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Anti-microbial and anti-inflammatory flavonoid constituents from the leaves of *Lawsonia inermis*

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ABSTRACT

A chemical investigation on the leaves of *Lawsonia inermis* led to the isolation and identification of five known and rare flavonoids, apigenin (**1**), 5, 7, 4′ - trihydroxy-6, 3′, 5′-trimethoxyflavone (**2**), pectolinarigenin (**3**), apigenin-7-O-β-D-glucoside (**4**), and pectolinarin (**5**). Their structures were established on the basis of chromatography and spectroscopic evidence and comparisons with literature data. Antimicrobial and carrageenan induced paw edema in male albino rats were determined. Compounds 4 and 5 showed the most powerful antimicrobial effect against Escherichia coli and *Aspergillus flavus* at the dose of 200 mg/kg. Compound 2 showed pronounced anti-inflammatory effects (3.17±0.15 after 3 h of injection), slightly higher than that of other four compounds.

Keywords: *Lawsonia inermis*, Flavonoids, Antimicrobial, Anti-inflammatory activity.

INTRODUCTION

Flavonoids have recently considerable interest because of their potential beneficial effects on human health [1]. Their occurrence is therefore widespread in the plant kingdom and about 500 types of flavonoids are known [2]. The searches for new biologically active compounds are most often based on hints coming from ethnobotany but there are still a huge number of unstudied plants. Plants are now considered as a valuable source of unique natural products for the development of medicines against various diseases and also for the development of industrial products. The present study describes the isolation and structure elucidations of five flavonoids obtained for the first time from the leaves of *Lawsonia inermis.* The known flavonoids: apigenin (**1**), 5, 7, 4′-trihydroxy-6, 3′, 5′-trimethoxyflavone (**2**), pectolinarigenin (**3**), apigenin-7-*O*-β-D-glucoside (**4**), pectolinarin (**5**) were identified by spectroscopic evidences and by comparison with literature data previously reported.

Lawsonia inermis is a much branched glabrous shrub or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds are also used in traditional medicine. The plant is reported to contain flavonoids, tannins, alkaloids, terpenoids and quinones, and reported to have analgesic, hypoglycemic, anti-inflammatory, antimicrobial, antioxidant, antifertility, and anticancer properties [3]. Based on these aspects we have studied the phytochemical constituents from the leaves of *L. inermis* and their antimicrobial and anti-inflammatory activities in a carrageenan induced paw edema bioassay in male albino rats.

MATERIAL AND METHODS

General experimental procedures

Melting points were determined on a Fisher Scientific melting point apparatus and are uncorrected. UV spectra were recorded on an ultraviolet spectrophotometer (UV2550, Shimadzu, Japan). IR spectra were measured on FT-IR spectrophotometer (Perkin Elmer, PE 1600, USA) with KBr tablets from 4000 to 400 cm^{-1} with resolution of 2 cm^{-1}. NMR experiments were performed on a Bruker AMX 400 instrument (Bruker Company, Faelladen, Switzerland) standard pulse sequences running at 400 MHz for ^{1}H NMR and ^{13}C NMR. Chemical shifts are given in δ (ppm), TMS was used as internal standard material and the coupling constants (*J*) are given in Hz. Column chromatography (CC) was performed on silica gel 60 as stationary phase (particle size 0.04-0.036 mm, 230-400 mesh, ASTM E. Merck, Germany) and activated by heating at 110 ºC for one hour prior to use. A Shimadzu HPLC system (Columbia, MD), was used with UV detection at 2800 - 350 nm, with a chromatographic system comprising a Spectra Physics P-200 series gradient pump (Fremont, CA, USA), and a rheodyne injector fitted with a 20-FL loop, a C18 column (250 x 4.6 mm) (phenomenex, Torrance, CA, USA) was used.

Plant material

The leaves of *L. inermis* were collected from the local area in and around Kumbakonam, Thanjavur District (India) and authenticated by Dr. N. Ramakrishnan, Head & Associate Professor, Department of Botany, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India with voucher specimen number GACBOT 501 was deposited at the Herbarium of the Department of Botany, in our institution. The plant leaves were air dried under shade and made into fine powder by using hand homogenizer and sieved through sieve No.40 and the fine powder was for extraction procedure and evaluation.

Extraction and isolation

The air-dried fresh leaves of *L. inermis* (3 Kg) were powdered and extracted with 90% MeOH (8 x 500 ml) at room temperature. The MeOH was evaporated *in vacuo* to give dark brown syrup (300 g). The syrup was suspended in water and extracted with petroleum ether and EtOAc, successively. The petroleum ether layer was concentrated to give a brown syrup (60.5 g) which was subjected to column chromatography on silica gel (60/230-400 mesh, Merck), and eluted with a gradient of petroleum ether and EtOAc (40:1, 30:1, 20:1) to give **1** (17.8 mg), **2** (16.2 mg), **3** (9.0 mg). The EtOAc layer was concentrated to give a brown syrup (30 g) which was subjected to chromatography on silica gel, and eluted with a gradient of $CHCl_3$ and MeOH (40:1, 20:1 MeOH) to give **4** (11.7 mg) and **5** (13.5 mg). The concentrated extract was applied to preparative paper chromatography using the solvent systems, BAW (n-BuOH / AcOH / H_2O - 4:1:5), 15, 30, 60% AcOH and then phenol saturated with water.

Apigenin (1): yellow powder; **mp.** 340-342°C; R_f = 0.88; RT: 10.2 min; **UV** λ_{max}^{MeOH} nm 266, 335; +NaOH 272, 332, 390; +$AlCl_3$ 303, 345, 380; +$AlCl_3$/HCl 298, 340, 381; +NaOAc: 274, 304, 378; +H_3BO_3 268, 300, 354. **FT-IR**: (KBr) cm^{-1} 3289, 3090 (OH), 2920, 1655(C=O), 1611 and 1504 (Ar), 1446, 1180, 832; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.90 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.91 (2H, d, *J* = 8.8 Hz, H-3′, H-5′), 6.79 (1H, s, H-3), 6.46 (1H, d, *J* = 1.6 Hz, H-8), 6.19 (1H, d, *J* = 1.6 Hz, H-6), 12.94 (1H, s, 5-OH), 10.82 and 10.36 (each 1H, s, 7-OH and 4′-OH); **^{13}C NMR** (400 MHz, $CDCl_3$,): *δ* 182.3 (C-4), 164.6 (C-2), 163.9 (C-7) , 160.8 (C-9), 159.4 (C-4′), 156.6 (C-5), 127.8 (C-6′, C-2′), 120.5 (C-1′), 115.2 (C-3′, C-5′), 104.2 (C-10) , 102.4 (C-3), 99.6 (C-6), 95.1 (C-8).

5, 7, 4′-trihydroxy-6, 3′, 5′-trimethoxyflavone (2): pale yellow powder; **mp**. 237-239°C; R_f =0.78; RT: 6.4 min; **UV** λ_{max}^{MeOH} nm 275, 352; **FT-IR**: (KBr)cm^{-1} 3452 (OH), 1654 (C=O), 1602, 1522 and 1473 (Ar), 1364, 1172, 1010, 831; **^{1}H NMR** (400 MHz,DMSO-d_6): δ 7.15 (2H, d, *J* = 2.0 Hz, H-2′, H-6′), 6.95 (1H, s, H-3), 6.62 (1H, s, H-8), 3.74, 3.77 (each 3H, s, 3′, 5′- OCH_3), 3.88 (3H, s, 6-OCH_3), 12.98 (1H, s, 5-OH), 10.81 (1H, s, 7-OH), 9.69 (1H, s, 4′-OH); **^{13}C NMR** (400 MHz, $CDCl_3$,): *δ* 181.9 (C-4); 163.4 (C-2); 159.5 (C-7); 154.1 (C-9); 153.6 (C-5); 152.1 (C-2′); 159.9 (C- 4′); 118.1 (C-5′); 112.3 (C-6′); 107.8 (C-3); 106.9 (C-1′); 105.6 (C-10); 116.9 (C-3′); 142.4 (C-6); 91.8 (C-8); 61.2 (OCH_3); 57.6 (OCH_3); 57.1 (OCH_3).

Pectolinarigenin (3): yellow needles; **mp**. 216-218°C; R_f =0.92; RT: 13.48 min; **UV** λ_{max}^{MeOH} nm 273, 328; +NaOH 275, 292, 370; +$AlCl_3$ 280, 303, 352; +$AlCl_3$ / HCl 278, 378; +NaOAc 278, 295, 370; +H_3BO_3 264, 340. **FT-IR**: (KBr) cm^{-1} 3292 (OH), 1651 (C=O), 1595 and 1504 (Ar); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.07 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 7.10 (2H, d, *J* = 8.8 Hz, H-3′, H-5′), 6.89 (1H, s, 3-H), 6.60 (1H, s, H-8), 3.74, 3.85 (each 3H, s, 4′-OCH3 and 6-OCH_3); **^{13}C NMR** (400 MHz, $CDCl_3$): 61.2 (OCH_3), 54.6 (OCH_3), *δ* 162.6 (C-2), 104.7 (C-3), 184.2 (C-4), 151.9 (C-5), 133.2 (C-6), 158.1 (C-7), 93.4 (C-8), 153.7 (C-9), 104.2 (C-10), 121.8 (C-1′), 128.1 (C-2′, C-6′), 112.1 (C-3′, C-5′), 160.1 (C-4′).

Apigenin-7-*O*-β-D-glucoside (4): pale yellow solid crystal; **m.p.** 239-240°C; R_f = 0.60; RT: 3.7 min; **UV** λ_{max}^{MeOH} nm 268, 336; +NaOH 272, 384, +$AlCl_3$ 276, 340, 365; +$AlCl_3$ / HCl 277, 343; +NaOAc 266, 392; +H_3BO_3 267, 348. **FT-IR**: (KBr) cm^{-1} 3405, 2920, 2848, 1738, 1630, 598. **^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.96 (2H, d, J = 8.9 Hz, H-6', H-2'), 6.95 (2H, d, *J* = 8.9 Hz, H-5', H-3'), 6.90 (1H, s, H-3), 6.82 (1H, d, *J* =2.1Hz, H-8), 6.42 (1H, d, *J* = 2.1Hz, H-6), 5.06 (1H, d, *J* = 7.2 Hz, H-1" Glc), 3.17 (1H, d, H-2″), 3.23 (1H, d, H-3″), 3.13 (1H, d, H-4″), 3.56 (1H, t, H-5″), 3.58 (1H, dd, J=11.5, 4.9 Hz, H_a-6, Glc), 3.84 (1H, dd, *J*=11.5, 2.5 Hz, H_b-6, Glc), 9.42 (1H, s, 4'-OH); **^{13}C NMR** (400 MHz, $CDCl_3$): 181.4 (C-4), 164.6 (C-2), 102.2 (C-3), 161.6 (C-5), 99.2 (C-6), 162.5 (C-7), 94.7 (C-8), 156.4 (C-9), 105.8 (C-10), 121.3 (C-1′), 128.9 (C-2′), 115.4 (C-3′), 161.9 (C-4′), 115.3 (C-5′), 128.1 (C-6′), 99.7 (C-1″), 73.3 (C-2″), 75.7 (C-3″), 69.9 (C-4″), 77.4 (C-5″), 64.8 (C-6″).

Pectolinarin (5): yellow needles; **m.p.** 260-262°C; R_f = 0.74; RT: 15.1 min; **UV** λ_{max}^{MeOH} nm 274, 338; +NaOH 277, 297, 371; +$AlCl_3$ 284, 302, 353; +$AlCl_3$ / HCl 279, 377; +NaOAc 274, 296, 371; +H_3BO_3 265, 338. **FT-IR**: (KBR) cm^{-1} 3395 (OH), 1665, 1620, 1604, 1510; **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 1.16 (3H, d, *J* = 6.3 Hz, H-6‴), 3.64 (1H, dd, *J* = 11.5, 4.9 Hz, H_a-6″), 3.72 (3H, s, 4 -OCH_3), 3.78 (1H, dd, *J* = 11.5, 2.5 Hz, H_b-6″), 4.01 (3H, s, 6-OCH_3), 4.53 (1H, s, H-1‴), 3.65 (1H, s, H-2‴), 3.46 (1H, dd, H-3‴), 3.30 (1H, dd, H-4‴), 3.40 (1H, m, H-5‴), 5.68 (1H, d, *J* = 7.3 Hz, H-1″), 3.19 (1H, d, H-2″), 3.26 (1H, d, H-3″), 3.12 (1H, d, H-4″), 6.81 (1H, s, H-3), 7.22 (2H, d, *J* = 9.0 Hz, H-3′ and H- 5′), 7.34 (1H, s, H-8), 8.01 (2H, d, *J* = 9.0 Hz, H-2′ and H-6′), 12.80 (1H, s, 5-OH), 5.37 (1H, s, 2″-OH), 5.21 (1H, s, 3″-OH), 5.17 (1H, s, 4″-OH), 4.65 (1H, s, 2‴-OH), 4.52 (1H, d, 3‴-OH), 4.70 (1H, s, 4‴-OH); **^{13}C NMR** (400 MHz, $CDCl_3$): 54.6 (OCH_3), 61.2 (OCH_3), 163.6 (C-2), 104.0 (C-3), 183.7 (C-4), 154.2 (C-5), 133.1 C-6),157.2 (C-7), 96.4 (C-8), 153.7 (C-9), 106.8 (C-10), 122.9 (C-1′), 129.2 (C-2′, C-6′), 114.9 (C-3′, C-5′), 162.2 (C-4′), 102.8 (C-1″), 75.1 (C-2″), 77.8 (C-3″), 70.7 (C-4″), 77.1 (C-5″), 67.9 (C-6″), 101.7 (C-1‴), 72.6 (C-2‴), 73.2 (C-3″), 74.2 (C-4‴), 69.7 (C-5‴), 18.8 (C-6‴).

Anti-microbial activity

Anti-microbial activity test was carried out with the following modification of the method originally described by Bauer *et al*., [4]. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 min and cooled to 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The isolated compound prepared at different dose individually was placed on each petriplates discs along with the control and standard (streptomycin and amphotericin) discs. The plates were incubated at 37°C for 24 h. After incubation period, the diameter of the inhibition zone around the paper disc was measured and reported in millimeters.

Anti-inflammatory activity

Carrageenan induced rat paw edema

The anti-inflammatory activity of the test compounds was evaluated in Wistar rats following a method published in the literature [5]. The different test concentrations at the dose of 100 and 200 mg/kg of isolated compounds and 300mg/kg of methanol extracts were administrated to the animals in the test groups by oral route. Animals in the standard group received Diclofenac sodium at dose of 100 mg/kg, by oral route. Control group animals received 2 % DMSO at the dose of 10 ml/kg body weight. The acute inflammation was induced by the sub-plantar administration of 0.1 ml of 2 % carrageenan in the right paw. Paw volume was measured by using digital plethysmometer (Ugo Basile-Italy) before administration of carrageenan and after 1, 2, and 3 h intervals [6]. The efficacy of the different drugs was tested on its ability to inhibit paw edema as compared to control group.

Volume of edema = Final Paw Volume - Initial Paw Volume

The Percentage inhibition of paw edema was calculated by the formula as below.

% Inhibition of Paw edema = [(VC – VT) / VC] X 100

Where, VC = Paw edema of control group and VT = Paw edema of treated group

Experimental animals

Male albino rats (150-200 g) were used in the study. They housed in cages and maintained under standard conditions at 26±2°C and relative humidity 44-56 % and 10 h light and 14 h dark cycles each day for one week before and during the experiments. All animals were fed with standard rodent pellet diet, and water *adlibitum*. Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Trichirappalli, Tamilnadu, India (Approval No. BDU/IAEC/2011/31/29.03.2011).

The animals were divided into 13 groups each group containing six rats.

Group - I: Served as control, received the vehicle only (2 %) carrageenan (10 mg/kg).

Group-II: Served as standard, received Diclofenac sodium at dose of 100 mg/kg.

Group-III to VII: Served as test, received compounds (**1-5**) isolated from the leaves of *L. inermis* at doses of 100 mg/kg respectively.

Group-VIII to XII: Served as test, received compounds (**1-5**) isolated from the leaves of *L. inermis* at doses of 200 mg/kg respectively.

Group-XIII: Served as test, received methanol extract of *L. inermis* at doses of 300 mg/kg respectively.

Statistical analysis

The experimental results were expressed as statistical comparisons of mean ± SEM which were carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test. P values less than 0.05 was considered as statistically significant

RESULTS AND DISCUSSION

Chemical constituents

In this paper, we report the isolation of five known flavonoids using column chromatography techniques from leaves of *L. inermis* being these apigenin (**1**), 5, 7, 4′-trihydroxy-6, 3′, 5′-trimethoxyflavone (**2**), pectolinarigenin (**3**), apigenin-7-*O*-β-D-glucoside (**4**), pectolinarin (**5**). All isolated compounds **1-5** (Figure 1), are soluble in methanol and when dissolved in dilute alkali gave an intense yellow solution, as well as, positive reaction with $FeCl_3$ [7]. The structures of the isolated compounds were established by melting point, R_f chromatographic behavior and by means of UV, IR, and NMR spectral analysis. The IR spectrum of compounds (1 - **5)** showed absorption bands corresponding to hydroxyl (3350 cm^{-1}), carbonyl (1650 cm^{-1}), and aromatic (1600, 1500 cm^{-1}) groups.

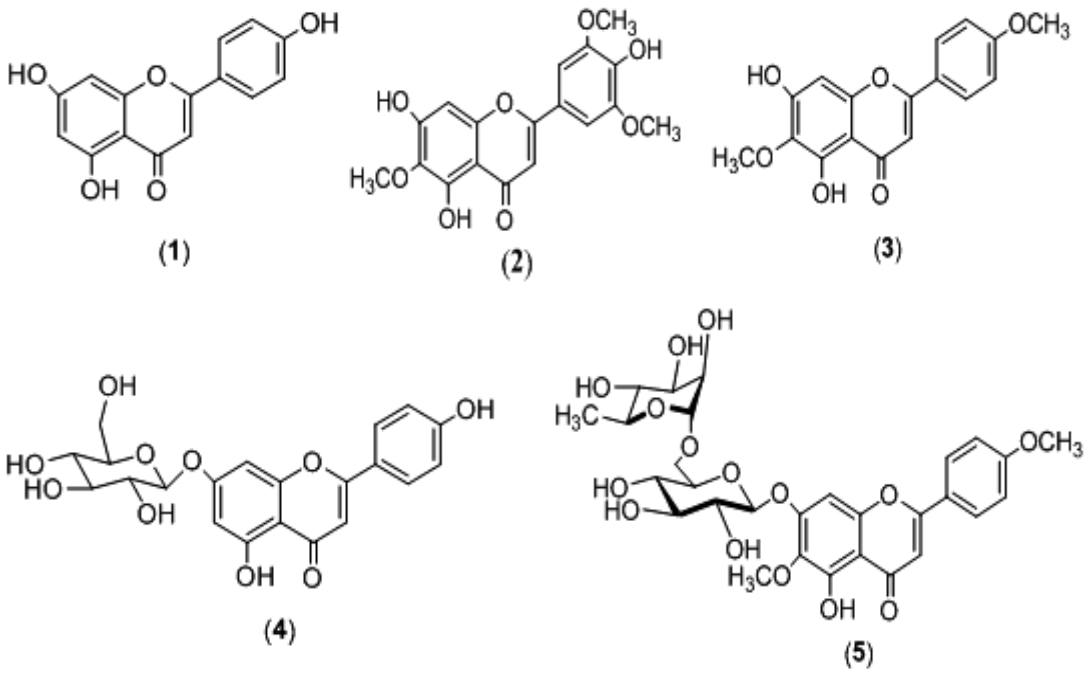

Figure 1 Compounds **1-5** isolated from leaves of *L. inermis*.

Compound **1** was isolated as a yellow solid; mp: 340-342°C. The UV spectrum λ_{max} (MeOH) showed bands at 266 and 335 nm (sh), deducing its flavone nature [8]. This compound showed 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 protons H-2′ and H- 6′ (δ 7.90, d, 8.8) and H-3′and H- 5′ (δ 6.91, d, 8.8), and signals for H-8 and H-6 (δ 6.46, 6.19). The spectral data of compound **1** were in good agreement with those reported for 4′, 5, 7- trihydroxyflavanone (apigenin) [9, 10].

Compound **2** obtained as a pale yellow powder, mp: 237-239 °C; 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 the free 5- and 7-hydroxyl groups. The ^{1}H NMR spectrum exhibited three singlets at δ 3.88 (3H), 3.77 (3H) and 3.74 (3H), while the ^{13}C NMR spectrum showed signals at δ 61.2, 57.6 and 57.1,proving the existence of three methoxy groups. The ^{1}H NMR showed a multiplet signal at δ 7.15, 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 12.98, 10.81 and 9.69 as singlets and they could be assigned to 5-OH, 7-OH and 4′-OH respectively which confirmed the UV data. The position of the methoxy groups signals at δ 3.88, 3.74 and 3.77 with carbon signals at δ 142.4 (C-6), δ 116.9 (C-3′) and 118.1 (C-5′) were determined by the ^{1}H and ^{13}C NMR spectra. The structure of compound **2** 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 [11].

Compound **3** obtained as yellow needles, mp: 216-218 °C; the UV spectral behavior of **3** with diagnostic reagents, such as NaOAc and $AlCl_3$ indicated the presence of the free 5- and 7-hydroxyl groups. The ^{1}H NMR spectrum showed an AA′ BB′ system at δ 6.94 and 7.77 (each 2H, J = 9.0 Hz) due to a 4′-oxygenated B ring [10]. The presence of methoxy groups was deduced by carbon resonances at δ 160.1 and 133.2 (C4′and C6) in the ^{13}C NMR spectrum. Thus, the structure of **3** was deduced as pectolinarigenin and confirmed by comparison of its spectral data with those in the literature [12].

Compound **4** was isolated as a pale yellow amorphous solid; mp: 239-240^{o}C. The UV spectrum λ_{max} (MeOH) showed bands at 268 and 336 nm (sh), deducing its flavone nature. This compound also gave positive color reactions for a hydroxyl flavone with several reagents [13]. The UV spectral data obtained in solution with methanol indicated that **4** was a flavone glycoside, while on addition of NaOAc indicated the 7th position to be occupied. Addition of H_3BO_3 indicated the presence of catecholic hydroxyl groups. Addition of $AlCl_3$ indicating the presence of free hydroxyl at C-5. The ^{1}H and ^{13}C NMR spectra were almost identical to those of **1**. In the ^{1}H NMR spectrum of **4**, the aromatic proton signals of two doublets at 6.82 and 6.42 (J= 2.1 Hz, 1H each) were attributed to H-8 and H-6 of ring A, respectively. Two vicinal coupled doublets at δ 7.96 and 6.95 (J = 8.8 Hz, 2H each) were assigned to H-2′/H-6′ and H-3′/H-5′, respectively, of ring B. Additionally, a singlet at δ 6.90 was ascribed to H-3. Additional resonances arising from a D-glucose unit in particular for the anomeric proton at H-1′′ (δ 5.06, 1H, d, J = 7.2 Hz,) and in the range δ 3.17-3.49, were observed in the **^{1}**H NMR data of **4**. The glucose moiety was allocated at δ 162.5 was attributed to C-7 based on ^{13}C-NMR data compared to those published in the literature (8-10, 14). Acid hydrolysis of **4** yielded the aglycone apigenin (**1**).

Compound **5** was isolated as yellow needles; m.p: 260-262°C; the UV spectrum λ_{max} (MeOH) showed bands at 274 and 338 nm (sh), deducing its flavone nature. Upon addition of sodium methoxide, the UV spectrum showed a shift indicating the presence of a free hydroxyl group at C-5`, while, the bathochromic shift with aluminum chloride confirmed the existence of 5-OH group. On the other hand, the absence of the shift with sodium acetate indicated the attachment of a sugar residue at C-7. The NMR signals of **5** were similar to those of **3.** The only difference was the appearance of additional signals for two sugar residues. The first sugar fragment corresponded to a glycosyl and the second one to a rhamnosyl. Also three of the six hydroxyl groups could be assigned to 2′′-OH, 3′′-OH and 4′′-OH at δ 5.37, 5.21 and 5.17 respectively in the glycosyl moiety. Similarly the other three hydroxyl group signals were assigned to 2′′′-OH, 3′′′-OH and 4′′′-OH at δ 4.65, 4.52 and 4.70 respectively in the rhamnosyl moiety. Acid hydrolysis of **5** yielded the aglycone pectolinarigenin (**3**). From these data the structure of compound **5** was unambiguously identified as pectolinarin and also confirmed by comparison of its spectral data with those in the literature [12, 15].

In the literature *L. inermis* has been reported to contain active compounds with different biological activities based on their use in traditional medicine. In the world there has been a revival of interest in herbal medicines as a result of increased awareness of the limited availability of synthetic pharmaceutical products to control major diseases, in addition to their higher incidence of adverse reactions compared to plant preparations. Therefore, it was deemed of interest to carry out certain pharmacological tests, such as antimicrobial and anti-inflammatory of the total plant extract and its successive fractions.

Anti-microbial Activity

The anti-microbial activity tests were carried out on MeOH extract and five isolated flavonoids from *L. inermis* using the disk diffusion method against Gram positive and Gram-negative bacteria. The diffusion test was applied to six microorganisms including three types of bacterial and three types of fungi namely *Escherichia coli*, *Staphylococcus aureus*, *Klebsella pneumonia*, *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* were chosen to investigate the anti-microbial capacity of *L. inermis.* The treated Petri disks were kept at 4 °C for 1 h, and incubated at 37 °C for 24 h. The anti-microbial activity was assessed by measuring the zone of growth inhibition surrounding the disks. Each experiment was carried out in triplicate. The results summarized in Table 1 showed that the crude extract from *L. inermis* as well as compounds **1-5** prevented the growth of all the tested microorganisms and it has been revealed that the medium diameter of inhibition zone increases proportionally with the increase of flavonoids concentration. The minimum inhibitory concentration is a measure to define the anti-microbial activity of an organism and is defined as the lowest concentration of drug that inhibits visible growth. Careful examination of the antimicrobial activity of the five compounds and the MeOH crude extract of *L. inermis* revealed that compounds **4** and **5** inhibited the growth of all microorganisms especially against *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus flavus* in which a better inhibition was noticed than over *Aspergillus niger*. On the other hand, compounds **1-3** showed less inhibitory activity against the treated microorganisms and fungi. Some flavonoids are formed as antimicrobial barriers in plants in response to microbial infection. There has been an enormous increase in the number of studies on flavonoids as potential antimicrobial agents [16].

Table 1: Anti-microbial activity of flavonoids isolated from *L. inermis*

Microorganisms	Zone of inhibition in mm											
	Compound										Methanol extract	Standard
	1		2		3		4		5			
	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	100 µg	
Staphylococcus aureus	06	09	04	07	08	11	15	21	18	24	13	30*
Escherichia coli	08	10	06	09	10	14	20	25	22	27	16	31*
Klebsella pneumonia	01	03	-	-	01	03	04	07	05	08	02	30*
Aspergillus niger	03	05	02	03	04	06	07	09	08	10	06	14**
Aspergillus flavus	02	04	01	02	03	06	08	11	09	12	07	14**
Candida albicans	-	02	-	-	02	04	03	06	04	07	03	14**

Bacteria Standard* - Streptomycin (100 µg); Fungal Standard** - Amphotericin – B (20 µg)

Anti-inflammatory Activity

In order to evaluate the anti-inflammatory activities of MeOH crude extract and flavonoids isolated from the leaves of *L. inermis*, the carrageenan-induced paw edema was carried out on male albino rats weighing (150-200 g). All rats were fed with the standard rodent pellet diet and water *adlibitum*. Edema was induced by injecting 0.2 ml of 2% carrageenan solution to 13 groups (six animals each) of rat hind paws. All groups showed a decrease in the thickness of edema of the hind paw in different percentages compared to the control group as indicated in (Table 2). The drug isolated from the leaves of *L. inermis* showed significant anti-inflammatory activity, when compared with the control, and a high rate of action against inflammation.

During the study of anti-inflammatory efficacy of MeOH crude extract and isolated flavonoids using carrageenan induced rat paw edema method, it was quite evident that, a gradual increase in paw volume was observed after carrageenan administration, which reached maximum at 3 hours as compared to control group. There were no significant differences in paw edema volume when standard was observed along with isolated flavonoids with 200 mg/kg dose level. The standard drug diclofenac sodium at a dose of 100 mg/kg inhibited the development of edema significantly from 1 h onwards. It showed maximum reduction (3.10±0.10) in paw edema at 3 h. It was found that isolated flavonoids at the dose of 100 and 200 mg/kg showed significant activity at 3 h. Methanol extract at the dose of 300 mg/kg body weight showed inhibition of paw edema at 3 h of 3.43±0.15. Among the five isolated flavonoids, at the same dose of 200 mg/kg, compound **2** showed pronounced anti-inflammatory effect of 3.17±0.15 after 3 h of injection. Compound **1** and **3** showed to inhibit the induced inflammatory response to carrageenan to a lesser extent than compound **2**. On the other hand, compounds **4** and **5** showed to be less potent as anti-inflammatory since they inhibited carrageenan-induced inflammatory by 3.45±0.10 and 3.48±0.14 respectively.

Table 2: Anti-inflammatory activity of flavonoids isolated from *L. inermis*.

Groups	Treatment	Anti inflammatory activity (cm) (M±SD)		
		1 h	2 h	3 h
I	Inflammatory Control (2% carrageenan)	3.87±0.10	3.65±0.13	3.46±0.15
II	Standard (Diclofenac sodium 100 mg/kg)	3.86±0.15	3.20±0.10	3.10±0.10
III - VII	Compound **1** (100 mg/kg)	3.82±0.12	3.60±0.05	3.40±0.14
	Compound **2** (100 mg/kg)	3.80±0.10	3.42±0.15	3.29±0.14
	Compound **3** (100 mg/kg)	3.87±0.15	3.55±0.10	3.36±0.12
	Compound **4** (100 mg/kg)	3.80±0.10	3.63±0.21	3.50±0.06
	Compound **5** (100 mg/kg)	3.87±0.10	3.70±0.12	3.54±0.05
VIII-XII	Compound **1**(200 mg/kg)	3.86±0.05	3.57±0.10	3.38±0.15
	Compound **2** (200 mg/kg)	3.87±0.14	3.35±0.12	3.17±0.15
	Compound **3** (200 mg/kg)	3.90±0.12	3.50±0.10	3.32±0.14
	Compound **4** (200 mg/kg)	3.83±0.06	3.61±0.12	3.45±0.10
	Compound **5** (200 mg/kg)	3.86±0.05	3.68±0.10	3.48±0.14
XIII	Methanol Extract (300 mg/kg)	3.84±0.12	3.61±0.15	3.43±0.15

Values are Mean ± S.E; n=6, ONE-Way: ANOVA p< 0.05.; Posttest: Dunnet's, compared all readings to normal control

CONCLUSION

To conclude, the present work highlights a new potential source of its occurrence in the leaves of *L. inermis* that are explores an opportunity for five known flavones on the biological activity to compare of crude MeOH extract. Bioactive substances from this plant were employed to develop drugs for treats inflammation and microbial disease.

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