SHORT REPORT

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Antibacterial and Cytotoxic Activities of Diterpenoids Isolated from Indian *Plectranthus coesta*

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Abstract: *Plectranthus* is known for its medicinal value. The paper describes the antibacterial and cytotoxic activity of diterpenoids *ent*-7-hydroxykaur-15,16-en-19-oic acid (**1**), 17-oxokaur-15,16-en-19-oic acid (**2**) and *ent*-7-hydroxy-15,16-epoxykauran-19-oic acid (**3**), isolated from the shade-dried and powdered leaves of Indian *Plactranthus coesta*. Diterpenoid **3** was isolated for the first time from Indian *P. coesta*. Diterpenoid **1**, shows potent activity against *E. coli* (15.6 µg/mL), *P. aureginosa* (17 µg/mL) and *K. planticola* (32.5 µg/mL), while diterpenoid **2**, also showed good activity against *E. coli* (61 µg/mL), *P. aureginosa* (61 µg/mL), *K. planticola* (61 µg/mL) and *S. epidermidis* (125 µg/mL). Diterpenoids **1-3** were found to be more efficacious on U87 cancer cells and less toxic on normal human HEK and HeLa cells.

Keywords: Antibacterial activity; cytotoxicity; diterpenoid; *Plectranthus coesta*.

1. Plant Source

In the course of phytochemical studies of medicinal plants from North Himalayan region of India, we investigated *Plectranthus coesta* (Buch-Ham, common name in India is Merudh or Molchara) which occurs throughout the hills between 3,000 and 7,500 feet [1, 2] from genus *Plectranthus*, which is the most important genus of Lamiaceae family and consists of some 300 species [3]. The chemistry of *Plectranthus* remains relatively unknown, but several plants have been studied chemically, and diterpenoids are the most common secondary metabolites of them [4, 5]. Here, we are reporting antimicrobial and cytotoxic activity of the diterpenoids *ent*-7-hydroxykaur-15,16-en-19-oic acid (**1**) [6, 7], 17-oxokaur-15,16-en-19-oic acid (**2**) [8, 9], and *ent*-7-hydroxy-15,16-epoxykauran-19-oic acid (**3**) [10, 11] along with their isolation, and characterization. Diterpenoid **3** was obtained for the first time from the Indian *P. coesta* (Figure **1**).

The dried leaves of *P. coesta* were collected from Jajardawal forest, Pithoragarh (PIN: 262501) district of Uttarakhand province in India on September 2003. The plant was identified by a renown botanist Dr. Y. P. S. Pangati, Professor, Department of Botany, D. S. B. Campus, Kumaun

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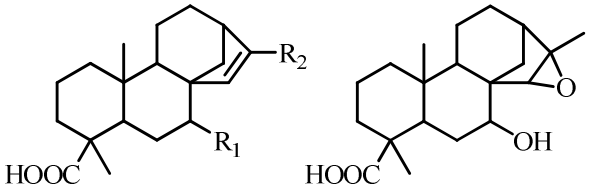
University, Nainital, Uttrakhand, India. A voucher specimen (No. 2174) was deposited in the Department of Botany, Kumaun University, Nainital, Uttrakhand, India.

2. Previous Studies

For thousands of years, natural products have played an important role throughout the world in treating and preventing human diseases. The natural product medicines have come from various natural resources [12-17]. Many natural products were obtained from *P. coesta* and most of them are biologically active species [18-24]. The crushed leaves paste of *P. coesta* is commonly used in the indigenous system of medicine for healing wounds and itches since ancient times. This species is also used for ethnobotanical uses *viz.* ornaments, and other medicinal uses like antiseptics, vermicides, purgatives, infections, toothache, stomachache and commonly used for the treatment of allergy [25]. The diterpenoids (**1-3**) isolated from *P. coesta* are structurally known compounds but less is known about their biological properties.

3. Present Study

Extraction and Isolation: Dried leaves (2.0 kg) of *P. coesta* were powdered followed by methanol extraction (3 L), which was stirred and macerated at room temperature for approximately 36 hr. This process was repeated four times. The resultant mixture was filtered and filtrate was evaporated under vacuo to afford 22.23 g of crude residue, which was stored at -10 °C prior to use. The residue was again stirred first with iso-octane then followed by chloroform and methanol (5×200 mL, each) respectively, and then filtered separately. All the fractions were evaporated to afford, iso-octane *Fr. 1* (5.03 g), chloroform *Fr. 2* (3.63 g) and methanol *Fr. 3* (12.80 g) and stored at -10 °C prior to use. Later, *Fr. 3* (MeOH extract) was used for further purification, which was subjected to column chromatography (CC) (SiO_2; 60-120 mesh, Hexane/EtOAc, 40:60 to 0:100) to afford a mixture of fractions *Fr. 3.1* (5.1 g). The mixture *Fr. 3.1* was again subjected to CC (SiO_2 (60-120 mesh); Hexane/EtOAc, 60:40 to 0:100) to afford *Fr. 3.1.1* (3.5 g, essential oil and other unidentified material, under investigation) and *Fr. 3.1.2* (0.860 g). Fraction *Fr. 3.1.2* was again subjected to CC (SiO_2 (100-200 mesh); Hexane/EtOAc, 40:60 to 0:100) to afford *Fr. 3.1.2.1* (80 mg), *Fr. 3.1.2.2*, (70 mg), *Fr. 3.1.2.3*, (72 mg), *Fr. 3.1.2.4* (200 mg). Then, after two times recrystallization of all the fractions *Fr. 3.1.2.1- Fr. 3.1.2.4* in EtOH separately, *Fr. 3.1.2.1* (20 mg), *Fr. 3.1.2.2*, (25 mg), *Fr. 3.1.2.3*, (18 mg) and *Fr. 3.1.2.4* (45 mg, under investigation) were obtained. After spectroscopic characterization fractions *Fr. 3.1.2.1*, *Fr. 3.1.2.2*, and *Fr. 3.1.2.3*, were identified as diterpenoids *ent*-7-hydroxykaur-15,16-en-19-oic acid (**1**), 17-oxokaur-15,16-en-19-oic acid (**2**), *ent*-7-hydroxy-15,16-epoxykauran-19-oic acid (**3**) respectively.



1, R_1 = OH, R_2 = CH_3
2, R_1 = H, R_2 = CHO

Figure 1. Structure of diterpenoids **1-3** isolated from Indian *P. coesta.*

In vitro Antibacterial activity: All the diterpenoids (**1-3**) have been screened *in vitro* against *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (BL21), and *Klebsiella planticola* (MTCC 2272), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (WHO 6) strains, where gentamycin (G) was used as a reference drug (Table **1**). Diterpenoid **1** was the most potent compound in this series, which was active against *E. coli*, *P. aeruginosa* and *K. planticola* with minimum inhibitory concentration (MIC) value of 15.6, 17.0, and 32.5 µg/mL, respectively. Diterpenoid **2** was

also found to be active against *E. coli*, *P. aeruginosa*, *K. planticola* with MIC value of 61 µg/mL each and *S. epidermidis* with MIC value of 125 µg/mL. Overall, diterpenoids **1**, and **2** are strongly active against *E. coli*, *P. aeruginosa* and *K. planticola* with MIC value ranges from 15.6 to 61 µg/mL, which assumed, diterpenoid **1** and **2** are 2-3 fold more potent than, the diterpenoid **3**. All diterpenoids **1-3** were also found to be quite active against *K. planticola* and *S. epidermidis* while, less effective against *S. aureus*. Thus, the present investigation points towards the presence of double bond in diterpenoid **1** and **2** might be playing crucial role for better activity, and on the other hand the oxirane ring present in diterpenoid **3** results in loss of potency due to oxirane ring opening.

Table 1. *In vitro* Antibacterial activity: MIC of isolated compounds on bacterial strains by Micro broth dilution method: All compounds were tested on bacteria at 10^6 cells/mL [Mean (± SD), n=3].

Compound	MIC(µg/mL)				
	E. coli	*S. aureus*	*P. aureginosa*	*K. planticola*	*S. epidermidis*
1	15.6	247(±5.7)	17(±2)	32.5	240(±10)
2	61 (±2)	243 (±11.5)	61 (±2)	61 (±2)	125 (±5)
3	122 (±5)	243 (±11.5)	118 (±7)	246 (±5)	233 (±15)
G	8(±1.7)	1.96(±0.02)	5.5(±1.1)	31(±1.2)	32.5

Cytotoxicity: Diterpenoids **1-3** were assayed against U87 gliomas, HeLa cervical cancer and HEK human tumor cell lines (Table **2**). For measuring cytotoxicity, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay has been used which is widely accepted as a reliable way to measure the cell proliferation rate. Diterpenoids **1-3** had good inhibitory effects on the growth of U87 brain and HeLa cervical cancer cells in dosage and time dependent manners. Diterpenoid **1**, effectively inhibited the U87 cell growth with an IC_{50} of 34-122 µg/mL. Diterpenoids **1-3**, could inhibit the cell growth at range of 34-250 µg/mL, after 24, 48 and 72 hrs of the treatment on U87 gliomas, while all the diterpenoids **1-3** could inhibit the cell growth at the range of 61-250 µg/mL, after 24, 48 and 72 hrs of the treatment on Hela cells. These diterpenoids **1-3** were found to be less cytotoxic than gentamycin (G) on the normal HEK cells (at moderate concentrations 250-0.45 µg/mL, IC_{50} 235-500 µg/mL).

Table 2. *In vitro* cytotoxicity: IC_{50} values (µg/mL) evaluated from MTT assay on U87 gliomas, Hela cervical cancer and HEK normal cells treated with complexes for 24, 48, 72 hr [Mean (± SD), n = 3].

Compound	U87			HeLa			HEK		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
1	122 (±3.5)	61 (±1.4)	34 (±5.4)	250	250	122 (±3.5)	237.5 (±17)	246.6 (±5.7)	450 (±28)
2	250	122 (±3.5)	34 (±5.4)	122 (±3.5)	61 (±1.4)	61 (±1.4)	235 (±21)	466 (±28)	500
3	250	122 (±3.5)	34 (±5.4)	122 (±3.5)	122 (±3.5)	61 (±1.4)	240 (±7)	500	500
G							9.2 (±1)	8.5 (±1.2)	4.7 (±0.6)

In conclusion, the diterpenoids (**1-3**) obtained from Indian *P. coesta* leaves have a potential as an antibacterial as well as antitumor agents. The present study suggests that these diterpenoids can be further explored as specific antibacterial as well as anticancer drugs due to their potent to moderate activity against all experimental strains. However, a thorough investigation relating the structure and the activity of the compounds as well as their stability under biological conditions is required. Related work is still under progress for further explorations.

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