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Chemical constituents from Rheum *emodi* and cytotoxic evaluation of individual constituents

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ABSTRACT

R. emodi has been used extensively in Ayurvedic and other traditional medicinal systems like homeopathic, Unani and Chinese systems. The extracts prepared from the roots, bark and leaves of this plant have been used as a laxative from ancient times and presently these are used in various herbal preparations. Herein we report the phytochemistry of this plant along with the cytotoxic activity of isolated constituents. Six compounds were isolated from Rheum emodi and were characterized via spectral techniques viz ESI-MS, H^1 NMR and C^{13}NMR. These characterized compounds were envisaged for cytotoxic evaluation. All the isolated compounds were screened against a panel of four different cancer cell lines viz. prostate (PC-3), colon (HCT-116 and Colo-205) and lung (A-549) Among all tested isolates, Compound 3 and 4 were found to be most active against lung cancer cell line A549 with IC_{50} values of 0.89 and 0.78 µM respectively and compound 1 and 5 were found to be active against both prostate (PC-3) and colo-205 with with IC_{50} values of 0.89, 0.98 and 0.78, 0.88 µM respectively. Compound 2 and 6 were least potent against all the tested cancer cell lines.

Key Words: R.emodi, Isolates, Cytotoxic activity, Cancer cell lines.

I INTRODUCTION

Rheum emodi (Polygonaceae) locally known as‘‘Pambchallan’’(in Kashmir)is a leafy perennial herb distributed in altitudes ranging from 2800 to 3800 m in the temperate and subtropical regions of Himalayas from Kashmir to Sikkim in India [1] . The plant has large woody roots, large leaves , branched leafy stems and usually the height of the plant is about 1.5-3.0 metres [2-5]. Roots and rhizomes constitute the main parts of the plant which are used as drugs. The rhizomes of the plant are collected in the month of October – November and the ones found in India are darker, inferior in aroma, coarser and untrimmed [6-7]. For the people living especially in rural and high altitudes of Kashmir, *R. emodi*in various forms constitutes an important source of food. The rhizomes of the plant are firstly cut into short pieces, threaded on a string and then dried either in the sun or by artificial heat. The dried pieces are then stored by the people and cooked during winters. In addition to it, the leaves of this plant locally called by the name of “Pambhaak” are also being cooked as a vegetable .The

leaf stalks are either eaten raw or boiled, then they are sprinkled with salt and pepper by the locals of Kashmir[8]. It has also been found that the flowers of the plant are also edible. In addition to it, *R. emodi* is also used for making pies that serve a number of functions like antipyretic, antihelminthic and are used in cases of constipation, jaundice and liver disorders [9]. *R. emodi* has been used extensively in Ayurvedic and other traditional medicinal systems like homeopathic, Unani and Chinese systems [10].The extracts prepared from the roots, bark and leaves of this plant have been used as a laxative from ancient times and presently these are used in various herbal preparations[11].*R.emodi* has been used traditionally for the treatment of number of ailments like fevers, ulcers, jaundice, bacterial and fungal infections. It is also used to treat kidney stones and other liver associated disorders like gout and jaundice [12-13].The rhizomes of the plant are used in the treatment of ailments like diabetes, atherosclerosis, ischemia. They are also used for the treatment of inflammation in Japanese and Chinese traditional medicine (Matsuda *et al*., 2001). The plant is also used to heal skin sores and scabs externally [14].In China, R. *emodi* is also used as an ulcer remedy .It is also considered as a bitter, dry herb which is used to clear heat from the liver, stomach and blood, to expel helminthes and to treat cancer, upper intestinal bleeding (ulcers), headache and toothache in China. [15-17].

II RESULTS AND DISCUSSION

Chemistry:

The roots of R. *emodi* were collected from the hilly areas of Dhaara , Harwan and Drang (Kahmir, India) .The air dried powdered material was then extracted with DCM:MeOH(1:1) and MeOH . The concentrated extracts were then subjected to column chromatography over silica gel. Repeated column chromatography of DCM: MeOH extract using varied solvent polarity (hexane: ethyl acetate) and recrystallization techniques afforded four compounds **(1-4).** Similarly, the repeated column chromatography of MeOH extract yielded two compounds **(5-6)**using chloroform-methanol as eluent with increasing polarity of 20 % and 30% MeOH respectively (Figure.1).

Figure.1. Molecular structures of the isolated compounds.

Biology:

All the isolated compounds were subjected to 3-(4,5-Dimethylthiazol-yl)-diphenyltetrazoliumbromide (MTT) cytotoxicity screening against a panel of four different cancer cell lines viz. prostate (PC-3), colon (HCT-116 and Colo-205) and lung (A-549). Among the isolated compounds Compound 3 and 4 were found to be most active against lung cancer cell line A549 with IC_{50} values of 0.89 and 0.78 µM respectively and compound 1 and 5 were found to be active against both prostate (PC-3) and colo-205 with IC_{50} values of 0.89, 0.98 and 0.78, 0.88 µM respectively. Compound 2 and 6 were least potent against all the tested cancer cell lines. The current study revealed the potential of these isolated compounds as potent cytotoxic agents.

Table.1. IC_{50} (µM) values of isolated compounds against Prostate(PC-3), lung (A-549), Colon (HT-29) and Colon (colo-205) cancer cell lines using MTT assay.

Entry	Prostate(PC-3)	Lung (A549)	Colon HT-29	Colon (colo-205)
	IC_{50} (µM)			
1	0.89	5.66	4.45	0.98
2	14.567	5.78	6.43	3.34
3	5.66	0.89	5.67	4.23
4	5.78	0.78	7.54	5.43
5	0.78	16.98	14.44	0.88
6	6.853	7.779	4.78	5.432
BEZ-235	0.056	0.20	0.404	0.0809

III CONCLUSION

In conclusion, the current study seems to be the first report involving isolation of six compounds and the cytotoxic activity studies using MTT assay. We have demonstrated the cytotoxic activity of six isolates, characterized by spectral data analysis. Among all tested isolates, Compound 3 and 4 were found to be most active against lung cancer cell line A549 with IC_{50} values of 0.89 and 0.78 µM respectively and compound 1 and 5 were found to be active against both prostate (PC-3) and colo-205 with IC_{50} values of 0.89 and 0.78 µM. Compound 2 and 6 were least potent against all the tested cancer cell lines. However further studies especially the *in vivo* studies need to be carried out for revealing the exact mechanism of action and further SAR of most active compound would prove beneficial for further assessment.

IV EXPERIMENTAL

4.1. General experimental procedures

^{1}H NMR and ^{13}C NMR spectra of the molecules along with their chemical shifts expressed in δ and their coupling constants in Hertz were recorded on Bruker DPX 500 instrument using $CDCl_3$ /MeOD/DMSO as the solvents with TMS as internal standard. Infrared spectra of the compounds were recorded as KBr pellets in cm^{-1} on a Hitachi 270-30 spectrophotometer. Melting points were being determined on a Buchi melting point apparatus. Column was run using silica gel (60-120 mesh). TLC plates were visualized under UV light and after exposure to iodine vapour in iodine chamber .The spraying reagent used was Cerric sulphate.

4.2.. Plant Material collection

The roots of*R.emodi*were collected from from the hilly areas of Dhaara , Harwan and Drang (Kashmir, India) . The plant was then properly identified by a plant taxonomist Mr. Akhtar and the specimen voucher number **1902 KASH** was deposited in Kashmir University Herbaria.

4.3. Extraction and Isolation

Air-dried and coarsely powdered plant material (root part, 3Kg) was divided into two parts. One part of the dried roots(about 1 kg) was extracted with DCM:MeOH (1:1)for 48 hours and the other part (about 2kg) was extracted with methanol for about 48 hours. The extracts thus obtained were concentrated under reduced pressure to give crude extract of 100.0 g for DCM:MeOH (1:1) as extracting solvent and 400.0 g for MeOH as an extracting solvent. The DCM:MeOH extract was then dissolved in minimum amount of methanol and adsorbed on silica gel to form slurry. The dried slurry was subjected to column chromatography over silica gel to afford compounds **1**(800.0 mg), **2**(1. 0 g), **3** (500.0 mg) and **4** (500.0 mg) using hexane-ethyl acetate as eluent with increasing polarity of 2 %, 10 %, 16 % and 30% Et OAC respectively. After this, MeOH extract was firstly dissolved in minimum amount of methanol and was then adsorbed on silica gel to form slurry. The dried slurry on subjecting to column chromatography over silica gel afforded compounds **5**(300.0 mg), **6**(500.0 mg) using chloroform-methanol as eluent with increasing polarity of 20 % and 30% MeOH respectively.

4.4. Spectral data of the isolated constituents

Chrysophanol (1): Orange crystalline solid; IR (KBr) ν_{max} cm^{-1}:3469 ,1719 ,1633;^{1}H NMR (400 MHz, DMSO) δ:11.91(s,1H,OH), 11.79(s,1H, OH), 7.80(dd, J_1=8.4, J_2 =7.6 Hz, 1H) , 7.71 (d, J=8 Hz, 1H) , 7.54 (s, br,1H), 7.38(d, J=8 Hz, 1H), 7.21(s, br,1H), 2.44 (s,3H,CH_3) .^{13}C NMR (101 MHz, DMSO) δ: 191.62, 181.49,161.57, 161.31,149.18,137.33, 133.31, 133.01, 124.40,124.08,120.55, 119.32, 115.85, 113.76, 21.62.

Emodin (2) : Yellowish powder;IR (KBr) ν_{max} cm^{-1} :3469 ,1719 ,1633;^{1}H NMR (400 MHz, DMSO) δ:12.07(s,1H,OH), 11.40(s, 1H, OH), 11.31(s, 1H, OH) ,6.76(s,1H) ,6.39 (s, 1H,) ,6.34 (s, 1H), 5.82 (s,1H),

2.50(s, 3H, CH_3) . ^{13}C NMR (101 MHz, DMSO) δ:191.14, 182.40, 166.53, 165.73, 162.72, 149.32, 136.26, 133.99, 124.82, 121.37, 114.29, 110.0, 109.62, 108.67, 22.12 .

oreugenin (3): Light brown powder; IR (KBr) v_{max} cm^{-1}: 3150 , 1647 cm;^1H NMR (400 MHz, DMSO) δ**:**12.97 (s, 1H, OH), 12.85(s, 1H, OH),6.48 (s, 1H), 6.32 (s, 1H) , 6.27 (s,1H) , 2.50 (s,3H, CH_3) . ^{13}C NMR (101 MHz, DMSO) δ:181.76, 167.67, 164.08, 161.51, 157.80, 107.94, 103.43, 98.74, 93.71, 19.91.

Physcion (4) : Yellowish powder; IR (KBr) v_{max} cm^{-1}:1681 ,1628;^1H NMR (400 MHz, $CDCl_3$) δ:12.31(s, 1H, OH) ,12.11(s, 1H, OH), 7.63(s, br, 1H), 7.37(d, J=2.4 Hz, 1H), 7.08(s, br, 1H), 6.69(d, J=2.4Hz, 1H), 3.94(s, 3H, OCH_3),2.46(s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 190.7, 181.9,166.5, 165.1, 162.4,148.4,135.2,133.2, 124.4, 121.2, 113.7,110.2,108.2, 106.8,56.1, 22.2.

Aloe emodin glycoside (5) : Brownish powder;IR (KBr) v_{max} cm^{-1}:3426 , 1672 ,1634 ;^1H NMR (400 MHz, DMSO) δ: 12.83(s, 1H, OH), 7.87(m, 2H), 7.70(d, J=8.0 Hz, 1H), 7.64(s, 1H), 7.26(s, 1H),5.60(d,J=8.0 Hz, 1H),5.10(m, 3H),4.60-4.65 (m, 3H).^{13}C NMR (101 MHz, DMSO) δ: 188.11, 182.38, 161.67, 158.34, 152.63, 136.29, 135.24,132.24, 122.59,121.54, 121.24, 120.56,116.20, 115.89,100.84, 77.79,76.74,73.43, 70.11,62.06, 60.70.

Chrysin(6) : Pale yellowish powder;IR (KBr) v_{max} cm^{-1}:1655,3403;^1H NMR (400 MHz, DMSO) δ: 12.77(s,1H,OH), 10.91(s, 1H, OH), 8.00-7.97(m, 2H), 7.55-7.48(m,3H), 6.89(s,1H), 6.45(d, J=1.5 Hz, 1H), 6.15(d, J=1.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ: 181.94, 164.50, 163.22, 161.53, 167.52, 131.09, 130.76, 129.20(2C), 126.46 (2C), 105.21, 104.03, 99.09, 94.20.

4.5. Biology

All the human cancer cell lines i.e., breast (HBL-100), lung (A-549), colon (HT-29) and colon (HCT-116) were obtained from National Center for Cell Science, Ganeshkhind, Pune-4111007 (India) and National Cancer Institute, Biological Testing Branch DTP/ DCTD/ NCI, Frederick Cancer Research and Development Center, Fairview Center, Suite 205, 1003 West 7th Street, Frederick, MD 21701-8527 (USA). RPMI-1640 medium, Penicillin, streptomycin, fetal calf serum, sodium bicarbonate, phosphate buffer saline, trypsin, gentamycin sulphate, tryphan blue, ethanol, DMSO, paraformaldehyde were purchased from Sigma Chemicals Co. Glacial acetic acid from Fischer scientific, PBS and trichloroactetic acid (TCA) from Merck specialties private limited.

4.6. Evaluation of cytotoxicity using MTT assay

All the compounds were evaluated against a panel of four different human cancer cell lines viz. prostate (PC-3), colon (HCT-116), leukemia (HL-60) and lung (A-549) using MTT assay in a 96 well plate. Cells were routinely maintained in RPMI 1640 (Sigma Aldrich) supplemented with 10% FBS (Merck) and 1% penicillin G and streptomycin (Sigma Aldrich) at 37^oC in a humidified incubator with 5% CO_2 and were subcultured at 1:5 ratio once a week. For antiproliferative activity, compounds were dissolved in cell culture grade DMSO. Briefly, cells (10^4 cells/well) were cultured in 96 well tissue culture plates and treated with different concentrations of compounds for 48 h. At the end of incubation, 20 µL of MTT (2.5 mg/mL) was added to the wells and incubated

for 4 h. Absorbance was recorded at 570 nm using Eliza Plate Reader. Inhibition of formation of coloured MTT formazan was taken as an index of cytotoxicity activity. The IC_{50} values on the cancer cells of different tissue origin used for screening were determined by non-linear regression analysis using graph pad software[18].

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