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13α-methyl-27-norolean-14-en-3β-ol, a Triterpeneisolated from the stem of *Euphorbia Hirta* (linn) Possess an Anti-Asthmatic Properties

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Abstract

The present study deals with the phytochemical screening of the alcoholic extract of stem of Euphorbia hirta revealed the presence of triterenoid which were subjected to physical, chemical and spectral identification using IR, 1H-NMR, 13C-NMR and FABMS. On the basis of the spectral data analysis and chemical reactions, the compounds have been established as 13α-methyl-27-norolean-14-en-3β-ol namelytaraxerol. The present study has been further designed to evaluate the anti-asthmatic activity of the compound taraxerol. The anti-asthmatic activity was carried out onhistamine induced bronchospasm in guinea pigs which showed that, the alcoholic extract of the stem significantlyinhibited ($p < 0.05$) the contractile effect of histamine. Thus the alcoholic extracts were found to possess potentantiasthmatic property against guineapig with p values less than 0.05, suggesting their potential for further investigation as anti-asthmatic agent.

Keywords: Euphorbia hirta, euphorbiaceae, triterpenoid, taraxerol, spectroscopic analysis, antiasthmatic activity.

Introduction

A number of herbal drugs have been used in the treatment of asthma and proven therapy in the treatment of this disease have been tried to give combined beneficial effects. The pharmacological actions of active principles isolated from various medicinal plants have been studied in our world[1].

Presently various synthetic drugs and physical medicine have been discovered and are being used on a large scale but the significant of the natural drugs have not been relegated. *Euphorbia hirta* Linnbelongs to natural order Euphorbiaceae. It is an annual herbaceous plant with a slender hairy stem. Its flowers are small and yellow in colour. The plant produces milky latex which is irritating to the mucus membrane[2,3].

The leaves of this plant have relaxing action upon the smooth muscles of lungs. It possesses great therapy in bronchial asthma. The whole plant is used for the treatment of diseases in children especially in warms,bowels complain and cough. Its juice is used in bronchial asthma affections and chronic coughs. The latex of this plant is used as an application for warts. The roots of this plant possess on anti-emetic properties. Its juice is used in dysentery, colic and also in warm infection[4,5]. The decoction of its leaves is used in bronchial asthma and chronic coughs. This plant has been found to possess anti-asthmatic, anti-histaminic and spasmolytic properties.

In view of its medicinal importance it was worthwhile to carry out systematic phytochemical investigation on the stem of this plant.

Material and Methods

The stems of the *Euphorbia hirta*Linn.were collected locally and identified by taxonomist of Botany Department of Dr. H.S. Gour University Sagar (M.P.). The plant material was collected in the month of September and October-2002 and herbarium specimen has been XXXV deposited in Chemistry Department room no. 36 of this University.

Extraction and Isolation: The air-dried powdered stem (4.0 kg) of the plant *Euhorbia*hirta was extracted with 95% ethanol. The total ethanolic extract was concentrated under reduced pressure to get a light brown viscous mass, which was successively and sequentially fractionated with various solvent from nonpolar to polar as petroleum ether (60–80°C), benzene, chloroform, ethyl acetate, acetone and methanol. The ethyl acetate extract on concentration under reduced pressure yielded a brown viscous mass, which on addition of excess of solvent ether gave a precipitate. The precipitate was then subjected to TLC[21] examination, using n-butanol: acetic acid: water (4 : 1 : 5) as solvent system and I_2 vapour as visualising agent when depicted two spots. Therefore, it was subjected to column chromatography over silica gel G for purification and eluted with benzene, benzene: chloroform (1 : 1), benzene : chloroform: ethyl acetate (1 : 1 : 1) and studied separately.

Compound**: C**olourless needles which analysed for the mf $C_{30}H_{50}O$, mp 280-282°C [M^+] 426, **IR** υ_{max}^{KBr} cm^{-1} 3422.8 (—OH group), 3018.6 (= C-H str), 2932.4 (–C–H str of CH_3), 2856.6 (–C–H str),1608.6 (–CH_2–CH group) ,1400.0, 13195 (triterpenoidal nature), 813 (> C = CH). **FABMS** m/z 426 [M^+] 408, 393, 284, 269, 204, (base peak)133. **^{13}C-NMR** (400 MHz,

CDCl3) **:** 39.5 (C–1), 33.8 (C–2), 216.8 (C–3), 47.3 (C–4), 54.6 (C–5), 38.6 (C–6), 36.6 (C–7), 36.5 (C–8), 52.7 (C–9), 38.9 (C–10), 51.6 (C–11), 58.1 (C–12), 37.6 (C–13), 156.5 (C–14), 119.2 (C–15), 38.3 (C–16), 35.5 (C–17), 48.2 (C–18), 20.1 (C–19), 28.7 (C–20), 33.2 (C–21), 35.2 (C–22), 21.5 (C–23), 26.2 (C–24), 16.2 (C–25), 26.7 (C–26), 19.5 (C–27), 30.2 (C–28), 33.7 (C–29), 29.9 (C–30).**^{1}H-NMR** (400 MHz, CDCl3)**:** 2.03 (1H, m, H–1a), 2.10 (1H, m, H–1b), 2.38 (1H, ddd, J= 15.2, 6.2, 2.4, H–2a), 2.63 (1H, ddd, J= 16.4, 11.0, 6.6, H–2b), 3.62(1H, t, H-3) , 1.29 (1H, m, H–5), 1.71 (1H, m, H–6), 1.17 (1H, m, H–7a), 1.46 (1H, m, H–7b), 1.02 (1H, d, J= 4.8, H–9), 3.14 (1H, t, J= 4.8, H–11), 2.80 (1H, d, J= 4.8, H–12), 5.53 (1H, dd, J= 8.0, 3.5, H–15), 1.69 (1H, dd, J= 14.6, 8.2, H–16a), 1.89 (1H, dd, J= 14.6, 3.4 H–16b), 1.17 (1H, m, H–18), 1.55 (1H, m, H–19), 1.42 (1H, m, H–19), 0.98 (1H, m, H–22a), 1.07 (1H, m, H–22b), 1.09 (1H, s, H–23), 0.93 (1H, s, H–24), 1.22 (1H, s, H–25), 1.10 (1H, s, H–26), 0.78 (1H, s, H–27), 0.85 (1H, s, H–28), 0.98 (1H, s, H–29), 0.95 (1H, s, H–30).

Results and Discussion

The air-dried powdered stem of the plant 'Euphorbia hirta' were extracted with ethanol (95%) and the total ethanolic extract was concentrated to light brown viscous mass. The ethylacetate soluble fraction of the ethanolic extract was chromatographed over Si-gel G column. On elution with benzene: $CHCl_3$: ethyl acetate (1:1:1), it gave a compound which analysed for mf $C_{30}H_{50}O$. [M+] 426 (FABMS) mp 282-283°C The significant peaks which appeared at 1400.0 $^{cm-1}$ and 1319.5 $^{cm-1}$ in the IR spectrum of the compound showed, it to be of triterpenoidal nature[16]. It responded to all the characteristic colour reactions of triterpenoid[6,7].

The IR spectrum of the compound shows a peak at 3422.8 $^{cm-1}$ which implied the presence of –OH group(s) in it. The compound was found to form acetyl derivative $C_{32}H_{52}O_2$, [M+] 468 mp.304-306°C. Estimation of acetyl group was found to be 91.8% as described by Wiesenberger method[8] as stated by Belcher and Go,[9] and so impressed the presence of one —OH group in the compound.

The compound on oxidation with CrO3/pyridine afforded a ketone of mf $C_{30}H_{49}O$, [M+] 425,mp 242-244°C. The compound gave positive Zimmerman test[10] for the C-3 keto group which confirmed the presence of one —OH group at C-3 and further indicated that this —OH group was secondary in nature. On the basis of above deliberations it has concluded that the C-3 —OH group of compound was secondary in nature and present at C-3 position.

An IR peak which appeared at 813^{cm-1} in the IR spectrum of compound indicated the presence of double bond in the compound. On catalytic hydrogenation with Pd/C, it afforded a dihydro derivative having mf $C_{30}H_{52}O$, mp.186-187°C. This indicated the presence of one double bond in it. In ^{1}HNMR spectrum of the compound olefin proton signal appeared at 553 as (dd) at low field which indicated the presence of vinyl proton at C–15 owing to the double bond between C–14 and C–15. This also suggested that this compound was a taraxerene type triterpene[11-17]. Further the double bond was confirmed by the reaction of compound with tetranitromethane (RUZICKA'S REACTION)**[26]** gave yellow colour which showed the presence of double bond in the nucleus.

An IR peak appeared at 2932.4 cm^{-1} for angular methyl groups which were estimated by Zeisel's method[18] (21.73%) and confirmed the presence of eight methyl groups in it. Various singlets are obtained in the ^{1}H-NMR spectrum at δ 109,δ107, δ 122, δ110, δ 0.78, δ 0.85 δ 0.98 δ and 0.95 indicated the presence of methyl groups. Chemical shift in ^{13}C-NMR spectrum at δ 21.5, δ 26.2, δ 16.2 , δ 26.7, δ 19.5, δ 30.2, δ33.7, δ 29.9 showed methyl groups in it .The compound was further identified by mass spectrum .The base peak at m/z 204 which originated from the fragmentation of ring C and D of an Δ^{14} taraxerene while another peak observed at m/z 284 originated from retro-Diels-Alder (RDA) fragmentation of ring D, which suggested the presence of unsaturation [19,20]. The above IR, 1HNMR, 13C-NMR and FABMS spectral data and their comparison with those described in the literatures showed the structure of isolated compounds are to be the Taraxerol (13α-methyl-27-norolean-14-en-3β-ol).

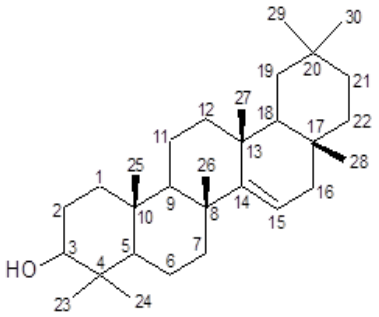


Figure-1
Taraxerol

Test Animals: Guinea pigs of either sex weighing 250-500 gm of pir bright white strain were used for the biological study.The animals were housed under standard conditions of temperature and humidity for 24 hrs and fasted with fresh leafy vegetables and carrots and with water adlibitum.

Induction of Histamine[23]: Animals were divided into four groups The guinea pigs of either sex were exposed to histamine aerosol (0.5% histamine diphosphate in saline) in an airtight histamine chamber by using a glass sprayer to induce the bronchospasm.

The guinea pigs after exposure to the histamine aerosol indicated the sign of progressive immobilization and bouts of coughing leading to convulsions when fell on its back.

The time taken by the guinea pigs to fall on its back after exposure to aerosol was called as exposition time which was noted for each animals Exposition time also known as PCD (pre-convulsive dyspnoea).

After recording the exposition time the animals were immediately removed from the chamber and placed in a fresh air so that the animals came back to its normal state.

Anti-Asthmatic Screening of Isolated Compounds: Anti-asthmatic screening of the isolated compounds were carried out on histamine induced bronchonstricted guinea pigs[22]. A detail method is elaborated below

Drug Treatment Schedule[24,25]: When the histamine was induced later after 1 hour the first three group of animals were administered separately with increasing doses of compounds and the fourth group animal was received salbutamol (050 mg/kg) by the intraperitoneal route. After 1 hour of drug administration the animal was again exposed to histamine and exposition times were noted. The fourth group animal was treated as a standard group of animal.

The animals in a first three groups were treated with isolated compounds. The animal of first group was given 50 mg/kg of compound second group was treated with 100 mg/kg and third group was administered with 200 mg/kg of compound Drug was administered intraperitoneally. Later after 1 hour each of the animals were exposed to aerosol and their exposition time were recorded.

Each time the animals were fed with fresh green leafy vegetables and tap water and adlibitum and made them refresh for one week. After then the animals were ejected intraperitoneally with an increasing doses. After 30 min of drugs administration they were again exposed to histamine and the exposition were noted for each animal.

The difference in the exposition time before and after extract administration was taken as a measure of the protective effect of the drugs.

Table- 1
^{1}H-NMR and ^{13}C-NMR Spectrum of the Triterpenoid in $CDCL_3$

S No	δ-Value	Pattern	Assignments
1	2.03	m	H-1a
2	2.10	m	H-1b
3	2.38	ddd	H-2a
4	2.63	ddd	H-2b
5	3.62	t	H-3
6	1.29	m	H-5
7	1.71	m	H-6
8	1.17	m	H-7a
9	1.46	m	H-7b
10	1.02	d	H-9
11	3.14	t	H-11
12	2.80	d	H-12
13	5.53	dd	H-15
14	1.69	dd	H-16a
15	1.89	dd	H-16b
16	1.16	m	H-18
17	1.55	m	H-19a
18	1.42	m	H-19b
19	0.98	m	H-22a
20	1.07	m	H-22b
21	1.09	s	H-23
22	0.93	s	H-24
23	1.22	s	H-25
24	1.10	s	H-26
25	0.78	s	H-27
26	0.85	s	H-28
27	0.99	s	H-29
28	0.95	s	H-30

S No	δ Value	Carbon No	Carbon type
1	39.5	C-1	CH_2
2	33.8	C-2	CH_2
3	21.68	C-3	CH
4	47.3	C-4	C
5	54.6	C-5	CH
6	38.6	C-6	CH_2
7	36.6	C-7	CH_2
8	36.5	C-8	C
9	52.7	C-9	CH
10	38.9	C-10	C
11	51.6	C-11	CH_2
12	58.1	C-12	CH_2
13	37.6	C-13	C
14	15.65	C-14	C
15	11.92	C-15	CH
16	38.3	C-16	CH_2
17	35.5	C-17	C
18	48.2	C-18	CH
19	20.1	C-19	CH_2
20	28.7	C-20	CH_2
21	33.2	C-21	CH_2
22	35.2	C-22	CH_2
23	21.5	C-23	CH_3
24	26.2	C-24	CH_3
25	16.2	C-25	CH_3
26	26.7	C-26	CH_3
27	19.5	C-27	CH_3
28	30.2	C-28	CH_3
30	29.9	C-30	CH_3

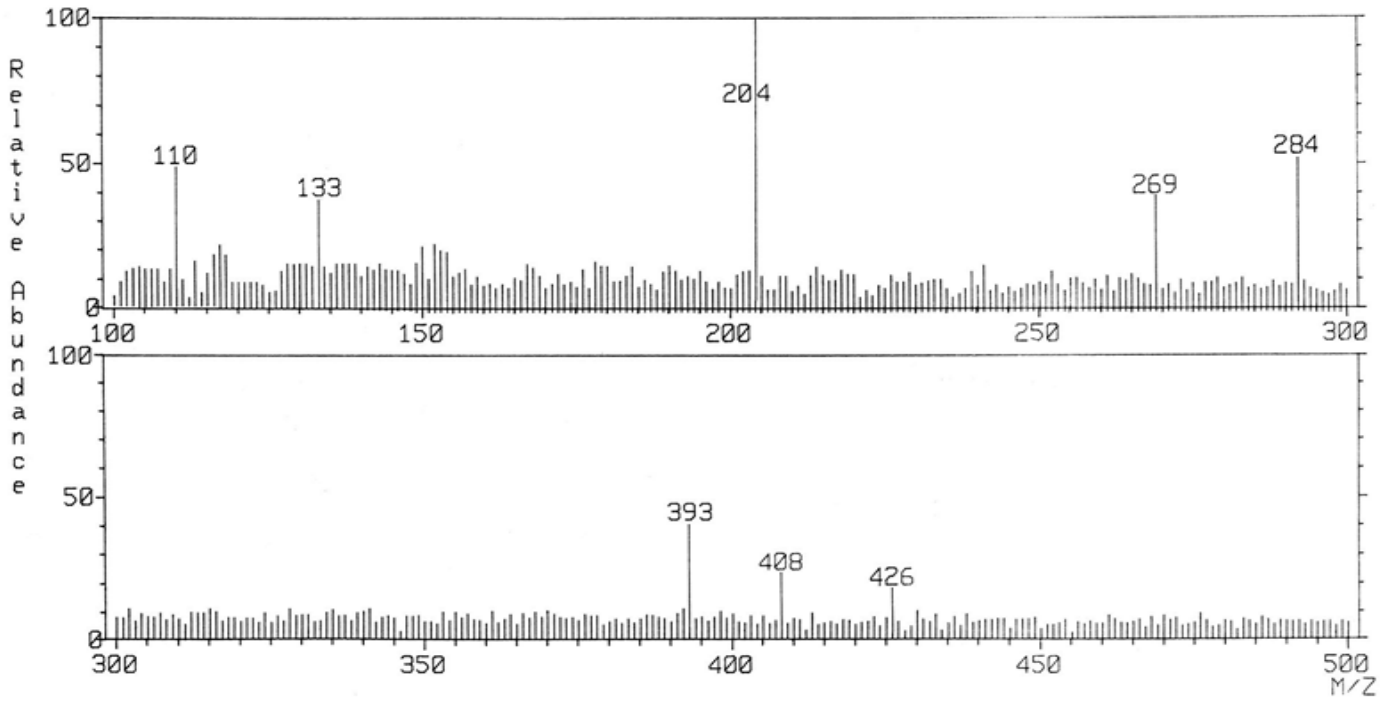
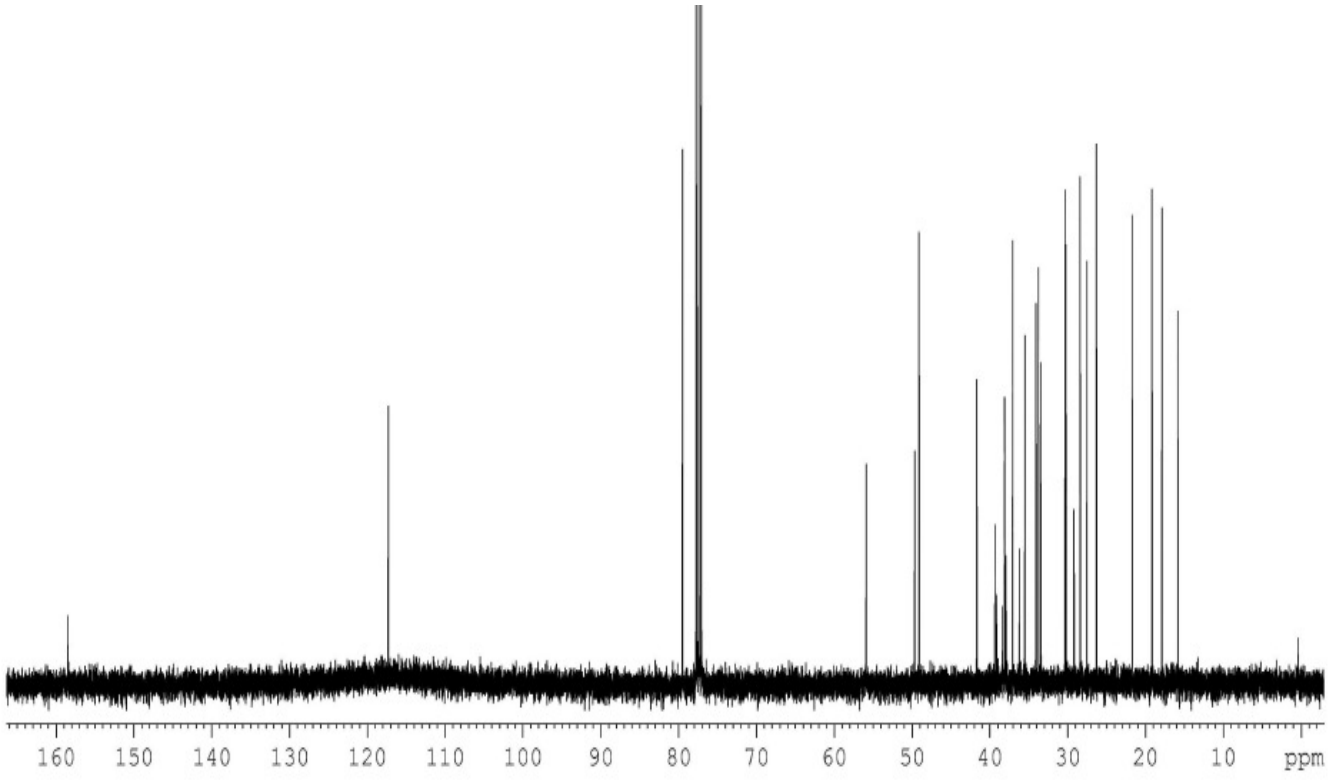


Figure-2
Mass Spectrum of Triterpene



Figure– 3
^{13}C-NMR Spectrum of the Triterpenoid in $CDCL_3$

Table-2
Effect of Compound Extract on Histamine Aerosol induced Bronchospasm in Guinea Pig

Treatment	Dose mg/kg ip	Pre-treatment exposition time in seconds ± SE	Post-treatment exposition time in seconds ± SE	% protection
Compound	50	115.26±10.06	130.21±10.95	11.48%
Compound	100	116.23±25.12	145.65±12.57	20.86%
Compound	200	122.23±10.99	230.35±46.93	30.71 %
Salbutamol	0.65	145.22±5.16	386.42±14.26	62.24 %

Calculation: The percentage protection offered by drugs in PCD was calculated by the formula

$$\text{Percentage protection} = \frac{\text{Eta} - \text{Etb}}{\text{Etb}} \times 100$$

(% Protection offered by the extract)

Where: Eta is the mean exposition time after administration of drug, Etb is the mean exposition time before administration of drug

Statistics: The results were expressed as mean ± SD The significance between post- treatment and pre-treatment exposition times was analysed by student test The values are considered to be significantly different when the p value was less than 0.05.

The compound offered the dose- dependent protection to 20.86 % and 30.71 % at doses 100 and 200 mg/kg respectively. Thus it can conclude that the plants drugs isolated from the stem of *Euphorbia hirta* Linn showed a significant protection against histamine challenge. Thus they can have a very potent anti-asthmatic effect. They produce bronchoprotective effect due to its H1-blocking effect.

Conclusion

13α-methyl-27-norolean-14-en-3β-ol, a taraxerol isolated from the extract of stem of Euphorbia hirta possess an antiasthmatic property.

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