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Isolation of metallic salts of cytotoxic clerodanes from medicinal plant *Polyalthia longifolia* var. pendula

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Abstract: Isolation of sodium and potassium salt of kolavenic acid (1,2), as a mixture of (3:1) and sodium and potassium salt of 16 oxo-cleroda-3,13(14)*E*-dien-15-oic acid (3, 4) as a mixture of (1:1) are first time reported form reddish black ripe and green unripe berries of *Polyalthia longifolia* var. pendula respectively. Three known constituents obtained, were identified as cleroda-3, 13(14) *E*-dien-15-oic acid (kolavenic acid) (5), 16(*R* and *S*)-hydroxy cleroda-3,13 (14)*Z*-dien-15,16-olide (6) and 16 oxo-cleroda-3,13(14) *E*-dien-15-oic acid (7). Structures of all these compounds have been determined through spectral studies while metal analyses were carried out to confirm the structure of the salts. Compounds 3, 4 and 7 possess cytotoxic activity against lung (NCI-H460), oral (CAL-27) and normal mouse fibroblast (NCI-3T3) cancer cell lines. Diterpenoid (7), a bioprivileged, compound shows potent cytotoxic activity against oral cancer cell line (CAL-27) with *IC*50 11.3±0.6µg/mL in comparison with the standard 5-flourouracil (IC_{50} 12.7±0.1µg/mL) and lungs cancer cell lines (NCI-H460) with IC_{50} 5.3±0.2µg/mL as compared to the standard drug cisplatin (IC_{50} 5.7±0.2µg/mL).

Keywords: *S*odium and potassium salts, kolavenic acid, 16-Oxo-cleroda-313 (14) *E*-dien-15-oic acid, cytotoxic activity, metal analysis.

INTRODUCTION

Polyalthia longifolia a medicinal plant which belongs to the family Annonaceae (Custard apple or Annona family) is included in the most primitive families of Angiosperms (Katkar *et al*., 2010). The genus name *Polyalthia* is originated from Greek words: 'poly' means much or many while 'althia' means cure. It includes 120 species which are mainly found in Africa (Wu *et al*., 1989, Yao *et al*., 2019). The plant which has immense therapeutic values, is used widely in Ayurveda due to the presence of bioactive alkaloids and clerodane diterpenoids in its different parts (Katkar *et al*., 2010).

P. longifolia var. pendula commonly known as "Ulta Ashok" is native to dried regions of India. It is an attractive, tall, perennial and ornamental tree with the drooping branches. It is different from *P. longifolia* (Seedha Ashok) due to its downward branching style. It is widely employed by Asians as folk remedy (Wu *et al*., 2014a). In the indigenous system of medicine, it has been used as an antipyretic and anthelmintic agent. It reduces fever, treat skin diseases and cure hypertension (Wu *et al*., 2014b, Bhattacharya *et al*., 2015). Moreover, recently three findings have been patented on *P. longifolia* describing it as antioxidant, cosmetics, and skin whitening and anti-aging agent (Tani *et al*., 2010, Suzuki *et al*., 2011a, Suzuki *et al*., 2011b). Previous phytochemical investigations on *P. longifolia* var. pendula has resulted in the isolation of various bioactive clerodane diterpenoids which possessed neuroprotective (Wu *et al*., 2014), antifungal (Bhattacharya *et al*.,2015), cytotoxic (Chang *et al*., 2006, Lee *et al*., 2009), antimicrobial (Faizi *et al*., 2008, Sashidhara *et al*., 2009), anti-inflammatory (Wu *et al*., 2014b, Chang *et al*., 2006), antimalarial (Gbedema *et al*., 2015, Annan *et al*., 2015), hypotensive (Saleem *et al*., 2005), anti-histaminic (Edmone *et al*., 2020) activities. The plant also contains antimicrobial alkaloids (Faizi *et al*., 2003), a cytotoxic cycloartane triterpenoid (Sashidhara *et al*., 2010) and some antioxidant constituents (Sashidhara *et al*., 2011). Recently, porphine and proaporphine clerodane hybrids possessing anti-dengue activity (Lo *et al*., 2022) as well as silver nanoparticles of *P. longifolia* have been also synthesized (Dashora *et al*., 2022).

MATERIALS AND METHODS

The IR spectra were recorded in KBr discs (1-4) and in $CHCl_3$ (5-7) on Shimadzu IR-460 and JASCO IRA-302 spectrophotometers respectively, while UV spectra were obtained using a Hitachi U-3200 spectrophotometer. The

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electron impact (EI) mass spectra were taken on a Finnigan MAT-112 instrument. HR EIMS spectra were recorded on a JEOL JMS HX-110 spectrometer. The ^{1}H-NMR spectra of compounds 1-5, 7 were recorded on Bruker Aspect AM-300 operating at 300 MHz and compound 6 was recorded on Bruker Aspect AM-500 spectrometer operating 500 MHz. NMR spectra were referenced as protio-deuterio solvent signals. Chemical shifts in ppm (δ) and coupling constants (J) are in Hz. Kieselgel 60 PF_{254} was used for vacuum liquid chromatography (VLC). All TLC analysis were taken at ambient temperature by using analytical grade silica gel $60F_{254}$ precoated cards (0.2 mm thickness) and visualized by UV light (254 and 366 nm). Metals (Na and K) in samples were analyzed on flame photometer (Jenway PFP7) and flame atomic absorption spectrophotometer (FAAS, PE-AAnalyst 700, Norwalk, CT, USA). The reddish black and green berries, and root-wood *P. longifolia* var. pendula, were collected from Karachi university campus in 2014 and 2018. The plant has been authenticated by plant taxonomist Dr. Rubina Dawar of the Department of Botany, University of Karachi and a voucher specimen (KUH GH # 63496) has been deposited in the herbarium of the same department.

Fresh, undried and uncrushed reddish black berries (0.300Kg) were extracted with petroleum ether at room temperature and filtered. During the process of extraction off-white solid deposited on the berries which was scratched out and collected. It was completely soluble in pyridine, sparingly soluble in distilled water and insoluble in chloroform, ethyl acetate and methanol. ^{1}H-NMR, EIMS, and IR spectral studies and Atomic Absorption techniques revealed that it was a (3:1) mixture of Na and K salts (1 and 2) of 5. The petroleum ether extract obtained, showed a major spot on TLC, which was purified through PTLC (Petroleum ether/EtOAc 9:1) affording a pure compound. Spectral studies determined its structure as 16(*R* and *S*)-hydroxy-cleroda-3,13(14)*Z*-diene-15,16-olide (6) which also contains its exo form (6a).

In another scheme extraction of fresh, undried and uncrushed green berries (0.300kg) were separately extracted with petroleum ether, ethyl acetate and methanol. Two layers were formed in first extract of petroleum ether which was separated. The lower whitish aqueous layer on concentration and filtration afforded a light brown substance which was identified as a (1:1) Na and K salts (3 and 4) of (7). The ethyl acetate extract also formed dual layered liquid which had been separated away. The upper organic layer was dried and freed of the solvent affording a residue (1.75g) which was subjected to vacuum liquid chromatography (VLC) [Kieselgel 60 PF_{254} (15.0 g), using petroleum ether and ethyl acetate in order of increasing polarity by 5% furnishing twenty-one fractions. Fraction no. 13 (Petroleum ether/EtOAc 4:6) yielded a pure compound which was identified as 16-oxo-cleroda-3,13*E*-dien-15-oic acid (7).

Fresh, undried and uncrushed root-wood (1.00Kg) was extracted twice with methanol at room temperature and concentrated under reduced pressure affording a thick residue (SPWM, 20.6g) which was fractionated by solvent separation technique which gave petroleum ether (SPWM1), dichloromethane (SPWM2), ethyl acetate (SPWM3) and methanol (SPWM4) soluble fractions as well as insoluble fraction (SPWM5). White crystals settled down in petroleum ether fractions (SPWM1), which were filtered and identified as known diterpene, kovalenic acid (5) (20 mg) Petroleum ether/EtOAc (7:3), R_f=0.68), which exists in endo(5) and exo(5a) forms (2:1).

For the determination of metals in the sample, 0.15g of each sample [*i.e*, salts of 5 (1 and 2) and salts of 7 (3 and 4)] was taken in a beaker separately, added 15ml of HNO_3 (65%, Merck, Germany) for digestion and heated the beaker on hot plate with continuously stirring through the glass rod. After dissolving the sample, filtered the solution and then transferred it into 50mL volumetric flask and deionized water was added till mark (Skoog *et al*., 1996, Yasmeen *et al*., 2016). Sodium (Na) in sample was analyzed on Flame Photometer with the standard sodium solutions from 4 to 100 ppm made from 1000 ppm stock solution (Merck, Germany). Na was also determined with K in each sample by Flame Atomic Absorption Spectrophotometer at the wavelength of 589.0 nm and 766.5 nm respectively.

The cytotoxic activity of compounds was assessed on lung cancer (NCI-H460), oral cancer (Cal-27) and normal mouse fibroblast (NIH-3T3) cells as defined earlier (Farooq *et al*., 2017). Cells were seeded at density 15,000 cells per well in 96 well micro titer plate and incubated overnight. After 24 hours of incubation, cells were treated with compounds at different concentrations in the range of 1.95-250µg/mL and incubated for 48h. After incubation, 10µl MTT dye was added in each well and incubated for further 4h to evaluate metabolically active cells. The formazan crystals were than solubilized in DMSO and absorbance was measured at 570 nm. Percent inhibition of cells was calculated by using following equation:

Cell proliferation inhibition=100-[($OD_{compound}$ - OD_{blank}) / ($OD_{control}$ - OD_{blank}) × 100]

EZ-Fit Enzyme Kinetics; Perrella Scientific had been used for evaluating the IC_{50} values of test compounds.

STATISTICAL ANALYSIS

Results were presented as ±S.D. and level of significance were analyzed by Student's t-test using SPSS software

version 21 (IBM, USA). The p values of <0.05 were considered significant.

Spectral characterization

Sodium and potassium salts of Kolavenic acid (1 and 2)

White solid; UV λ_{max} (MeOH) nm: 214, 229, 279 nm; IR ν_{max} (KBr) cm^{-1}: 2926, 2858 (C-H stretch), 1738, 1705 (-COO^-M^+), 1692 (C=C stretch), 1638 (C=C stretch), 1436 (C-H bending), 1252 (C-O) cm^{-1}; EIMS *m/z* (rel. intensity, %): 326 [M, $C_{20}H_{31}O_2Na]^+$ (10), 303 [M-Na, $C_{20}H_{31}O_2]^+$ (20), 287 (10), 239 (7), 191 (92), 190 (27), 189 (73), 121 (71), 107 (90), 95 (100); ^{1}H NMR (300MHz, Pyridine-d_5, δ) 6.12 (brs, 1H, H-14), 5.14 (brs, 1H, H-3), 2.36 (s, 3H, CH_3-16), 1.70 (td, 1H, J = 12.0 Hz, J = 3.0 Hz, H-6eq), 1.57 (brs, 3H, CH_3-18), 1.16 (ddd, 1H, J = 12.0 Hz, J = 12.0 Hz, J = 5.0 Hz, H-6ax), 0.98 (s, 3H, CH_3-19), 0.80 (d, J = 6 Hz, 3H, CH_3-17) and 0.71 (s, 3H, CH_3-20) (table 1).

***Sodium and potassium salts of 16-oxo-cleroda-3,13E-dien-15-oic acid* (3 and 4)**

Brown solid; IR ν_{max} (KBr) cm^{-1}: 2965 (C-H stretch), 2735 (α, β-unsaturated aldehydic CH stretch), 1732, 1709 (α, β-unsaturated CHO), 1642 (C=C stretch) and 1253 (C-O) cm^{-1}; EIMS *m/z* (rel. intensity, %): 317 [M-Na or K, $C_{20}H_{29}O_3]^+$ (12), 300 (10), 285 (15), 264 (7), 236 (8), 203 (5), 191 (100), 149 (30), 135 (24), 121 (36), 107 (45), 95 (77), 81 (46), 69 (50) and 55 (68); ^{1}HNMR (300MHz, DMSO-d_6, δ) 9.27 (s, 1H, H-16), 6.68 (s, 1H, H-14), 5.12 brs ($W_{1/2}$ =9.0,1H, H-3), 1.70 (td, 1H, J=12.0 Hz, J=3.0 Hz, H-6eq), 1.57 (brs, 3H, CH_3-18), 1.18 (m, 1H, H-6ax), 0.99 (s, 3H, CH_3-19), 0.84 (d, J =6.0 Hz, 3H, CH_3-17) and 0.67 (s, 3H, CH_3-20) (table 1).

RESULTS

On extraction with petroleum ether off-white solid deposited on the ripe, reddish black berries, which were scratched out from them. It was found to be insoluble in chloroform, ethyl acetate and methanol, soluble in pyridine while sparingly soluble in distilled water, pointing to its nature as a glycoside or salt of an organic compound. Its IR spectrum exhibited bands at 2926, 2858, 1738, 1705, 1692, 1638 cm^{-1} for C=C and 1252 cm^{-1} for C-O moiety. Strong peaks at 1705 cm^{-1} and 1738 cm^{-1} showed the substitution of proton of carboxylic acid with the metals due to which stretching vibration of carboxylic acid has diminished, indicating that it might be a metallic salt of an organic compound. The EIMS spectrum showed a peak at *m/z* 326 for $[M]^+$ ion corresponds to molecular formula $C_{20}H_{31}O_2Na$ along with the stable fragment at *m/z* 303 $[M\text{-}Na]^+$ which indicated the presence of sodium metal in the compound. Its ^{1}H NMR spectrum (300 MHz, Pyridine-d_5, table. 1) exhibited two olefinic proton singlets at δ_H 6.12 (1H, brs, H-14) and 5.14 (1H, br s, H-3), while olefinic methyl appeared at 2.36 (3H, s, H-16) these protons resonated slightly up-field as compared to those of diterpenoid 5. Other methyls appeared at δ_H = 0.71 (3H, s, H-20), 0.80 (3H, d, J = 6.0 Hz, H-17), 0.98 (3H, s, H-19) and 1.57 (3H, brs, H-18) these data with those observed for 5, however methyls at carbon 5, 8 and 9 resonated down-field (table. 1). These facts disclosed that the insoluble material is a salt of 5, which was confirmed by the metal analyses. Thus, analysis of Na-metal was performed on flame photometer, which indicated the presence of sodium metal in the compound and 58.86 ppm of sodium was determined by calibration curve and regression analysis method (table. 2, fig. 1). For the detection of Na and K metals; flame atomic absorption spectrophotometer was used. It showed the presence of 59.35 ppm Na and 21.07 ppm of K (table. 3). Hence, these results confirmed that it was a mixture of Na and K salts with the ratio of 3:1. The overall spectral data and metal analysis established the structure of the off-white solid as Na and K salts (1 and 2) of 5, which is the first report of its metallic salt. It is important to note that, the white solid does not deposit on green berries and might be formed due to the ripening of fruits. The petroleum ether extract of these berries contained 5 and 6 with its exo-form (6a) as major diterpene (Misra *et al*., 2010).

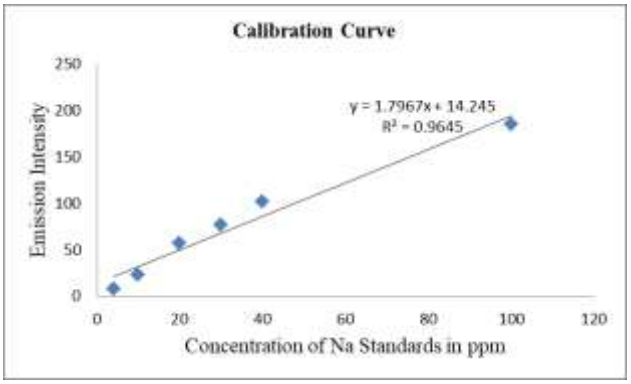

Fig. 1: Calibration curve between emission intensities versus concentration of Na standards to calculate concentration of sodium salt of kolavenic acid (1) by using regression line equation (y=1.7967x+14.245)

The petroleum ether extract of fresh green berries gave two layers which were separated into upper petroleum ether and lower aqueous phases. The whitish aqueous layer was concentrated and filtered, giving a light brown solid which was found to be a 1:1 mixture of Na and K salts (3 and 4) of 7. It was insoluble in chloroform, ethyl acetate and methanol and completely soluble in pyridine, DMSO and water. Its IR spectrum unveiled bands at 2735 cm^{-1} for aldehydic group. The peaks at 1709 and 1732 cm^{-1} indicated the substitution of proton of carboxylic acid with the metals. In the EI mass spectrum a peak was observed at *m/z* 317 $[M\text{-}Na/K]^+$ which gave the formula of $C_{20}H_{29}O_3$. In the ^{1}H NMR spectrum (300MHz, DMSO-d_6, table-1) H-14 and H-3 resonated at δ_H 6.68 (brs) and 5.10 (W1/2 =9.0 Hz) while aldehydic proton appeared at

δ_H 9.27 as a singlet. Signals at δ_H 0.67 (3H, s, H-20), 0.84 (3H, d, *J* = 6.0 Hz, H-17), 0.99 (3H, s, H-19) and 1.57 (3H, brs, H-18) were assigned to the methyl protons. These chemical shifts are comparable to those observed for diterpenoid 7, however, all the protons resonated down-field except for aldehydic proton which appeared up-field (table 1). These data revealed that it is a metallic salt of 7. Metal analysis through flame photometer indicated the presence of 175.58 ppm Na in the compound by calibration curve and regression analysis method (table. 4, fig. 2). Moreover, flame atomic absorption spectrophotometer confirmed the presence of 178. 88 and 137.78 ppm of Na and K respectively (table. 3). These results disclosed that it was a 1:1 mixture of Na and K salts. Spectral data together with metal analysis established the structures as Na and K salts (3 and 4) of 7. It is the first instance of isolation of metallic salts of 7. It is important to note that it is the first report of isolation, characterization along with metal analysis of any naturally occurring salts of diterpenoids. Recently, the isolation of Na and K salts of glycoside of apiuronic acid from the bark of *Cinnamomum cassia* was published in literature (Chang *et al*., 2021).

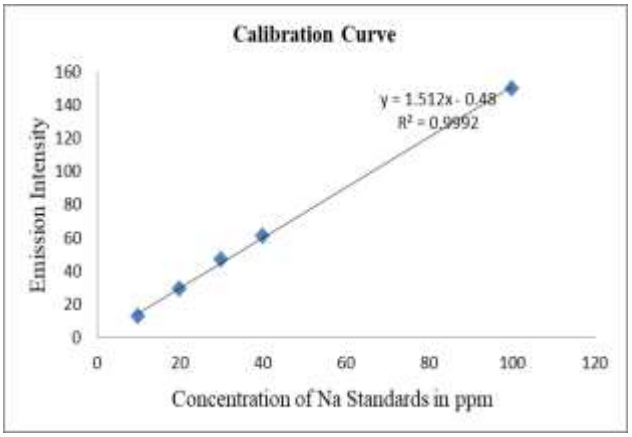

Fig. 2: Calibration curve between emission intensities versus concentration of Na standards to calculate concentration of sodium salt of 16-oxo-cleroda 3,13(14) *E*-dien-15-oic acid (3) by using regression line equation (y=1.512x-0.48)

DISCUSSION

In the present study four new natural products were obtained, a mixture (3:1) of Na and K salts (1, 2) of 5, and a mixture (1:1) of Na and K salts (3, 4) of 7 from reddish black, ripe and green, unripe berries respectively. Moreover, three known constituents were obtained and identified through spectral studies as cleroda-3,13(14)*E*-dien-15-oic acid (kolavenic acid) (5) (Faizi *et al*., 2003, Khan., 2004, Ikram., 2015, Misra *et al*., 2010, Phadnis *et al*., 1988, Khan *et al*., 2017) isolated from root-wood, 16(*R* and *S*)-hydroxy cleroda-3,13(14)*Z*-dien-15,16-olide (6) (Khan, 2004, Ikram, 2015, Misra *et al*., 2010) from reddish black berries, and 16-oxo cleroda-3,13(14)*E*-dien-15-oic acid (7) a bio-privileged molecule (Faizi *et al*., 2008, Phadnis *et al*., 1988, Khan *et al*., 2017) from green berries. Compounds 3, 4 and 7 showed cytotoxic activity against lung (NCI- H460), oral (CAL- 27) and normal mouse fibroblast (NIH- 3T3) cancer cell lines. The main isomers contain $\Delta^{3,4}$ (5 and 6) and minor isomers have $\Delta^{4,18}$ (5a and 6a) bond in A ring [fig. 3(a) and fig. 3(b)] which is of rare occurrence in nature (Li *et a*., 2016).

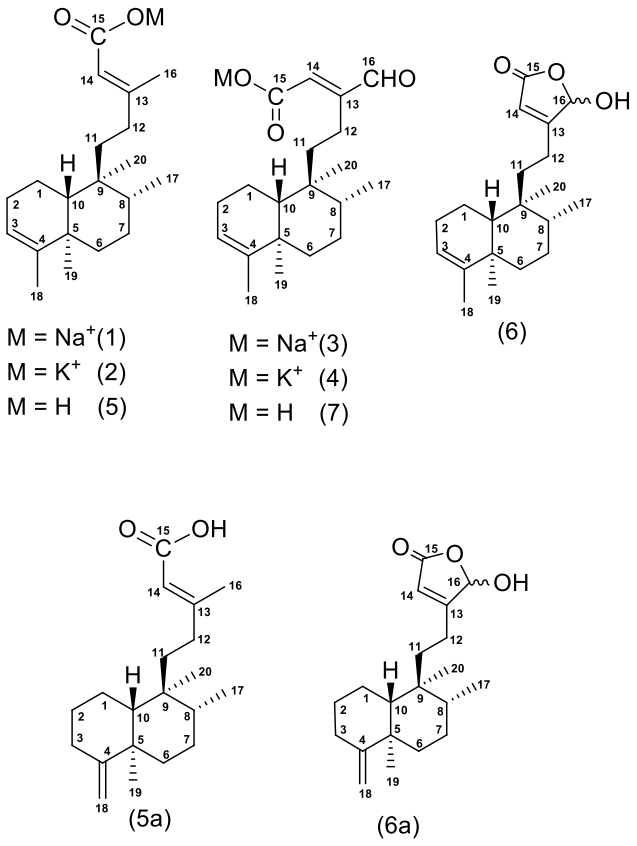

Fig. 3(a): Structures of isolated compounds

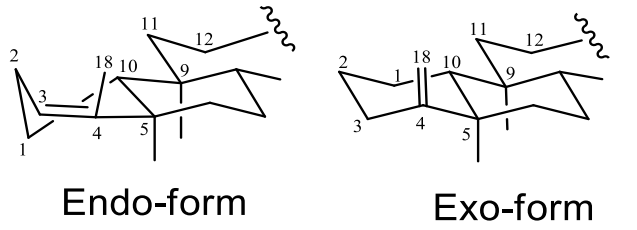

Fig. 3(b): Chair conformation of endo (5 and 6) and exo (5a and 6a) forms.

Compounds 3, 4 and 7 were found to be active against lung (NCI-H460), oral (CAL-27) and normal mouse fibroblast (NIH-3T3) cancer cell lines. 7 possesses potent cytotoxic activity against oral cancer (CAL-27) cell lines in comparison with standard 5- fluorouracil –and lung cancer (NCI-H460) cell line as compared to standard cisplatin. Salts (3, 4) were found to be less active against

Table 1: ^{1}H NMR characterization of compounds 1-7

Assignment	δ_H / ppm (J / Hz)				
	1 and 2	5[a]	3 and 4	7	6
	Pyridine-d_5 300 MHz	Pyridine-d_5 300 MHz	DMSO-d_6 300 MHz	DMSO-d_6 300 MHz	$CDCl_3$ 500 MHz
1ax	1.47 m	1.48 m	1.44 m	1.34 m	1.48 m
1eq	1.47 m	1.48 m	1.44 m	1.34 m	1.48 m
2ax	2.05 m	2.06 brm	2.11 m	1.98 m	2.05 m
2eq	2.05 m	2.06 brm	2.08 m	2.03 m	1.97 m
3	5.14 br s	5.18 br s	5.12 br s ($W_{1/2}$ =9.0)	5.10 br s ($W_{1/2}$ = 9.0)	5.15 br s ($W_{1/2}$ = 8.6)
6ax	1.16 ddd (12, 12, 5)	1.10 m	1.18 m	1.10 m	1.16 dt (12, 12, 5)
6eq	1.70 td (12, 3, 3)	1.62 m	1.70 td (13, 3, 3)	1.68 m	1.70 m
7	1.41 m	1.44 m	1.42 m	1.31 m	1.42 m
8	1.41 m	1.44 m	1.42 m	1.30 m 0.75 d (6.6)	1.42 m
10	1.30 dd (12, 2)	1.31 d	1.25 m	1.17 m	1.25 dd (12, 2)
11a	1.51 m	1.53 m	1.75 m	1.63 m	1.64 m
11b	1.35 m	1.36 m	1.75 m	1.58 m	1.64 m
12a	1.98 m	1.99 m	2.54 m	2.35 m	2.09 m
12b	1.98 m	1.97 m	2.60 m	2.32 m	2.28 m
13	-	-	-	-	-
14	6.12 brs	6.13 brs	6.68 s	6.60 s	5.80 s
15	-	-	-	-	-
16	2.36 s	2.40 s	9.27 s	9.47 s	5.99 s
17	0.80 d (6)	0.73 d (5.8)	0.84 d (6)	0.75 d (6.6)	0.79 d (6)
18	1.57 brs	1.57 brs	1.57 brs	1.49 brs	1.56 d (1)
19	0.98 s	0.95 brs	0.99 s	0.89 s	0.97 s
20	0.71 s	0.65 s	0.67 s	0.58 s	0.74

a - Exo-form (5a) H-18, 4.51 s, H-19, 1.05 s
b - Exo-form (6a) H-18, 4.50 brs, H-19, 1.05 s

Table 2: Emission intensities of Na standard solutions and mixture of salts of Kolavenic acid (1 and 2). Performed on flame photometer (Jenway PFP7)

Concentration of Na Standards (ppm)	Emission Intensities
4	8
10	23
20	57
30	77
40	102
100	185
58.86 Concentration of Na in a mixture of salt of Kolavenic acid (1 and 2) (Found through calibration curve fig. 1)	120

lung and oral cancer cell lines as compared to standard drugs (table. 5). Earlier 7 was reported as cytotoxic agent against MCF-7 and A549 cell lines, in that study doxorubicin and paclitaxel were used as standards (Lee *et al*., 2009), while its cytotoxic activity was not determined against lung (NCI-H460), oral (CAL-27) and normal mouse fibroblast (NIH-3T3) cancer cell lines. On the other hand, compound 6 was reported to show antiproliferative activity against human leukemia HL-60 cell line with IC_{50} value of 13.7 μM (Sari *et al*., 2013).

The cytotoxic activity of 5 and 7 was reported by shrimp lethality bioassay. Both the compounds showed the activity with LC50 3.16 and 2.52μg/mL respectively (Islam *et al*., 2001). *Z*-isomer of compound 7 (polyalthialdoic acid) was also reported as a most active constituent in the brine shrimp bioassay with ED50 values 6×10^{-1} μg/mL in the *In vitro* tumor culture (Zhao *et al*., 1991). Moreover, cytotoxicity of novel semi-synthetic derivative of 7 was reported against neuroblastoma SH-SY5Y cell line with IC_{50} 12.5 (Hussain *et al*., 2018). Cytotoxicity of 1+2 and 5 could not be determined due to paucity of these compounds, However, for comparative studies they will be re-isolated.

CONCLUSION

The present investigation was carried out on reddish black and green berries, and root-wood of *P. longifolia* var. pendula on the basis of the remarkable significance of plant as it contains a variety of natural products possessing extensive biological significance. Of which clerodane diterpenoids are predominant while alkaloids are present as minor components. Present investigation headed to the isolation of seven chemical constituents of which four were identified as new natural products, two

Table 3: Concentration (ppm) of Na and K in the salts of Kolavenic acid (1 and 2) and 16-oxo-cleroda-3,13(14) *E*-dien-15-oic acid (3 and 4). Performed on flame atomic absorption spectrophotometer (FAAS, PE-AAnalyst 700, λmax (Na) = 589.0 nm λmax (K) =766.5 nm)

Mixture of salts	Concentration of Na (ppm)	Concentration of K (ppm)
Salt of kolavenic acid (1 and 2)	59.35	21.07
Salt of 16-oxo-cleroda-3,13(14) *E*-dien-15-oic acid (3 and 4)	177.88	137.78

Table 4: Emission intensities of Na standard solutions and mixture of salts of 16-oxo-cleroda-3,13(14) *E*-dien-15-oic acid (3 and 4) Performed on flame photometer (Jenway PFP7).

Concentration of Na Standards (ppm)	Emission Intensities
10	13
20	29
30	47
40	61
100	150
175.58 Concentration of Na in a mixture of salt of 16-Oxo-cleroda-3,13(14)*E*-dien-15-oic acid (3 and 4) (Found through calibration curve fig. 2)	265

Table 5: Anticancer activity of sodium and potassium salt of 16-oxo-cleroda-3,13(14)*E*-dien-15-oic acid (3,4) and 16-oxo-cleroda-3,13(14) *E*-dien-15-oic acid (**7**).

Compounds	IC_{50}±S. D. / µg mL^{-1}		
	CAL-27	NCI-H460	NIH-3T3
Sodium and potassium salt of 16-oxo-cleroda-3,13(14) *E*-dien-15-oic acid (3,4)	45.22 ± 5.4	89.44 ± 0.3	-
16-Oxo-cleroda-3,13(14) *E*-dien-15-oic acid (7)	11.32 ± 0.6	5.34 ± 1.4	12.1 ± 0.7
Cisplatin	-	5.75 ± 0.2	-
5 Fluorouracil	12.7 ± 0.1	-	-

NCI-H460 = Lung cancer, CAL-27 = Oral cancer and NIH-3T3 = Normal Mouse Fibroblast cells

from black berries, Na- and K- salts (1, 2) of 5 and two from green berries, Na- and K- salts (3, 4) salts of 7. Compounds 5 and 7 are predominant in root woods and green berries respectively and possess valuable biological activities. Compound 7, a bioprivileged diterpenoid possesses potent cytotoxic activity against lung (NCI-H460) and oral cancer (CAL-27) cell lines, whereas the mixture of Na and K salt (3, 4) of 7 is less active against these cell lines as compared to compound 7 and standard drugs.

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