Flavonoids Isolated from Vitex grandifolia: An Underutilized Vegetable, Exert Monoamine A & B Inhibitory and Anti-Inflammatory Effects and Structure Activity Relationship (SAR)

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INTRODUCTION: Vitex grandifolia belongs to Lamiaceae or Labiatae family, it consists of flowering plants and it is also called mint-family and the Yoruba people of South-West in Nigeria called it "Oriri or Efo oriri". This plant is classified as one of underutilized vegetable, little is known about its phytochemistry and its biological evaluations.
METHODS: The methanol extracts of the dried leaves and stem of the plant was subjected to fractionation and isolation using vacuum layer and column chromatography methods. The structures of the compounds were elucidated using spectroscopic techniques including IR, 1D and 2D-NMR and by comparison with the data reported in the literature. They were evaluated in vitro for the inhibition of monoamine recombinant human MAO-A & B and anti-inflammatory activities.
RESULTS: Three known flavonoids were isolated from its methanolic part of the leaves of Vitex grandifolia for the first time to the best our knowledge i.e. isoorientin (1), orientin (2) and isovitexin (3). Most of the isolated compounds showed selective inhibition of monoamine oxidase B, inhibition of MAO B by Isoorietin (1) and orientin (2) were 9-fold more potent (IC50 (μg/mL) of 11.08 and 11.04) compared to the inhibition of MAO A (IC50 (μg/mL) of >100) while clorgyline and deprenyl were used as positive standards. The isolated flavonoids displayed good activity against NF-Kb assay with IC50 (μg/mL) of 8.9, 12 and 18. The study establishes the link between the structure and the biological activities on the basis of the different patterns of substitution particularly C2=C3 double bond and position of glucose moiety.
DISCUSSION AND CONCLUSION: This study is the first to establish the phytochemistry of the polar part of Vitex grandifolia, the anti-inflammatory and neurodegenerative protective role of these isolated compounds.

Keywords: Vitex grandifolia, underutilized vegetable, Lupeol, MAO-A and B, neurodegenerative.

Introduction

Pathological and neurodegenerative paths, Alzheimer's disease, cancer, coronary and Parkinson diseases are the result of free radical-mediated reactions and reactive oxygen species from the human body [1, 2]. In the many epidemiological studies and investigations carried out, it has been discovered that there is strong connection between group of people whose diets are rich in fresh fruits and leafy

vegetables and low incidence of cardiovascular diseases, neurodegenerative diseases and some particular forms of cancer [3] . Many studies and investigations are mostly dedicated to antioxidant displayed of compounds in fruits, medicinal plants and vegetables so as to improve human health and regulate physiological functions. Monoamine oxidases (MAO-A and MAO-B), are mitochondrial enzymes which oxidative deaminate monoaminergic neurotransmitters and (potentially harmful) dietary monoamines. Their major purpose is the regulation of noradrenaline, dopamine, serotonin, and adrenaline in the brain [4]. The study of MAO-A and B have been of significant pharmacological attention recently and their inhibitors (MAOIs) have found extensive medical use for the management of numerous neurological and psychiatric maladies. These enzymes remove as well as they catalyze of exogenous amines. The MAO A inhibitors are important in taking care of anxiety and depression while the MAO B inhibitors are effective to inhibit and treat Alzheimer and Parkinson's disease [5, 6, 7]. Medicinal plants i.e. vegetables, botanical extracts and herbal products from natural sources have been viewed as an important and primary basis for MAOs' inhibitors and this opinion validate the cultural application of many botanicals as substitute for the management of depression, Parkinson's disease and other neuropsychiatric as well as neurological disorders[8]. Flavonoids as secondary metabolites are one of the most popular polyphenols present in medicinal plants, they are broadly distributed in many plant species and can be found in various parts of these plants i.e. bark, flowers, fruits, leaves and stems [9, 10, 11, 12]. Flavonoids have been reported to display a large variety of biological activities, some of these are antioxidants, enzyme inhibitors, others have anti-inflammatory, anticancer, antihyperglycaemia and hepatoprotective activities [13, 14, 15, 16].

In present times, research on medicinal plants has globally increased tremendously, and volumes of reputable evidence have been gathered to portray the enormous prospects of medicinal plants used in traditional systems [17, 18]. Many of these herbal plants have been identified and studied using current scientific methods and ways, the results revealed the immense promise of medicinal plants in the field

of medical science [19]. *Vitex grandifolia* which belongs to *Lamiaceae* family, bears fruits which is edible and used to make an alcoholic drink by the locals, the bark is used in the treatment of stomach ache, to treat diarrhea, bronchial complaints, rickets, sore, and fever. In the treatment of colic, infections of the umbilical cord, toothache, rheumatism, and orchitis. Epidi and Odili (2009) reported the biocidal effect of the powdered leaf of *V. grandifolia* against *Tribolium castaneum* in stored groundnut *Arachis hypogaea* [20]. The plant is a shrub or small tree about 10–12 cm in length and 5–7 cm in width, trunk to 60 cm girth bearing a spreading crown, in high deciduous forest or secondary jungle. Local names of this plant species; Oori ọdan (Yoruba, Nigeria), ofonma (Egun, Republic of Benin), or ofofrin (Setangun, Republic of Benin) [21]. Surprisingly, this plant's phytochemistry have not being looked into though it is a vegetable. So, its study and that of the isolated compounds i.e. biological activities from this plant will be considered worthwhile to investigate. Hence, the present study reports the isolation, characterization and the *in-vitro* inhibition of MAO A and B of the constituents from the polar extract of the *Vitex grandifolia*.

Materials and Methods

Collection of Plant's Sample

The leaves with the stem of *Vitex grandifolia* was collected in April-October 2015 from Ilorin metropolis, Kwara State, Nigeria. The collected plant was identified by a taxonomic botanist in the department of Plant Biology, University of Ilorin, Ilorin where voucher number was obtained after the deposit of the specimen. The leaves and stem were air dried, powdered and stored for further analysis.

Extraction of the Plant Materials

The air-dried and powdered plant material was defatted with hexane then was prepared by maceration (1.5 kg), with 7 L of methanol at ambient temperature for 24 h. The process was repeated three times, and the filtrates were combined and evaporated under vacuum to dryness.

General experimental procedure

Pre-coated TLC plates (AluO); Silica gel 60 F_{254}; layer thickness 0.25 mm (Merck). Pre-coated TLC plates (Glass); RP-18 F_{254}; layer thickness 0.25 mm (Merck); Silica gel 60, 40-63 μm mesh size (Merck); RP-18 40-63 μm mesh size; Sephadex LH 20; 25-100 μm mesh size (Merck). Silica gel 60 and RP C-18, Diaion HP-20 was used for column chromatography. ^{1}H NMR, ^{13}C NMR and 2D NMR were recorded on 400, 500 and 600 (MHz) instrument (Agilent and Bruker Inc., California). Chemical shifts were expressed in parts per million (δ) using TMS as internal standard. Values of coupling constant *J* are reported in Hz. Infra-Red spectroscopy was done using Perkin Elmer FT-IR Spectrum. Two spectrometer and the mass of the compounds were determined using Agilent 1260 liquid chromatography (Agilent, USA) equipped with a quaternary solvent delivery system, and Triple quad 6410 MS system and Agilent technologies 6540 UHD Accurate Mass Q-TOF Liquid chromatography-mass spectrometer (Agilent, USA).

Fractionation and Isolation

The extract of *V. grandifolia* was defatted using hexane, then extracted with methanol. Methanol (MeOH) extract of *V. grandifolia* was added to Reverse Phase silica gel (RP-18) using Vacuum Layer Chromatography (VLC) for fractionation. Water with increasing MeOH used as eluting solvent, the eluates were collected and concentrated on rotavapor. TLC was used to check and monitor the isolates and combine the eluates. Eleven (11) fractions were obtained, the first fraction (H_2O only) was picked for further fractionation because the TLC revealed promising compounds. This fraction was subjected to Diaion HP-20 Column for isolation. The column was eluted with water first, then with increasing MeOH and the eluates were collected and concentrated. Twelve (12) fractions were obtained, all fractions were collected, concentrated and monitored by TLC. The eighth fraction was loaded unto column in CH_3Cl using 65:35:10 (CH_3Cl: MeOH: H_2O) as eluting solvent. Six fractions were obtained; the third fraction gave compound (**3**) which was purified using CC in CH_3Cl with 65:35:10 (CH_3Cl:

MeOH: H_2O) as eluting solvent. Fractions 1 & 2 were combined using TLC and two compounds (**1** & **2**) were isolated from the first fraction after further purification with CC in CH_3Cl with 8:2:0.5 (CH_3Cl: MeOH: H_2O) as eluting solvent. Compounds **1** (19 mg), **2** (14.7 mg) and **3** (10.5 mg) (Fig. 1) were isolated in pure form after purification.

Monoamine Oxidase Inhibition Assays (MAO)

To evaluate the outcome of the isolated compounds from *V. grandifolia* on monoamine oxidase A and B, the kynuramine deamination assay was used for 96-well plates as expressed previously [22]. The method used was adapted from the reported literature [23, 24]. The isolated constituents did not display any meddling with fluorescence measurement, but clorgyline, deprenyl were used as positive control for the experiment.

Anti-inflammatory Activity

Inhibition of iNOS activity

The assay was performed using mouse macrophages 915 (RAW 264.7, obtained from ATCC). Cells were cultured in phenol 916 red free RPMI medium supplemented with 10 % bovine calf serum and 100 U/mL penicillin G sodium, and 100 µg/mL streptomycin at 37 °C in an atmosphere of 5 % CO_2 and 95 % humidity. Cells were seeded in 96-well plates at 5×10^4 cells/well and incubated for 24 h. Test compounds diluted in serum free medium were added to the cells. After 30 minutes of incubation, LPS (5 µg/mL) was added and the cells were further incubated for 24 h. The concentration of nitric oxide (NO) was determined by measuring the level of nitrite released in the cell culture supernatant by using Griess reagent [25]. Percent inhibition of nitrite production by the test compound was calculated in comparison to the vehicle control. IC_{50} values were obtained from dose curves. Parthenolide was used as positive control [26, 27].

Inhibition of NF-kB activity

The assay was performed in human chondrosarcoma (SW1353, obtained from ATCC) cells as described earlier. Cells were cultured in 1:1 mixture of DMEM/F12 supplemented with 10 % FBS, 100 U/mL penicillin G sodium and 100 μg/mL streptomycin at 37 ° C in an atmosphere of 5 % CO_2 and 95 % humidity. Cells (1.2 x 10^7) were washed once in an antibiotic and FBS-free DMEM/F12, and then reintroduced in 500 μL of antibiotic-free DMEM/F12 containing 2.5 % FBS. NF-κB luciferase plasmid construct was added to the cell suspension at a concentration of 50 μg/mL and incubated for 5 min at room temperature. The cells were electroporated at 160 V and one 70-ms pulse using BTX disposable cuvettes model 640 (4-mm gap) in a BTX Electro Square Porator T 820 (BTX I, San Diego, CA). After electroporation, cells were plated to the wells of 96-well plates at a density of 1.25×10^5 cells per well. After 24 h, cells were treated with different concentrations of test compound for 30 min prior to the addition of PMA (70 ng/mL) and incubated for 8 h. Luciferase activity was measured using the Luciferase Assay kit (Promega). Light output was detected on a Spectra-Max plate reader. Percent inhibition of luciferase activity was calculated as compared to vehicle control and IC_{50} values were obtained from dose curves. Sp-1 was used as a control transcription factor which is unresponsive to inflammatory mediators (such as PMA). This is useful in detecting agents that nonspecifically inhibit luciferase expression due to cytotoxicity or inhibition of luciferase enzyme activity [27].

Results

MAO A and B

Most of the isolated compounds showed selective inhibition of either of monoamine oxidase A or B as shown in Table **1**. The inhibition of MAO B both Isoorietin (**1**) and orietin (**2**), two of the flavonoids isolated from *Vitex grandifolia* and were 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). Isovitexin (**3**), a flavonoid isolated from this wild vegetable for the first time displayed a fair selective activity against MAO A (IC_{50}

(μg/mL) of >100) to MAO B (IC_{50} (μg/mL) of 21.3) like the other two flavonoids while clorgyline and deprenyl were used as positive standards.

Anti-inflammation

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

Isolated Compounds

Compound 1

Compound 1 (Fig. 1) was isolated as a yellow solid. Thus, its molecular formula was deduced to be $C_{21}H_{20}O_{11}$ from a combination of ^{1}H NMR and ^{13}C NMR data. ^{1}H NMR (600 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 (150 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"). Compound 1 was identified as isoorientin, by NMR analysis, and comparison with its literature data [28].

Compound 2

Compound 2 (Fig. 1) was obtained as a yellow solid. Thus, its molecular formula was deduced to be $C_{21}H_{20}O_{11}$ from a combination of ^{1}H NMR and ^{13}C NMR data. ^{1}H NMR (400 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 (100 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"). Compound 2 was identified as orientin by NMR analysis, and comparison with its literature data [29].

Compound 3

Compound 3 (Fig. 1) (19 mg) was isolated as a yellow amorphous powder and the melting point is 219 - 221^{O} C; IR v max (cm-1): 3320 (-OH), 1697 (C=O), 1645 (C=O); the molecular formula was deduced to be $C_{21}H_{19}O_{10}$ from a combination of ^{1}H NMR and ^{13}C NMR data. ^{1}H-NMR (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 (500 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"). Compound 3 was identified as isovitexin by NMR analysis, and comparison with its literature data [30, 31, 32].

Structure Activity Relationship

Anti-inflammatory Activity

The following preliminary structure-activity relationship (SAR) profile is proposed based on the anti-inflammatory effects of the flavonoids that were isolated from *V. grandifolia;* these are summarized as following: (a) the C_2=C_3 double bond might contribute to the activity of these flavonoids as anti-inflammatory agents, Wang *et al.,* (2018) reported that C_2=C_3 double bond might contribute to molecular planarity, this was discovered from the more significant anti-inflammatory effect displayed

by diosmetin than hesperetin, its nonexistence (C_2=C_3 double bond) resulted into a greater volume/ratio [33, 34] (b) Hydroxylation substitution (-OH) noticed at both C-3' and 4' i.e. tertiary increases activity especially at ring B moiety i.e. isoorientin (**1**), orientin (**2**); Wang *et al.* (2018) reported the hydroxylation pattern, its importance in C- 3'-hydroxylation in the case of fisetin and 5-hydroxylation in the case of isoflavones, gives significant effects for inducing cell delineation (apigenin vs. chrysin) especially ring B moiety [33, 34] (c) the position of the sugar moiety, glycosides on ring A gives a better anti-inflammatory activity than on ring B and C, Isoda *et al.,* (2014) gave an assessment that glycosides with a significant hydrophilicity displayed lower anti-inflammatory activity, which may be due to lower hydrophobicity as well as sterical interference, lessening membrane permeability [35].

MAO A and B

The following preliminary structure-activity relationship (SAR) profile is proposed based on the inhibitory effects of the flavonoids against MAO A and B, that were isolated from *V. grandifolia;* these are summarized as following: (a) Hydroxylation subsutution pattern (-OH) noticed at both C-3' and 4' i.e. tertiary increases inhibitory activity of MAO A and B mostly at ring B moiety i.e. isoorientin (**1**), orientin (**2)** but a reduced activity was noticed in Isovitexin **(3)** though Spencer *et al.,* (2012) attested to the fact that both unsaturation degree of C_2=C_3 double bond and the hydroxylation pattern on ring B moiety have a great significant on the anti-neurodegenerative effects on flavonoids in general [36].

Discussion

Compounds **1 - 3** (Fig. 1) were known based on their 1D and 2D NMR; and by comparison of their NMR data with those reported in the literature [28, 29]. Isoorientin (**1**), Orientin (**2**) and Isovitexin (**3**) were isolated from *V. grandifolia* for the first time to the best of our knowledge. The observation in UV spectrum was an indication of the presence of hydroxyl groups at C-4', C-5 and C-7. Compound (51) showed hydroxyl (3376 cm^{-1}), carbonyl (1660 cm^{-1}) and aromatic groups C=C, CH_2, C-H bending at 1561, 1446, 845, 800 cm^{-1} absorptions in its IR spectrum. The ^{1}HNMR spectrum of the compound

1 indicated that B ring protons, H-2'and H-6' gave multiplet at δ 7.80 and H-5' proton signal was observed at 6.8 ppm as a doublet (J=9 Hz). H-8 and H-3 protons were at δ 6.75 (1H, s) and δ 6.25 (1H, s) respectively. Anomeric proton was observed at δ 4.93 (1H, d, J=7 Hz, C-glucosyl H-1") and sugar proton signals overlapped at δ 3.5 - 4.8. The spectroscopic results were well correlated to the reported data, hence the compound 1 is Isoorientin. The complete ^{1}H and ^{13}C NMR assignments of compound 2 were made by a combination of ^{1}H, ^{13}C, ^{13}C DEPT, COSY, HSQC and HMBC experiments and by comparing with assignments published in the literature as orientin. The presence of –OH group at C-5' and C-4´ in flavonoid was confirmed. In the ^{13}CNMR spectrum, **2** displayed one anomeric carbon signal at *ä* 104.8 and other sugar moiety signals due to glucopyranoside, indicating that there was one glucose unit. UV spectrum in MeOH showed absorption at 345 nm, which is a characteristic absorption of Orientin [37, 38, 39]. The compound 3 was elucidated by a combination of ^{1}H, ^{13}C, ^{13}C DEPT, COSY, HSQC and HMBC experiments and by comparing with assignments previously reported for Isovitexin [40, 41]. The UV spectrum of the compound 2 was an indication of hydroxyl groups at C-4', C-5 and C-7 because of the presence of a bathochromic shift with NaOMe, NaOAc and $AlCl_3$/HCl in its UV spectrum. The IR spectrum of the compound 3 showed absorptions at 3440 cm-1(OH), 1660 cm-1 (C=O), 1620 cm-1 (C=C), 1523, 1475 cm-1 (Aromatic group), 1017 cm-1 (C-O). The ^{1}HNMR spectrum of the compound 3 showed six aromatic proton resonances from δ 6-8. A set of four AA'BB' proton signals at δ 7.62 (2H, *d, J*=8 Hz, H-2' and H-6') and 6.84 (2H, *d, J*=8 Hz, H-3', H-5') located on the ring B, H-8 and H-3 protons were observed as singlets at δ 6.5 (1H) and 6.25 (1H) respectively. The anomeric proton showed a doublet at 4.88 ppm (1H, *d, J*=8 Hz, glucosyl H-1"). Sugar proton signals overlapped at 3.5 - 4.7 ppm. This ion was formed by the loss of $C_5H_{10}O_5$ from the molecular ion. On the basis of UV, IR, NMR, EI mass data, which was correlated with the literature, compound 3 has been identified as isovitexin.

Extracts and concoctions from medicinal plants still play vital functions in managing primary health requirements in most developing countries. Most of the world's population (80%) depends on these herbs and botanicals as reported by the World Health Organization, there are active chemical constituents present in these plants responsible for the biological activity [42]. It is therefore of immense concern to evaluate these plants, in order to validate their employment in old-age medicine and to reveal the secondary metabolites responsible for pharmacological activity. Most of these medicinal plants have been reportedly contain flavonoids, various flavonoids of been isolated from such i.e. apigenin, galangin, kaempferol, quercetin, luteolin, naringenin and other flavonoids, many are MAO inhibitors [43]. Zarmouh *et al.,* (2015) reported that the selective MAO-B inhibitors of isolated compounds from the ethanolic extract of *Psoralea corylifolia* seeds, a medicinal plants known for its antiaging effects. In this work, human recombinant MAO-B and MAO-A iso-enzymes were employed for the inhibition of enzymes studies. The authors discovered that out of the eight compounds isolated, only two flavonoids i.e. bavachinin and genistein showed a significant selectivity of MAO-B inhibition; these two flavonoids showed significant reduction in H_2O_2 produced by MAO-B as compared to MAO-A [44]. Lee *et al.,* (2001) isolated four flavonoids namely isoquercitrin, quercitrin, quercetin and rutin from the leaves of *Melastoma candidum* D. Don for the first time. These flavonoids displayed a selective inhibitory activity against MAO-B with IC_{50} values of 19.06, 11.64, 3.89, and 10.89 μM respectively [45]. Monoamine oxidase inhibitors (MAOIs) differ by their selectivity of the MAO receptor. Some MAOIs inhibit both MAO-A and MAO-B equally. Other MAOIs have been developed and found to target one over the other [46]. Some studies here corroborate our work that some flavonoids from natural sources can selectively inhibits the MAO-B.

Conclusion

There is a growing attention in the assessment of medicinal plants especially for inhibition of MAO, owing to the likely daily and cultural use as food and vegetables. Chemical constituents in medicinal

plants helps in management of the disorders associated with nervous system together with their likely connections with medicines and the diet abundant in dietary-monoamines. The present study ascertained that the isolated compounds from *V. grandifolia* are moderate MAO-B inhibitor, and this result could be of importance for better application of this wild vegetable in traditional neuropharmacological use. The use of *V. grandifloia* as vegetable is widely accepted though tagged as "poor man" food, this study hopes to give cognize to its pivotal role, hence as a source for the development of nutraceutical products. No work or study has reported the inhibition of MAO-A and B by constituents of this plant with their anti-inflammatory activity.

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Conflicts of interest statement

There is no conflict of interests declared by the authors.

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Tables

Table 1: IC_{50} Values of Isolated Compounds as MAO A&B inhibitory agents

	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
7	Clorgyline	1.6	NT
8	Deprenyl	NT	0.48

Table 2: IC_{50} Values of Isolated Compounds as Anti-inflammatory agents

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

Figures

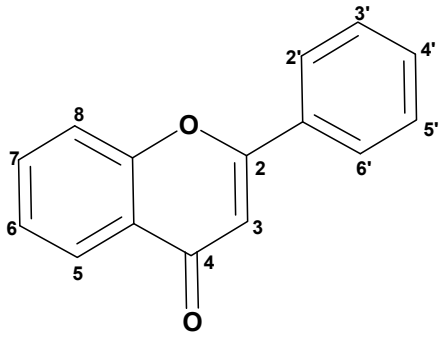

Figure 1: Basic Skeleton or Structure of Flavonoids

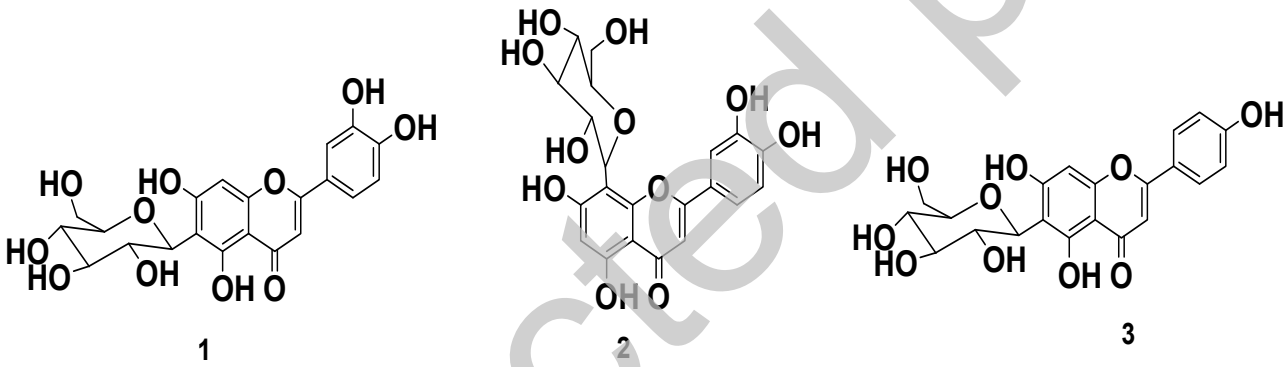

Figure 2: Isolated Flavonoids from *Vitex grandifolia*