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## Research Article

# A New Flavanone from *Flemingia strobilifera* (Linn) R. Br. and its Antimicrobial Activity

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## Abstract

***Purpose:*** *To carry out a bioactivity guided fractionation and isolation of the antimicrobial constituent(s) of the roots of Flemingia strobilifera against some bacteria and fungi.*
***Methods:*** *The root of F. strobilifera was extracted with methanol, butanol and dichloromethane. Antimicrobial activity of the extracts was determined against both bacteria and fungi while the isolation and characterization of compounds of the extracts was done by column chromatography,2D spectroscopic studies and spectral data (U.V, I.R, NMR and MS).*
*Results: Flemingiaflavanone (8, 3'-diprenyl-5, 7, 4'-trihydroxy flavanone), Genistin (5, 4'-dihydroxy isoflavone 7-O-glucoside) and β - sitosterol-D glucoside were isolated from the extracts. Flemingiaflavanone showed significant antimicrobial activity against Gram-positive (S. aureus, S. epidermidis, MRSA), Gram-negative bacteria (Ps. aeruginosa, E. coli) and fungi (C. albicans). Genistin showed moderate activity against Gram-positive, Gram-negative bacteria and fungi.*
***Conclusions:*** *The isolation and antimicrobial activity of presence of Flemingiaflavanone is being reported for the first time.*

***Key words****: Flemingia strobilifera, Flemingiaflavanone (8, 3'-diprenyl-5, 7, 4'-trihydroxy flavanone), Genistin (5, 4'-dihydroxy isoflavone 7-O-glucoside), MeOH (Methanol), BuOH (Butanol), Antimicrobial activity, MIC (Minimum inhibitory concentration).*

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## INTRODUCTION

*Flemingia strobilifera* (R.Br.), an important medicinal plant, commonly known as Kusrunt and belongs to the *Leguminosae* family [1, 2]. The plant is found in Sind, Rajputana, Bengal, South India and Andamans[3]. The roots of this plant have been indigenously used in epilepsy and hysteria and the leaves are reported to be used as vermifuge[4]. Previous phytochemical investigations reported various chalkones[4, 5], flavonoid glycosides[6], aurone glycosides[7] and epoxy chromenes[8].

This paper reports the isolation and structural elucidation of a new flavanone from the roots of *Flemingia strobilifera* characterized as 8, 3'-diprenyl-5, 7, 4'-trihydroxy flavanone **(1)** that is being reported for the first time from the genus *Flemingia* along with two known compounds and their antimicrobial activity.

### *Experimental*

General Melting point was determined on a Buchi Melting Point B-540 (Switzerland) apparatus. U.V was performed in U.V-Perkin Elmer double beam U.V Spectrophotometry (Germany). I.R spectra were recorded on a Jasco FT/IR 410 (U.S.A) in KBr. NMR spectra were measured in Bruker 400 MHz Ultrashield, Advance 400 (Germany) spectrometer, using TMS as internal standard. NMR experiment included the HSQC, HMBC, and COSY pulse sequences. Coupling constants (J values) were given in Hz. Quatro Micro Mass; Waters (U.S) was used to record Mass experiment. Silica gel used for column chromatography was normal phase 60-120 mesh size while TLC was carried out on silica gel GF254 sheets (Merck, Germany).

### *Plant material*

The roots of *F. strobilifera* were collected from forests of Shann Power House, Joginder Nagar, (Distt Mandi) Himachal Pradesh in October 2006. The identity of the plant material was verified by Dr. B. Naag Ex-Botanist Research Institute of Ayurveda, Joginder Nagar, (H.P) and Dr. H.B Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi and a voucher specimen number NISCAIR/RHMD/Consult/06/757/74 was deposited at the Herbarium of National Institute of Science Communication and Information Resources, New Delhi.

### *Extraction and isolation*

The root (2.94 Kg) of *F. strobilifera* were air-dried, ground and extracted with five liters of methanol for 24 h by maceration. The mark left was repeatedly extracted five times similarly, for complete extraction. The MeOH extract was evaporated in rota-vapor to yield a semisolid (1000 g), 900 g of which was suspended in five liters of water and partitioned with ten liters of DCM for five times and also partitioned with ten liters of *n*-BuOH for five times to yield 18 g and 34 g of extracted material, respectively. The DCM fraction (16 g) was column chromatographed over silica gel using petroleum ether (PE) and ethyl acetate (EtoAc), step gradient as eluents to yield compound **1** and **3.** The PE and EtoAc (85:15) fractions were collected and these fractions (203.9 mg) were further chromatographed using DCM and MeOH (95:5) over silica gel to yield compound **1,** (6.7 mg) and compound **3** (81.8 mg), β- sitosterol-D glucoside was eluted from PE: EtoAc (40:60) eluents. The *n*-BuOH fraction (25 g) was subjected to column chromatography on silica gel eluted with chloroform ($CHCl_3$): MeOH (85: 15) to yield compound **2,** (58.5 mg).

### *Antimicrobial activity method*

The minimal inhibitory concentration (MIC) of extracts and isolated compounds were determined by the broth microdilution method according to National Committee for Clinical Laboratory Standards guidelines[9] as well as for non-filamentous fungi in 96-well microtitre plates with MHB (Muller Hinton broth) made in-house. 96-well microtitre plates contained the antimicrobial agents in serial twofold dilutions from 136 to 0.53 µg/ml, depending on the antimicrobial agent being tested. Inocula were prepared in MHB from cultures grown on tryptic soya agar. The final concentration was $1 \times 10^5$ CFU/ml. All microtitre plates were prepared in duplicate and incubated at 35°C for 24 hrs. The susceptibility of the standard

drugs vancomycin, linezolid, fluconazole and itraconazole were defined as the lowest concentration of drug that resulted in total inhibition of microbial growth. The MIC was defined as the minimum inhibitory concentration of the extract or compound that resulted in total inhibition of microbial growth.

## RESULTS

The DCM fraction was column chromatographed over silica gel using PE and EtoAc to yield compound **1** and **3.** The *n*-BuOH fraction was column chromatographed over silica gel using $CHCl_3$: MeOH to yield compound **2.** Compound **1** showed $R_f$ value of 0.52 in DCM: MeOH (95:5) & showed $R_f$ value of 0.58 in $CHCl_3$: MeOH (95:5) solvent system. It gave orangish yellow colour with 10% methanolic sulfuric acid and pink colour with Shinoda confirmed the presence of flavanone. Compound **2** showed $R_f$ value of 0.61 in $CHCl_3$: MeOH (70:30**)** solvent system and showed $R_f$ value of 0.52 in PE: EtoAc (75:25**)** solvent system. It gave yellow colour with 10% methanolic sulfuric acid and green-brown colour with alcoholic $FeCl_3$.

Compound **3** showed $R_f$ value of 0.50 in EtoAc: MeOH: $H_2O$ (10: 1: 0.5) and showed $R_f$ value of 0.54 in $CHCl_3$: MeOH (80:20) solvent system and gave purple colour with 10% methanolic sulfuric acid and also showed positive Molisch test with formation of violet ring.

The antimicrobial activity of compound **1** has shown the most significant activity against Gram-positive, Gram-negative bacteria and fungi.

Compound **2** showed moderate activity against Gram-positive, Gram-negative bacteria and fungi.Compounds **2** and **3** are known and identified as Genistin (**2**) and β -sitosterol-D glucoside (**3**) by comparisons of their spectral data (U.V, I.R, NMR and MS) with those reported previously[10, 11, 12, 23, 24].The compound **1** was isolated for the first time from the plant and showed MIC of 17µg/ml against *Staphylococcus aureus, Staphylococcus epidermidis, Methicillin resistant Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*.

## DISCUSSION

**Compound 1** Compound **1** designated as 8, 3'-diprenyl-5, 7, 4'-trihydroxy flavanone was obtained as pale yellow crystals. Its molecular formula was established as $C_{25}H_{28}O_5$ due to parent ion at m/z 408 $[M]^+$ and [M + 1] at 409. The I.R spectrum showed strong absorptions at 1630$cm^{-1}$ (chelated C=O group) and 3300$cm^{-1}$ (OH). The characteristic U.V absorption bands [$\lambda_{max}^{MeOH}$ nm = 228 sh, 293,339 + NaoMe, 248, 285, 333; + $AlCl_3$, 221, 316, 392; + NaoAc, 287, 297,334 + $H_3BO_3$] suggested a flavanone structure. That was confirmed by the detection of $^1$H-NMR signals characteristic at $\delta_H$ 5.40 (1H, dd, J = 12.1, 2.7 Hz, $C_2$-βH), 3.20 (1H, dd, J = 17.4, 6.6Hz, $C_3$-αH), 2.73 (1H, dd, J = 17.0, 2.9 Hz, $C_3$-βH) attributed to the flavanone C-ring protons and at $\delta_C$ 78.1 and 41.8 in its $^{13}$C-NMR spectrum **(Table 1).**

It also indicated the presence of two-prenyl unit at $\delta_H$ 1.65, 1.54 [(each 6 H) s ($CH_3$ × 4)], 3.07, 3.17 (each 2H, d, J = 4.6 Hz, Ar-C$\underline{H_2}$-CH = × 2), 5.08, 5.26 (each 1H, t, J = 6.6 Hz, $CH_2$-C$\underline{H}$ = × 2), three hydroxyl groups [$\delta_H$12.00, 6.20 and 5.32 (each 1H, s) which shifted in DMSO-$d_6$ to $\delta_H$ 12.10, 10.74 and 9.40]. Aromatic protons at $\delta_H$ 5.95 (1H, s) were assigned to the H-6 in A ring[13]. A characteristic ABX system at $\delta_H$ 6.78, 7.16 and 7.11 indicated the presence of a C-3', 4' disubstitution on the B-ring moiety.

Positive U.V shifts after the addition of sodium acetate and aluminium chloride indicated that the three hydroxyl groups at $C_5$, $C_7$ and $C_{4'}$ were free and therefore the prenyl group in the A ring must be at C-8[14]. Since the $^1$H-NMR spectrum (B-ring) of compound showed ABX type proton signals of the aromatic ring, the prenyl group in the B-ring must be located at C-3'. These data indicated the substitution pattern of the A ring was 5-hydroxy substituted and 8-prenylated, and that of the B ring was 4'- hydroxylated and 3'- prenylated and the later three signals were assigned to

**Table 1:** $^{1}$H-NMR and $^{13}$C-NMR Spectroscopic Data of Compound 1, δ (ppm) in DMSO-$d_6$

| Position | $^{1}$H-NMR[a] | $^{13}$C-NMR/HSQC | DEPT[b] | COSY | HMBC[c] $^{3}J_{CH}$ |
|---|---|---|---|---|---|
| 2β | 5.40 dd (2.7, 12.1) | 78.1 | CH | | |
| 3α | 3.20 dd (6.6,17.4) | 41.8 | CH | | |
| 3 β | 2.73 dd (2.9,17.0) | 41.8 | CH | | |
| 4 | | 196.6 | | | |
| 4a | | 101.7 | C | | |
| 5-OH | 12.10 s | 161 | C | | C-6, C-4a, C-8 |
| 6 | 5.95 s | 95.2 | CH | | C-5, C-4a, C-8 |
| 7-OH | 10.74 s | 164.2 | C | | C-8 |
| 8 | | 106.9 | C | | |
| 8a | | 159.6 | C | | |
| 1' | | 129.1 | C | | |
| 2' | 7.16 d (2.4) | 127.7 | CH | | C-4' |
| 3' | | 127.3 | C | | |
| 4'-OH | 9.40 s | 155 | C | | C-1' |
| 5' | 6.78 d (8.2) | 114.5 | CH | H-5'-H-6' | C-4' |
| 6' | 7.11 dd (8.2, 2.4) | 125 | CH | | C-4' |
| 1'' | 3.07 d (4.6) | 21.2 | $CH_2$ | H-1''-H-2'' | C-2'', C-3'', C-8 |
| 1''' | 3.17 *d* (4.6) | 28 | $CH_2$ | | C-2'', C-3'' |
| 2'' | 5.08 t (6.6) | 122.6 | CH | H-2''-H-4'' | |
| 2''' | 5.26 t (6.4) | 122.6 | CH | | |
| 3'' | | 130.1 | C | | |
| 3''' | | 131.3 | C | | |
| 4''/4''' | 1.65 s | 17.5 | $CH_3$ | | C-5''/C-5''', C-2''/C-2''', C-3''/C-3''' |
| 5''/5''' | 1.54 s | 25.5 | $CH_3$ | | C-5''/C-5''', C-2''/C-2''', C-3''/C-3''' |

the C-5', C-2' and C-6' protons, respectively from their chemical shifts and coupling patterns.

The $^{13}$C-NMR spectrum of **1** showed 25 carbon atoms that were classified as four methyl carbons at $\delta_C$ 17.5/17.5, 25.5/25.5 (C-4"/4"" and C-5"/5""), three methylene carbons at δc 21.2, 28 (C-1"/C-1""), 41.8 (C-3), seven quaternary carbon at δc 106.9, 159.6, 101.7, 130.1, 129.1, 127.3, 131.3 (C-8, C-8a, C-4a, C-3", C-1', C-3', C-3""), seven methines at δc 78.1, 95.2, 122.6, 127.7, 125.0, 114.5, 122.6 (C-2, C-6, C-2", C-2', C-6', C-5', C-2"") and three- hydroxylated carbons at δc 161.0, 164.2, 155.0 (C-5, C-7, C-4'), with one carbonyl carbon at 196.6 using distortion less enhancement by polarization transfer (DEPT 90º and 135º) spectral analysis.

This was confirmed by the HMBC experiment; long-range correlations were observed between the following protons and carbons: 5-OH and 6, 4a, 8-C; 7-OH and 8-C; 4'-OH and 3', 1'-C. In the HMBC spectrum (**Fig.1**), the proton at $\delta_H$ 5.95 (1H, s, H-6) was correlated with C-5, 4a, 8, ($\delta_C$ 161, 101.7, 106.9), suggesting that one prenyl unit was located at C-8 and the other prenyl unit will be attached at C-3' of the ABX system of 'B' ring. The H-5' proton of Ring-B showed correlation with H-6' in $^1$H-$^1$H COSY spectrum and H-1" showed correlation with H-2" in $^1$H-$^1$H COSY spectrum. The absolute configuration at C-2 was established as S by comparing the optical rotation value with literature data of euchrestaflavanone A[15]. This is the first report of a flavanone (**1**) in a *Flemingia* species. The related 8, 3'-diprenyl-5, 7, 4'-trihydroxy flavanone (**1**) (Euchrestaflavanone A) is found in *Euchresta japonica*[15], *Sophora moorcroftian*[16], *Lupinus luteus*[17], *Euchresta formonsa*[18], *Azadirachta indica*[19]and Lespedezaflavanone B is found in *Lespedeza davidii*[20] *Glycyrrhiza glabra*[21 22].

From the above discussion the structure of **1** was concluded to be (S) - 8, 3'-diprenyl-5, 7, 4'-trihydroxy flavanone.

**8, 3'-diprenyl 5, 7, 4'-trihydroxy flavanone (1):** Green-brown with $FeCl_3$, Pink colour with Shinoda (Mg-HCl), Pale yellow crystal; m.p 155ºC; U.V $\lambda_{max}$ [MeOH, nm (log ε)]: 228 sh, 293, 339; + NaOMe, 248, 285, 333; + $AlCl_3$, 221,316, 392; + NaOAC, 287, 297, 334 (+ $H_3BO_3$); IR (KBr) $cm^{-1}$: 3300(OH), 1630 (C=O), 1000, 1500 (arom C=C), 1390, 1370 ($CH_3$). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta_H$ and $^{13}$C-NMR (400 MHz, DMSO-$d_6$) δc given in **Table 1**. MS m/z (rel.int): 409 $[M+H]^+$ $C_{25}H_{28}O_5$ (100), 408 $[M]^+$ (20).

**Genistin (2):** Green-brown with $FeCl_3$, No Pink colour with Shinoda (Mg-HCl), Yellow colour after spray with 10% MeOH-$H_2SO_4$. Properties and spectra were identical to those reported earlier[10, 11].

**β-sitosterol-D glucoside (3):** Violet ring formation with Molisch reagent. Purple colour after spray with 10% MeOH-$H_2SO_4$, obtained as white crystals. Properties and spectra were identical to those reported earlier[12].

Compound **1** and **2** were tested for its *in vitro* antimicrobial activity by measuring their MIC [9] against selected test organisms (**Table 2).** 8, 3'-diprenyl 5, 7, 4'-trihydroxy flavanone (**1**) showed the higher activity against Gram-positive, Gram-negative bacteria and fungi. Genistin (**2**) showed moderate activity against Gram-positive and Gram-negative bacteria, and fungi. The DCM and *n*-BuOH fractions of the plant also showed potent activity against these selected test organisms. Compound **(3),** already known compound and its activity have been reported in the literature[23, 24]. Vancomycin and Linezolid showed potent activity against Gram-positive bacteria in comparison to Gram-negative bacteria. Fluconazole and Itraconazole were active at MIC of>64 and >16 µg/ml respectively against fungi.

## CONCLUSION

The present study has identified the isolation and characterization of a new flavanone for the first time from the *Flemingia* species. The antimicrobial activity of compound **(1)** has shown the most significant activity against Gram-positive, Gram-negative bacteria and fungi. Compound **(2)** showed moderate activity against Gram-positive, Gram-negative bacteria and fungi. The DCM and *n*-BuOH extracts of the plant also showed potent activity against these selected test organisms. The antimicrobial activity of these compounds and extracts has not been reported earlier.

**Table 2:** Antimicrobial activities of Compound (1), (2), DCM extract and BuOH extract on selected test organism

| Test organism [a] | MIC µg/ml | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Test Compounds and Extracts[b] | | | | Standards[c] | | | |
| | Cpd. (1) | Cpd. (2) | DCM ext. | BuOH ext. | V | L | F | I |
| **Gram-positive** | | | | | | | | |
| *S. aureus* ATCC 25923 | 17 | 34 | 2.1 | 34 | 1 | 2 | | |
| *S. epidermidis* ATCC 12228 | 17 | 34 | 2.1 | 34 | 2 | 1 | | |
| *MRSA* 562 | 17 | 34 | 2.1 | 34 | 1 | 2 | | |
| **Gram-negative** | | | | | | | | |
| *P. aeruginosa* ATCC 7853 | 17 | 136 | 34 | 136 | 16 | 16 | | |
| *E. coli* ATCC 25922 | 17 | 146 | 17 | 136 | 2 | 2 | | |
| **Non-filamentous fungi** | | | | | | | | |
| *C. albicans* ATCC 1122 | 17 | 136 | 17 | 68 | | | >64 | >16 |

*Test organisms[a]: S. aureus; Staphylococcus aureus, S. epidermidis; Staphylococcus epidermidis MRSA; Methicillin resistant Staphylococcus aureus, Ps. aeruginosa; Pseudomonas aeruginosa, E. coli; Escherichia coli, C. albicans; Candida albicans*
*Test Compounds and Extracts[b]: Cpd. (1): Compound (1); Cpd. (2): Compound (2); DCM ext.: Dichloromethane extract; BuOH ext.: n-Butanol extract.*
*Standards[c]: V: Vancomycin; L: Linezolid; F: Fluconazole, I: Itraconazole*

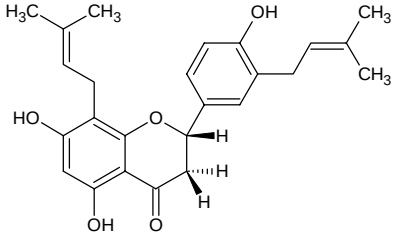


**Fig 1: Compound 1**: (8, 3'-diprenyl-5, 7, 4'-trihydroxy flavanone).

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