Malaysian Journal of Analytical Sciences
(MJAS)
Published by Malaysian Analytical Sciences Society

FLAVONOIDS WITH MONOAMINE OXIDASE A AND B INHIBITORY AND ANTI-INFLAMMATORY EFFECTS FROM *Vitex grandifolia*

(Flavonoid Bersama Perencat Moniamin Oksidase A dan B dan Kesan Anti-Radang dari *Vitex grandifolia*)

Tijjani Ali*, Bello Oluwasesan Michael, Abiodun B. Ogbesejana

Department of Applied Chemistry,
Federal University Dutsin-Ma, Katsina State, Nigeria

**Corresponding author: tali@fudutsinma.edu.ng*

Received: 18 July 2019; Accepted: 20 July 2020; Published: xx December 2020

Abstract

Vitex grandifolia is a plant that belongs to the *Lamiaceae* or *Labiatae* family. It is classified as an underutilised vegetable with little known phytochemistry. Methanol fraction of the leaves of the plant afforded the isolation of three known flavonoids: isoorientin (**1**), orientin (**2**), and isovitexin (**3**). Spectroscopic techniques, namely NMR and IR and comparison with data in the literature were used to assess the structures of the isolated compounds. The molecules isolated were tested in vitro for monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) human recombinant, and anti-inflammatory activities. Mostly, the isolated compounds showed selective activity towards MAO-B. Inhibition of MAO-B by isoorietin (**1**) and orietin (**2**) was 9-fold more potent (IC_{50} [μg/mL] of 11.08 and 11.04) compared to the inhibition of MAO-A (IC_{50} [μg/mL] of >100), while clorgyline and deprenyl were used as the positive standards. All flavonoids isolated from the plant displayed good activity against NF-Kb with IC_{50} (μg/mL) of 8.9, 12, and 18. The study shows a strong relationship between the structures of the flavonoids isolated with its biological activities based on the different patterns of substitution, particularly in C_2=C_3 bond and the positions of glucose in the isolates. Furthermore, the study is the first of its kind to determine the phytochemistry of the polar fraction of *V. grandifolia*, its anti-inflammatory, and neurodegenerative protective roles.

Keywords: *Vitex grandifolia*, underutilised vegetable, flavonoids, MAO-A and B, neurodegenerative

Abstrak

Vitex grandifolia ialah sejenis tumbuhan yang tergolong dalam keluarga Lamiaceae or Labiatae. Ia diklasifikasikan sebagai sayur-sayuran yang kurang digunakan dengan sedikit maklumat fitokimia. Pecahan metanol daun tumbuhan ini dapat memencilkan tiga jenis flavonoid: isoorientin (**1**), orientin (**2**), dan isovitexin (**3**). Teknik spektroskopi iaitu NMR dan IR dan perbandingan dengan data dari kajian literatur digunakan untuk menilai struktur sebatian yang dipencilkan. Molekul yang dipencilkan diuji secara *in vitro* terhadap rekombinan manusia monoamine oxidase A (MAO-A) dan monoamine oxidase B (MAO-B), dan aktiviti anti-radang. Sebatian yang dipencilkan kebanyakannya menunjukkan aktiviti memilih terhadap MAO-B. Perencatan MAO-B oleh isooretin (**1**) dan orietin (**2**) adalah 9 kali ganda lebih poten (IC_{50} [μg/mL] sebanyak 11.08 dan 11.04) berbanding perencatan terhadap MAO-A (IC_{50} [μg/mL] > 100), dengan menggunakan clorgyline dan deprenyl sebagai piawaian positif. Semua flavonoid yang dipencilkan daripada tumbuhan menunjukkan aktiviti yang baik terhadap NF-Kb with IC_{50} (μg/mL) sebanyak 8.9, 12, dan 18. Kajian ini menunjukkan hubungan yang kuat antara struktur flavonoid yang dipencilkan dengan aktiviti biologi berdasarkan pelbagai corak penggantian, terutamanya pada ikatan C_2=C_3 bond dan kedudukan glukosa dalam pencilan tersebut. Tambahan pula, kajian ini merupakan kajian pertama seumpamanya yang menentukan fitokimia pecahan berkutub *V. grandifolia,* berserta fungsi anti-radang dan perlindungan rosotan neuro.

Kata kunci: *Vitex grandifolia,* sayur-sayuran yang kurang digunakan, flavonoids, MAO-A dan B, rosotan neuro

Introduction

Pathological and neurodegenerative pathways, Alzheimer's disease, cancer, coronary, and Parkinson diseases are the results of free-radical mediated reactions and reactive oxygen species (ROS) from the human body [1, 2]. Many epidemiological studies have shown a strong correlation in the group of people whose diet is rich in fresh fruits and leafy vegetables and a lower risk of heart disease, neurodegenerative diseases, and some particular forms of cancer [3]. Many studies are mostly dedicated to antioxidant exhibited by compounds in fruits, medicinal plants, and vegetables. Monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) are enzymes found in the mitochondria. These enzymes deaminate (via oxidation) monoaminergic neurotransmitters and dietary monoamines that are potentially harmful. In addition, they also regulate noradrenaline, dopamine, serotonin, and adrenaline in the brain [4-7].

Medicinal plant vegetables and other herbal products were identified to be the essential and primary basis for MAOs' inhibition, and this may validate the folkloric applications of many plants as a replacement to manage neuropsychiatric disorders, neurological problems (e.g. Parkinson's disease), and depression [8]. Secondary metabolites such as flavonoids are among the prevalent polyphenols present in medicinal plants. They are often found in many plant species and obtainable in various parts of plants, for example, in the bark, flowers, fruits, leaves, and stems [9-12]. Flavonoids were reported to show different types of biological activity, including antioxidants, enzyme inhibitors, anti-inflammatory, anticancer, antihyperglycemic, hepatoprotective activities, among others [13-16].

Nowadays, plant-based medicinal research is growing tremendously, and evidence has been gathered to demonstrate the immense potential of medicinal plants used in traditional systems [17, 18]. Many of these botanicals have been described and studied using modern scientific methods, with findings showing immense promise of medicinal plants in the field of medical-related sciences [19].

Vitex grandifolia (*V. grandifolia*) belongs to the Lamiaceae family, has edible fruits that the locals used in making the alcoholic drink. The plant bark is locally used to treat fever, bronchial complaints, diarrhoea, rickets, sore, and stomach-ache. Other uses include treatment of toothache, rheumatism, orchitis, colic, and umbilical cord infections. There was also a report by Epidi and Odili [20] on the biocidal effects of *V. grandifolia* powdered leaf against *Tribolium castaneum* Herbst in stored groundnut, *Arachis hypogaea* [20]. *V. grandifolia* is a small tree or shrub that is approximately 10–12 cm long and 5– cm width. Its trunk has 60 cm girth that bears a spreading crown and usually inhabits secondary jungle or high deciduous forest. Local names of this plant species are ooriọdan (Yoruba, Nigeria), ofonma (Egun, Republic of Benin), or ofofrin (Setangun, Republic of Benin) [21]. Surprisingly, this plant's phytochemistry was not investigated before this study. Therefore, the need to isolate compounds with biologic activity from this plant emerged. Hence, this is a report of isolation and structure elucidation of flavonoids with in vitro MAO-A and MAO-B activity (inhibition) from the leaf extract of *V. grandifolia*.

Materials and Methods

Plant sample

The whole plant of *V. grandifolia* was collected from Ilorin Metropolis, Illorin L.G.A. Kwara State, Nigeria. The collected plant was identified in the Department of Plant Biology, University of Ilorin, where voucher number was obtained after the deposition of the specimen. The leaves were air-dried, powdered, and stored in the refrigerator before further analyses.

Extraction

Following air-drying and powdering, 1.5 kg of the leaves were macerated with n-hexane (10 L) and methanol (7 L) sequentially in this order for 24 h. The extraction process was repeated three times and the extracts obtained were evaporated, under reduced pressure, to dryness.

General experiments

Reverse Phase (RP-18) with 40-63 µm mesh size silica gel and silica gel 60 was used in the column chromatography. Furthermore, Diaion HP-20 was also used for chromatographing. Pre-coated thin layer chromatography (TLC) plates coated with silica gel 60 F_{254} with the layer thickness of 0.25 mm (Merck) were used to monitor fractions obtained from the column chromatography, as a supplementary experiment. NMR spectroscopy was done on either 600 or 500 or 400 (MHz) machines (Agilent and Bruker Inc., California) for ^{1}H NMR, ^{13}C NMR, and 2D NMR experiments. Chemical shifts expression was noted in parts per million (δ) using tetramethylsilane (TMS) as the internal standard and *J* values (coupling constant values) measured in Hz. Infra-red spectroscopy was carried out using Fourier-transform infrared (FTIR; Perkin Elmer) spectrometer.

Isolation

Methanol (MeOH) extract of *V. grandifolia* was loaded onto Reverse Phase silica gel (RP-18) using Vacuum Layer Chromatography (VLC) for fractionation. Distilled water with increasing MeOH was used as eluting solvents. The eluates collected were concentrated on the rotary evaporator. TLC was used to monitor eluates. A total of 11 fractions were collected, and the first fraction (H_2O only) was picked for further fractionation because its TLC revealed spots that looked promising. This fraction was subjected to Diaion HP-20 Column for isolation. The column was eluted with water first, then with increasing concentration of MeOH and the eluates obtained were concentrated. About 12 fractions were obtained. The collected fractions were concentrated and monitored by TLC.

The eighth fraction was loaded into the column in CH_3Cl using 65:35:10 (CH_3Cl: MeOH: H_2O) as the eluting solvent, and six fractions were obtained. The third fraction produced compound (**3**; Figure 2), which was purified using CC in CH_3Cl with 65:35:10 (CH_3Cl: MeOH: H_2O) as the eluting solvent. Fractions 1 and 2 were combined based on their TLC profile and two compounds (**1** and **2**; Figure 2), were isolated from the first fraction of the repeated column after further purification with CC in CH_3Cl with 8:2:0.5 (CH_3Cl: MeOH: H_2O) as the eluting solvent. Confirmation of purity of compounds **1**, **2**, and **3** was achieved by observing a single spot each on the chromatogram.

Monoamine oxidase (MAO) assays

Isolates from *V. grandifolia* were assayed for MAO-A and MAO-B activity. Kynuramine deamination assay was chosen using 96-well plates, as reported previously [22]. The method used was adapted from previous studies [23, 24]. The isolated molecules did not display any meddling with fluorescence measurement. Clorgyline and deprenyl were used as positive controls for the experiment.

Anti-inflammatory test

The anti-inflammatory test was performed using mouse macrophages 915 (RAW264.7) from ATCC. Cell culture was carried out in phenol 916 red-free RPMI medium q with bovine calf serum (10%), penicillin G sodium (100 U/mL), and streptomycin (100 µg/mL) at 37 °C in CO_2 (5%) and humidity (95%). Subsequently, incubation of cells was done for one day (24 hours) after seeding in 96-well plates to obtain 5×10^4 cells/well. Then, compounds isolated were added to the cells after dilution in serum-free medium. LPS (5 µg/mL) was added after 30 minutes of incubation, and afterwards, the cells were again incubated for 24 hours. Determination of nitric oxide (NO) concentration was done by measuring the level of nitrite released in the supernatant using Griess reagent [25]. The percentage of nitrite production inhibition by the test flavonoids were calculated and compared with the vehicle control. Meanwhile, IC_{50} values were determined from the dose curves, and the positive control used was parthenolide [26, 27].

NF-kB activity

The NF-kB inhibition assay was carried out in human chondrosarcoma (SW1353) from ATCC. Culturing of cells was achieved in 1:1 mixture of DMEM/F12 together with FBS (10%), penicillin G sodium (100 U/mL), and streptomycin (100 µg/mL) at 37 °C in CO_2 (5%) and humidity (95%). Antibiotic and FBS-free DMEM/F12 were used to wash the cells (1.2×10^7 cells /60 mm dish) once, and then transferred into antibiotic-free DMEM/F12 (500 µL) that contained FBS (2.5%). This was followed by the addition of NF-κB luciferase plasmid construct to the cell suspension at a concentration of 50 µg/mL. Next, incubation was done for 5 minutes at room temperature. Electropermeabilisation of the cells was performed at 160 V and 70-ms pulse using cuvettes (BTX disposable) with model no. 640 and the 4-mm gap in a BTXI Electro Square Porator (T 820; San Diego, California). Subsequently, the cells were seeded into 96-well plates (1.25×10^5 cells/well density). After 24 hours, the cells were treated with different concentrations of the isolated molecules for 30 min before the addition of 70 ng/mL PMA and incubation for 8 hours. Determination of luciferase activity was done using the Luciferase Assay kit (Promega). Spectra-Max plate reader was used for the detection of light output. Luciferase activity (percentage inhibition) was calculated compared to the control vehicle, while the IC_{50} values were determined from the dose curves. The control transcription factor used was Sp-1 because it does not respond to inflammatory mediators (for example, PMA). This was done to allow easy detection of cytotoxic substances that non-specifically inhibit luciferase expression [27].

Results and Discussion

MAO-A and MAO-B assay

The isolates showed selective activity by inhibiting either MAO-A or MAO-B (Table 1). Isoorietin (1) and orietin (2) inhibited MAO-B with 9-fold potency (IC_{50} [µg/mL] of 11.08 and 11.04) compared to its inhibition against MAO-A (IC_{50} [µg/mL] of >100). Isovitexin (3), a flavonoid isolated from the wild vegetable for the first time displayed moderate selective activity against MAO-A (IC_{50} [µg/mL] of >100) compared to MAO-B (IC_{50} [µg/mL] of 21.3) just like the other two flavonoids. Meanwhile, clorgyline and deprenyl were used as positive standards.

Anti-inflammation

All isolated flavonoids displayed good activity against NF-Kb assay with IC_{50} (µg/mL) of 8.9, 12, and 18. Orientin (**2**) showed moderate activity against Sp-1 assay (IC_{50} of 23 µg/mL), while others displayed poor activity when compared with the positive standard (IC_{50} of 8 µg/mL), as shown in Table 2. Isovitexin (**3**) exhibited moderate activity against iNOS assay while others (isoorientin [**1**] and orientin [**2**]) displayed poor activity (IC_{50} of 48 and 54 µg/mL). The positive standard used in this study was parthenolide.

Isolated compounds

Compound 1 (Figure 1) is a yellow solid (21 mg) and its melting point ranges from 233–235 °C. ^{1}H NMR (400 MHz, DMSO-d6) d: 13.2 (1H, brs, 5-OH), 7.55 (1H, *dd*, *J* = 2.5, 9.0 Hz, 60-H), 7.45 (1H, *d*, *J* = 2.5 Hz,20-H), 6.85(1H, *d*, *J* = 9.0 Hz, 50-H), 6.63 (1H, s, 3-H), 4.7 (1H, *d*, *J* = 9.7 Hz, 100-H). ^{13}C NMR (100 MHz, DMSO-d6) d:163.24 (C-2), 102.68 (C-3), 182.32 (C-4), 160.74 (C-5), 108.88(C-6), 164.41 (C-7), 98.53 (C-8), 156.36 (C-9), 102.68 (C-10),122.24 (C-1′), 114.34 (C-2′), 146.22 (C-3′), 150.16 (C-4′),116.00 (C-5′), 119.71 (C-6′), 73.76 (C-1″), 70.13 (C-2″), 79.12(C-3″), 70.06 (C-4″), 82.35 (C-5″), 62.01 (C-6″). The molecular formula was taken as $C_{21}H_{20}O_{11}$ based on the combined data of ^{1}H NMR and ^{13}C NMR. Analyses of the NMR results coupled with comparison with available data in literature [28] enabled the determination of Compound 1 as isoorientin.

Compound 2 (Figure 2) is a yellow solid (15 mg) and its melting point ranges from 265–267 °C. ^{1}H NMR (600 MHz, DMSO-d6) d: 3.22–3.88 (6H, m, glucosyl-H), 4.69 (1H, *d*, *J* =9.9 Hz, H-1″), 6.25 (1H,s, H-6), 6.64 (1H, s, H-3), 6.86 (1H, *d*, *J* = 8.4 Hz, H-5′), 6.86(1H, *dd*, *J* = 2,8.4), 7.48 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.54 (1H, *dd*, *J* = 2.0, 8.4 Hz, H-6′), 13.20 (1H, s, 5-OH). ^{13}C NMR (150 MHz, DMSO-d6) d:164.4 (C-2), 102.7 (C-3), 182.3 (C-4), 160.74 (C-5), 98.53(C-6), 163.24 (C-7), 104.91 (C-8), 156.86 (C-9), 104.28 (C-10), 122.24 (C-1′), 114.34 (C-2’), 146.22 (C-3’), 150.16 (C-4’),116.00 (C-5′), 119.71 (C-6′), 73.76 (C-1″), 71.13 (C-2″), 79.12(C-3″), 71.06 (C-4″), 82.35 (C-5″), 62.04 (C-6″). The molecular formula of the compound was determined to be $C_{21}H_{20}O_{11}$ from the combination of ^{1}H NMR and ^{13}C NMR data. Comparison of acquired NMR data with those in literature [29] lead to identification of compound 2 as orientin.

Compound 3 (Figure 2) is a yellow powder (19 mg) and its melting point ranges 219–221 °C. ^{1}H-NMR (600 MHz, DMSO-d6):δ_H: 13.55 (1H, brs, 5-OH), 7.93 (2H, *d*, *J* = 8.8 Hz, 3′, 5′ -H), 6.93 (2H, *d*, *J* = 8.4 Hz, 2′, 6′ -H), 6.78 (1H, s, 3-H), 6.51 (1H, s, 8-H), 4.55 (1H, *d*, *J* = 9.8 Hz, 1″ - H),3.11—4.03(6H, m, glucosyl H). ^{13}CNMR (150 MHz, DMSO-d6) δ_C: 164.36 (C-2), 104.25 (C-3), 182.81 (C-4), 161.52 (C-5), 109.75 (C-6), 164.17 (C7), 94.48 (C-8), 157.08 (C-9), 103.65 (C-10), 121.96 (C-1′), 129.34 (C-2′, 6′), 116.84 (C-3′, 5′), 162.04 (C-4′), 73.91 (C1″), 71.47 (C-2″), 79.80 (C-3″), 71.06 (C-4″), 82.46 (C-5″), 62.34 (C-6″). Based on acquired data from the results of ^{1}H NMR and ^{13}C NMR, molecular formula of the compound was determined to be $C_{21}H_{19}O_{10}$. NMR data analyses compared with information from literature [30, 31] enabled the identification of compound 3 to be the known flavonoid isovitexin.

Some structure-activity relationship (SAR) suggestions following the anti-inflammatory activity of the flavonoids obtained from *V. grandifolia* were made. They are as summarised: firstly, the presence of C_2=C_3 may confer the isolates anti-inflammatory effects [32]. It was reported that C_2=C_3 double bond could also contribute to the planarity of molecules [32]. This was discovered from the more significant anti-inflammatory effects displayed by diosmetin more than hesperetin (a compound without C_2=C_3 double bond) which resulted in a higher volume/ratio [32, 33]. Secondly, hydroxylation substitution (-OH) noticed at both C-3′ and 4′ tertiary increases the activity, especially at ring B moiety for isoorientin (**1**) and orientin (**2**). Wang et al. [32] reported the hydroxylation pattern and its importance in C-3′ hydroxylation [32, 33]. Thirdly, the position of the sugar moiety, glycosides, on ring A gives a better anti-inflammatory activity than on ring B and C [34].

Some SAR suggestions based on the inhibitory effects of the isolates against MAO-A and B were made. These are summarised as follows: (a) Hydroxyl group (-OH) substitution pattern noticed at C-3′ and C-4′ rises the inhibitory activity of MAO-A and B mostly, especially at ring B moiety of isoorientin (**1**) and orientin (**2**); but a decrease in activity was observed in isovitexin **(3)**. However, Spencer et al. [35] attested that both unsaturation degree of C_2=C_3 bond coupled with hydroxylation pattern on ring B moiety has a great significant role on the anti-neurodegenerative effects on flavonoids in general [35].

Structures of compounds **1–3** (Figure 2) were determined based on their 1D and 2D NMR, and also by comparing the acquired NMR results with the data in the literature [28, 29]. To the best of our knowledge, the isolates isoorientin (**1**), orientin (**2**), and isovitexin (**3**) were obtained from *V. grandifolia* for the first time. For instance, FTIR spectrum of compound 1 displayed absorption bands due to O-H (3376 cm^{-1}), C=O (1660 cm^{-1}), and C-C stretch in-aromatic ring at 1561and 1446, and also =C-H bend at 845 and 800 cm^{-1}. Proton NMR data of compound **1** showed that ring B protons, H-2′ and H-6′, generated a multiplet at 7.80 ppm. A doublet (J = 9 Hz) typical of H-5′ was present at 6.8 ppm. Signals at 6.75ppm (1H, s) and 6.25 ppm (1H, s) for H-8 and H-3 protons, were observed. Signal for anomeric proton due to C-glucosyl was present at 4.93 ppm (1H, d, J=7 Hz, H-1″). Overlapping sugar proton signals appeared at 3.5–4.8 ppm. The spectroscopic results compared well with information reported; hence, molecule (1) was inferred to be isoorientin [36, 37]. Assignments for compound 2 were completely achieved by combining the data from ^{1}HNMR, ^{13}CNMR, DEPTS, 2D-NMR (COSY, HSQC, and HMBC) experiments together with assignments published in the literature [37].

For example, data acquired from^{13} C NMR enabled the confirmation of the presence of –OH group at C-5′ and C-4′ in the compound. Another different moiety in the molecule was the anomeric carbon for glucopyranoside with a signal that appeared at 104.8 ppm, which indicated a glucose unit in orientin [36, 37, 38]. Therefore, based on the information obtained majorly from the NMR results and also that from supporting literature, compound **2** was inferred to be the flavonoid known as orientin. Structure of compound **3** was established using data acquired from ^{1}HNMR, ^{13}CNMR, DEPTS, COSY, HSQC, and HMBC experiments, and by also comparing with data already reported for isovitexin structure [39]. For example, FTIR results for compound **3** indicated absorptions for OH, C=O, C=C, C-C (in-aromatic ring), and C-O at 3440 cm^{-1}, 1660 cm^{-1}, 1620 cm^{-1}, 1523 cm^{-1}, and 1017 cm^{-1} respectively. Data from ^{1}H NMR experiment for compound 3 showed signals at 6–8 ppm for flavonoid aromatic protons. There exist the resonances for the sugar moiety at 3.5-4.7 ppm. Also, the anomeric proton's signal was available at 4.88 ppm. From the mentioned spectroscopic data, which compared well with the data in the literature, compound **3** was concluded to be isovitexin.

Concoctions and extracts of medicinal plants are still the backbones of primary health care management requirements in many developing nations. According to World Health Organisation (WHO), about 80% of people over the world depends on the herbs for their day-to-day health management due to various biologic effects present in the plants [40, 41]. Therefore, it is paramount to scientifically assess the plants for possible validation of the reason for their use in age-old medicine and to acquire phytocompounds with pharmacologic effects. According to previous reports [42], most of these medicinal plants contain flavonoids, and some of these flavonoids were isolated from plants source that exhibited MAO inhibitory effects [42]. For instance, Zarmouh et al. [42] reported the selective MAO-B inhibition of some compounds from the seeds extract of *Psoraleacory lifolia*. Another study revealed that human recombinant MAO-B and MAO-A iso-enzymes were employed for the inhibition of the enzymes (MAO-B and MAO-A). The authors of that study discovered that out of the three compounds studied, only two (bavachinin and genistein) showed selective inhibition of MAO-B significantly. It was due to a significant reduction of H_2O_2 by the compounds that were produced by MAO-B compared to MAO-A [42, 43]. Monoamine oxidase inhibitors (MAOIs) differ by the MAO receptor selection; some inhibit MAO-A and MAO-B in the same manner, while others only target one type [44]. These studies corroborate with our work that some flavonoids from natural sources can selectively inhibit the MAO-B.

Table 1. IC_{50} Values of isolated compounds as MAO-A&B inhibitory agents

SN	**Sample Name**	**MAO-A (IC_{50})**	**MAO-B (IC_{50})**
1	Isoorientin (1)	>100	11.08
2	Orientin (2)	>100	11.04
3	Isovitexin (3)	>100	21.3
4	Clorgyline	1.6	NT
5	Deprenyl	NT	0.48

Table 2. IC_{50} Values of Isolated Compounds as Anti-inflammatory Agents

SN	Test Compounds	NF-kB	SP-1	iNOS	% cell death at 100 µg/mL
1	Isoorientin (1)	8.9	63	48	63.89
2	Orientin (2)	12	23	54	
3	Isovitexin (3)	18	41	21	
4	Parthenolide	0.9	6.5	0.18	
5	Parthenolide	0.6	8	0.15	

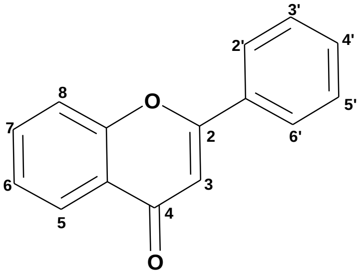
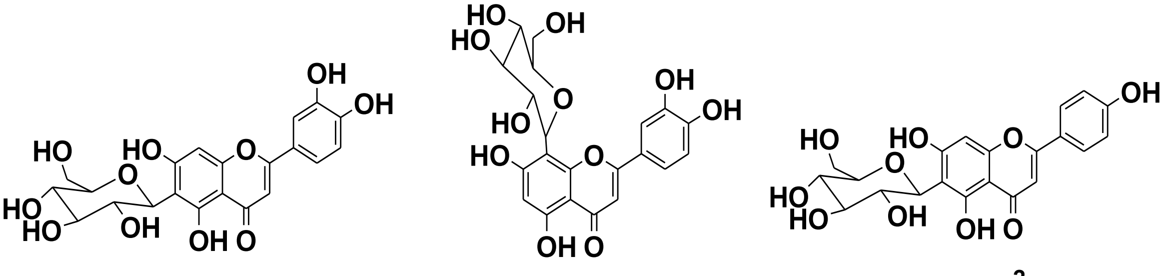


Figure 1. Nucleus of flavonoids



1

2

3

Figure 2. Isolated flavonoids from *V. grandifolia*

Conclusion

The need to assess medicinal plants for its biological properties, especially its MAO inhibitory effects, is increasing attention due to the regular use of plants as vegetable. The constituents of medicinal plants help to treat neurological disorders due to the likely association with medicines and a diet rich in dietary monoamines. The present study found that isolated flavonoids from *V. grandifolia* moderately inhibit MAO-B, and this outcome may be of significance for improved use of this wild vegetable in traditional neuropharmacological use. The use of *V. grandifolia* as vegetable is widely accepted despite being tagged as “poor man’s” food. This study hopes to recognise its pivotal role as a source for the development of nutraceutical products. Prior to this work, there was no report on the inhibition of MAO-A and B by constituents of this plant with their anti-inflammatory activity.

Acknowledgement

We wish to thank Federal University Dutsin-Ma for finding us worthy of nomination for sponsorship to the National Tertiary Institution (TETFUND) sponsorship. Many thanks go TETFUND for its Sponsorship.

References

1. Ames B. N., Shigenaga, M. K. and Hagen, T. M. (1993). Oxidants, antioxidants and degenerative diseases of aging. *Proceeding National Academy Science,* 90: 7915-7922.
2. Shih, J. C., Chen, K. and Ridd, M. J. (1999). Monoamine oxidase: From genes to behaviour. *Annual Review Neuroscience*, 22:197-217.
3. Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Bolwell, G. P. and Rice-Evans, C. (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain breaking antioxidants. *Archives of Biochemistry and Biophysics*, 322: 339-346.
4. Yuh-Hwa, L., Wen-Chun, W., Yeh-Lin, L., Ying-Jang, L. and Wen-Chi, H. (2010). Antioxidant and amine oxidase inhibitory activities of *Hydroxyurea*. *Bioscience, Biotechnology, and Biochemistry*, 74(6):1256-1260.
5. Silverman, R. B., Ding, C.Z. and Gates, K. S. (1993). Design and meachanism of monoamine oxidase inactivators from an organic chemical perspective. In: Testa, B., Kyburz, E., Fuhrer, W., Giger, R. (Eds.), Perspectives in Medicinal Chemistry. Verlag Helvetica ChomicaActa. Basel, Weinheim, New York: pp. 73-86.
6. Kumazawa, T., Minatogawa., Matsuba, S. and Sato, J. I. (2000). An effective synthesis of isoorientin: the regioselective synthesis of a 6-C-glucosylflavone. *Carbohydrate Research*, 329: 507.
7. Abell, C.W. and Kwan, S.W. (2009). Molecular characterization of monoamine oxidase A and B. *Progress in Nucleic Acid Research and Molecular Biology,* 65:129-156.
8. Vina, J., Sastre, J., Pallardo, F. and Borras, C. (2003). Mitochondrial theory of aging: Importance to explain why females live longer than males. *Antioxidant and Redox Signalling,* 5: 549-556.
9. Bratkov V. M., Shkondrov A. M., Zdraveva P. K. and Krasteva, I. N. (2016). Flavonoids from the genus *Astragalus*: Phytochemistry and biological activity. *Pharmacognosy Review,* 10: 11-32.
10. Yadav P. and Malpathak N. (2016). Estimation of antioxidant activity and total phenol, flavonoid content among natural populations of caper (*Capparismoonii*, Wight) from Western Ghats region. *Indian Journal of Pharmaceutical Education Research*, 50: 495-501.
11. Oliveira F. G., de Lima-Saraiva S. R., Oliveira A. P., Rabêlo S. V., Rolim L. A. and Almeida, J. R. (2016). Influence of the extractive method on the recovery of phenolic compounds in different parts of *Hymenaeamartiana* Hayne. *Pharmacognosy Research*, 8:270-275.
12. El-gizawy H. A. and Hussein, M. A. (2017). Isolation, structure elucidation of ferulic and coumaric acids from *Fortunella japonica*s wingle leaves and their structure antioxidant activity relationship. *Free Radical Antioxidant*. 7: 23-30.
13. Venkatesan, A., Kathirvel, A., Prakash, S. and Sujatha, V. (2017). Antioxidant, antibacterial activities and identification of bioactive compounds from *Terminalia chebula* bark extracts. *Free Radical Antioxidant*, 7: 43-49.
14. Dutta, S. and Das, S. (2010). A study of the anti-inflammatory effect of the leaves of *Psidiumguajava* Linn. on experimental animal models. *Pharmacognosy Research*, 2: 313-317.
15. Middleton E, Jr, Kandaswami, C. and Theoharides T. C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacology Review*, 52: 673-751.
16. Havsteen, B. H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacology and Therapeutics*, 96: 67-202.
17. Fiala. E. S., Reddy, B. S. and Weisburger, J. H. (1985). Naturally occurring anticarcinogenic substances in foodstuffs. *Annual Review of Nutrition*, 5:291-321.
18. Tapsell, L.C., I. Hemphill, L., Cobiac, C. S. P. and Sullivan, D. R. (2006). Health benefits of herbs and spices: The past, the present, the future. *Medical Journal Australia*, 185: S4-S24.

19. Triggiani, V., Resta, F., Guastamacchia, E., Sabba, C., Licchelli, B., Ghiyasaldin, S. and Tafaro, E. (2006). Role of antioxidants, essential fatty acids, carnitine, vitamins, phytochemicals and trace elements in the treatment of diabetes mellitus and its chronic complications. *Drug Targets*, 6: 77-93.
20. Epidi, T.T. and Odili, E.O. (2009). Biocidal activity of selected plant powders against *T. castaneum* (Herbst).in stored groundnut (*Arachishypogaea L*.). *African Journal Environment Science Technology*, 3: 1-5.
21. Burkill, H. M. (1995). The useful plants of West Tropical Africa. 2nd edition. Families J-L. Royal Botanic Gardens, 3: pp. 857.
22. Samoylenko, V., Rahman, M. M., Tekwani, B. L., Tripathi, L. M., Wang, Y. H., Khan, S. I., Khan, I. A., and Muhammad, I. (2010). *Banisteriopsiscaapi*, a unique combination of MAO inhibitory and antioxidative constituents for the activities relevant to neurodegenerative disorders and Parkinson's disease. *Journal of Ethnopharmacology*, 127: 357-369.
23. Narayan D. Chaurasiya, Mohamed A. Ibrahim, Ilias Muhammad, Larry, A. W. and Babu, L.T. (2014). Monoamine oxidase inhibitory constituents of propolis: kinetics and mechanism of inhibition of recombinant human MAO-A and MAO-B. *Molecules*, 19: 18936-18952.
24. Bankova, V., Popova, M. and Trusheva, B. (2014). Propolis volatile compounds: Chemical diversity and biological activity: A review. *Chemistry Central Journal,* 8: 28.
25. Quang, D. N., Harinantenaina, L., Nishizawa, T., Hashimoto, T., Kohchi, C., Soma, G. and Asakawa, Y. (2006). Inhibition of nitric oxide production in RAW 264.7 cells by azaphilones from Xylariaceous fungi. *Biology Pharmaceutical Bulletin*, 29: 34-37.
26. Zhao, J., Khan, S. I. and Wang, M. (2014). Octulosonic acid derivatives from Roman chamomile (*Chamaemelumnobile*) with activities against inflammation and metabolic disorder. *Journal Natural Production*, 77: 509-515.
27. Al-Taweel, A. M., El-Shafae, A. M., Perveen, S., Fawzy, G. A. and Khan, S. I. (2015). Anti-inflammatory and cytotoxic constituents of *Bauhinia retusa*. *International Journal of Pharmaceutical*, 11:372-376.
28. Li, Y., Ma, S., Yang, Y., Ye. S. and But, P. P. (2002). Antiviral activity of flavonoids and organic acid from *Trollius chinensis* Bunge. *Journal of Ethnopharmacology*, 79(3): 365-368.
29. Song, D. M. and Sun, Q. S. (2004). Chemical studies on constituents of *Trollius altaicus* CA Mey. *Medical Chemistry,*14: 233-235.
30. Ju, Y., J. N. and Sacalis, C. C. S. (1998). Bioactive flavonoids from endophyte-infected blue grass (Poaampla). *Journal of Agriculture Food Chemistry*, 46: 3785.
31. Cheng, G., Bai, Y., Zhao, Y., Tao, J., Liu, Y., Tu, G., Ma, L., Liao, N. and Xu, X. (2000). Flavonoids from *Ziziphusjujuba* Mill var. spinose. *Tetrahedron*, 56: 8915.
32. Wang, T., Li, Q. and Bi, K. (2018). Bioactive flavonoids in medicinal plants: Structure activity and biological fate. *Asian Journal of Pharmaceutical Sciences*, 13(1): 12-23.
33. Celik, H. and Kosar, M. (2012). Inhibitory effects of dietary flavonoids on purified hepatic NADH-cytochrome b5 reductase: structure-activity relationships. *Chemico-Biological Interaction*, 197(2–3): 103- 109.
34. Isoda H, Motojima H, Onaga S, Samet I, Villareal, M. O. and Han, J. (2014). Analysis of the erythroid differentiation effect of 574 flavonoid apigenin on K562 human chronic leukemia cells. *Chemico-Biological Interactions*, 575: 269-277.
35. Spencer, J. P., Vafeiadou K., Williams, R. J. and Vauzour, D. (2012). Neuroinflammation: modulation by 635 flavonoids and mechanisms of action. *Molecular Aspects of Medicine*, 33(1): 83-97.
36. Xin, Z., Jinyong, P., Guorong, F. and Yutian, W. (2015). Isolation and purification of flavonoid glycosides from *Trolliusledebouri* using high-speed counter-current chromatography by stepwise increasing the flow-rate of the mobile phase. *Journal of Chromatography A*, 1092: 216-221.

37. Sharma, K. K., Sharma, A. K., Sharma M. C. and Tanwar K. (2014). Isolation of orientin (51) and vitexin from stem bark of *parkinsoniaaculeata* (caesalpiniaceae) and their successive blending on sheep wool fiber. *International Journal of Pharmaceutical and Phytochemical Research*, 6(3): 557-561.
38. Jinfeng, Y. A., Yong, S. K. B. and Myong Jo, K. (2015). Isolation and characterization of bioactive compounds from *Lepisorus thunbergianus* (Kaulf.) *Arabian Journal of Chemistry*, 8: 407-413.
39. Peng, J., Fan, G., Hong, Z., Chai, Y. and Wu, Y. (2005). Preparative separation of isovitexin and isoorientin from *Patriniavillosa*Juss by high-speed counter-current chromatography. *Journal of Chromatography A*, 1074: 111-115.
40. Endo Y, Hayashi,H, Sato T, Maruno M, Ohta, T. and Nozoe, S. (1994). Confluentic acid and 2′-O-methylperlatolic acid, monoamine oxidase B inhibitors in a Brazilian plant, Himatanthussucuuba. *Chemical Pharmaceutical Bulletin*, 42: 1198-1201.
41. Lin R. D., Hou W. C., K.Y. Yen, and Lee, M. H. (2003). Inhibition of monoamine oxidase B (MAO-B) by Chinese herbal medicines. *Phytomedicine*, 10: 650-656.
42. Zarmouh, O. N., Mazzio, A. E., Elshami, M. F., Messaha, S. S., Eyunmi, V. K. S. and Soliman, F. A. K. (2015). Evaluation of inhibitory effects of bavachinin and bavachin on human monoamine oxidases A and B. *Evidence-Based Complementary and Alternative Medicine*, 2015: 14.
43. Lee, M., Lin, R., Shen, L., Yang, L., Yen, K. and Hou, W. (2001). Monoamine oxidase B and free radical scavenging activities of natural flavonoids in *Melastoma candidum* D. Don. *Journal Agriculture Food Chemistry*, 49(11): 5551-5555.
44. Ficarra, R., Ficarra, P., Tommasini, S., Carulli, M., Melardi, S., Di Bella, M. R., Calabro, M. L. and Casuscelli, F. (1997). Isolation and characterization of *Guierasenegalensis* J. F. Gmel. active principles. *Bollettino Chimico Farmaceutico*, 136: 454-459.