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Research Article

In vitro Anti-Leishmanial and Anti-Tumour Activities of a Pentacyclic Triterpenoid Compound Isolated from the Fruits of *Dregea volubilis* Benth *Asclepiadaceae*

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Abstract

Purpose: *Dregea volubilis Benth, commonly known as Jukti in Bengal, is used in the treatment of boils and abscesses from ancient times. The purpose of this study is to elucidate the active compounds and as well as their anti-leishmanial and anti-tumour activities.*

Methods: *Dried and crushed fruits of Dregea volubilis were extracted by petroleum ether (40 - 60°C); the best solvent system had first been verified by analytical Thin Layer Chromatography (TLC). The extract was subjected to TLC and column chromatography (CC) to isolate the pure compounds. Spectra data were obtained by Infra Red pectroscopy, Mass Spectroscopy and Nuclear Magnetic Resonance - Proton Magnetic Resonance (PMR), Carbon Magnetic Resonance (CMR) and Distortionless Enhancement by Polarization Transfer (DEPT) - for structure elucidation of the isolated compound(s). One of the compounds isolated was screened for anti-leishmanial activity against promastigotes of Leishmania donovani and anti-tumour activity on K562 leukemic cell line.*

Results*: A pentacyclic triterpenoid compound was isolated and designated as taraxerone, and then characterized as d-friedoolean-14-en, 3 one together with ß-sitosterol and a long chain lipid fraction.. This compound showed in vitro anti-leishmanial activity against promastigotes of Leishmania donovani (strain AG 83) and anti-tumour activity on K562 leukemic cell line.*

Conclusion: *A pentacyclic triterpenoid compound designated as taraxerone and characterized as D-friedoolean-14-en, 3 one together was successfully isolated. The structure was determined on the basis of spectral analysis (IR, MASS, NMR (PMR, CMR and DEPT) and the compound demonstrated in vitro anti-leishmanial and anti-tumour activities.*

Keywords: *Dregea volubilis*, Pentacyclic triterpenoid, Taraxerone, Spectroscopy (IR, MASS, CMR, PMR, DEPT), Anti-leishmanial, Anti-tumour.

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Introduction

Dregea volubilis (Linn. f.) Benth ex. Hook f. Syn: *Wattakaka volubilis* (Linn. f.) Stapf; *Marsedenia volubilis* (Cooke) belongs to the family Asclepiadaceae and is commonly known as "Jukti" in Bengal. It is a tall woody climber with height of 11 m and a girth of 95 cm with densely lenticulate branches. It occurs throughout the hotter parts of India and Car Nicober Islands ascending to an altitude of 1500m[1]. The parts of the plant are used traditionally as medicines. The juice of the plant is used as a sternutatory and the leaves are employed in the treatment of boils and abscesses. The roots and tender stalks are used as emetic and expectorant. It has been reported that the ethyl alcohol (50%) extract of the plant has activity on the central nervous system as well as anti-cancer activity against Sarcoma 180 in mice[1], the reported maximum tolerated dose being 500 mg/kg body weight of albino mice. Two pregnane glycosides, dregeosides Ap1 and A_{01}, isolated from this plant, collected from Thailand, showed anti-tumor activities against melanoma B-16 in mice[1]. Reichstein and co- workers[2] studied the components of the seeds of the plant and deduced the structure of drevogenins A, B, D and P. Subsequently, the isolation and characterization of twelve polyhydroxy C/D cis-pregnane glycosides from the same plant collected from Thailand was reported[2,3]. Isolation of β- sitosterol, kaempherol-3-galactoside, a 2-deoxy sugar, drevogenin A, drevogenin P, D–cymarose and L-olendrose from the plant has also been reported[4,5].

The objective of this work is to undertake the isolation and characterization of a novel pentacyclic triterpenoid compound from the extract of *Dregea volubilis* in order to evaluate its anti-leishmanial activity as well as its anti-tumour activity on K562 leukemic cell line. Previously taraxerone was isolated from the plant, *Myrica rubra*[5,6], and shown to have inhibitory activity on reverse transcriptase on human immunodeficiency virus and of kinesin motor proteins[7].

Experimental

Plant material

The plant material, *Dregea volubilis,* was collected from Indian Botanic Garden, Howrah, West Bengal, India. A voucher specimen (voucher no CNH/I-I/(267)/2008) was preserved for future reference at Bengal Institute of Pharmaceutical Sciences, Kalyani, Nadia, India.

Extraction and isolation

The shade-dried powdered fruits of *Dregea volubilis* (2.4 kg) were extracted successively with petroleum ether (3×8L) at 40 - 45°C temperature. The combined extract was concentrated and 18g of the extract was applied to a column of silica gel 60 (400g) and washed with 100% petroleum ether. Gradient elution was carried out with a mixture of petroleum ether and chloroform (1:9, 1:4, 3:7, 2:3 and 1:1, respectively). A total of 72 fractions (50ml) were collected. Fractions giving similar spots on TLC (obtained by spraying with Libermann - Buchard reagent) were combined. Fractions eluted with chloroform-petroleum ether (1:4) were also combined and subjected to re-chromatography over silica gel (20 g). The fractions (15 ml lots) were eluted with chloroform-petroleum ether mixture (3:7) to furnish taraxerone (1.5 g).

Thermal and spectroscopic studies

All melting points were measured on Yanagimoto micromelting apparatus and are uncorrected. Infra-red (IR) spectra were determined using JASCO 7300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. Nuclear magnetic resonance (NMR) spectra – both proton magnetic resonance (PMR) and carbon magnetic resonance (CMR) - were recorded at 500 and 300 MHz, respectively, using a Jeol ECP-500 spectrometer in C_5D_5N with TMS as the

internal standard. High resolution fast atom bombardment mass spectroscopy (MASS) was performed on a JEOL MS-700 mass spectrometer. TLC was carried out on silica gel $60F_{254}$ and the spots were visualized by spraying with Libermann- Buchard's reagent followed by heating. Silica gel (silica gel 60-120 mesh, Merck) was used for column chromatography (CC).

Parasite culture and growth conditions for anti-leishmanial activity

Leishmania donovani strain AG 83 was collected[8] and maintained in golden hamsters by serial passage. After 2 months, the hamster was sacrificed and its spleen was isolated and macronized. Approval (no: 367001/C/CPCACA) for the animal experiment was given by the Ethical Committee of Jadavpur University, Kolkata, West Bengal, India. The spleenic culture was made in Medium-199 (L-glutamine with Hepes buffer without $NaHCO_3$) supplemented with 10% fetal bovine serum of pH 7.2. The logarithm phases of promastigotes (2×10^6 cells/ml) were incubated with or without the compounds along with Medium-199 at 22 °C. The tested compounds were dissolved in 0.2% dimethyl sulphoxide (DMSO), these compounds were added to the culture in graded dose of 3 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml and 30 µg/ml. After 2 h of treatment, the tubes were centrifuged at 8000 g for about 10 min. The supernatant was decanted and the pellets were washed with 20mM phosphate buffer saline (PBS). Each pellet was dissolved in 100µl (2mg/ml) MTT solution, the tubes incubated at 22°C for 4 h and then centrifuged at 8000 g for 10 min. The resulting pellets were dissolved in 500µl 0.2% DMSO and the absorbance measured spectrophotometrically at 570nm. Lysis of promastigotes (%) by the compounds was calculated by the standard formula of Tim Mosmann[8], as shown in Eq 1.

% lysis = 100 – {(test – positive control)/(control – positive control)} × 100 ...(1)

The IC_{50} dose was evaluated by linear regression analysis using Graph Pad Prism 3 software.

Cell culture and growth conditions for anti-tumour activity

The human chronic myelogenous leukemia cell line K562 was obtained from a patient in blast crisis of chronic myeloid leukemia. The cells were grown in RPMI-1640 medium supplemented with 10% foetal calf serum (Gibco,USA), air and 5% CO_2. The tested compound contained 1×10^6 cells/ml at graded 2mML glutamine and 50 µg/ml gentamycin at 37°C in a fully humidified atmosphere of 95% dose. After 18 h of incubation at 37°C in 5% CO_2 incubator, all tubes were centrifuged at 8000 g for 10 min. The supernatant was decanted and the pellets were washed with 20mM of PBS. Each pellet was dissolved in 100µl (2mg/ml) MTT solution incubated at 22°C for 4 h and centrifuged at 8000 g for 10 min. The resulting pellets were dissolved in 500µl DMSO and the absorbance measured spectrophotometrically at 500nm. Lysis (%) of K562 cells by the compound was calculated by standard formula[8] (Tim Mosmann), the formula is [100 – {(test – positive control)/(control – positive control)}] × 100. The IC_{50} dose was evaluated by linear regression analysis using Graph Pad Prism 3 software.

Results

In vitro anti-leishmanial activity against *Leishmania donovani* (strain AG83) and anti-cancer activity on K562 leukemic cell line have been shown from the isolated compound in the petroleum ether extract of *Dregea volubilis* fruits. The active fraction of the extract of *Dregea volubilis* fruits and identified as a pentacyclic triterpenoid compound, namely, taraxerone whose structure is shown Fig 1. The compound was crystallized from methanol as colorless crystalline needles with a melting point (m.p.) of 239 - 242ºC, [α]D+9.72 (c, 1.04, $CHCl_3$). The compound gave a brownish violet color for the

Libermann-Burchard test, indicating the presence of triterpene skeleton. The compound exhibited in its **IR** spectrum absorption bands at 1708, 1473, 1375, 996 and 818 cm-1 attributed to a keto group and an olefinic double bond. MASS spectrum displayed a sodiated molecular ion peak at m/z 447.39, indicating the molecular weight of the compound to be 424 and its molecular formula, $C_{30}H_{48}O$. The ^1H NMR spectrum of the compound displayed eight methyl signal (all singlets) at δ 0.83, 0.90, 0.91, 0.95, 1.06, 1.08, 1.08 and 1.14. The spectrum also showed one double doublet at δ 5.56 (J = 7.8 and 2.7 Hz) ascribed to an olefinic proton. The ^{13}C NMR spectrum of the compound indicate the presence of thirty carbons in the compound. All the carbon resonances were assigned by multiplicity information obtained from distortionless enhancement by polarization transfer (DEPT). The spectrum revealed the presence of eight methyls, ten methylenes, four methines and eight quaternary carbons, including signals appropriate for one trisubstituted double bond and a carbonyl function. The singlet at δ 217.9 unambiguously demonstrated the presence of a carbonyl group attached to a six-member ring. The singlet at δ 158.0 and doublet at δ 117.6 can be assigned to C-14 and C-15, respectively, indicating that the compound belongs to taraxerone or D- friedooleananes group.

From the foregoing evidence, it can be said that the triterpene core of the compound is D-friedoolean-14- en, 3 one as illustrated in Figure 1.

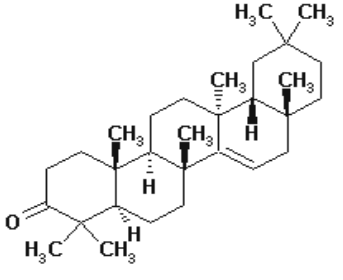

Figure 1: Structure of taraxerone (D-friedoolean-14- en, 3 one)

Table 1: ^{13}C NMR data

Carbon number	Taraxerone
1	217.9(s)
2	158.0(s)
3	117.6(s)
4	56.2(d)
5	49.2(d)
6	49.1(d)
7	48.0(s)
8	41.0(t)
9	39.3(s)
10	38.7(t)
11	38.1(t)
12	38.1(s)
13	37.9(s)
14	37.1(t)
15	36.2(s)
16	35.5(t)
17	34.5(t)
18	34.0(t)
19	33.8(q)
20	33.5(t)
21	30.3(q)
22	30.2(q)
23	29.2(s)
24	26.5(q)
25	26.0(q)
26	21.9(q)
27	21.7(q)
28	20.4(t)
29	17.8(t)
30	15.2(q)

Anti-leishmanial activity

Table 2 shows that taraxerone inhibited growth of *Leishmania promastigotes* in a dose-depended manner

Anti-tumour activity

The results of the anti-tumour test are indicated in Table 3. As the dose of the drug increased, the proportion of cells lysed rose from 38 to 87%.

Table 2: Activity of taraxerone against *Leishmania promastigotes* culture (2×10^6 cells/ml)

Drug dose (µg/ml)	Lysis (%) with respect to control (0.1% $CHCl_3$)	IC_{50} value for Taraxerone
3	51.34	
5	57.2	
10	66.34	3.18
15	72.7	
30	90.8	

Table 3: Anti-tumour data for taraxerone

Drug dose (µg/ml)	Lysis (%) with respect to control (0.1% $CHCl_3$)	IC_{50} value for taraxerone
5	38.4	
10	61.3	7.401
15	87.4	

Discussion

Our studies indicate that the non-polar content of the fruits of *Dregea volubilis* Benth yielded a pentacyclic triterpenoid designated as taraxerone and characterized as D-friedoolean-14-en, 3 one the structure was determined on the basis of IR, MASS, NMR (PMR, CMR and DEPT) spectroscopic analysis On the other hand, previous studies on the flowers of this plant showed the presence of polyoxy- and polyhydroxyl pregnane glycosides[1,2,3] which constitute the steroid moiety.

That the isolated compound exhibited *in vitro* anti-leishmanial activity against promastigotes of *Leishmania donovani* (strain AG 83) and anti-cancer activity on K562 leukemic cell line, is instructive. This work buttresses the need to continue to investigate traditional remedies with a view to isolating their active constituents since an estimated 60% of people living in developing countries depend on traditional medicine for their primary health care. Modern therapy derived from medicinal herbs promises a practical approach to development of effective and affordable drugs. However, further work (including *in vivo* studies) will still be required to establish the effectiveness and full potentials of the taxerone isolated from *Dregea volubilis* Benth.

Conclusion

The non-polar portion of the fruit extract of *Dregea volubilis* Benth contains a pentacyclic triterpenoid compound, taraxerone. The compound demonstrated anti-leishmanial and anti-tumour activities.

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