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Pharmacologically Active C-19 Diterpenoid Alkaloids from the Aerial parts of *Aconitum laeve* Royle

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Abstract: Two new lycoctonine type C19-diterpenoid alkaloids, swatinine-A (**1**), and swatinine-B (**2**), along with four known C19-diterpenoid alkaloids, foresticine (**3**), neoline (**4**), delvestine (**5**), and chasmanine (**6**), were isolated from the aerial parts of the *Aconitum laeve* Royle. The structures of compounds **1** and **2** were deduced on the basis of spectroscopic techniques (EI-MS, HREI-MS, ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC). Biological activities like inhibition of the multifunctional copper-containing enzyme tyrosinase, anti-oxidant and anti-inflammatory have also been carried out for all the compounds and reported herein. The structure-activity relationships for the inhibition of the enzyme tyrosinase by the compounds have been discussed.

Keywords: *Aconitum laeve* Royle; C19-diterpenoids alkaloids chemistry; swatinine-A; swatinine-B; antioxidant; anti-inflammatory; tyrosinase inhibitors.

1. Introduction

The genus *Aconitum* is a rich source of diterpenoid alkaloids, many of which exhibited a broad spectrum of biological activities. Lappaconitine hydrobromide has been used as an antiarrhythmic drug [1]. The methyllycaconitine perchlorate is used in curaremimetic preparation [2]. Some aconitine and mesaconitine derivatives possess potent analgesic and anti-inflammatory activities [3]. The methyllycaconitine and lycaconitine exhibited neuronal nicotinic acetylcholine receptor

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affinity [4]. Lycaconitine, obtained from several *Aconitum* species, was found to be effective against multi-drug resistance cancers. *Aconitum* plants are widely used in Chinese and Indian traditional systems of medicine [5-6]. Turkish *Aconitum* species are used externally in the treatment of rheumatic pain and sciatica and also against body lice [7-8]. We have reported many diterpenoid and norditerpenoid alkaloids from *Aconitum* and *Delphinium* species [9-12].

Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a multifunctional copper-containing enzyme widely distributed in plants and animals. Tyrosinase inhibitors may therefore be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation and also important in cosmetics for whitening and depigmentation after sunburn [13].

Free radicals play an important role in carcinogenesis through their involvement in breaking of DNA strands [14]. They are known to be involved in inflammation processes, cardiovascular disease [15-17], rheumatoid arthritis, neurodegenerative disease and the aging process [18-19]. The harmful actions of free radicals can be blocked by anti-oxidants. During this study, the diterpenoid alkaloids isolated from *A. laeve* have been tested for their anti-oxidant activities.

Neutrophils are essential elements for the hosts defense. The uncontrolled release of reactive oxygen species (ROS) is suspected to be responsible for certain pathological conditions such as heart attacks, septic shocks, rheumatoid arthritis and ischemia reperfusion injury [20]. In these cases the administration of agents that can decrease the accumulation of neutrophils in the inflamed area might be a remedy for these conditions. A cell-based *in vitro* bioassay [21] has been used in this study to examine the anti-inflammatory activities of the compounds isolated from *A. laeve*.

In the continuation of our study on *A. laeve* Royle, we report herein the isolation and structure determination of the two new C19-diterpenoid alkaloids, swatinine-A **(1)** and swatinine-B **(2),** along with four known C19-diterpenoid alkaloids, foresticine (**3**) [22], neoline (**4**) [23], delvestine (**5**) [24], and chasmanine (**6**) [25]. In the present study inhibitory potentials of these C19-diterpenoid alkaloids against the multifunctional copper-containing enzyme tyrosinase, their anti-oxidant and the anti-inflammatory activities have been performed and reported. The structure-activity relationships (SARs) for the inhibition of the enzyme tyrosinase by the compounds have been discussed.

2. Materials and Methods

2.1. Equipments

Optical rotations were measured on a JASCO DIP 360 polarimeter. IR spectra were recorded on a JASCO 302-A spectrophotometer. EI-MS and HREI-MS were recorded on Jeol JMS HX 110 with data system and on JMS-DA 500 mass spectrometers. The ^{1}H- and ^{13}C-NMR spectrum were recorded on Bruker NMR spectrometers, operating at 500 and 400 MHz (100 and 125 MHz for ^{13}C). The chemical shifts values are reported in ppm (δ) units and the coupling constants (*J*) are given in Hz.

2.2. Chromatographic conditions

For thin layer chromatography (TLC) precoated aluminium sheets (silica gel G-60F-254, E. Merck) were used. Visualization of the TLC plates was achieved under ultra-violet (UV) at 254 and 366 nm and by spraying with Dragendor's reagent. Solvent system *n*-hexane-acetone-diethylamine (7:3:5 drops) was used.

2.3. Plant material

The aerial parts, (5 kg dry wt) of *A. laeve* Royle were collected from Loweri top, near Ziarat village of the Chitral district of Pakistan at an elevation of 1200 m in August 2007 and was identified by Prof. Dr. Habib Ahmad, Department of Genetics, University of Hazara, Mansehra, Khyber Pakhtunkhwa, Pakistan**.** A voucher specimen (RA-01) has been deposited at the department herbarium.

2.4. Extraction and Isolation

The air-dried powdered plant material (5 kg) was first exhaustively extracted with *n*-hexane and 11.5 g gummy residue was obtained. The remaining plant material was extracted with 90% ethanol. The ethanolic extract was concentrated and acidified with 0.5 *N* H_2SO_4 and extracted with $CHCl_3$. The acidic aqueous solution was basified with 10% KOH solution and extracted with $CHCl_3$ to obtain 10.3 g of a crude alkaloidal mixture. This crude alkaloidal mixture was fractionated by vacuum liquid chromatography (VLC) using a column packed with basic alumina (200 g Al_2O_3 EM 1085) and eluted with petroleum ether, and gradients of CH_2Cl_2 and MeOH. A fraction obtained on elution with 2% MeOH–CH_2Cl_2 was fractionated further by silica gel column chromatography, using an isocratic solvent system of 5% acetone in n-hexane with 5-drops of diethyl amine per 100 mL. Fractions 5–14 (0.6 g) were combined and again subjected to column chromatography to obtain foresticine (3) (13 mg), neoline (4) (10 mg) and delvestine (5) (14.5 mg), Fractions 15–30 (1 g) contained swatinine-A (1) (5 mg), swatinine-B (2) (7 mg) and chasmanine (6) (15.6 mg). The known compounds were identified by comparing their spectral data and physical properties with the reported values.

Swatinine-A **(1)** white amorphous powder (5 mg); melting point 145-147^o C; $[\alpha]^{30}_D$ + 63.0^o (*c* = 1.0, $CHCl_3$); IR ν_{max} cm^{-1} ($CHCl_3$): 3429 (OH), 1115 and 1087 (C-O), 864 and 1006 (inner ether); ^1H, ^{13}C-NMR δ (see Table-1); EIMS *m/z* 465, HREI-MS *m/z* 465.5802 ($C_{25}H_{39}NO_7$, calcd. 465.5814)

Swatinine-B **(2)** white amorphous powder (7 mg); melting point 173-177^o C; $[\alpha]^{30}_D$ + 28.3^o (*c* = 1.0, CH_3OH); IR ν_{max} cm^{-1} ($CHCl_3$): 3462 (OH), 3355 (NH), 1695 (C = O), 1620 and 1595 (C = C), 1080 (simple ether); ^1H, ^{13}C-NMR (see Table-1); EIMS *m/z* 586, HREI-MS *m/z* 586.71631 ($C_{25}H_{39}NO_7$, calcd. 586.71643)

2.5. Tyrosinase inhibition assay

Tyrosinase inhibition assay was performed in a 96-well microplate format using a SPECTRAmax™ 340 (Molecular Devices, CA, USA) microplate reader according to the method developed by Hearing [26]. Briefly, first the compounds were screened for the *o*-diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All the active compounds from the preliminary screening were being subjected to IC_{50} studies. Compounds were dissolved in methanol to a concentration of 2.5%. Thirty units of mushroom tyrosinase (28 nM from Sigma Chemical Co., USA) was first pre-incubated with the test compounds in 50 nM Na-phosphate buffer (pH 6.8) for 10 min at 25 °C. Then the L-DOPA (0.5 mM) was then added to the reaction mixture and the enzyme reaction was being monitored by measuring the change in absorbance at 475 nm (at 37 °C) due to the formation of the DOPAchrome for 10 min. The percent inhibitions of the enzyme have been calculated as follows, by using MS Excel®™ 2000 (Microsoft Corp., USA) based program developed for this purpose:

$$\text{Percent inhibition} = [B - S/B] \times 100$$

Here the *B* and *S* are the absorbances for the blank and samples, respectively. After screening of the compounds, median inhibitory concentrations (IC_{50}) were also calculated. All the studies have been carried out at least in triplicates and the results represents the mean ± S.E.M. (standard error of the mean). Kojic acid and L-mimosine were used as standard inhibitors for the tyrosinase and have been purchased from Sigma Chem. Co., USA.

2.6. Anti-oxidant assay: DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging activity

The reaction mixture containing 5 µL of test sample (1 mM in DMSO) and 95 µL of DPPH (Sigma, 300 µM) in ethanol was taken in a 96-well microtiter plate and incubated in the plate reader SPECTRAmax™ 340 (Molecular Device, CA, USA) at 37 °C for 30 min. The absorbances were being measured at 515 nm. Percent radical scavenging activity was determined by comparison with a DMSO containing control (shown in Table 3). The values of IC_{50} represent concentrations of compounds to

scavenge 50% of DPPH radicals. The 3-*t*-butyl-4-hydroxyanisole (BHA) has been used as a positive control. All the chemicals used were of analytical grade (Sigma, USA).

2.7. Anti-inflammatory assay

Heparinized fresh venous blood was being drawn from healthy volunteers in a local blood bank and neutrophils were then isolated by the method of Siddiqui et al. 1995 and Daeseok et al., 1995 [27-28]. Briefly whole blood was mixed with Ficoll or Dextran (6%) with the ratio of 1:3 and allowed to settle down. Buffy coat was collected and a layer on the bed of the Ficoll (3 mL) was centrifuged at 1500 rpm for 30 min. The pellets were collected and washed with PBS Buffer (pH 7.4). The RBCs were lysed with ammonium chloride solution and then centrifuged. The pellets were then washed with PBS and then resuspended with the same buffer at the concentration of 1×10^6 cells/mL.

To determine anti-inflammatory activity of a compound, the modified assay of Berridge et al. was used [21]. This *in vitro* assay is based on the reduction of a highly water soluble tetrazolium salt WST-1 in presence of activated neutrophils. Anti-inflammatory activity was determined in a total volume of 200 µL PBS (pH 7.4) containing $0.5–1.0 \times 10^4$ neutrophils/mL, 750 µM WST-1, and various concentrations of test compounds. The control only contained buffer, neutrophils and WST-1. All compounds were equilibrated at 37 °C for 10 min and the reaction was then initiated by adding Zymosan Activated Serum (ZAS), prepared as described previously [28]. Indomethacin has been used as positive control.

Absorbances were measured at 450 nm using a SPECTRAmax™ 340 (Molecular Device, CA, USA) microplate reader for 30 min. Each value is the mean of reactions in six wells for a single compound placed in a 96-well plate. The IC_{50} was calculated by comparing with DMSO as a blank and expressed as % inhibition of the superoxide produced.

3. Results and Discussion

3.1. Structure elucidation

Swatinine-A **(1)** was isolated from the chloroform fraction of *A. laeve* Royle, as a white amorphous powder. The mass spectrum (MS) of the compound **1** is characteristic of those alkaloids with a lycoctonine skeleton [29]. The compound **1** was assigned the formula $C_{25}H_{39}NO_7$ on the basis of ion peak at *m/z* 465.5802 $[M+1]^+$ (calcd. 465.5814) in high resolution electronic ionization mass spectrometry (HREI-MS), and nuclear magnetic resonance (NMR) spectral data. The infrared (IR) spectrum of compound **1** showed absorption bands at 3429 (OH), 1115 and 1087 (C-O), 864 and 1006 cm^{-1} (inner ether) [30-31].

The ^{1}H-NMR spectrum of swatinine-A (**1**) (Table-1) exhibited signals for *N*-ethyl, four methoxy groups, and several methine protons geminal oxygen substituents. The overall spectral data of compound **1** was similar to that of 18-methoxygadesine [30], except the presence of a methoxy group instead of a hydroxyl group at C-14. In the down field region of the spectrum, a triplet of one proton integration at δ 3.57 (J = 4.7 Hz) was observed which is characteristic for H-14 methine proton. In the upfield region, a triplet of three proton integration at δ 1.01 (J = 7.1 Hz) was assigned to the methyl of *N*-ethyl. Two singlets each of one proton integration at δ 3.82 and 4.04 were assigned to the H-6 and H-19 methine protons. The signals appeared at δ 3.41, 3.38, 3.31 and 3.22 were assigned to the methoxy groups present in the molecule of the compound **1**.

The ^{13}C-NMR spectrum (Broad Band decoupling (BB), Distorsionless Enhancement by Polarization Transfer (DEPT)) (Table-2) showed twenty five signals, including four methoxy, one methyl, six methylene, ten methine, and four quaternary carbons. The ^{1}H-^{13}C correlations were determined by hetero nuclear multiple quantum coherence (HMQC) spectrum, while the long range ^{1}H-^{13}C connectivities were obtained through the hetero nuclear multiple bond connectivity (HMBC) technique (Figure 1). In the HMBC spectrum, the C-6 methine proton (δ 3.82) showed correlations with C-5 (δ 45.8), C-6 (δ 90.6), C-7 (δ 84.2) and C-11 (δ 51.9). The C-5 methine proton showed correlations with C-6 (δ 90.6), C-7 (δ 84.2), C-18 (δ 69.6), C-11 (δ 51.9), and C-4 (δ 38.1). The C-1 methine (δ 3.61) protons showed correlations with the C-1 (δ 83.6), C-2 (δ 18.4) and C-11 (δ 51.9). Similarly C-14 methine proton showed correlations with C-13 (δ 43.7), C-9 (δ 45.1), and C-14 (δ

83.9). Furthermore, C-19 methine proton showed HMBC interactions with C-4 (δ 38.1), C-5 (δ 45.8), and C-3 (δ 31.0). Thus, the structure of compound **1** was deduced as 14,18-dimethoxygadesine (swatinine-A).

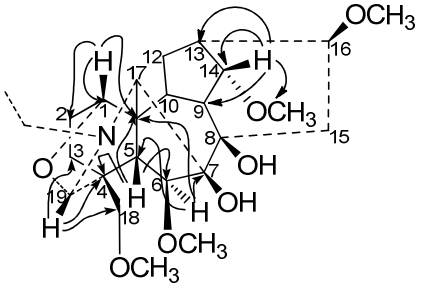

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Figure 1. Selected HMBC correlations of compound **1**.

Swatinine-B (**2**) was isolated from the chloroform fraction of *A. laeve* Royle, as a white amorphous powder, had a molecular formula $C_{32}H_{46}N_2O_8$ (*m/z* 586.71631, calcd. 586.71643) in the HREI-MS, and NMR spectral data. The IR spectrum of compound **2** exhibited absorption bands at 3462 (OH), 3355 (NH_2), 1695 (C = O), 1620, 1595 (C = C) and 1080 cm^{-1} (simple ether bonds). The mass fragmentation of compound **2** was characteristic of alkaloids with a lycoctonine skeleton. The base peak was that of the (M^+ -31) ion which is indicative of the presence of an *α*-methoxy group at C-1 [29].

The ^{1}H-NMR spectrum of swatinine-B (**2**) (Table 1) exhibited signals for *N*-ethyl, four methoxy groups, and several methine protons geminal oxygen substituents. The overall spectral data of compound **2** was similar to that of anthranoyllycoctonine [32], except the presence of an *α*-methoxy group instead of *β* methoxy group at C-1.

The ^{13}C-NMR spectrum (BB, DEPT)) (Table-2) of compound **2** in chloroform exhibited thirty two signals, including four methoxy, one methyl, seven methylene, thirteen methine, and seven quaternary carbons, showed a closed resemblance to that of anthranoyllycoctonine [32], except the signal at C_1 and C_3. The upfield shift of C_1 at δ 77.2 and C_3 at δ 28.7 ppm supports the presence of a methoxy group at C_1 in the *α*-configuration. Norditerpenoid alkaloids with C_1-*β*-methoxy group exhibited a sharp increase in the intensity of the M^+ -15 peak as compared with their *α*-counterpart [33-35]. Compound **2,** with *α*- methoxy group, exhibited a distinct M^+ -15 peak and a very weak peak in the chemical ionization mode due to the loss of one methoxy group from the base peak (*m/z* 421).

On the basis of these data, swatinine-B has been suggested as a C_1-epimer of anthranoyllycoctonine and structure **2** has been assigned. The ^{1}H-^{13}C correlations were determined by HMQC spectrum, while the long range ^{1}H-^{13}C connectivities have also been obtained through the HMBC technique (Figure 2). Thus, the structure of compound **2** was deduced as *α*-methoxyanthranoyllycoctonine (swatinine-B).

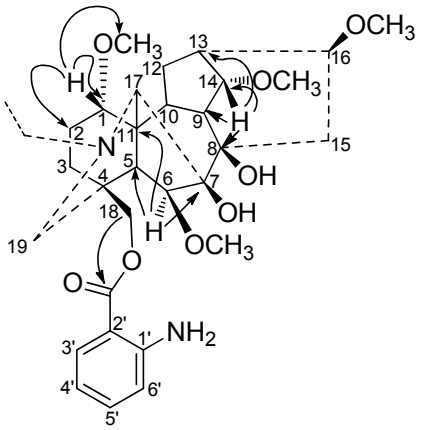

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Figure 2. Selected HMBC correlations of Compound **2**.

Table 1. ^{1}H NMR data of Compounds **1** and **2** (at 400 MHz in $CDCl_3$, δ in ppm, J in Hz)

Position (H)	**1**	**2**
1	3.61, brd. d, J = 11.14	3.21, t, J = 11.41
2	1.88, m	2.09, m
3	1.65, brd. s	1.75, m
4	-	-
5	1.89, s	2.15, brd, s
6	3.82, s	3.88, brd, s
7	-	-
8	-	-
9	2.42, 2.39, dd, J = 4.47	2.37, t, J = 3.89
10	2.56, m	2.31, m
11	-	-
12	2.21, m	2.58, m
13	2.29, m	3.05, m
14	3.57, t, J = 4.7	3.57, t, J = 4.5
15	2.21, m	1.64, m
16	3.05, t, J = 5.14	3.19, t, J = 4.98
17	2.89, s	2.92, brd, s
18	3.18, brd, s	4.09, brd, s
19	4.04, s	1.74, brd, s
$N-CH_2$	2.77, m	2.54, m
CH_3	1.01, t, J = 7.1	1.05, t, J = 7.2
OCH_3	3.41, s	3.37, s
OCH_3	3.38, s	3.34, s
OCH_3	3.31, s	3.31, s
OCH_3	3.22, s	3.23, s
1′	-	-
2′	-	-
3′	-	6.66, m
4′	-	7.25, m
5′	-	6.61, m
6′	-	7.77, m
C = O	-	-

*The ^{1}H- connectivities were deduced according to HMQC.

Besides two new compounds, we have isolated four known compounds from the same plant. These are foresticine, ($C_{24}H_{39}NO_6$) (**3**) [22], previously reported from *A. forestii,* neoline ($C_{24}H_{39}NO_6$) (**4**) [23], previously reported from *A. nagarum,* delvestine ($C_{32}H_{46}N_2O_8$) (**5**) [24], previously isolated from *Delphinium vestitum,* and chasmanine ($C_{25}H_{41}NO_6$) (**6**) [25], previously isolated from *A. chasmanthum.*

Tyrosinase inhibition studies on all alkaloids (**1**–**6**) have been conducted. The compounds **1**, **2**, **5** and **6** exhibited mild inhibition against the enzyme (shown in Table 3). The compounds swatinine-A

(**1**), swatinine-B (**2**), delvestine (**5**) and chasmanine (**6**) were found to be active against the enzyme tyrosinase having IC_{50} values of 115.17, 83.28, 96.33 and 137.21 μM, respectively, whereas the reference inhibitors L-mimosine and kojic acid showed potent inhibition against tyrosinase having IC_{50} values of 3.68 and 16.67 μM, respectively.

Table 2. ^{13}C NMR data of Compounds **1** and **2** (in $CDCl_3$ at 100 MHz, δ in ppm)

Position (C)	**1**	**2**
1	83.6 (CH)	77.2 (CH)
2	18.4 (CH_2)	26.3 (CH_2)
3	31.0 (CH_2)	28.7 (CH_2)
4	38.1 (C)	37.5 (C)
5	45.8 (CH)	46.1 (CH)
6	90.6 (CH)	90.8 (CH)
7	84.2 (C)	88.4 (C)
8	80.4 (C)	77.6 (C)
9	45.1 (CH)	50.3 (CH)
10	38.1 (CH)	38.2 (CH)
11	51.9 (C)	49.1 (C)
12	28.7 (CH_2)	32.2 (CH_2)
13	43.7 (CH)	43.2 (CH)
14	83.9 (CH)	83.9 (CH)
15	33.5 (CH_2)	33.6 (CH_2)
16	82.6 (CH)	82.5 (CH)
17	64.8 (CH)	64.5 (CH)
18	69.6 (CH_2)	68.2 (CH_2)
19	87.5 (CH)	51.1 (CH_2)
$N-CH_2$	51.1 (CH_2)	52.4 (CH_2)
CH_3	13.2 (CH_3)	14.1 (CH_3)
OCH_3	58.8 (CH_3)	57.9 (CH_3)
OCH_3	57.9 (CH_3)	57.8 (CH_3)
OCH_3	57.8 (CH_3)	56.3 (CH_3)
OCH_3	56.4 (CH_3)	55.8 (CH_3)
1`	-	110.3 (C)
2`	-	150.7 (C)
3`	-	116.8 (CH)
4`	-	134.3 (CH)
5`	-	116.3 (CH)
6`	-	130.7 (CH)
C = O	-	167.8 (C)

*The ^{13}C connectivities and multiplicities were deduced according to HMQC and DEPT experiments.

Compound **1** has an ether linkage between C-1 and C-19 and a OCH_3 group at the position 14, whereas compounds **3** and **6** have differences in the same positions; probably because of these differences are the reasons for the inactivation of compound **3** against the enzyme tyrosinase and less inhibitory action of compound **6** (IC_{50} = 137.21 μM) when compared with the activity of the compound **1** (IC_{50} = 115.17 μM) against tyrosinase. Similarly, differences between compounds **2** and **5** are present, compound **2** having an α-OCH_3 functional group at C-1 instead of OH group, and an OH group instead of OCH_3 at C-8, and stereochemically different methoxy group at C-6 as β-OCH_3, probably led to compound **2** to be a better inhibitor (IC_{50} = 83.28 μM) than that of compound **5** (IC_{50} = 96.33 μM).

Table 3. Tyrosinase inhibitory activities of Compounds **1**–**6**

Compounds	IC_{50} (mean ± SEM[a]) (in μM)
Swatinine-A (**1**)	115.17 ± 0.1739
Swatinine-B (**2**)	83.28 ± 0.1527
Foresticine (**3**)	NA[c]
Neoline (**4**)	ND[d]
Delvestine (**5**)	96.33 ± 0.1432
Chasmanine (**6**)	137.21 ± 0.19645
Kojic acid[b]	16.67 ± 0.5190
L-Mimosine[b]	3.68 ± 0.02234

[a] SEM is the standard error of the mean.
[b] The standard inhibitors (KA and LM) of the enzyme tyrosinase.
[c] Is not active against Tyrosinase.
[d] Not done due to insufficient quantities.

Table 4. Anti-oxidant activities of Compounds **1**–**6**

Compounds	DPPH radical % scavenging activity (at 1 mM)
Swatinine-A (**1**)	63.4
Swatinine-B (**2**)	33.4
Foresticine (**3**)	51.4
Neoline (**4**)	65.3
Delvestine (**5**)	32.2
Chasmanine (**6**)	35.1
3-*t*-Butyl-4-hydroxyanisole	92.5

The free-radical scavenging action is known as an important mechanism of anti-oxidation. Compounds **1**–**6** have been screened for their anti-oxidant activities using DPPH radicals (shown in Table 4). Among the compounds screened, only swatinine-A (**1**) (63.4%) and neoline (**4**) (65.3%) exhibited significant DPPH radical scavenging activities at concentrations of 1 mM, while standard anti-oxidant BHA inhibited 92.1% at the same concentration.

Anti-inflammatory activities of all the compounds have also been determined by an *in vitro* approach. Only compounds **2** (38.71%) and **4** (34.12%) exhibited significant anti-inflammatory activity (Table 5) when compared with standard anti-inflammatory agent indomethacin (42.02%) at concentration of 100 μg/mL.

Table 5. Anti-inflammatory activities of Compounds **1**–**6**

Compounds	% Inhibition (at 100 μg/mL)
Swatinine-A (**1**)	25.82
Swatinine-B (**2**)	38.71
Foresticine (**3**)	ND
Neoline (**4**)	34.12
Delvestine (**5**)	28.10
Chasmanine (**6**)	19.31
Indomethacin	42.02

4. Conclusions

In the present study, aerial parts of *A. laeve* royle have been investigated phytochemically. Two new C19-diterpenoid alkaloids were isolated and characterized in addition to four known alkaloids isolated for the first time from this plant. The inhibitory potentials of these C19-diterpenoid alkaloids against the multifunctional copper-containing enzyme tyrosinase along with their anti-oxidant and anti-inflammatory activities have been conducted. Swatinine-B (38.71 %) and neoline (34.12 %) demonstrated very good anti-inflammatory activity in comparison to the standard drug (indomethacin 42.02 %). The structure-activity relationships (SARs) for the inhibition of the enzyme tyrosinase by the compounds have also been discussed.

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