RESEARCH ARTICLE

Inhibition of prolyl oligopeptidase by flavonoids isolated from the roots of *Allexis obanensis* (Baker f.) Melch

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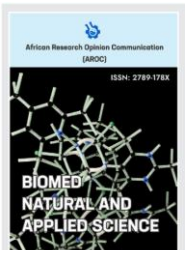

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ABSTRACT

Background: Prolyl oligopeptidase is a cytosolic serine peptidase that hydrolyzes peptides containing proline at the carboxy terminus of proline residues. It has been associated with several neurodegenerative diseases. Therefore, it is a target in the management of these disease conditions. **Methods:** *Allexis obanensis* was taken through cold extraction, subjected to column chromatography and flavonoids isolated via high-performance liquid chromatographic technique. The flavonoids obtained were investigated for their *in vitro* prolyl oligopeptidase inhibitory activity. **Results:** The flavonoids isolated include: 4.4'''- dimethoxylophirone A **[1]** and 7-hydroxy-3-(3-hydroxy-4 méthoxyphenyl)-5-méthoxy-4H chromen-4-one **[2]**. They inhibited prolyl oligopeptidase at low IC_{50} concentrations of 7.201±3.021 µM and 6.223±2.002 µM respectively. **Conclusion:** The results obtained from this study proves the potential of these flavonoids as prolyl oligopeptidase inhibitors, by inference, their potentiality in the management of neuropsychiatric disorders.

Keywords: *Allexis obanensis*, Flavonoids, Prolyl oligopeptidase, Inhibition

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1.0 Introduction

Prolyl oligopeptidase (POP) is widely distributed in body organs, as well as the brain [1-3]. Previous studies have shown that POPs act in certain key physiological functions, such as learning and memory, cell division and differentiation, and signaling transduction, as well as in certain psychiatric disorders [4].

In recent years, POP has gained prominence as a treatment target for schizophrenia (SZ), bipolar affective disorder (TB), and cognitive impairment, such as those present in Alzheimer's disease (AD), primarily due to of its involvement in metabolism, which is a key molecule in the neuropeptide signaling transduction cascade [5]. POP also participated in the treatment of neuropeptide precursors [6]. In addition to this, neuroprotective and cognition-enhancing effects of POP inhibitors in laboratory animals have been reported [4,7].

The use of natural substances for therapeutic purposes continues to expand internationally. Medicinal plants are an important source of bioactive compounds that can serve as a basis in drug research and development because they produce chemically variable molecules with a wide range of activities. Some natural products are known POP inhibitors in the micromolar

range such as the baicalin flavonoids [8] and oroxylin [9], berberine alkaloid [10] and 6-(8´Zpentadecenyl) salicylic acid [11].

Allexis obanensis (Baker f.) Melch, a species of plant in the Violaceae family is a shrub about 9 m high. It is commonly found in Nigeria and southern Cameroon [12] more precisely in Campo where it is traditionally called "Mont des Elephants", The stem is gray, smooth, with a diameter of between 3 and 6 cm [13]. The leaves of the plant are thin, leathery and have a dark green top, the veins are alternate and the petiole has a sheath at its base. In traditional medicine, the barks of *Allexis obanensis* are used to treat fever and syphilis. The aerial parts are traditionally used in dermatological affections, acne, scarring and in oral hygiene, and orally this plant is indicated as antispasmodic and as antitussive [13], and the methanolic extract of all the different part of *Allexis obanensis* has presented the inhibition of human prolyl oligopeptidase (POP)[14]. The aim of this study was to elucidate the possible POPs inhibitory effect of two flavonoids isolated from *Allexis obanensis*.

2.0 Materials and Methods

2.1 Plant material

Allexis obanensis was collected on 7th June 2014 at Bidou II, 20 km from the town of Kribi (South Cameroon). The plant was authentication by a botanist, Mr. NANA at the National Herbarium of Cameroon and was given a specimen number 31839/HNC.

2.2 Extraction and isolation

Dried and powdered root of *Allexis obanensis* (1 kg) was extracted with MeOH (3L) at room temperature and evaporated under vacuum to yield a crude extract. 100g of this extract was dissolved in MeOH-H_2O (8:2) and partitioned with n-hexane (3×150 mL) and ethyl acetate (3×200 mL). The ethyl acetate portion (35g) was subjected to column chromatography over silica gel eluting with gradients of CH_2Cl_2/MeOH to produce 79 fractions of 250 mL each. These fractions were combined based on their TLC profiles into 3 major fractions: A (5.2 g,1–40); B (3.6g, 41–55); C (3g, 56–79). Fractions A (CH_2Cl_2/MeOH 50:1); B (CH_2Cl_2/MeOH 40:1); C (CH_2Cl_2/MeOH 30:1). The compounds **[1]** and **[2]** were isolated from the fractions A and C respectively.

2.3 Structure elucidation

Melting points were determined on an Electro thermal I A 9000 series digital melting point apparatus and were uncorrected. The UV spectra were recorded on UV-570/VIS/NIP and Shimadzu UV-24012A double-beam spectrophotometers. IR measurements were obtained on a PerkinElmer (model 1600) FTIR spectrometer. The 1D (1 H, 13C, DEPT) and 2D (COSY, NOESY, HSQC and HMBC) NMR spectra were recorded in DMSO-d6 and MeOH-d4 using a Bruker 600 (600 MHz for 1H NMR, 150 MHz for 13C NMR) spectrometer. ESIMS were obtained using an MSQ Thermofinnigan instrument. Chemical shifts are stated in parts per million (ppm) from tetramethylsilane (TMS) internal standard. Flash column chromatography was performed using silica gel 60 (Merck, 0.040–0.063 mm). TLC was conducted on pre-coated Merck Kieselgel 60 F254 plates (20×20 cm, 0.25 mm). Spots were checked on TLC plates under UV light (254 nm), and developed with sulphuric acid (50 %), followed by heating.

2.4 Protease inhibition assay

The POP inhibition assays were carried out using a bacterial expressed POP enzyme as described in previous studies [14-16]. All experiments were performed in 96- microtiter black bottom plates. The protease activity was quantified as fluorescence from the proteolytically cleaved amino-coumarin fluorophore with excitation 380 nm and emission 420 nm on a Biotek Synergy H4 plate reader. The fluorogenic substrate provides elevated fluorescence by the delocalization of electrons after the amide bond cleavage and release of the 7 amino coumarin

moiety. All samples for protease inhibition assays were prepared in ddH_2O. Concentration-dependent inhibition studies were performed with the isolated flavonoids (0.1 –60 µM) as described in previous study [14]. A substrate background was used for correction of all measurements. KYP-2047, a specific POP inhibitor, was used as positive control. For the graphical illustration, the inhibition data of isolated flavonoids were normalized to the maximum response.

2.5 Data analysis

All data are represented as mean of three independent experiments ± standard deviation. Non-linear regression curve fits were obtained using GraphPad Prism v5.0 fitting algorithms using top constraints 'must be equal or less than 100%'. Four parameter non- linear regression curve fits were obtained using GraphPad Prism v5.0 fitting algorithms with equation YBottom + (Top-Bottom)/(1 +10^((LogIC_{50}-X)) in which X is the log dose and Y the measured response.

3.0 Results and Discussion

3.1 Identification of flavonoids compounds isolated from *Allexis obanensis*

Following extraction, two known compounds were isolated: 4, 4'''-dimethoxylophirone A **[1]** and 7-hydroxy-3-(3-hydroxy-4-méthoxyphenyl)-5-méthoxy-4H chromen-4-one **[2]** through HPLC technique (figure 1). The compounds were obtained by elution with a gradient of 2 %/min of the solvent system hexane/ethyl acetate. The compounds **[1]** and **[2]** have a retention time of 9.7 ppm and 11.5 ppm respectively.

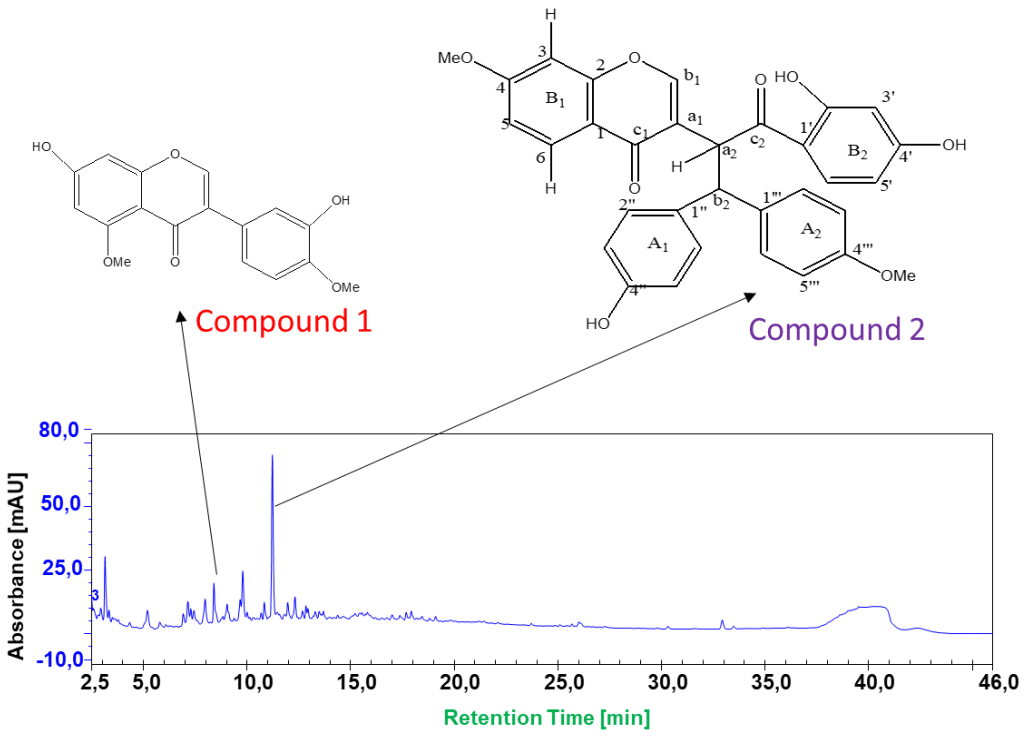

Figure 1: HPLC Chromatogram of dimethylcholride/methanol fractions of *Allexis obanensis.* Compound 1: 4, 4'''-dimethoxylophirone A **[1]**; Compound 2: 7-hydroxy-3-(3-hydroxy-4-méthoxyphenyl)-5-méthoxy-4H chromen-4-one **[2].**

3.2. Effects of isolated flavonoids on the activity of human prolyl oligopeptidase

Allexis have been given little attention in medicinal research. To date, phytoanalysis on Allexis led to the identification of antimicrobial molecules, which have been obtained by organic solvent extraction [17]. In contrast, the scope of the current study was to extract plant metabolites with medium hydrophobicity[16]. The plant extracts of four Allexis species were evaluated for the inhibition of human prolyl oligopeptidase (POP). Since all four species inhibited POP activity, a bioactivity-guided fractionation approach was performed and they have presented a good inhibition [14]. The flavonoids revealed a concentration-dependent inhibition of human POP activity in the tested concentration range of 0.1-60 µM. Interestingly, the two flavonoids isolated in this study have been previously isolated in our laboratory from *Allexis batangae* [17], which is of the same genus as the plant of interest but of different species. These tested compounds showed a good inhibitory activity on POP (compound 1 with an IC_{50} value of 7.201 ± 3.021 µM (Figure 2A), and compound 2 with an IC_{50} value of 6.223 ± 2.002 µM (Figure 2B), comparatively to the reference, KP-2047 who has a value of IC_{50}, 0.0322 ± 0.031 µM (Figure 2C). Nevertheless, POP has been inhibited by compound 1 with a percentage value of 100%, whereas, compound 2 with a percentage of 88.9% (figure 2).

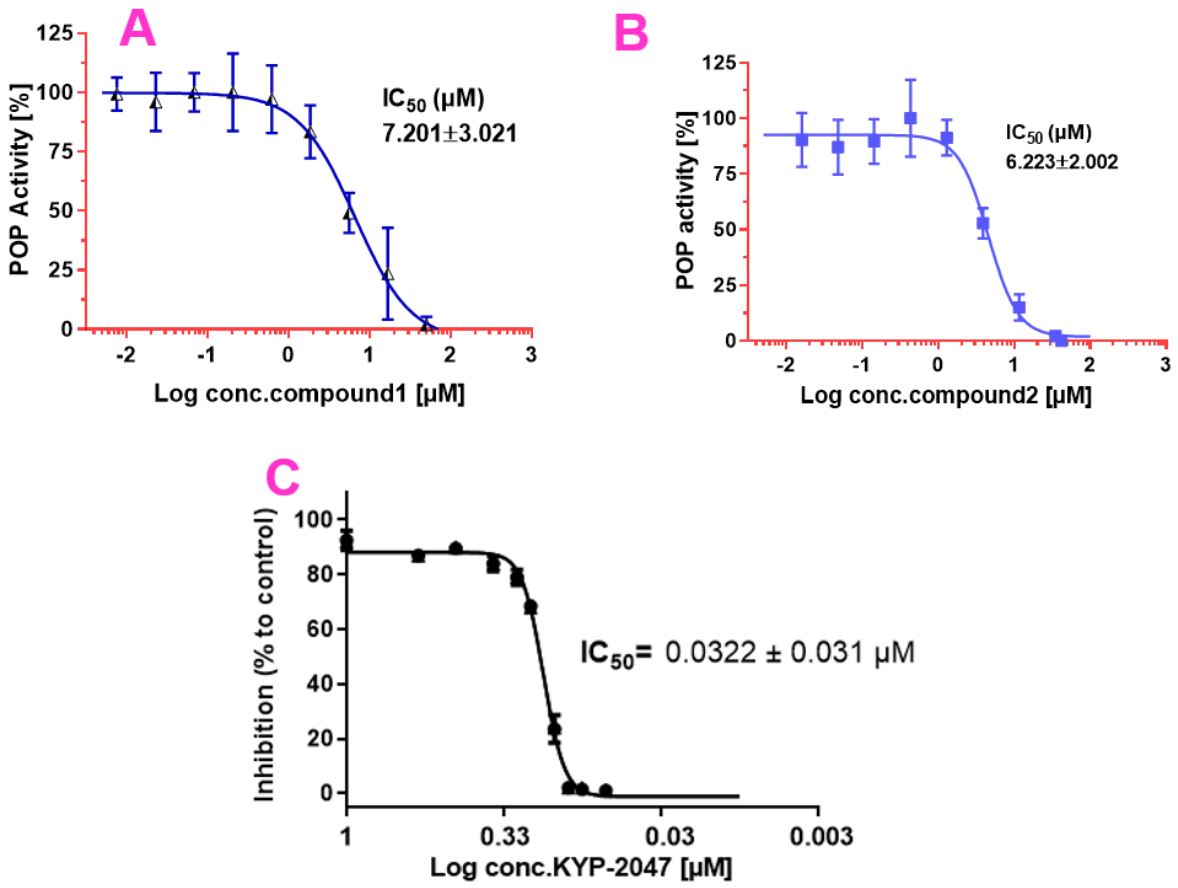

Figure 2: Flavonoids 4, 4'''-dimethoxylophirone A **[1]** and 7-hydroxy-3-(3-hydroxy-4- méthoxyphenyl)-5-méthoxy-4H chromen-4-one **[2]** for concentration dependent inhibition of POP activity. All experiments were performed in three biological experiments, respectively and the data are shown as mean ±standard deviation. The inhibition was quantified to full enzyme activity and normalized to the highest measured data value for the shown plot of dose response data.

Recent evidence has pointed to the involvement of POP in cancer and tumor growth. The POP business model was studied in a large series of human neoplastic tissues [18]. The increased POP activity in renal clear cell carcinoma (CCRCC), Urothelial carcinoma of the renal pelvis (UCRP), Head and neck squamous cell carcinoma (HNSCC) and colorectal adenomatous polyp suggests this enzyme may be also involved in these malignant tumors [19]. Consequently, our compounds of interest could be suggested as therapeutic agents for these diseases. Although the mechanism of inhibition of POP of the compounds tested has not been elucidated, the results obtained from this study sufficiently proves the potential of these flavonoids as POP inhibitors, by inference, their potentiality in the treatment of neuropsychiatric disorders.

4.0 Conclusions

This study showed that flavonoids, 4, 4'''-dimethoxylophirone A and 7-hydroxy-3-(3-hydroxy-4-méthoxyphenyl)-5-méthoxy-4H chromen-4-one, isolated from *Allexis obanensis* roots have inhibitory effect on prolyl oligopeptidase. Therefore, these flavonoids show great promise in the management of neuropsychiatric disorders.

Conflict of interest: The authors declare no conflict of interest.

Authors Contributions: Author O.N.W: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Authors S.D., E.N.T.Z., and B.N: Supervised this work, analyzed data and drafted the manuscript. Author O.T.B: Reviewed the entire work and contributed to the writing of the paper. All authors read and approved the final version of the manuscript.

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