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A Pentacyclic Triterpenoid Possessing Anti-inflammatory Activity from the Fruits of *Dregea volubilis*

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ABSTRACT

The aim of the study is to find anti-inflammatory activity of the compound obtained from the petroleum ether extract of the fruits of *Dregea volubilis*.

Fruits of *Dregea volubilis* were extracted by petroleum ether and through column chromatography led to obtain a compound. The structure of the compound was determined on the basis of IR, MASS, NMR (PMR, CMR and DEPT) spectroscopic analysis. The compound was screened for anti-inflammatory activity in albino rats using acute carageenan induced paw oedema.

The petroleum ether extract of the fruits of *Dregea volubilis* Benth led to isolation and characterization of a pentacyclic triterpenoid compound designated as taraxerol and characterized as D- friedoolean- 14- en, 3 ol (Fig.1). It has shown significant anti-inflammatory activity in albino rats.

Anti-inflammatory activity in albino rats has been shown by taraxerol obtained from the petroleum ether extract of the fruits of *Dregea volubilis*.

KEYWORDS: *Dregea volubilis* Benth, pentacyclic triterpenoid, taraxerol, anti-inflammatory activity.

1. 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, 11 m. of height and 95 cm. in girth with densely lenticulate branches, occurring throughout the hot climatic parts of India and Car Nicober Islands ascending to an altitude of 1500m. The parts of the plant are used traditionally as medicines. The juice of the plant is used as stimulant and leaves are employed in application for boils and abscesses, in other way the roots and tender stalks are used as emetic and expectorant (1). It is reported that an ethanol (50%) extract of the plant has shown activity on the central nervous system as well as anticancer activity against Sarcoma 180 cell line in mice. The maximum tolerated dose was found to be 500 mg/kg body weights of albino mice (2). Two pregnane glycosides dregeosides Ap1 and A_{01} isolated from this plant collected from Thailand have shown antitumor activities against melanoma B-16 in mice (3). Reichstein and co-workers studied the components of the seeds of the plant and deduced the structure of drevogenins A, B, D and P. Previous investigation, reported the isolation and characterization of twelve polyhydroxy C/D cis-pregnane glycosides from the same plant collected from Thailand have been reported (4). 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 collected from Shibpur, Howrah, West Bengal (4).

Present work is based on the chemical studies on naturally occurring bioactive triterpene. It is reported herein the isolation and characterization of a pentacyclic triterpenoid (5) designated as taraxerol having anti-inflammatory activity, from the petroleum ether extract of the fruits of this medicinal plant for the first time. Previously taraxerol (6) was isolated from the plant *Myrica Rubra* and had shown its inhibitory activity on reverse transcriptase on human immunodeficiency virus and of kinesin motor proteins (7).

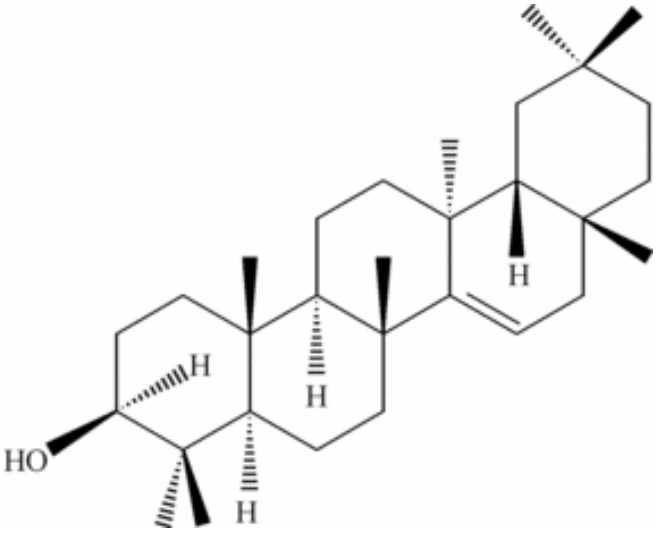

Figure 1: D – friedoolean – 14 – en 3- ol or Taraxerol

2. MATERIALS AND METHODS

2.1 General procedure

All melting points were measured on Yanagimoto micromelting apparatus and are uncorrected. IR spectra were determined using JASCO 7300FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively using a Jeol ECP-500 spectrometer in C_5D_5N with TMS as internal standard. HRFABMS was performed on a JEOL MS-700 mass spectrometer. TLC was carried out on silica gel $60F_{254}$ and spots were visualized by spraying with Libermann-Burchard's reagent followed by heating. Silica gel (silica gel 60, Merck) was used for column chromatography.

2.2. Plant material

The plant material was collected and authenticated from Indian Botanic Garden, Howrah, West Bengal, India. A voucher specimen has been preserved for any future reference.

2.3. Extraction and isolation

The shade dried powdered fruits of *Dregea volubilis* (2.4kgs) was extracted with Petroleum ether solvent at 40–45°C temperature. The extract was concentrated and 18gms of extract was applied to a column of silica gel 60 (400gm) and washed with 100% Petroleum ether. Gradient elution was carried out with mixture of petroleum ether- chloroform (1:9, 1:4, 3:7, 2:3 and 1:1). A total of 72 fractions (50ml) were collected and fractions giving similar spots on TLC were combined. Fractions eluted with chloroform-petroleum ether (1:4) were combined and subjected to re-chromatography over silica gel (20g), the fraction contained taraxerol along with β-sito sterol and a long chain lipid fraction. Fractions (collected 15ml lots) eluted with chloroform-petroleum ether mixture (1:1) furnished Taraxerol (1.5g).

2.4. Anti-inflammatory Activity
Carrageenan induced rat paw oedema:

Eighteen rats were divided in 3 groups of 6 rats each for various treatments as shown in Table 1. The concentration of carrageenan was selected 0.1ml of 1% and was injected subcutaneously 30 minutes after administration of the compound (5 mg/kg p.o) into the planter region of right hind paw to induce oedema. The paw volume was measured initially and at 1, 2, 3 and 4 h after carrageenan injection using plythesmometer (8–9). Indomethacin 10 mg/ kg injected through i.p route as standard drug. Percentage inhibition of edema was calculated by the formula,

$$\% \text{ inhibition} = (1 - vt/vc) \times 100.$$

Where vt and vc indicate mean relative changes in paw volume of the test and control respectively (table -1).

3. RESULTS

The petroleum ether extract of *Dregea volubilis* fruits has shown moderate in- vitro anti-inflammatory activity. The active fraction eluted with petroleum ether-chloroform mixture from the silica gel chromatography led to isolation of the pentacyclic triterpenoid.

Taraxerol(1) was crystallized from methanol as colorless shiny needles mp.276–278°C, $[\alpha]_D$ +0.55°. The compound gave positive Libermann-Burchard's test indicating the triterpene nature of the compound. The IR spectrum exhibited an absorption band at 3482 cm-1 attributable to

Table 1. CMR Data: Mentioned in the following Table:

Carbon No.	Taraxerol
1	38.4(t)
2	27.3(t)
3	79.2(d)
4	39.1(s)
5	55.7(d)
6	19.0(t)
7	35.3(t)
8	38.9(s)
9	48.9(d)
10	37.9(s)
11	17.7(t)
12	35.9(t)
13	37.9(s)
14	158.1(s)
15	117.0(d)
16	36.9(t)
17	38.1(s)
18	49.4(d)
19	41.4(t)
20	29.0(s)
21	33.9(t)
22	33.2(t)
23	28.1(q)
24	15.6(q)
25	15.6(q)
26	30.1(q)
27	26.0(q)
28	30.1(q)
29	33.5(q)
30	21.5(q)

hydroxyl group and an olefinic double bond at 1638 cm-1. It displayed a quasi-molecular ion peak at m/z 449[M+Na]+ in the MALDI-TOF mass spectrum indicating the molecular weight to be 426 gm. This information coupled with the ^{13}C NMR and DEPT spectral analysis suggested the molecular formula as $C_{30}H_{50}O$.

The ^{1}H NMR spectrum of the compound displayed eight methyl signals resonated at δ 0.80, 0.82, 0.90(2×CH3), 0.92, 0.95, 0.97, and 1.09. Additional signals observed include those described to an olefinic proton at δ 5.25 and one oxymethine proton at δ 3.2.

The compound displayed 30 signals in its ^{13}C NMR spectrum accounted for seven singlets, five doublets, ten triplets and eight quartrates. The signal appeared at δ 79.5(doublet) indicated the presence of a hydroxyl group at C-3 of the triterpene skeleton and is β-oriented (equatorial). While the olefinic carbons resonated at δ 158.5(singlet) and 117.0(doublet) indicated the position of the double bond between C-14 and C-15 in ring D. Finally from the fore going evidences it was concluded that the triterpene core of compound 1 was D-friedoolean-14- en, 3 ol or Taraxerol.

4. ANTI-INFLAMMATORY ACTIVITY

The compound has shown significant anti-inflammatory effect. The compound at a dose of 5 mg/ kg reduced significantly ($p<0.001$) paw volume, which is compared with standard drug. After 4 h of treatment taraxerol had shown % inhibition of 82.52% which is less than standard drug produced 88.83%. But the

% inhibition of the compound is close to standard drug and significant as described in Table 2.

5. DISCUSSION

Inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases (10). A number of natural products are used in various traditional medical systems to treat relief of symptoms from inflammation. The compound obtained from the petroleum-ether extract of fruits of *Dregea volubilis* demonstrated significant anti-inflammatory effect in carrageenan induced inflammation model. Carrrageenan induced paw edema model is known to sensitive to cyclooxygenase inhibitors. It has been used to evaluate the effect of NSAIDS, which primarily inhibits the cyclooxygenase involved in prostaglandin synthesis (11–13). There are two phases in inflammatory reaction in carrageenan-induced paw edema model in rats : first phase and second phase. The first phase, which occurs between 0 to 2.5 h after injection, has been attributed to the release of histamine or serotonin (14). The second phase of inflammatory reaction which is measured

Table 2. Anti-inflammatory effect of test compound on carrageenan induced paw oedema in albino rats and the mean % of inflammation at time.

Treatment	1hr	2hr	3hr	4hr	% Inhibition
Control (Carrageenan)	0.73± 0.0881	1.4 ± 0.5774	1.8 ± 0.5774	2.06 ± 0.0881	-
Standard (Indomethacin)	0.2±0.05774**	0.5±0.05774***	0.36±0.03333***	0.23±0.03333***	88.83
Test (Taraxerol)	0.23±0.3333**	0.7 ± 0.1000**	0.5 ± 0.05774***	0.36±0.03333***	82.52

Values are mean ± SEM; n= 6 in each group;

**indicates P value is <0.01, One way ANOVA with Tukey-Kramer multiple comparison post test.

***indicates P value is <0.001 when compared to control.

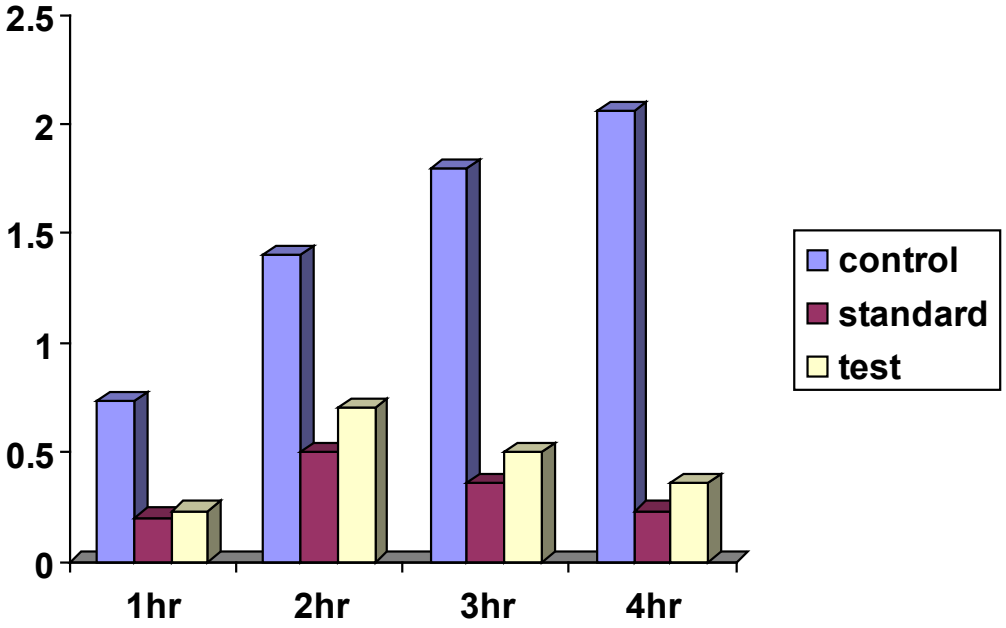

Figure 2. Graphical representation of anti-inflammatory activity of Taraxerol.

after 3 h is caused by the release of bradykinin, protease, prostaglandin and lysosome (14).

The compound had shown a significant inhibitory effect as an anti-inflammatory agent. The compound had inhibited 82.52% which is less than standard drug (88.83%). But it is very close and comparable with standard indomethacin. Therefore, it can be inferred that the inhibitory effect of taraxerol on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Thus the results presents current study of triterpinoid taraxerol isolated from Fruits of *Dregea volubilis* has potent and significant anti-inflammatory activity. However a more detail study is require to identify the exact mechanism of action.

5. CONCLUSION

The compound taraxerol obtained from the petroleum ether (40°–60°C) extract of the fruits of *Dregea volubilis* has shown anti-inflammatory activity in albino rats.

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