

Three New Pentacyclic Triterpenes and Some Flavonoids from the Fruits of an Indian Ayurvedic Plant *Dendrophthoe falcata* and Their Estrogen Receptor Binding Activity

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Extensive chromatographic screening of extracts of the fruits of the Indian Ayurvedic plant, *Dendrophthoe falcata*, resulted in the isolation of three new triterpenes, 3 β -acetoxy-1 β -(2-hydroxy-2-propoxy)-11 α -hydroxy-olean-12-ene (1), 3 β -acetoxy-11 α -ethoxy-1 β -hydroxy-olean-12-ene (2) and 3 β -acetoxy-1 β -hydroxy-11 α -methoxy-olean-12-ene (3) along with nine known compounds, 3 β -acetoxy-1 β ,11 α -dihydroxy-olean-12-ene (4), 3 β -acetoxy-1 β ,11 α -dihydroxy-urs-12-ene (5), 3 β -acetoxy-urs-12-ene-11-one (6), 3 β -acetoxy-lup-20(29)-ene (7), 30-nor-lup-3 β -acetoxy-20-one (8), (20*S*)-3 β -acetoxy-lupan-29-oic acid (9), kaempferol-3-*O*- α -L-rhamnopyranoside (10), quercetin-3-*O*- α -L-rhamnopyranoside (11), and gallic acid (12). The structures of these compounds were determined using 1D and 2D NMR and high resolution electrospray mass spectrometry. These compounds were assayed for binding to estrogen receptors- α and β and kaempferol-3-*O*- α -L-rhamnopyranoside (10) was found to be a ligand for both receptors with greater affinity for β . The triterpenes (1–9) are reported for the first time in the genus *Dendrophthoe* and assumes taxonomic significance.

Key words *Dendrophthoe falcata*; Loranthaceae; triterpene; flavonoid; estrogen receptor

Dendrophthoe falcata (LINN. f.) ETING. (Syn: *Loranthus longiflorus* DESR., *Loranthus falcatus* LINN. f.; Fam. Loranthaceae) is known as 'Vanda' in the Indian Ayurvedic System of Medicine.¹⁾ *D. falcata* is a large bushy parasitic plant that grows on a variety of host plants in deciduous forests throughout India.²⁾ The entire plant is medicinally important and is used extensively in traditional medicine as an aphrodisiac, astringent, narcotic, diuretic, and for the relief of asthma, menstrual disorders, and pulmonary tuberculosis.³⁾ It is interesting to note that the medicinal properties of this parasitic plant are greatly influenced by the host plant. For example, when grown on *Calotropis gigantea*, this parasitic plant is considered useful for improving cognitive function. However, when *D. falcata* is grown on *Tamarindus indicus*, it is used to treat impotence,⁴⁾ and the plant grown on *Shorea robusta* (Sal tree) is used to treat paralysis by the tribes of the Bihar state of India.⁵⁾ In addition to its medicinal value, the fruit of *D. falcata* tastes sweet and is consumed as a food.⁶⁾

Boonsong *et al.*⁷⁾ reported the isolation of three cardiac glycosides, viz. strosposide, odoroside F and neritaloside, from the leaves of *D. falcata* growing on *Nerium oleander*. Anjeneyulu *et al.*⁸⁾ obtained oleanolic acid, its acetate and methyl ester acetate, β -sitosterol, and stigmasterol from the stems of *D. falcata* growing on *Mangifera indica*. In addition, Indrani *et al.*⁹⁾ reported (+)-catechin, leucocyanidin, gallic acid, ellagic acid, and chebulinic acid from the leaves and bark of *D. falcata* growing on *Terminalia tomentosa*. In a comparative evaluation of this parasitic plant grown on various host plants, Nair *et al.*¹⁰⁾ reported the accumulation of kaempferol, quercetin, myricetin, and their glycosides. To the best of our knowledge, the fruits of *D. falcata* have not yet been chemically examined. In view of the above, we have taken up its detailed chemical screening and herein report the isolation of nine taxonomically significant triterpenes from the *n*-hexane extract and three phenolic metabolites from the

methanolic extract of the fruits of *D. falcata* growing on *Shorea robusta* (Sal tree) (Chart 1). Among the triterpenes, three are new compounds (1–3).

Results and Discussion

Compound 1 was obtained as a colorless oil. Its molecular formula was determined as C₃₅H₅₈O₅ by the presence of sodiated mass at *m/z* 581.4157 (Calcd 581.4182) using HR-EMS. The presence of an acetoxy group was indicated by the peaks at 1732 cm⁻¹ (IR), δ 2.16 (¹H) and δ 20.5 and δ 171.0 (¹³C) (Table 1).¹¹⁾ The ¹³C and DEPT spectra showed the presence of 35 carbons comprising 11 methyl, 8 methylene, 3

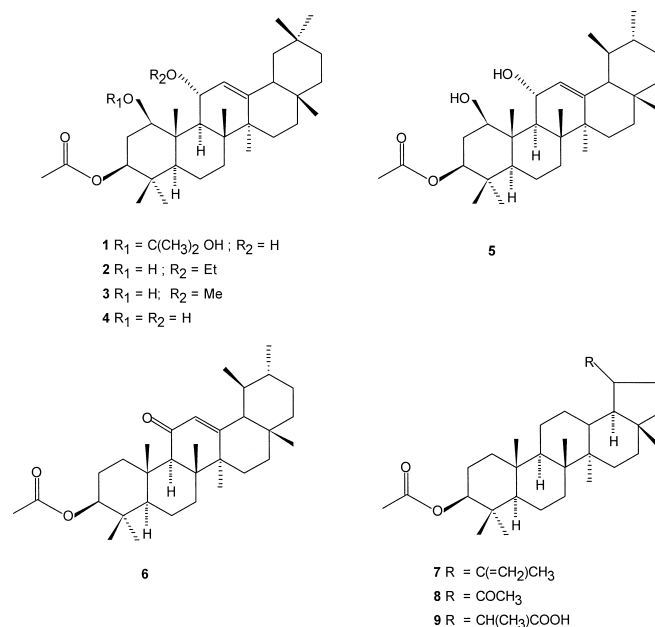


Chart 1. Structures of the Compounds Isolated from *D. falcata*

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methine, 1 olefinic methine, 3 oxymethine, 7 quaternary carbons (one oxygenated), 1 carboxyl carbon and 1 fully substituted olefinic carbon. Valence bond calculations reveals that there are 7 degrees of unsaturation present in the compound, which, can be accounted for 1 acetoxy carbonyl, 1 double bond and 5 ring systems. Detailed 1D and 2D NMR (^1H , ^{13}C , ^1H - ^1H , HMBC, HMQC) analysis of compound **1** and comparison with literature data¹²⁾ suggested that it is an oleanane type pentacyclic triterpene.

The HMQC experiments revealed the presence of an olefinic system between C_{12} - C_{13} , a β -acetoxy at C_3 , two oxygenated functionalities at $\text{C}1(\beta)$ and $\text{C}11(\alpha)$ and a doubly oxygenated quaternary carbon with two methyl groups.^{13,14)} Loss of acetone from sodiated mass (m/z 523) and acetone and water from the protonated mass (m/z 483) reveals that an isopropoxy substituent is present in compound **1**.

As compound **1** contains only two hydroxyl groups and the substitutions at all carbons were known except for C-1 and C-11, one hydroxyl group must be located on either C-1 or C-11, and the other must be on an isopropoxy substituent bound to the oleanane skeleton through an ether oxygen. This assignment was supported by the low field chemical shift of the quaternary carbon (δ 100.0) in the ^{13}C -NMR. Based on the above data, the side substitution was determined to be a 2-hydroxy-2-propoxy group, and its position at C-1 was then confirmed on the basis of HMBC and COSY correlations with its neighbors. In the HMBC spectrum, the quaternary di-oxygenated carbon (δ 100.0) showed correlations with H-1 (δ 3.80) and the methyl group singlets at δ 1.41 and 1.42, respectively, which correlated with carbons at δ 25.2 and 24.8, respectively, in the HMQC spectrum (Fig. 1). Thus, compound **1** was identified as 3β -acetoxy- 1β -(2-hydroxy-2-propoxy)- 11α -hydroxy-olean-12-ene.

Compound **2** was isolated as a colorless solid. The IR spectrum indicated the presence of hydroxyl (3346 cm^{-1}) and carbonyl (1721 cm^{-1}) functional groups. The molecular formula of **2** was determined to be $\text{C}_{34}\text{H}_{56}\text{O}_4$ using exact mass measurement with high resolution electrospray mass spectrometry. Specifically, the $[\text{M}+\text{Na}]^+$ ion was detected at m/z 551.4075 (Calcd m/z 551.4076). Fragment ions were observed in the HR-MS at m/z 505.3691 ($\text{C}_{32}\text{H}_{50}\text{O}_3\text{Na}$), 483.3893 ($\text{C}_{32}\text{H}_{51}\text{O}_3$), 465.3697 ($\text{C}_{32}\text{H}_{49}\text{O}_3\text{Na}$), and 423.3659 ($\text{C}_{30}\text{H}_{47}\text{O}$), which corresponded to $[\text{M}+\text{Na}-\text{C}_2\text{H}_6\text{O}]^+$, $[\text{MH}-\text{C}_2\text{H}_6\text{O}]^+$, $[\text{MH}-\text{C}_2\text{H}_6\text{O}-\text{H}_2\text{O}]^+$, and $[\text{MH}-\text{C}_2\text{H}_6\text{O}-\text{HOAc}]^+$, respectively. Using a low resolution mass spectrometer, one additional fragment ion was observed at m/z 405 (see the Experimental) which probably corresponded to loss of ethanol, acetic acid, and water from the protonated molecule of **2**. The ^1H - and ^{13}C -NMR spectral data for compound **2** were similar to those of **1** (Table 1) with the exception that the signals for the 2-hydroxy-2-propoxy group were missing, and signals for an ethoxy group were detected instead. In the ^1H -NMR spectrum signals were detected at δ 1.15, 3.66 and 3.50, which corresponded to the carbons at δ 15.7 and δ 59.3 in the HMQC experiment. In the ^{13}C -NMR spectrum of compound **2**, the chemical shifts of the carbons C-9, C-11 and C-12 were observed at δ 50.6, 74.0 and 123.0 ppm, which shifted -3.7 , $+3.8$ and -1.5 ppm, respectively, compared with the corresponding signals of 3β -acetoxy- 1β , 11α -dihydroxy-olean-12-ene.¹³⁾ This indicated that the ethoxy group was located at C-11. This assignment was

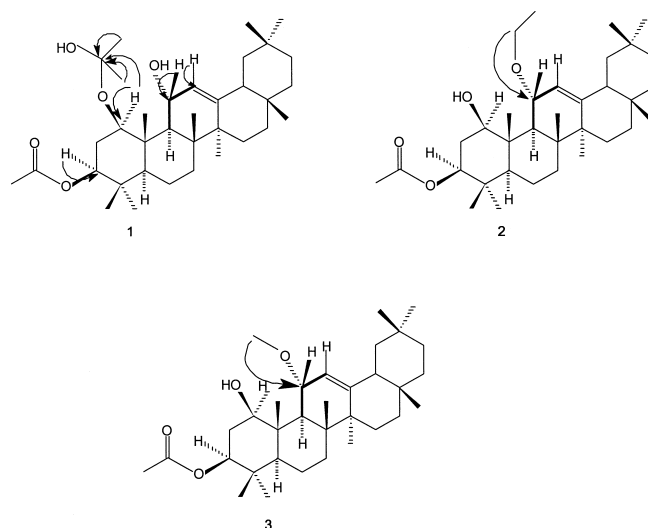


Fig. 1. Selected HMBC/HMQC (\rightarrow) and COSY (\dashrightarrow) Correlations of Compounds **1**—**3**

also supported by the cross correlation in the HMBC spectrum of the methylene proton peaks at δ 3.66 and δ 3.50 with the C-11 carbon at δ 74.0 and the methyl carbon (δ 15.7) of the ethoxy group in the HMBC spectrum (Fig. 1). Therefore, the structure of compound **2** was determined to be 3β -acetoxy- 11α -ethoxy- 1β -hydroxy-olean-12-ene.

Compound **3** was isolated as colorless shining needles. The IR spectrum indicated the presence of a hydroxy group (3346 cm^{-1}) and a carbonyl group (1732 cm^{-1}). The molecular formula of **3** was determined to be $\text{C}_{33}\text{H}_{54}\text{O}_4$ by HR-MS. Specifically, the $[\text{M}+\text{Na}]^+$ ion of **3** was measured at m/z 537.3892 (Calcd m/z 537.3920). In addition, fragment ions were observed at m/z 505.3691 ($\text{C}_{32}\text{H}_{50}\text{O}_3\text{Na}$), 483.3803 ($\text{C}_{32}\text{H}_{50}\text{O}_3$), 465.3785 ($\text{C}_{32}\text{H}_{49}\text{O}$), 423.3659 ($\text{C}_{30}\text{H}_{47}\text{O}$), and 405.3533 ($\text{C}_{30}\text{H}_{45}$). These fragment ions corresponded to $[\text{M}+\text{Na}-\text{CH}_4\text{O}]^+$, $[\text{MH}-\text{CH}_4\text{O}]^+$, $[\text{MH}-\text{CH}_4\text{O}-\text{H}_2\text{O}]^-$, $[\text{MH}-\text{CH}_4\text{O}-\text{HOAc}]^+$, and $[\text{MH}-\text{CH}_4\text{O}-\text{HOAc}-\text{H}_2\text{O}]^+$, respectively. The ^1H - and ^{13}C -NMR spectra of compound **3** were similar to those of **2** (Table 1) with the exception that the signals for the 11-ethoxy group were absent. Instead, signals for a methoxy group were detected at δ 3.28 (3H, s) in the ^1H -NMR spectrum, which corresponded to the carbon at δ 51.4 in the HMQC experiment. In the ^{13}C -NMR spectrum of compound **3** the chemical shifts of carbons C-9, C-11 and C-12 were observed at δ 49.7, 74.1 and 121.5 ppm, respectively, which were shifted -4.6 , $+3.9$ and -3.5 ppm compared to the corresponding signals of 3α -acetoxy- 1β , 11α -dihydroxy-urs-12-ene and 3β -acetoxy- 1β , 11α -dihydroxy-olean-12-ene.¹³⁾ This indicated that the methoxy group of **3** was located at C-11. This assignment was supported further by the methyl proton signals at δ 3.28 that cross correlated with the carbon at C-11 at δ 74.1 in the HMBC spectrum (Fig. 1). Therefore, compound **3** was identified as 3β -acetoxy- 1β -hydroxy- 11α -methoxy-olean-12-ene.

Compounds **1**—**3** are new with a rare C-1 and C-11 dioxygenation functionalities. It is noteworthy to mention here that recently five 11α -ethoxy, 11α -methoxy and 11α -hydroperoxy 3β -acetoxy-urs and olean-12-enes were reported from a plant species, *Ficus microcarpa*.¹⁴⁾ However, these compounds lack the additional oxygen functionality at C-1.

The other known compounds 3 β -acetoxy-1 β ,11 α -dihydroxy-olean-12-ene (**4**),¹⁵ 3 β -acetoxy-1 β ,11 α -dihydroxy-urs-12-ene (**5**),¹³ 3 β -acetoxy-urs-12-ene-11-one (**6**),¹⁶ 3 β -acetoxy-lup-20(29)-ene (**7**),¹⁷ 30-nor-lup-3 β -acetoxy-20-one (**8**),¹⁸ (20*S*)-3 β -acetoxy-lupan-29-oic acid (**9**),¹² kaempferol-3-*O*- α -L-rhamnopyranoside (**10**), quercetin-3-*O*- α -L-rhamnopyranoside (**11**), and gallic acid (**12**) were also isolated from the fruits of *D. falcata* (Chart 1) and identified using high resolution mass spectrometry and 1D and 2D NMR. ¹H, ¹³C, DEPT, HMQC, and HMBC NMR analyses were carried out to confirm the structures of these compounds. To the best of our knowledge, this is the first report of the occurrence of the triterpenes **1**–**9** in the genus *Dendrophthoe* and assumes taxonomic significance. It is noteworthy to mention here that the host plant, *Shorea robusta* reported to accumulate pentacyclic triterpenes (resin)^{19,20} and phenolics, phenolic acids and flavonoids (heart wood and seeds).^{21–23} Interestingly all these three classes of compounds were now found in *D. falcata* fruits.

As the pentacyclic triterpenes and flavonoids were tested earlier for Estrogen Receptor binding activities,²⁴ all the isolated compounds were screened for ER binding activities using the recently developed ultra filtration LC-MS methodology.²⁵ Hence, compounds **1**–**12** were assayed for binding to estrogen receptor- α (ER- α) and ER- β based on competitive binding with radio labeled estradiol.²⁶ Among these compounds, kaempferol-3-*O*- α -L-rhamnopyranoside (**10**), was found to be a weak ligand for the estrogen receptor *in vitro* with a greater affinity for estrogen receptor- β .

Experimental

General Melting points were determined using a standard capillary melting point apparatus and are uncorrected. The optical rotations were measured using a JASCO (Tokyo, Japan) P-1020 digital polarimeter, and IR spectra were recorded on a JASCO FTIR-5300 spectrometer. NMR spectra were measured using Bruker (Rheinstetten, Germany) Avance 360 and DPX-300 spectrometers. The chemical shift values are reported in parts per million (ppm) relative to the internal standard TMS, and the coupling constants are in Hz (in parentheses). For the NMR spectra, multiplicities were determined by DEPT experiments (DEPT 90 and DEPT 135). Positive ion electrospray low and high resolution mass spectra were obtained using a Micromass (Manchester, U.K.) Quattro II triple quadrupole spectrometer and a Q-TOF-2 hybrid mass spectrometer, respectively. Samples dissolved in methanol/water/formic acid (50:49:1) were infused into the mass spectrometer at 20 μ l/min at an ion source temperature of 140 °C.

Plant Material The fruits of *D. falcata* growing on *Shorea robusta* (Sal tree) were collected from the Baliguda forest (20°07'N, 83°05'E) of Orissa, India, during April, 1999. Voucher specimens (010499) were deposited at the Traditional and Herbal Drugs Department, Regional Research Laboratory, Bhubaneswar, India.

Extraction and Isolation The shade dried and powdered fruits (1.0 kg) of *D. falcata* were extracted successively with *n*-hexane (31 \times 12) and then methanol (31 \times 12) using a soxhlet extractor. The solvent was removed under vacuum to yield the respective *n*-hexane (190 g) and methanol (110 g) extracts. A portion of the *n*-hexane extract (100 g) was chromatographed over a silica gel column (100–200 mesh, 95 \times 7.5 cm) with a solvent gradient from *n*-hexane to ethyl acetate to yield six major fractions: A (*n*-hexane/EtOAc, 97:3), B (*n*-hexane/EtOAc, 92.5:7.5), C (*n*-hexane/EtOAc, 80:20), D and E (*n*-hexane/EtOAc, 65:35), and F (*n*-hexane/EtOAc, 1:1).

Fractions D (0.3 g) and F (6.8 g) on repetitive column chromatographic purifications afforded compound **1** (4 mg) and compounds **2** (5 mg), **3** (12 mg), **4** (7 mg) and **5** (22 mg) respectively. Similarly, fractions B (1.2 g), A (7.6 g) and C (2.8 g) were also individually purified to yield compound **6** (16 mg), **7** (190 mg) and **8** (42 mg) respectively. However, fraction E (0.65 g) on re-crystallization afforded compound **9** (150 mg).

The methanol extract (85 g) was rechromatographed on a smaller silica gel column (85 \times 6 cm) using a gradient solvent system from 100% chloroform to 100% methanol. Three known phenolic compounds were isolated,

10 (30 mg), **11** (120 mg) and **12** (99 mg).

3 β -Acetoxy-1 β -(2-hydroxy-2-propoxy)-11 α -hydroxy-olean-12-ene (**1**): Colorless oil. $[\alpha]_D^{25} +301^\circ$ ($c=0.1$, CHCl₃). IR (neat, cm⁻¹) λ_{max} : 3325 (–OH), 1732 (C=O, acetate), 1244 (C–O, acetate), 1078 (ether). ¹H- and ¹³C-NMR, see Table 1. Positive ion electrospray MS m/z (%): 581.4157 [M+Na]⁺ (Calcd 581.4182, 10), 563.4050 [M+Na–H₂O]⁺ (20), 541.4276 [MH–H₂O]⁺ (10), 523.3738 [M+Na–acetone]⁺ (5), 483.3893 [MH–acetone–H₂O]⁺ (100), 465.3697 [MH–acetone–2H₂O]⁺ (30).

3 β -Acetoxy-11 α -ethoxy-1 β -hydroxy-olean-12-ene (**2**): Colorless solid. mp 182–184 °C. $[\alpha]_D^{25} +224^\circ$ ($c=0.1$, CHCl₃). IR (KBr, cm⁻¹) λ_{max} : 3346 (–OH), 1721 (C=O, acetate), 1248 (C–O, acetate), 1061 (ether). The ¹H- and ¹³C-NMR, see Table 1. Positive ion electrospray MS m/z (%): 551.4075 [M+Na]⁺ (20), 505.3691 [M+Na–EtOH]⁺ (5), 483.3893 [MH–EtOH]⁺ (100), 465.3697 [MH–EtOH–H₂O]⁺ (15), 423.3659 [MH–EtOH–HOAc]⁺ (45).

3 β -Acetoxy-1 β -hydroxy-11 α -methoxy-olean-12-ene (**3**): Colorless shining needles. mp 228 °C. $[\alpha]_D^{25} +212.7^\circ$ ($c=0.25$, CHCl₃). IR (KBr, cm⁻¹) λ_{max} : 3346 (–OH), 1732 (C=O, acetate), 1248 (C–O, acetate), 1049 (ether). ¹H- and ¹³C-NMR, see Table 1. Positive ion electrospray MS m/z (%): 537.3892 [M+Na]⁺ (10), 505.3691 [M+Na–MeOH]⁺ (15), 483.3803 [MH–MeOH]⁺ (100), 465.3785 [MH–MeOH–H₂O]⁺ (20), 423.3659 [MH–MeOH–HOAc]⁺ (45), 405.3533 [MH–MeOH–HOAc–H₂O]⁺ (15).

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