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Structure Elucidation of New Flavone and Antioxidant, Anti-Inflammatory Activities from Leaves of *Vitex negundo* Linn.

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A medicinally active chemical investigation on the leaves of *Vitex negundo* induce the confinement and identification of flavones apigenin (**1**), kaempferol (**2**) and one new flavone 5,7,4′-trihydroxy-3,3′,6,8-tetramethoxy flavone (**3**) and one known and rare flavone 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**4**). These compounds composition were well-established on account of chromatography and spectroscopic sign and correlation with literature data. Antioxidant and carrageenan-induced paw edema in male albino rats were determined. Compounds **1** and **2** exhibited the most potent antioxidant effect at a dose of 200 mg/kg. Compound **1** produced noticeable anti-inflammatory effects (2.66 ± 0.08 at 3 h of injection) lightly off that of other compounds.

Keywords: Flavones, *Vitex negundo* Linn., Antioxidant, Anti-inflammatory.

INTRODUCTION

Vitex negundo Linn. (Verbenaceae), locally known as 'Nirgundi/Nochi' is an essential medicinal plant and is used for the analysis of a large health disorders in traditional and folk medicine. All parts of the plant especially its leaves accommodated numbers of secondary metabolites such as alkaloids, phenols, flavonoids, glycosidic iridoids, tannins and terpenes. On account of the excellences in phytochemicals, the plant is associated to acquire a number of therapeutic uses *e.g.*, antimicrobial, anti-inflammatory, diuretic, emmenagogue, anticancer and hepatoprotective, *etc*. [1]. The natural antioxidants are rich in various plant origins, free from after effects and these substances behead a precautionary role in assure against the generation of free radicals. Various studies presented that flavonoids and phenolic compounds have added to the antioxidant activities of natural products [2]. The role of free radicals and reactive oxygen species has indicated in a number of diseases such as cancer, cardiovascular disease, cataracts, wound healing, gastrointestinal inflammatory diseases and other inflammatory processes [3]. Inflammation is a complicated process, which is frequently associated with pain and involves occurrence including the increase of vascular permeability, an increase of protein and membrane alteration. There are different chemical classes of NSAIDs each with a specific mechanism of action and certain pharmacokinetics [4,5]. In the present study, plant was screened for antioxidant and anti-inflammatory study by carrageenan-induced paw edema. The plants were selected on the basis of the traditional use of plant or pharmacological activities reported in the literature.

EXPERIMENTAL

Melting points were determined on a Fisher Scientific melting point apparatus and are uncorrected. GC-MS Ionizes compounds and measures their mass numbers. The analyses of volatile constituents were run on a Perkin-Elmer Clarus 500 GC-MS system. The fused - silica HP-5 MS capillary column (30 m - 0.25 mm ID, a film thickness of 0.25 mm) was directly connected to the MS. The UV spectra study was accomplished on a UV-3010 Ultraviolet spectrometers (Perkin-Elmer spectrophotometer) scanning from 200 to 700 nm. IR spectra were measured on the FT-IR spectrograph (Perkin-Elmer Spectrophotometer) with KBr tablets from 4000 to 400 cm^{-1}. Supporting evidence for the structure of compound is provided by ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR (500 MHz, $CDCl_3$) spectra were recorded on a Bruker AMX 500 NMR spectrometer (Bruker Company, Faelladen, Switzerland).

Plant materials: Fresh *Vitex negundo* (Nochi) leaves were collected in the month of November to December 2017, from local areas of Kollidam riverside, Kumbakonam (Taluk), India and accurately disinfected to remove any adhering foreign matters and then washed with water. The leaves were air-dried under shade within a temperature range of 37-40 ºC and authenticated by Dr. R. Murugan, Department of Botany, Government Arts College (Autonomous), Kumbakonam, India. A voucher specimen (GACBOT-308) was deposited in the herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, India for future reference.

Extraction and isolation: The air-dried leaves were ground to make coarse powder and macerated with 90% aqueous ethanol (8 × 500 mL) during 72 h at 30 ºC filtration, an excess of solvent was distilled under reduced pressure using rotary vacuum evaporator at temperature below 40 ± 1 ºC and dry residues were redissolved in 90% aqueous methanol. A crude extract was recovered and concentrated at room temperature, which was dissolved in H_2O, and then partitioned successively with diethyl ether (1.5 L), ethyl acetate (1.5 L) and, the solvents recovered by simple distillation and were evaporated to dryness at room temperature and percentage yield was calculated and stored in a refrigerator. The petroleum ether layer was concentrated to give a brown syrup (30.5 g) which was subjected to column chromatography on silica gel (60/230-400 mesh, Merck) and eluted with a gradient of methanol and ethyl acetate (60:1, 40:1, 30:1, 20:1) to give compounds **1** (21.8 mg), **2** (18.3 mg), **3** (16,9 mg) and **4** (12.8 mg). The dried extracts were stored in the dark at 4 ºC until use. The fractions were collected and tested for the components by using thin layer chromatography. TLC spot was identified by spraying a 5% w/v alcoholic solution of H_2SO_4 as a spraying reagent. The sprayed plates were heated at 100 ºC for 5-10 min and the numbers of constituents present in each fraction were found. The toluene:EtAcO:HCOOH = 60:30:10 (v/v); showed R_f value 0.68 and 50:30:20 (v/v): R_f value 0.88 and chloroform-methanol (60:40; 60:20) ratios give the fraction was found to be similar and showed an R_f value 0.48 (compound **3)** and R_f value 0.78 (compound **4**). Thus, it was recrystallized from methanol and compounds (**3** and **4**) were used for pharmacological studies by suspending a weighed amount of compound in normal saline (95 mL):Tween 80 (5 mL) ratio.

DPPH free radical scavenging method: A case solution of material (50 µL) at four concentrations (0.125, 0.25, 0.5, and 1.0 mg/mL) has different with a freshly adapted methanolic solution of DPPH (634 µM) and grant to stand for 30 min at room temperature. The absorbance was measured at 515 nm using a spectrophotometer. The free radical DPPH had determined using the following formula:

$$\text{Inhibition (\%)} = \frac{A_o - A_{sample}}{A_o} \times 100$$

Anti-inflammatory activity: The anti-inflammatory activity of the test compositions was checked out in male albino rats administer the methods [6,7]. Animals have fasted overnight and branched into control, standard and different test groups each consisting of six animals. Control group animals have collected 1% DMSO at the dose of 10 mL/kg body weight. They housed in cages and conserved under standard conditions at 26 ± 2 ºC and relative humidity 60-65 % and 12 h light and 14 h dark cycles each day for one week before and during the experiments. The acute inflammation was convinced by sub-plantar administration of 0.1 mL of 1% carrageenan in the right paw. A paw volume was consistent by using a digital plethysmometer (Ugo Basile, Italy) before legislation of carrageenan and after 1, 2 and 3 h intervals. The percentage inhibition of paw edema is calculated by using the following formula:

$$\text{Inhibition of paw edema (\%)} = \frac{V_C - V_T}{V_C} \times 100$$

where V_C = Paw edema of a control group and V_T = paw edema of the treated group.

Animals: The albino rats (wistar strain) of any sex were used as per experimental protocols after consent from the Institutional Animal Ethical Committee (IAEC), Bharathidasan University, Thiruchirapalli, India (Approval No. BDU/IAEC/2011/31/29.03.2011). The animals (weighing 180-220 g) were house in standard environmental conditions (25 ± 2 ºC and humidity 50 ± 5 %) and 12 h light/dark cycle and they were provided standard rodent chow/feed and water *ad libitum* The animals were fasted for 12 h before the experiment and allowed free access to tap water to ensure uniform hydration and to minimize variability in edematous response. Each group comprises six rats.

Statistical analysis: The data of pharmacological experiments were expressed as mean ± standard error of mean (SEM). Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, USA). Data of paw volume, were analyzed using one way analysis of variance (ANOVA) followed by Dunnett′s test. A value of $p < 0.05$ was considered to be statistically significant.

Apigenin (1): yellow powder; m.p.: 340-342 ºC; R_f = 0.68; EI-MS *m/z*: [M+H] + 271. UV λ_{max}^{MeOH} (log ε) nm 268, 335; +$AlCl_3$ 302, 345, 382; +$AlCl_3$/HCl 298, 342, 380; +NaOAc: 275, 305, 378; +H_3BO_3 268, 302, 352. FT-IR: (KBr, ν_{max}, cm^{-1}): 3290, 3095 (OH), 2925, 1660(C=O), 1610 and 1505 (Ar), 1445, 1180, 830; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.92 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.90 (2H, d, *J* = 8.8 Hz, H-3′, H-5′), 6.80 (1H, s, H-3), 6.20 (1H, d, *J* = 1.6 Hz, H-6), 6.48 (1H, d, *J* = 1.6 Hz, H- 8), 12.95 (1H, s, 5-OH), 10.80 and 10.35 (each 1H, s, 7-OH and 4′-OH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 182.4 (C-4), 165.1 (C-2), 164.0 (C-7) , 161.0 (C-9), 159.2 (C-42), 156.8 (C-5), 128.0 (C-62, C-22), 121.1 (C-12), 115.5 (C-32, C-52), 104.6 (C-10), 102.6 (C-3), 99.8 (C-6), 95.4 (C-8).

Kaempferol (2): Yellow amorphous powder. m.p.: 276-277 ºC; R_f: 0.88; ESI-MS *m/z*: 286.087 [M]+, UV λ_{max}^{MeOH} (log ε): 266-362 nm; IR (KBr, ν_{max}, cm^{-1}): 3422 (OH *str*.), 1680 (C=O), 1622 (C-C), 1385 (C-H), 1175 (C-O), 830; ^{1}H NMR (500 MHz, CD_3OD, δ ppm): 8.06 (2H, d, *J* = 8.8 Hz, H-2′,6′), 6.94 (2H, d, *J* = 8.8 Hz, H-3′,5′), 6.49 (1H, d, *J* = 2.0 Hz, H-8), 6.25 (1H, d, *J* = 2.0 Hz, H-6), 9.88 (1H, s, 3-OH), 12.52 (1H, s, 5-OH), 10.69 (1H, s, 7-OH), 10.22 (1H, s, 42-OH). ^{13}C NMR (125 MHz, CD_3OD): δ 146.6 (C-2), 135.9 (C-3), 175.8 (C-4), 161.6 (C-5), 98.8 (C-6), 164.2 (C-7), 94.2 (C-8), 157.4 (C-9), 103.8 (C-10), 122.8 (C-1′), 126.4 (C-2′), 115.8 (C-3′), 159.6 (C-4′), 116.8 (C-5′), 128.4 (C-6′).

5,7,4′-Trihydroxy-3,3′,6,8-tetramethoxyflavone (3): Colourless powder, m.p.: 180-182 ºC, ESI-MS (*m/z*): 390.145; R_f: 0.48; UV λ_{max} (nm): MeOH 280 and 356 nm (sh); IR (KBr, ν_{max}, cm^{-1}): 3320-3610 (O-H, free hydroxyl group), 2955-2870 (C-H *str.*), 1660 (C=O *str.*), 1500-1380 (C-C ring *str.*), 1280-1180 (C-C ring *str.*), 1120-820 (O-H, out of plane bend). ^{1}H NMR; 7.49 (1H, d, *J* = 1.77 Hz, 2′), 7.78 (2H, d, *J* = 2.0 Hz, 6′), 6.89 (1H, dd, *J*= 8.44 Hz, 5′), 3.89 (3H, s; OCH_3, 3), 3.78 (3H, s; OCH_3, 6), 3.68 (3H, s; OCH_3, 8), 3.66 (3H, s, OCH_3, 3′), 12.80 (1H, s, 5-OH), 10.91 (1H, s, 7-OH), 9.86 (1H, 4′-OH); ^{13}C NMR; 153.6 (C-2), 139.8 (C-3), 177.9 (C-4), 153.1 (C-5), 137.2 (C-6), 134.8 (C-7), 148.1 (C-8), 150.2 (C-9), 105.8 (C-10), 122.4 (C-1′), 114.8 (C-2′), 149.6 (C-3′), 147.4 (C-4′), 116.6 (C-5′), 122.8 (C-6′), 56.8- 61.8 (OCH_3).

5,7,4′-Trihydroxy-6,3′,5′-trimethoxyflavone (4): Pale yellow powder; mp. 238-239 ºC; R_f = 0.78: 6.4 min; UV λ_{max}^{MeOH} (log ε) nm 275, 350; FT-IR: (KBr, ν_{max}, cm^{-1}) 3450 (OH), 1652 (C=O), 1600, 1520 and 1475 (Ar), 1362, 1170, 1010, 830; ^{1}H NMR (400 MHz,DMSO-d_6): δ 7.18 (2H, d, *J* = 2.0 Hz, H-2′, H-6′), 6.92 (1H, s, H-3), 6.60 (1H, s, H- 8), 3.75, 3.78 (each 3H, s, 3′, 5′-OCH_3), 3.86 (3H, s, 6-OCH_3), 12.95 (1H, s, 5-OH), 10.82 (1H, s, 7-OH), 9.64 (1H, s, 4′-OH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 182.0 (C-4); 163.2 (C-2); 160.0 (C-7); 154.2 (C-9); 153.5 (C-5); 152.2 (C-2′); 159.8 (C-4′); 118.2 (C-5′); 112.4 (C-6′); 107.9 (C-3); 106.8 (C-1′); 105.4 (C-10); 116.8 (C-3′); 142.2 (C-6); 92.1 (C-8); 61.4 (OCH_3); 57.8 (OCH_3); 57.2 (OCH_3).

RESULTS AND DISCUSSION

Two new flavones have been isolated from the leaves of *Vitex negundo* in this study. The petroleum ether soluble fraction from the crude methanol extract of *Vitex negundo* was subjected to column chromatography. The structure of the isolated compounds was analyzed using UV, IR, ^{1}H and ^{13}C NMR and MS spectra. Finally, the structure was confirmed by comparison with the reference data and the compounds were identified as apigenin (**1**), kaempferol (**2**) 5,7,4′-trihydroxy 3′,3,6,8-tetramethoxy flavone (**3**) and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**4**) (Fig. 1).

Compound 1: The UV spectrum λ_{max} (MeOH) showed bands at 268 and 335 nm (sh), deducing its flavone nature [8]. This compound exhibited bathochromic shifts with sodium methoxide and aluminum chloride respectively, at band I and bathochromic shift at band II with sodium acetate, giving indication for free hydroxyl groups at C-4′, C-5 and C-7. The ^{13}C NMR spectrum was quite informative, indicating the presence of 15 carbon signals and the ^{1}H NMR spectrum of compound **1** was consistent with a flavone structure and it exhibited a characteristic AA′ BB′ resonance system with the aromatic proton resonance at δ 7.92 (d, 8.8; H-2′ and H-6′) and δ 6.90 (d, 8.8; H-3′ and H-5′), and signals observed at δ 6.20, 6.48 (H-6 and H-8). The spectral data of compound **1** were in good agreement with those reported for 4′,5,7-trihydroxyflavanone (apigenin) [9,10].

Compound 2: Molecular ion peak exhibited at *m/z* 286.087 acquired molecular formula $C_{15}H_{10}O_6$. UV MeOH λ_{max} 266-362 nm. The FTIR spectrum reported a broad absorption bands at 3422 cm^{-1} which represents to OH stretching. The absorption band appeared at 1680 cm^{-1} to carbonyl group (C=O) and the absorption bands at 1622 cm^{-1} (C-C), 1385 cm^{-1} (C-H) and 1175 cm^{-1} presented C-O stretching. The ^{1}H NMR spectrum presented the characteristic peak a doublet at δ 8.06 (H-2′ and H-6′) with *ortho* coupling of 8.8 Hz whereas similar *ortho* coupling appeared doublet at δ 6.94 (H-3′ and H-5′).^{1}H NMR spectrum of compound **2** further showed a aromatic proton signals as a doublet at δ 6.25 (H-6) and at δ 6.49 (H-8) displayed *meta* coupling of 2.0 Hz. The ^{13}C NMR spectrum of compound **2** displayed signals of nine quaternary and six methine carbons; all data of compound **2** were in good agreement with the reported for kaempferol [11].

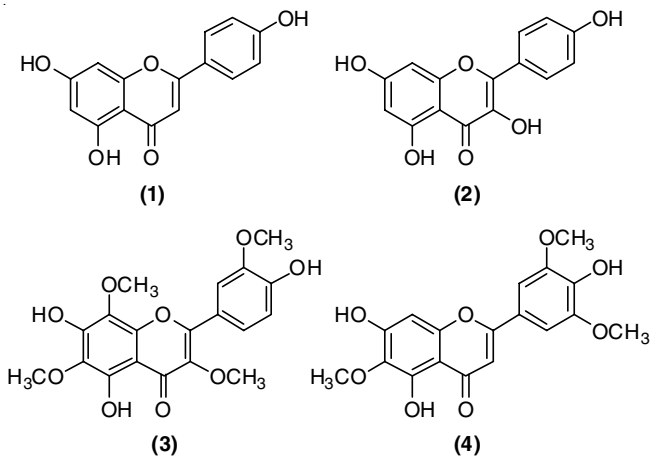

Fig. 1. Structure of apigenin (**1**) kaempferol (**2**) 5,7,4'-trihydroxy-3',3,6,8-tetramethoxy flavone (**3**) and 5,7,4'-trihydroxy-6,3',5'-trimethoxy-flavone (**4**): isolated from *Vitex negundo*

Compound 3: UV spectrum λ_{max} (MeOH) exhibited bands at 280 and 356 nm (sh). UV spectral properties of compound **3** shown to be flavone with free free-OH at C-5 in the glycoside which was noticeable from its positive response to Wilson′s boric acid test, which is also supported by the fact that it conferred an intense fluorescence under UV [12]. The same can also be implicit from a bathochromic shift of 52 nm noticed in the $AlCl_3$ spectra. A bathochromic shift of + 50 nm noticed on the addition of NaOMe was suggestive of the presence of free -OH at C-4′. A bathochromic shift of + 7 nm in band II of the spectrum on addition of NaOAc indicates the presence of a free -OH at C-7. The EI-MS spectrum exhibited the molecular ion peak at *m/z* 390.198, which was in conformity with the molecular formula of $C_{19}H_{18}O_9$ (*m/z* = 390.198 (100) [M+]. The IR spectrum of isolated compound **3** had shown the absorption bands at 3610-3320 cm^{-1} (O-H, free hydroxyl group), 2955-2870 cm^{-1} (C-H *str.*), 1660 cm^{-1} (C=O *str.*), 1500-1380 cm^{-1} (C-C ring *str.*), 1280-1180 cm^{-1} (C-C ring *str.*), 1120-820 cm^{-1} (O-H out of plane bend), respectively. Analysis of ^{1}H & ^{13}C NMR data revealed that the aromatic signals were close to those reported for 5,7,4′-trihydroxy-3′,3,6,8-tetramethoxy flavones. The ^{1}H NMR spectrum showed an ABX pattern in the B-ring was revealed by the three protons resonances at δ 7.49 (1H, dd, *J* = 8.2 Hz and 1.7 Hz), δ 7.78 (1H, *J* = 1.7 Hz) and δ 6.89 (1H, d, *J* = 8.2 Hz), which could be assigned to H-2′, H-6′ and H-5′, respectively, as found in 3′,4′-oxygenated flavonoids [13]. Four methoxy signals were observed at δ 3.89 (OCH_3-3, s), 3.68 (OCH_3-3′, s), 3.78(OCH_3-6, s), 3.66 (OCH_3-8, d), while

the ^{13}C NMR spectrum showed a considerable downfield chemical shifts at δ 61.8, 60.9, 59.6 and 56.8 ppm, proving the existence of four methoxy groups and also suggested that these groups were attached to di-*ortho*-substituted carbons [14] and possibly located on the A and C-ring. Three hydroxyl protons were observed at δ 12.80, 10.91 and 9.86 ppm as singlets and they could be assigned to 5-OH, 7-OH and 4′-OH, respectively which confirmed the UV data. The ^{13}C NMR spectrum of an isolated compound **3** showed three aromatic methoxy groups with considerable downfield chemical shifts at δ 61.8-56.8 ppm that suggest these groups were attached to di-*ortho*-substituted carbons [14] and possibly located on the A-ring and C-ring. The resonance at δ 56.8- 61.8 ppm could be assigned to the methoxy groups located at C-3′,3,6,8 considering this chemical shift is a characteristic of an aromatic methoxy group attached to carbon bearing one or no *ortho*-substituent. The occurrence of a flavone skeleton in the molecule could be easily deduced from ^{1}H & ^{13}C NMR spectra. The structure of the isolated compound **3** was deduced as 3′,3,6,8-tetramethoxy-4′,5,7-trihydroxy flavones.

Compound 4: The UV spectrum λ_{max} (MeOH) showed bands at 275 and 352 nm (sh) and also with diagnostic reagents such as NaOAc and $AlCl_3$ indicated the presence of free 5- and 7-hydroxyl groups. The IR spectrum indicated the presence of 3452 (OH), 1654 (C=O), 1602, 1522 and 1473 (Ar), 1364, 1172, 1010, and 831 cm^{-1}. The EI-MS spectrum exhibited the molecular ion peak at *m/z* 360.175, which was in conformity with the molecular formula of $C_{19}H_{18}O_9$ (*m/z* = 360.175 (100) [M+]. Analysis of ^{1}H & ^{13}C NMR data revealed that the aromatic signals were closed to those reported for 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone. The ^{1}H NMR spectrum exhibited three singlets at δ 3.86 (3H), 3.78 (3H) and 3.75 (3H) ppm, while the ^{13}C NMR spectrum showed signals at δ 61.4, 57.8 and 57.2 ppm proving the existence of three methoxy groups. The ^{1}H NMR showed a multiplet signal at δ 7.18 corresponding to two aromatic protons, which were assigned to H-6′ and H-2′ in-ring B and three hydroxyl protons were observed at δ 12.95, 10.82 and 9.64 ppm as singlets and they could be assigned to 5-OH, 7-OH and 4′-OH, respectively which confirmed the UV data. The position of methoxy groups signals at δ 3.86, 3.78 and 3.75 ppm with carbon signals at δ 142.4 (C-6), δ 116.9 (C-3′) and 118.1 (C-5′) ppm were determined by ^{1}H & ^{13}C NMR spectra. The structure of compound **4** was deduced as 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone which was confirmed by comparison of its spectral data with those in the literature [15].

Free radical scavenging activity: The DPPH (1,1-diphenyl-2-picrylhydrazyl) antioxidant estimate is based on the competence of DPPH an established free radical and decolourize in the sustenance of antioxidants. The percentage of free radical scavenging activity of ascorbic acid calculated at various concentrations (0.125, 0.25, 0.5 and 1.0) and at upper concentration it was found to be 6.64 ± 1.25, 18.44 ± 1.62, 40.83 ± 1.92 and 48.25 ± 1.99 percent for ascorbic acid (Table-1). The DPPH free radical scavenging activity of compound **3** of *Vitex negundo* showed the highest activity (% inhibition 5.36 ± 1.24, 25.25 ± 1.62*, 32.16 ± 1.91* and 45.74 ± 1.98* at 0.125; 0.25; 0.5; and 1.0 mg/mL, respectively) followed by compound **4** (3.43 ± 1.19, 16.38 ± 1.65, 19.49 ± 1.93*, 30.72 ± 1.98* at 0.125; 0.25; 0.50 and 1.0 mg/mL, respectively). Methanolic extract exhibited least DPPH radical ability with % inhibition 1.36 ± 0.98, 12.40 ± 1.59, 18.59 ± 1.82* and 28.87 ± 1.91* at 0.125; 0.25; 0.50 and 1.0 mg/mL, respectively. The results of the free radical scavenging activity of *Vitex negundo* determined by the DPPH test and amount of the sample needed for 50% inhibition of free radical activity, IC_{50} values are summarized in Table-1. Lower IC_{50} value suggests a higher antioxidant activity. Based on the results found the antioxidant activity of *Vitex negundo* compound **3** (IC_{50}: 77.45 µg/mL) has comparable with standard antioxidant of L-ascorbic acid (IC_{50}: 74.88 µg/mL).

Anti-inflammatory activity: The anti-inflammatory activity of compounds **3** & **4** and methanolic extract of *Vitex negundo* was determined in carrageenan-induced paw edema of rats and found to be significant compared to diclofenac sodium used as standard (Table-2). Carrageenan-induced paw edema was tested on male albino rats (weighed 180-220 g) in a suitable experimental animal model for evaluation of anti-edematous effect on natural products [16]. Inflammation-induced *via* carrageenan involves three distinct phases of the discharge of the mediator; as well as serotonin and histamine in the primary phase (0-2 h); kinins released in the second phase (3 h) and PG in the 3rd phase (> 4 h) [17]. The rats were divided into eight groups (six animals each) served as control and tested animals. Acute inflammation was produced by sub-plantar injection of 0.1 mL of 1% suspension of carrageenan in normal saline in the left hind paw of the rats, 1 h after the oral administration of drugs. All tested groups decreased the thickness of edema of hind paw compared to the control group.

A change in paw volume in the group of animals treated with *Vitex negundo* methanolic extract 500 mg was 3.36 ± 1.26 and for compound **4** 200 mg/kg was 3.08 ± 0.09 and compound **3**, 200 mg/kg was 2.66 ± 0.08 at 3 h. A paw volume was compared with that of standard diclofenac sodium 100 mg/kg and showed a percentage paw volume decrease of 2.38 ± 0.09. The anti-edematous response was also significantly

TABLE-1
DPPH FREE RADICAL SCAVENGING ACTIVITY OF FLAVONES ISOLATED FROM *Vitex negundo*

Samples	Concentration (mg/mL)				
	0.125	0.25	0.5	1.0	IC_{50}
	Radical scavenging effect (%)				
Compound **1**	5.36 ± 1.24	25.25 ± 1.62*	32.16 ± 1.91*	45.74 ± 1.98*	77.45
Compound **2**	3.43 ± 1.19	16.38 ± 1.65	19.49 ± 1.93*	30.72 ± 1.98*	88.62
Methanolic extract of *Vitex negundo*	1.36 ± 0.98	12.40 ± 1.59	18.59 ± 1.82*	28.87 ± 1.91	96.09
L-Ascorbic acid	6.64 ± 1.25	18.44 ± 1.62*	40.83 ± 1.92*	48.25 ± 1.99*	74.88

Values are expressed in Mean ± Standard deviation (M ± SD).

TABLE-2
DETERMINATION OF PAW VOLUME OF RATS FOR FLAVONES ISOLATED FROM *Vitex negundo*

Groups	Initial paw volume	Paw volume at different time interval (mL)		
		1 h	2 h	3 h
Compound **1** (100 mg/kg)	1.13 ± 0.24	1.93 ± 0.29	2.78 ± 0.04	2.88 ± 0.06
Compound **1** (200 mg/kg)	1.16 ± 0.25	1.98 ± 1.24	2.62 ± 0.02	2.66 ± 0.08
Compound **2** (100 mg/kg)	1.10 ± 1.20	2.18 ± 1.26	2.98 ± 1.26	3.19 ± 0.22
Compound **2** (200 mg/kg)	1.12 ± 1.23	2.97 ± 1.25	3.01 ± 1.23	3.08 ± 0.09
MeOH extract (250 mg/kg)	1.13 ± 1.12	2.29 ± 1.22	3.26 ± 1.14	3.52 ± 0.28
MeOH extract (500 mg/kg)	1.12 ± 1.13	2.07 ± 1.24	3.16 ± 1.28	3.36 ± 1.26
Control (1 % DMSO)	1.16 ± 1.15	1.84 ± 0.16	2.86 ± 1.08	3.24 ± 0.06
Diclofenac sodium (100 mg/kg)	1.12 ± 0.22	1.88 ± 0.23	2.36 ± 0.08	2.38 ± 0.09

Data expressed as Mean ± SEM, n = 6 in each group by one way ANOVA followed by Dennett's test.

decreased in rats pre-treated with diclofenac sodium, a known COX inhibitor. It is noted that carrageenan-induced inflammation by intensify PE2 release and leukocyte movement. It moreover increases the expression of COX-2 in skeletal muscle, epidermis and inflammatory cells, propose that the management of prostaglandin E2 is linked through an expression of cyclooxygenase-2 [18,19]. Both compounds **3** and **4** exhibited excellent inhibition in paw edema volume at doses *i.e.* 100 and 200 mg/kg p.o. The compound **3** exhibited maximum activity in comparison with compound **4** and methanolic extract of *Vitex negundo*. The present study showed a significant anti-inflammatory activity of *Vitex negundo* Linn. as compared to standard diclofenac sodium.

Conclusion

In present investigation, apigenin (**1**), kaempferol (**2**), 5,7,4′-trihydroxy-3,3′,6,8-tetramethoxy flavone (**3**) and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**4**) isolated from *Vitex negundo* Linn. was reported. Among the obtained flavones, two new flavones *viz*. 5,7,4′-trihydroxy-3,3′,6,8-tetramethoxy flavone (**3**) and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**4**) were isolated first time. It was also concluded that test formulation showed better antioxidant and anti-inflammatory activities as compared to the control group.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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