0021 mg/mL. The mixture (**1**+**2**) showed the highest antiradical scavenging activity

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CONTACT Joël Abel Gbaweng Yaya ygbaweng@yahoo.fr Department of Chemistry, Faculty of Science, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon; Jean Noël Nyemb nyembjeannoel@gmail.com Department of Refining and Petrochemistry, National Advanced School of Mines and Petroleum Industries, The University of Maroua, Kaele, P.O. Box 08, Maroua, Cameroon

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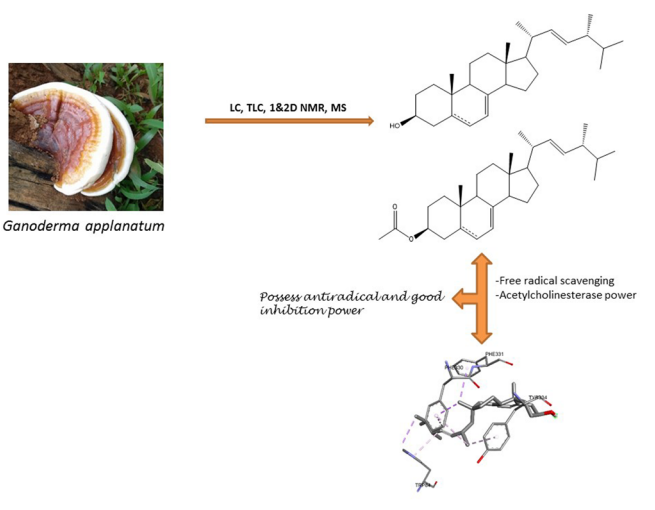
with IC_{50} of 0.0770 mg/mL. The results obtained demonstrated that the acetylation has reduced the antiradical scavenging potential. Concerning the acetylcholinesterase inhibition power, the DCM extract and the mixture (**1** + **2**) showed a very good power with an inhibition percentage of 89%. The acetylation has also reduced the activity of the obtained derivative. The results provide insights into the potential efficacy of these compounds as acetylcholinesterase inhibitors. The binding interactions of the isolated and acetylated derivatives against acetylcholinesterase protein (PBP 3i6m) of *Torpedo californica* were studied using Autodock software. Ergosterol (−11.9 kcal/mol) binds better to the protein biding site through significant pi-sigma interactions.



1. Introduction

The World Health Organisation (WHO) estimates that one billion people were over the age of 60 in 2020, and that this age category will double to 2.1 billion people by 2050. Two-thirds of whom will be living in lower- and middle-income countries (WHO 2023a). The number of people aged 80 years or older is meanwhile expected to triple during the same time frame to reach 426 million (WHO 2023a). As our societies age, the number of people living with dementia across the world is expected to rise from 55 million in 2019 to 139 million in 2050, according WHO (2023b). The costs associated with dementia are also expected to more than double from US 1.3 trillion per year in 2019 to 2.8 trillion dollars by 2030 (WHO 2023b). Between all causes of dementia, Alzheimer disease (AD) is the most common cause accounting for an estimated 60% to 80% of cases (Brenowitz et al. 2017). Alzheimer's disease is a type of brain disease caused by damage to nerve cells (neurons). Brain's neurons are essential to all human activity, including thinking, talking and walking. In Alzheimer's disease, the neurons damaged first are those in parts of the brain responsible for memory, language and thinking (Jack et al. 2009; Reiman et al. 2012; Villemagne et al. 2013; Brenowitz et al. 2017). When the symptoms become severe enough to interfere

with a person's ability to perform everyday tasks, a person is said to have Alzheimer's dementia (Bateman et al. 2012). The vast majority of people who develop Alzheimer's dementia are age 65 or older. This is called late-onset alzheimer's dementia. Experts believe that Alzheimer dementia, like other common chronic diseases and conditions, develops as a result of multiple factors rather (age, genetics and family history) than a single cause (Saunders et al. 1993; Farrer et al. 1997; Green et al. 2002; Hebert et al. 2010; Byard and Langlois 2019; NIA 2023). There are no drug treatments that can cure AD or any other common type of dementia. Two types of enzymes are associated with the disease: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). However, Alzheimer research is focusing on the cholinergic system and mostly on acetylcholinesterase (AChE) inhibitors. Medicines have been developed for Alzheimer's disease that can temporarily alleviate symptoms, or slow down their progression, in some people. This medication is classified as acetylcholinesterase inhibitors (often shortened to just 'cholinesterase inhibitors) and NMDA receptor antagonists. The enzyme acetylcholinesterase (AChE) catalyses the hydrolysis of the ester bond of acetylcholine (ACh) to terminate the impulse transmitted action of ACh through cholinergic synapses (Fratiglioni et al. 1993). Although the basic reason of Alzheimer's disease (AD) is not clear so far, AD is firmly associated with impairment in cholinergic transmission. A number of AChE inhibitors have been considered as candidates for the symptomatic treatment of AD as the most useful relieving strategy (Stryer 1995). Principal role of acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease (Anonymous 2000; Brenner 2000; Howes et al. 2003). There are a few synthetic medicines, e.g. tacrine, donepezil, and the natural product based rivastigmine for treatment of cognitive dysfunction and memory loss associated with AD (Rahman and Choudhary 2001). These compounds have been reported to have severe adverse effects including gastrointestinal disturbances and problems associated with bioavailability (Schulz 2003; Oh et al. 2004), which necessitates the interest in finding better AChE inhibitors from natural resources. In this work, we report the isolation of compounds from the methylene chloride (DCM) extract of *Ganoderma applanatum* (Pers.) Pat (Ganodermataceae), with some of their acetylated derivative and the evaluation of their antiradical scavenging activity and antiacethylcholinesterase potential and a molecular docking study. *G. applanatum* (Pers.) Pat., belong to the family Ganodermataceae, class Basidiomycota. It has stipe and its perennial and bracket form fruiting body are hard, greyish brown to red brown, and with a woody-textured and concentric or irregular striations on its upper side. Its underside has white spores soon turns brown by any rubbing or scratching. It broadly distributed and has been reported on the woods and trees several plants worldwide (Elkind 2010). The fungus has been reported to possess several activities among with anticancer, antioxidant, hepatoprotector, antibacterial, and antimalarial activities (Ma et al. 2011; Shamameh et al. 2019; Khalilova et al. 2022; Katarzyna et al. 2023). Previous mycochemical evaluation of *G. applanantum* led to the isolation of triterpenes, steroids, and polysaccarides (Dilip et al. 2006; Basnet et al. 2017; Hoang et al. 2018; Hai-Guo et al. 2021; Xing-Rong et al. 2022; Monika et al. 2014). The current study involved the isolation of secondary metabolites from methylene chloride

extract of *G. applanatum* (Pers.) Pat, the evaluation of the antioxidant and anti-AChE activity of the extract and compounds; and provide insights into the mechanism of such interactions by a virtual screening through molecular docking.

2. Results and discussion

2.1. Results of the phytochemical study

The phytochemical evaluation of the DCM extract of *Ganoderma applanatum* (Pers.) Pat., yield to the isolation of five compounds identified by the mean of 1 & 2D NMR and MS data as a (1:1) mixture of ergosterol (**1**) (Venditti et al. 2017) and stellasterol (**2**) (Ge et al. 2017), palmitic acid (**3**) (Moradali et al. 2006), ganodermanondiol (**4**) (Vega-Mendoza et al., 2015), lucidumol B (**5**) (Min et al. 1998) and lupeol (**6**) (Mahamat et al. 2021; Sakava et al. 2024). Ergosterol (**1**) and stellasterol (**2**) in mixture along with ganodermanondiol (**4**) have been acetylated leading to their corresponding acetylated derivatives: 3-*O*-acetylergosterol (**7**) and 3-*O*-acetylstellasterol (**8**) as a mixture, and 24-acetylganodermanondiol (**9**) (Figure 1).

2.2. Results of biological activities

2.2.1. Total polyphenol amount result

The DCM, EtOAc and n-BuOH extracts were evaluated for their amount in polyphenol using the method describe by Hatami et al. (2014). The results obtained (Supplemental Table S1) showed that amongst all the tested samples, DCM extract demonstrated the higher amount in polyphenol with a value of 133.9512 mg EAG/g EX, followed by the EtOAc with 91.7286 mg EAG/g EX.

2.2.2. DPPH antiradical scavenging activity

The DCM extract, the mixture of ergosterol (**1**) and stellasterol (**2**), ganodermanondiol (4) and their acetylated derivatives were evaluated for their antiradical potential using

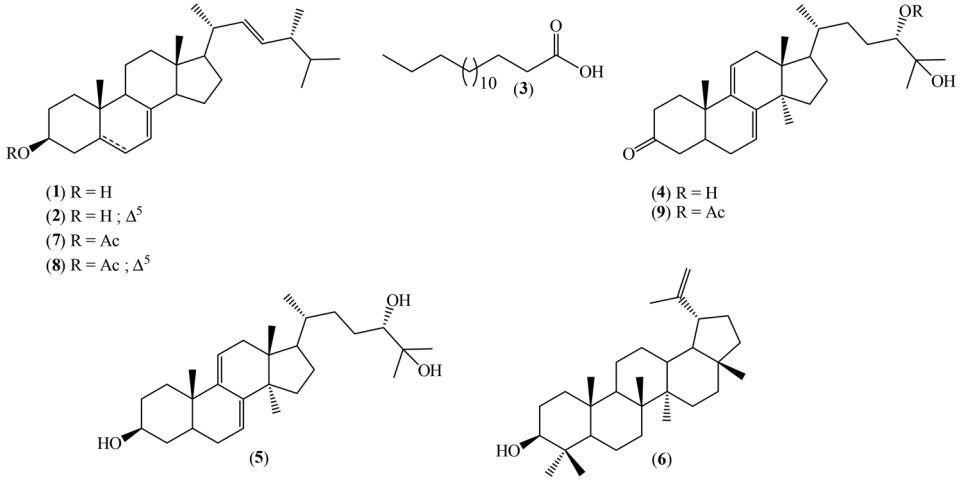


Figure 1. Isolated and hemisynthetic compounds from *G. applanatum*.

the method describe by Daouda et al. (2017). The results obtained are shown in Table 2. According to Path Canada (1994), the antiradicalar potential of extracts and compounds are classified as follow: very good when $0 < IC_{50} < 0.01$ mg/mL, good when $0.01 < IC_{50} < 0.05$ mg/mL, moderate when $0.05 < IC_{50} < 0.1$ mg/mL, weak when $0.25 < IC_{50} < 0.5$ mg/mL and very weak when $IC_{50} < 0.5$ mg/mL. Amongst all the tested samples DCM extract demonstrated a very good antiradical potential with an IC_{50} of 0.0021 mg/mL very near of the IC_{50} of the ascorbic acid used as reference. Concerning the obtained and evaluated compounds from this extract, the mixture (**1** + **2**), and compound (**4**) showed a moderate antiradical potential with IC_{50} of 0.0770 mg/mL and 0.0997 mg/mL respectively. The two acetylated derivatives (**7** + **8**) and **9** showed weak antiradical potential with IC_{50} of 0.1987 mg/mL and 0.2375 mg/mL, respectively confirming as mentioned by some authors that the acetylation reaction reduces the antiradical potential of samples.

2.2.3. Inhibition of acetylcholinesterase power

The DCM extract, two isolated compounds and one acetylated derivative were evaluated for their power to inhibit acetylcholinesterase using spectrophotometric method reported by Ferreira (Ferreira et al. 2020) with some modification. The results obtained are showed in Supplemental Table S3. According to Vinutha et al. (2007), extracts and compounds are considered as good inhibitor of AChE when the inhibition percentage is higher than 50%, moderate when the inhibition percentage is between 30 and 50%, and weak when the inhibition percentage is less than 30%. DCM extract and the mixture (**1** + **2**) showed a very good inhibition power of AChE of 89% at 2 mg/mL. Compound (**4**) was the less active sample. As for the antiradical scavenging activity, the acetylation reaction has reduced the potential, the acetylated compound from the mixture (1 + 2) showed an AChE inhibition power of 69% at a concentration of 1 mg/mL. The DCM extract can be considered as a good inhibitor of AChE and could be target for the production of phytomedecine. Due to the fact that combination therapy is more encouraged by WHO, the mixture of ergosterol (**1**) and stellasterol (**2**) could also be targeted for its *in vivo* antialzheimer potential.

2.3. Result of the molecular docking study

A virtual screening through molecular docking was perform to evaluate the binding affinities of the isolated and semi-synthetic compounds against an enzymatic target. The crystal structure of acetylcholinesterase from Torpedo californica (TcAChE) with PDB code 3i6m was used as the target macromolecule. The co-crystallized ligands (L0) served as reference and galantamine was used as control drug of the proposed inhibitors for 3i6m as it is a known acetylcholinesterase inhibitor. The docking scores indicate that several compounds exhibit strong binding affinities to both targets compared to the co-crystallized ligands and reference compounds (Supplemental Table S4). The co-crystallized ligand (L0) showed the highest affinity (−12.1 kcal/mol) with the target 3i6m, followed by several compounds like 3-acetylergosterol (−11.8 kcal/mol) and ergosterol (−11.9 kcal/mol), which engaged in significant pi-sigma interactions crucial for binding stability. The presence of multiple types of interactions (e.g.

conventional hydrogen bonds, pi-alkyl) across different ligands suggests that these compounds may effectively compete with the co-crystallized ligand for the active site (Figure 2).

3. Experimental

3.1. General methods

The melting points of the isolated compounds were measured using an Electrothermal IA9000 Series digital melting point apparatus (Bibby Scientific, Great Britain). NMR data were acquired using AVANCE II 500 and NEO 600 (Bruker) spectrometer, employing tetramethylsilane (TMS) as the standard for ^{1}H and ^{13}C NMR; IR spectra were obtained using FT-IR Spectrum 100 of Perkin Elmer spectrometer and UV spectra were obtained using UV/Vis Lambda 35 of Perkin Elmer spectrometer. Mass spectrometry was conducted using QTOF-MS-LD^{+} equipment. Column chromatography (CC) was carried out using silica gel (Merck, particle size 230–400 mesh) as the adsorbent, while thin-layer chromatography (TLC) was performed on silica gel pre-coated aluminium sheets (Merck KGaA).

3.2. Plant material

Whole medicinal fungus *Ganoderma applanatum* (Pers.) Pat (Ganodermataceae) was collected in Dang, Adamawa region of Cameroon and authenticated by Dr Fawa, a botanist at the Faculty of Science-University of Ngaoundere. The ITS sequences of *G. applanatum* is deposited in GenBank under the number JN008873.

3.3. Extraction and isolation

After treatments of *G. applanatum*, 0.93 kg of powder was extracted by simple maceration using 15 L of methanol for 72 h. The filtrate was concentrated using a Bioevopeak REV-200B rotavapor. The extraction was repeated 3 times. At the end, 106 g of crude methanolic extract was obtained. During this extraction the compound mixture (**1** + **2**, 10.3 g) was obtained has a white solid. This extract has been dissolved

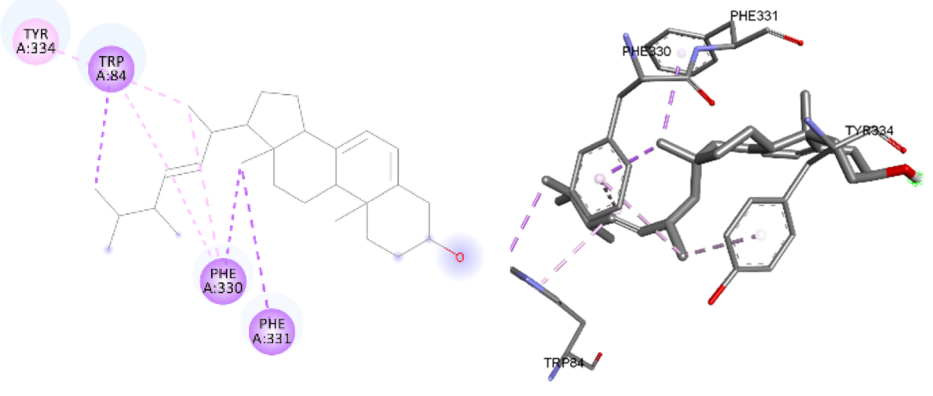


Figure 2. 2D And 3D representations of 3i6m-ergosterol interaction.

in 500 mL of water. The obtained solution has been introduced in a conical flask and 2 L of DCM added. After a liquid-liquid extraction 53 g of the DCM fraction was obtained. The water residual solution has been treated successively with EtOAc and *n*-BuOH to obtain 16 g of the EtOAc fraction and 14 g of *n*-BuOH fraction.

47 g of *G. applanatum* DCM extract was then subjected to column chromatography of silica gel (Merck, 230–400 mesh) and eluated using a gradient of hexane, hexane-DCM and DCM-MeOH. TLC was used to regroup fraction with same profile together. At hexane-DCM (9: 1) solvent system compound **3** (white powder, 8 mg) was obtained, at hexane-DCM (1:1) solvent system compound **4** (124.5 mg), white powder was obtained. Compounds **5** (white powder, 6.5 mg) and **6** (white powder, 5 mg) were obtained after the treatment of 9.5 g of fractions obtained at hexane-DCM (1:1) by column chromatography on silica gel and Sephadex LH-20.

3.3.1. Data of hemisynthetic derivatives

3.3.1.1. 3-acetylergosterol (7). $C_{30}H_{46}O_2$, MS, calc: 438.3.^{1}HNMR ($CDCl_3$, 600 MHz); δ_H: 4.65 (1H, m, H-3), 1.80 (3H, s, H-30); ^{13} C NMR (150 MHz, $CDCl_3$) δ_C: 37.9 (C-1), 28.1 (C-2), 72.8 (C-3), 37.9 (C-4), 138.6 (C-5), 120.2 (C-6), 116.3 (C-7), 141.5 (C-8), 46.0 (C-9), 36.6 (C-10), 21.1 (C-11), 39.0 (C-12), 43.3 (C-13), 54.5 (C-14), 23.0 (C-15), 28.3 (C-16), 55.7 (C-17), 12.1 (C-18), 17.6 (C-19), 40.4 (C-20), 21.1 (C-21), 135.6 (C-22), 132.0 (C-23), 40.5 (C-24), 33.1 (C-25), 19.7 (C-26), 20.0 (C-27), 116.2 (C-28), 170.6 (C-29), 21.2 (C-30).

3.3.1.2. 3-acetylstellasterol (8). $C_{30}H_{48}O_2$, MS, calc: 440.4,^{1}H NMR ($CDCl_3$, 600 MHz); δ_H: 4.63 (1H, m, H-3), 2.2 (3H, s, H-30), ^{13} CNMR (150 MHz, $CDCl_3$); δ_C: 37.9 (C-1), 28.3 (C-2), 73.5 (C-3), 37.9 (C-4), 40.5 (C-5), 129.5 (C-6), 117.3 (C-7), 139.5 (C-8), 42.8 (C-9), 37.1 (C-10), 21.1 (C-11), 39.0 (C-12), 43.3 (C-13), 55.0 (C-14), 23.0 (C-15), 29.5 (C-16), 55.9 (C-17), 12.1 (C-18), 17.6 (C-19), 40.5 (C-20), 21.4 (C-21), 135.6 (C-22), 132.0 (C-23), 40.5 (C-24). 33.1 (C-25), 19.9 (C-26), 19.6 (C-27), 117.6 (C-28), 170.6 (C-29), 21,4 (C-30).

3.3.1.3. 24-acetylganodermanondiol (9):. $C_{30}H_{48}O_3$, white powder, MS, LR-ESI-TOF: 456. ^{1}HNMR ($CDCl_3$, 600 MHz); δ_H: 1.34 (2H, m, H-1), 5.41 (1H, d, J = 6.3 Hz, H-7), 5.53 (1H, d, J = 6,5 Hz, H-11), 0.60 (3H, s, H-18), 1,11 (3H, s, H-19), 0.92 (1H, d, J = 6.0 Hz, H-21), 4.8 (1H, dd, J = 12 Hz, H-24), 1.15 (3H, s, H-26), 1.27 (3H, s, H-27), 0.92 (3H, s, H-28), 1.11 (3H, s, H-29), 1.15 (3H, s, H-30), 2.21 (3H, s, H-1′). ^{13}CNMR ($CDCl_3$, 150 MHz); δ_C: 36.6 (C-1), 34.9 (C-2), 216.9 (C-3), 47.5 (C-4), 50.3 (C-5), 23.7 (C-6), 119.9 (C-7), 142.9 (C-8), 144.5 (C-9), 37.8 (C-10), 117.3 (C-11), 37.2 (C-12), 43.7 (C-13), 50.7 (C-14), 28.7 (C-15), 28.7 (C-16), 51.0 (C-17), 15.7 (C-18), 22.1 (C-19), 36.5 (C-20), 18.6 (C-21), 31.5 (C-22), 33.5 (C-23), 79.6 (C-24), 73.3 (C-25), 25.5 (C-26), 25.4 (C-27), 23.2 (C-28), 26.6 (C-29), 22.5 (C-30), 22.1 (C-1′), 171.3 (C-2′).

3.4. Biological activities

3.4.1. Total phenolic content

Total phenol content was assessed using the Folin and Ciocalteu (FC) method decribe by Hatami et al. (2014) with some modifications. The concentrations (0.02–0.15 mg/mL) of standard (gallic acid) and plant extract (0.02–0.15 mg/mL) were prepared with the

reaction mixture containing 100 µL of gallic acid or extract, 500 µL of FC reagent, and 400 µL of 7.5% Na_2CO_3. The mixture was then incubated at room temperature for 10 min and the absorbance was read at 730 nm using a spectrophotometer. The concentration of total phenol compounds in gallic acid equivalent was determined from the calibration curve of gallic acid and expressed in mg gallic acid equivalent (GAE)/g of plant extract. The total phenolic contents in all samples, was calculated using the formula:

$$C = cV / m$$

Where, C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in mL, m = mass of extract in gram.

3.4.2. DPPH antiradical scavenging evaluation

The *in vitro* radical-scavenging activity of extracts as well as isolated compounds was performed according to Daouda et al. (2017) with slight modifications. Briefly, different concentrations (62.5–500) µg/mL of extracts/compounds and of the ascorbic acid were prepared. After preparation of samples, 0.1 mL of each sample solution was mixed with 1.9 mL of methanolic DPPH solution (10 mg/L) in test-tubes. The resulting solution was incubated for 30 min at room temperature before the optical density (OD) was measured at 517 nm. The percentage radical scavenging activity was calculated from the following formula:

$$\%\,\text{Scavenging}[\text{DPPH}] = \left[\left(A_0 - A_1 \right) / A_0 \right] \times 100.$$

Where A_0 was the absorbance of the negative control (methanolic DPPH solution) and A_1 was the absorbance in the presence of the samples. IC_{50} value was determined from the graph obtained using standard vitamin C by using the "$y=mx+c$" formula from the slope of the graph. All the analyses were carried out in triplicate.

3.4.3. In vitro inhibition of AchE

The acetylcholinesterase (AChE) inhibition was performed using spectrophotometric method reported by Ferreira (Ferreira et al. 2020) with some modification. The tank used as a blank to control for the nonenzymatic hydrolysis of acetylcholine contained a mixture of 500 µL of 3 mM DTNB solution (in 0.1 M potassium phosphate pH 8), 100 µL of 15 mM ACh (in water), 275 µL of 0.1 M potassium phosphate pH 8, and 100 µL of extract solutions (50 mg/mL, 100 mg/mL, 200 mg/mL, and 300 mg/mL). In the reaction tank, 275 µL of buffer was replaced by AChE solution 0.16 U/mL. The resulting solutions were placed in a spectrophotometer. The thiocholine formed during the hydrolysis of acetylcholine reacts rapidly with DTNB and a yellow compound is formed. The reaction was monitored for 5 min at 405 nm and the absorbance registered every minute. Velocities of reaction were calculated; enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer solution instead of inhibitor (extracts). The assays were performed in triplicate. The reaction velocity (v) equals (V_{max} [A])/(K_m + [A]) as described by the Michaelis-Menten equation where V_{max} is the

maximal velocity, [*A*] is the substrate concentration, and K_m is the Michaelis constant, or the substrate concentration at half maximal velocity. The activity was express in µmol of acetylthiocholine iodide hydrolysed/g of tissu/minute.

3.4.4. Molecular docking study

3.4.4.1. Preparation of ligands. The 3D molecular structures of isolated compounds were generated using Chem3D 15.0 running a windows workstation with an Intel(R) Core (TM) i5-3340M processor. The 3D structures were then saved in .pdb format. They were imported to the workspace and preparation was done for docking studies.

3.4.4.2. Preparation of enzymes. To perform docking of compounds, the target proteins was selected based on their function. 3D structures were obtained from protein data bank (http://www.rcsb.org) in .pdb format. The AutoDockTools (ADT) was used to prepare the ligand and receptor structures, add appropriate Gasteiger and Kollman charges, identify and modify ligand rotatable bonds. The potential binding sites of target were calculated using the Lamarckian GA (4.2) algorithm implemented in Autodock4. The population size, maximum number of evaluation (medium) and maximum number of generations were set at 150, 27,000 and 2,500,000 respectively. The search space of the simulation exploited in the docking studies was studied as a subset region of the active site. The water molecules were removed from the enzyme to decrease interactions between functional group of ligands and water molecules.

AutoDock program performs the research and evaluation of the different ligand configurations. It is possible to use several techniques to obtain the configurations (by simulated annealing, genetic algorithm or by Lamarckian genetic algorithm). A grid-based method was used to enhance the quick evaluation of the binding energy of conformations of the complexes formed. The grid boxes were centred using coordinates of a virtual centre of mass atom for the enzymes. For each protein, the grid box was determined respectively in *x*, *y* and *z* dimension according to amino acids which formed active site. The affinity of the docked complexes was described using binding energy based on a semi empirical force field.

3.5. Statistical analysis

GraphPad Prism software version 5.00 was used for data analysis. Each experiment was repeated at least thrice. Data from 3 or more groups with one variable were analysed by ANOVA followed by Dunnett's post-hoc test for multiple comparison. Statistical significance was set at p value < 0.05.

4. Conclusion

Four pure compounds along with a mixture have been isolated and identified from methylene chloride (DCM) extract of *Ganoderma applanatum* (Pers.) Pat (Ganodermataceae). The mixture of 3-acetyl-ergosterol (**7**) and 3-acetylstellasterol (**8**) along with 24-acetyl-ganodermanondiol (**9**) were obtained from the acetylation reaction of the mixture of ergosterol (**1**) and stellasterol (**2**), along with ganodermanondiol (**4**). These acetylated

derivatives are reported here for the first time. DCM extract exhibited the highest total phenolic content with a very good DPPH antiradical scavenging potential. The same extract showed a very good acetylcholinesterase inhibition power. Amongst all the tested compounds, compound the mixture of ergosterol (**1**) and stellasterol (**2**), showed a good antiradical scavenging activity with a very good acetylcholinesterase inhibition power. The results obtained demonstrated that the acetylation has reduced the antiradical scavenging potential. Molecular docking study has identified several promising compounds with favourable docking scores against the 3i6m enzymatic target. The results suggest that some of these compounds may serve as effective inhibitors or modulators of enzyme activity compared to the reference ligands Galantamine and Dacomitinib. Future work should focus on experimental validation of these findings to confirm their inhibitory effects and elucidate their mechanisms of action in biological systems, paving the way for potential therapeutic applications.

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Disclosure statement

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ORCID

Roméo Toko Feunaing http://orcid.org/0009-0003-8690-9660
Joël Abel Gbaweng Yaya http://orcid.org/0000-0003-4666-2627
Jean Noël Nyemb http://orcid.org/0000-0001-5069-6737
Hervé Landry Ketsemen http://orcid.org/0009-0007-1874-690X
Céline Henoumont http://orcid.org/0000-0002-3280-2441
Antoine Kavaye Kandeda http://orcid.org/0000-0003-4358-6593
Alessandro Venditti http://orcid.org/0000-0003-1492-6739
Sophie Laurent http://orcid.org/0000-0002-2589-3250

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