

SHORT COMMUNICATION

Isolation and *in vitro* cytotoxic activity of 11-methylxoside isolated from bark of *Randia dumetorum* Lamk.

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Summary

Randia dumetorum (family *Rubiaceae*) is highly reputed ayurvedic medicinal tree commonly known as the Mainphal. A large deciduous thorny shrub grows up to 5 m of height. It occurs almost throughout India up to 1200 m of altitude. It is found in Himalaya from Jammu East ward ascending to 400 m and from Kashmir to East ward up to 1200 m. 11-methylxoside (compound 1), an iridoid glucoside, was isolated from the bark of this plant. The structure was characterized by using spectroscopic methods including 1D-¹H-NMR, ¹³C-NMR and 2D-NMR (HSQC, HMBC, DQF-COSY) experiments and confirmed by comparison of their NMR data with those from the literature. This compound has been reported for the first time in *Randia dumetorum* bark. The 11-methylxoside was subjected to cytotoxic activity against MDA-MB-231 (breast cancer cell line) and SK-MEL-2 (human skin melanoma cell line), BE(2)C (neuroblastoma cell line derived from human bone marrow) and U87MG (human neuronale glioblastoma (astrozytom) cell line showed appreciable cytotoxic effect with IC₅₀ value 63.10 µg/ml concentration for SK-MEL-2 (human skin melanoma cell line).

Key words: *Randia dumetorum*, *Rubiaceae*, iridoid glucoside, 2D-NMR, cytotoxic activity

INTRODUCTION

Randia dumetorum is a large deciduous thorny shrub belonging to family *Rubiaceae*. It is also known as *Catunaregam spinosa* (Thumb.). Leaves simple, obovate, wrinkled, shiny and pubescent. Flowers white, fragrant, solitary, seen on at the end of short branches. Fruits globose, smooth berries with longitudinal ribs, yellow when ripe. Seeds many, compressed, embedded in the dark fetid pulp[1]. *Randia dumetorum* Lamk. has been reported as an emetic, antidysentric agent. It has anti-bacterial, anti-allergic, anti-inflammatory, analgesic and immunomodulatory activity [2]. Different workers [3-10] have isolated saponins and iridoid from the fruits and bark of this plant. Herein we report the isolation of 11-methylxoside, an iridoid glycoside. This compounds is reported for the first time to exist in this plant's bark. The compound showed effective cytotoxic activity at 400 $\mu\text{g/ml}$ of concentration of the compound against MDA-MB-231(Breast cancer cell line) with cell cytotoxicity of 70.01%, IC_{50} value 338.8 $\mu\text{g/ml}$. The cytotoxic effect of compound was also studied against SK-MEL-2 (human skin melanoma cell line) showed 47.51% cell cytotoxicity at 40 $\mu\text{g/ml}$ which increased to 53.93% at 120 $\mu\text{g/ml}$ concentration with IC_{50} value 63.10 $\mu\text{g/ml}$, against BE(2)C (neuroblastoma cell line derived from human bone marrow) showed 39.44% cell cytotoxicity at 80 $\mu\text{g/ml}$ concentration which increased to 54.68% cell cytotoxicity with 150 $\mu\text{g/ml}$ concentration with IC_{50} value 123 $\mu\text{g/ml}$ and against U87MG (human neuronale glioblastoma (astrozytom) cell line) showed 38.94% cell cytotoxicity at 400 $\mu\text{g/ml}$ concentration which increased to 50.83% at 800 $\mu\text{g/ml}$ with IC_{50} value of 741.3 $\mu\text{g/ml}$ (fig. 1) compound concentration.

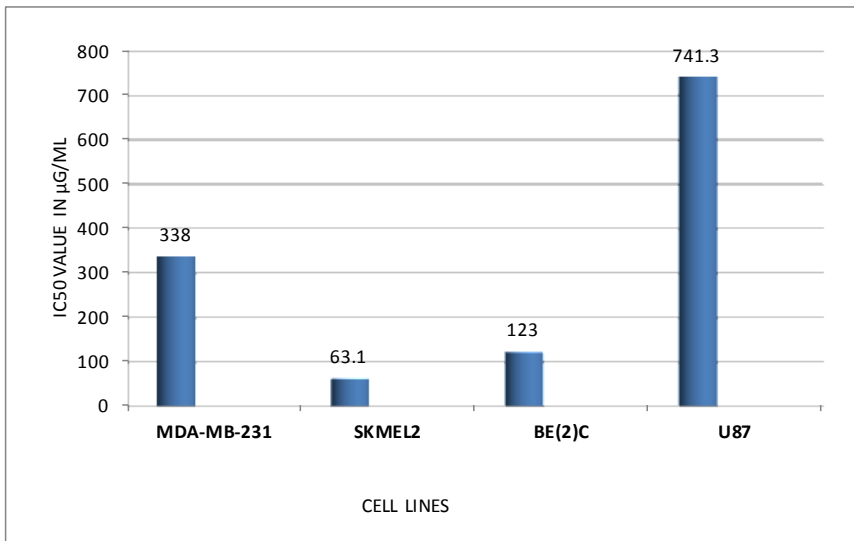


Figure 1.

Ic_{50} values for different cell lines

MATERIAL AND METHOD

Plant bark (3 kg) was collected from Ghansali (Tehri Garhwal, U.K.) and was air-dried in shade. After complete drying, it was subjected to defatting. Defatted bark was exhaustively extracted with 100% absolute ethanol solvent (5 l) until the extract became colorless. Ethanol extract was subjected to distillation under reduced pressure to liberate the crude extract from ethanol solvent. Then, ethanolic crude extract was evaporated to near dryness by leaving overnight. Crude ethanolic extract (250 g) was adsorbed on Silica gel and subjected to column chromatographic separation using CHCl_3 followed by increasing proportions of MeOH in CHCl_3 (v/v) as eluent, collecting 150 ml fractions, CHCl_3 :MeOH (85:15 v/v) eluent gave sample (1), 800 mg (white needles) crystallized from 100% MeOH solvent. The $^1\text{H-NMR}$ spectrum in CDCl_3 showed that the sample was not a pure compound although TLC showed a single spot. For this reason a portion of sample was subjected to further purification by RP-HPLC with a water 590 series pumping system equipped with a water R401 refractive index detector, a μ -Bondapak C18 column (300 \times 7.8 mm i.d.) and a U6k injector using MeOH- H_2O (9:1) as mobile phase (flow rate-2.0 ml/min.) giving compound (1) (t_r 15.3 min).

The NMR experiments, a Bruker DRX-600 NMR spectrometer using the UXNMR software package, NMR 600 MHz for $^1\text{H-NMR}$ and 150 MHz for $^{13}\text{C-NMR}$, solvent CD_3OD , values relative to TMS reference. Chemical shift expressed in δ (parts per million) values, solvent peak δ_H 3.34 and δ_C 49.0 for CD_3OD ; coupling constant (J) are expressed in Hz. 1D- and 2D-NMR experiments were carried out using conventional pulse Sequence[11].

Melting points are uncorrected. The FABMS were: solvent glycerol, accelerating voltage 2 KV, gas Xe. Column chromatography was on silica gel (Merck) and TLC on Kieselgel 60G (Merck), spot on TLC was visualized by spraying with 20% H_2SO_4 and heating at 120 $^\circ\text{C}$ for a few minutes. M.P.216-217 $^\circ\text{C}$, $\text{UV}^{\text{meoh}}_{\text{max}}$ nm:220, IR:1715,1655,1620 cm^{-1} , FABMS: m/z: 805, 643, 565, 465, 425, 403, 385, 315, 251, 224, 193. EIMS(negative ion) 2 ev, m/z: 240, 222, 194, 149, 138 (found: C, 50.63, H, 53.5, $\text{C}_{17}\text{H}_{22}\text{O}_{11}$ requires: C,50.74,H, 5.47%).

$^1\text{H-NMR}$ indicated protons for aglycone part at δ_H 5.72 ppm (d, J =4.5Hz,H-1), δ_H 7.52 ppm(d, J =4.5 Hz,H-3), δ_H 3.33 ppm (m,H-5), δ_H 2.42,2.94 ppm (m, H-6), δ_H 6.89 ppm (m, H-7), δ_H 3.22 ppm (m, H9), δ_H 3.73 ppm (- OCH_3) and for glycone part δ_H 4.65 ppm (d, J =7.6 Hz, Glc-1), δ_H 3.19 ppm (m, Glc-2), δ_H 3.29 ppm (m, Glc-3), δ_H 3.29 ppm (m, Glc-4), δ_H 3.41 ppm (m, Glc-5), and for Glc-6 at δ_H 3.69 ppm (dd, J =11,4.0 Hz) and δ_H 3.89 ppm (dd, J =11,3.3 Hz) (fig. 2) and $^{13}\text{CNMR}$ peaks for aglycone part δ_C 96.2 ppm (C-1), δ_C 152.7 ppm (C-3), δ_C 112.5 ppm (C-4), δ_C 34.6 ppm (C-5), δ_C 39.6 ppm (C-6), δ_C 146.5 ppm (C-7), δ_C 135.8 ppm (C-8), δ_C 46.8 ppm (C-9), δ_C 176.8 ppm (C-10), δ_C 169.5 ppm (C-11) and δ_C 51.2 ppm (- OCH_3). $^{13}\text{CNMR}$ peaks for glycone part showed peaks at δ_C 99.6 ppm (Glc-1), δ_C 73.9 ppm (Glc-2), δ_C 78.0 ppm (Glc-3), δ_C 70.8 ppm (Glc-4), δ_C 77.4 ppm (Glc-5), δ_C 62.4 ppm (Glc-6). These assignments were confirmed by 2D COSY, HSQC (fig 3). HMBC correlation

were established for H3-C5, C1, C4, C11; H7-C5, C6, C9, C8, C10 and H1-C5, C9, Glc-C1, C8, C10 and Glc-H1-C1, Glc-C3 (fig. 4).

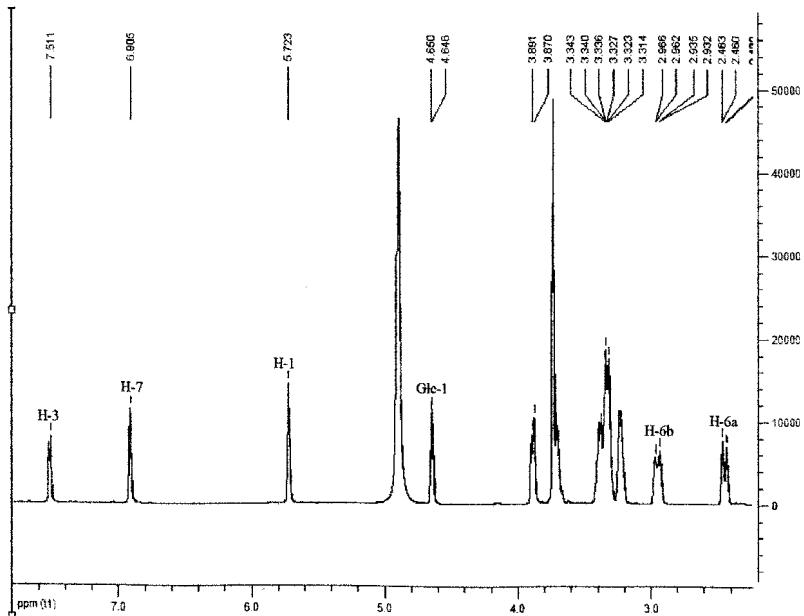


Figure 2.

^1H NMR spectra of compound 1

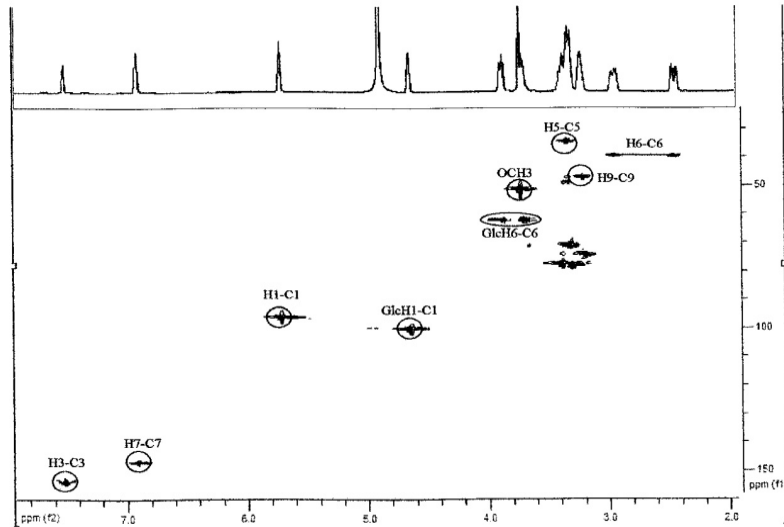


Figure 3.

HSQC spectra of compound 1

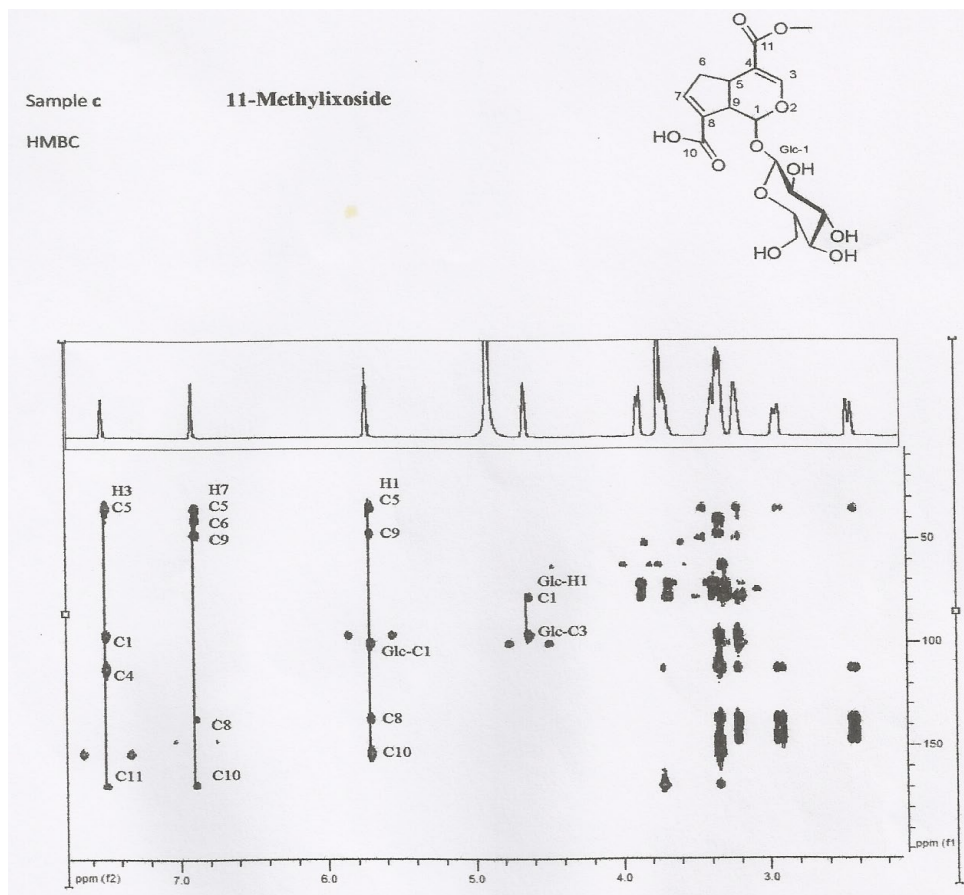


Figure 4.
The HMBC spectra of compound 1

Cell viability assay preparation

Cells were grown in a 96-well plate in Delbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum and anti-biotic (Streptomycin, Penicillin-G, Amphotericin). About 1 ml of each cell suspension type (10^5 cells/ml) was seeded in each well and incubated at 37°C for 48 hours in 5% CO₂ for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various dilutions of extract. The cell viability was measured using MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) with MTT (5 mg/ml) and DMSO. This tetrazolium salt is metabolically reduced by viable cells to yield a blue insoluble Formazan product measured at 540 nm

spectrophotometrically. Controls were maintained throughout the experiment (untreated wells as a control) the assay was performed in triplicate for each of the concentration. The mean of the cell viability values was compared to the control in order to determine the effect of compound on cells and % cell viability was plotted against concentration values.

The IC_{50} , the concentration of compound required to inhibit 50% cell growth, was determined by plotting a graph of Log (concentration of compound) vs % cell inhibition. A line drawn from 50% value on the Y axis meeting the curve and interpolated to the X axis. The X axis value gave Log (concentration of compound). The antilog of that value gave IC_{50} value. Percentage inhibition of compound against all cell lines was calculated using the following formula:

$$\% \text{ cell survival} = (At - Ab) \times 100 / (Ac - Ab)$$

where At – absorbance of test, Ab – absorbance of blank (media) and Ac – absorbance of control (cells)

$$\% \text{ cell inhibition} = 100 - \% \text{ cell survival.}$$

RESULT AND DISCUSSION

An aqueous ethanolic extract of the bark on repeated column chromatography afforded 11-methylxoside, molecular weight of 11-methylxoside is 402, as concluded from the FAB-Mass spectra which showed peaks at m/z 565(M+H+glucose)⁺, 642(M+H+aglycone)⁺ and 805(2M+H)⁺. The EIMS (negative ions) showed peaks at m/z 240 (aglycone), 222 (aglycone-H₂O) and 194 (aglycone-CO). Its UV spectrum showed an intense absorption band at 230 nm and IR spectrum ν^{KBr}_{max} (cm⁻¹) showed band at 1710 and 1645 cm⁻¹. This data showed an conjugated carbonyl functional group ($\nu_{C=C=O}$). The ¹H-NMR spectrum normal mode 600 MHz showed a doublet (J=5.72 Hz, d(4.5) for C-1 proton at 96.2 ppm δ_c , a doublet (J=7.52 Hz) at δ_c 152.7 ppm for the C₃ proton characteristic of iridoids. The interpretation of the proton spin-spin coupling pattern was done with the aid of 2D-NMR, HSQC and HMBC experiments. This gave more unequivocal results. Comparison of the ¹H-NMR and ¹³C-NMR with those from the literature [12-13] confirmed the structure of compound (1) as 11-methylxoside (fig. 1).

Cytotoxic activity

Compound 1 was subjected to cytotoxic activity against MDA-MB-231 (breast cancer cell line) and the results are shown in Table 1. The percentage viability was 85.75% on treating cell line at 50 $\mu\text{g/ml}$ which decreased to only 29.99% with further increasing concentration to 400 $\mu\text{g/ml}$ with IC_{50} value 338.8 $\mu\text{g/ml}$.

Table 1.

Cytotoxic activity of compound 1 on MDA-MB-231(breast cancer cell line)

Concentration	O.D. average	% Cell viability	S.D.	% Cell cytotoxicity
Control	0.426	100	3.642	00
50 $\mu\text{g/ml}$	0.365	85.75	5.929	14.25%
100 $\mu\text{g/ml}$	0.252	59.12	2.803	40.88%
200 $\mu\text{g/ml}$	0.232	54.58	2.22	45.42%
400 $\mu\text{g/ml}$	0.128	29.99	2.4	70.01%

Compound 1 subjected against SK-MEL-2 (human skin melanoma cell line) (tab. 2) showed % cell cytotoxicity of 47.51% at 40 $\mu\text{g/ml}$ of concentration) which increased to 53.93% at 120 $\mu\text{g/ml}$ concentration with IC_{50} value of 63.10 $\mu\text{g/ml}$.

Table 2.

Cytotoxic activity of compound 1 on SK-MEL-2 (human skin melanoma cell line)

Concentration	O.D average	% Cell viability	S.D.	% Cell cytotoxicity
Control	0.956	100	2.401	0
5 $\mu\text{g/ml}$	0.846	88.49	10.562	11.51%
10 $\mu\text{g/ml}$	0.67	70.07	11.669	29.93%
20 $\mu\text{g/ml}$	0.609	63.76	10.77	36.24%
40 $\mu\text{g/ml}$	0.502	52.49	4.949	47.51%
120 $\mu\text{g/ml}$	0.4404	46.07	4.212	53.93%

Compound 1 subjected to BE (2)C (neuroblastoma cell line) Table 3 showed maximum 39.44% at 80 $\mu\text{g/ml}$; 54.68% at 150 $\mu\text{g/ml}$ with IC_{50} value of 123 $\mu\text{g/ml}$.

Table 3.

Cytotoxic activity of Compound 1 on BE(2)C is an neuroblastoma cell line

Concentration	O.D. average	% Cell viability	S.D.	% Cell cytotoxicity
Control	0.488	100	2.869	0
5 $\mu\text{g/ml}$	0.396	81.22	0.385	18.78%
10 $\mu\text{g/ml}$	0.39	79.85	0.392	20.15%
40 $\mu\text{g/ml}$	0.372	76.3	1.479	23.70%
80 $\mu\text{g/ml}$	0.2955	60.56	1.453	39.44%
150 $\mu\text{g/ml}$	0.2211	45.32	1.356	54.68%

Compound 1 against U87MG (Human Neuronale Glioblastoma (Astrozytom) cell line (tab. 4) showed 38.94% cell cytotoxicity at 400 $\mu\text{g/ml}$ concentration and 50.83% cell cytotoxicity at 800 $\mu\text{g/ml}$ with IC_{50} value of 741.3 $\mu\text{g/ml}$ concentration.

Table 4.

Cytotoxic activity of compound 1 on U87MG (human neuronale glioblastoma (astrozytom) cell line

Concentration	O.D. average	% Cell viability	S.D.	% Cell cytotoxicity
Control	0.378	100	6.006	0%
50 µg/ml	0.344	91.02	3.543	8.08%
100 µg/ml	0.295	77.89	1.037	22.11%
200 µg/ml	0.2646	70.01	5.52	29.09%
400 µg/ml	0.231	61.06	2.703	38.94%
800 µg/ml	0.185	49.17	4.98	50.83%

CONCLUSION

The occurrence of 11-methylxoside in *Randia dumetorum* Lamk. confirms the presence of iridoids in genus *Randia*. The natural occurrence of this compound can be conclusive for the chemotaxonomic characterization of this genus. With respect to numerous uses of this plant as folk medicines and great experiments that have been accomplished to investigate its biological activity, we investigated chemical constituents and their cytotoxic activity to get potential cytotoxic agents.

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IZOLACJA I DZIAŁANIE CYTOTOKSYCZNE *IN VITRO* 11-METYLIKSOZYDU WYIZOLOWANEGO Z KORY *RANDIA DUMETORUM* LAMK.

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Streszczenie

Randia dumetorum (rodzina *Apiaceae*) jest rośliną cenioną w medycynie ajurwedyjskiej, znaną pod nazwą Mainphal. Duży, liściasty, ciernisty krzew osiąga wysokość 5 m. Występuje praktycznie w całych Indiach do wysokości 1200 m. Można go znaleźć w Himalajach od Jammu (400 m) do Kaszmiru (1200 m). Z kory tej rośliny wyizolowano glukozyd irydoidowy 11-metyloksyd (składnik 1). Jego strukturę zbadano za pomocą metod spektroskopowych obejmujących 1D-¹HNMR, ¹³C-NMR i 2D-NMR (HSQC, HMBC, DQF-COSY) i potwierdzono poprzez porównanie jego NMR danych z danymi literaturowymi. Obecność tego składnika stwierdzono po raz pierwszy w korze *Randia dumetorum*. Działanie cytotoksyczne 11-metyliksozydu było badane przeciw MDA-MB-231 (linia komórkowa raka sutka) i SK-MEL-2 (linia komórkowa czerniaka), BE(2)C (linia komórkowa neuroblastomy uzyskana z ludzkiego szpiku kostnego) i U87MG (linia komórkowa ludzkiej glioblastomy (gwiazdziak). Wykazano znaczne działanie cytotoksyczne z wartością IC₅₀ w stężeniu 63.10 μg/ml przeciw SK-MEL-2 (linia komórkowa czerniaka).

Słowa kluczowe: *Randia dumetorum*, *Rubiaceae*, glukozyd irydoidowy, 2D-NMR, działanie cytotoksyczne