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Research Article

Preliminary Phytochemical Screening and Isolation of Lupeol from *Euphorbia hirta*

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Abstract: *Euphorbia hirta* is commonly known as Dudhi and is found in tropical countries, belongs to euphorbiaceae family. Traditionally the whole herb is used for the treatment of diarrhoea, peptic ulcers, vomiting, dysentery, cold and cough, menstrual problems etc. the various secondary metabolites, such as tannins, flavonoids, terpenoids and saponins have been previously isolated. In the present study an attempt has been made to assess the preliminary phytochemical screening of ethanolic extract of *E. hirta* and further isolation of lupeol was carried out by using chromatographic method. The isolated compound was characterized by modern analytical technique.

Keywords: Lupeol, triterpenoid, Euphorbia, Dudhi, IR & NMR.

INTRODUCTION

India which is land of one of the oldest and eminent civilization [1]. Plants has been suitable source of medicines for past era. These medicines primarily took the form of crude drugs [2]. Medicinal plants play a vital role to preserve human health [3]. India is well known as the "*Emporium of Medicinal Plants*". Due to their great importance, demand of medicinal plants has increased numerous folds [4]. *Euphorbia hirta* (*E. hirta*) L., belongs to the family Euphorbiaceae. It is a small annual herb common to tropical countries. It is a slender- stemmed, annual hairy plant, spreading upto 40 cm in height, reddish or purplish in color. Leaves are opposite, elliptic - oblong to oblong- lanceolate, acute or subacute, 1- 2.5 cm long, blotched with purple in the middle, and toothed at the edge. The fruits are yellow, three- celled, hairy, keeled capsules, 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds [5].

Fig-1: ***Euphorbia hirta***

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E. hirta is a very popular herb amongst practitioners of traditional medicine, widely used as a decoction or infusion to treat various ailments including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, sterility and venereal diseases. Moreover, the plant is also used to treat affections of the skin and mucous membranes, including warts, scabies, tinea, thrush, aphthae, fungal afflictions, measles, Guinea-worm and as an antiseptic to treat wounds, sores and conjunctivitis. The plant has a reputation as an analgesic to treat severe headache, toothache, rheumatism, colic and pains during pregnancy. It is used as an antidote and pain relief of scorpion stings and snakebites. The latex used to facilitate removal of thorns from the skin [6, 7]. In the present study an attempt has been made to assess the preliminary phytochemical screening of ethanolic extract of *E. hirta* and further isolation of lupeol was carried out by using chromatographic method. The isolated compound was characterized by modern analytical technique.

MATERIALS AND METHODS

Collection and authentication of plant material

Whole herb of *E. hirta* was collected in the month of February from local region of Sehore district, Madhya Pradesh (India). Herbarium specimen was prepared, identified and authenticated. The plant material was shade dried and coarsely powdered by using mechanical grinder. The powder was passed through sieve no. 40 and stored in airtight container for the extraction.

Extraction and Fractionation

All the solvents used for extraction were of technical grade and distilled before use. The solvents used for column chromatography and preparative TLC was of Analytical grade. The Silica Gel used for column chromatography was Silica Gel G 60-120 (Merk).

Preliminary qualitative test of ethanolic extract of *E. hirta*

The ethanolic of extract of *E. hirta* was subjected to preliminary qualitative phytochemical investigation as standard procedure [8-11].

Procedure for extraction and Isolation

Air dried powdered whole herb of *E. hirta* (4000g) were defatted with petroleum ether (60-80ºC) and the left residue was again extracted with ethanol (70%v/v) and concentrated under reduced pressure to get ethanolic extract (41.8g) by means of rotary evaporator. Ethanolic extract was chromatographed on silica gel column (70cmX15cm, 60-120mesh, 3kg) chromatography and preparative TLC. Column was first eluted with chloroform, then polarity of mobile phase was gradually increased by adding methanol in different concentrations (100:0, 95:5, 90:10, 85: 15, 80:20, 70:30 v/v) [12]. 240 fractions each of 50mL were collected and TLC was performed of each fraction individually and elutes were monitored for the presence of various constituents. Fractions were pooled on the basis of their TLC profile, pooled fractions (41-56were selected for the isolation of constituents. Further purification was performed by preparative TLC of isolated constituent.

Characterization of isolated compound

Thin Layer chromatography was performed on precoated TLC plates. IR spectra was recorded on FTIR (ATR Bruker), ^{1}H NMR and ^{13}C NMR spectra was recorded on Bruker (500MHz) in $CDCl_3$. TMS was used as internal standard. ESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA).

RESULTS AND DISCUSSION

E. hirta is well known herb amongst practitioners of traditional medicine, widely used as a decoction or infusion to treat various ailments including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis etc. The preliminary phytochemical screening of the ethanolic extract of *Euphorbia hirta* revealed the presence of various secondary metabolites such as alkaloids, sterols, glycosides, triterpenoids, phenolic compounds, tannins, flavonoids (Table-1). The isolation of lupeol from the ethanolic extract was carried out by chromatographic technique. Further characterization of isolated compound was carried out modern analytical methods. TLC profile showed R_f value 0.7 (Fig-2). The IR studied revealed that the presence of OH bond vibration of hydroxyl group, carbonyl stretch & C=C vibrations. ^{1}H NMR spectrum showed the presence of seven tertiary methyl protons at δ0.67, δ0.69, δ0.76, δ0.78, δ1.01, δ1.17, and δ1.19. A sextet of one proton at δ2.37 corresponds to 19 β –H is characteristic of lupeol. H-3 proton appeared as multiplet at δ3.17 while two broad singlets at δ 4.56 and δ4.68 due to two exomethylene protons attached at C29. ^{13}C NMR spectrum showed seven methyl groups at δ14.56 (C-23), δ15.38 (C-28), δ15.98 (C-25), δ16.13 (C-26), δ18.02(C-24), δ18.33(C-27), and δ19.32 (C-30). The deshielded signals at δ79.05 were due to presence of hydroxyl group at C-3. The comparison of the spectral data with standard reported led us to propose the structure as lupeol. Mass spectrum of isolated compound showed molecular ion m/z 271.3[M+1] corresponding to the molecular formula $C_{30}H_{50}O$.The result was tabulated in Table 2 & Fig 2-5.

Table-1: Results of preliminary phytochemical analysis

Sr. No	Name of the Test	Ethanolic extract
1	**Tests for sterols**	
	1. Salkowski's Test	+
	2. Libermann Burchard's Test	+
2	**Test for glycosides**	
	1. Baljet's Test	+
	2. Keller–Killiani Test	+
	3. Legal's Test	+
3	**Tests for saponins**	
	1. Foam Test	+
	2. Haemolysis Test	+
4	**Test for carbohydrates**	
	1. Molish's Test	-
	2. Barfoed's Test	-
	3. Benedict's Test	+
5	**Tests for alkaloids**	
	1. Mayer's Test.	+-
	2. Wagner's Test.	+-
	3. Dragendorff's Test	
	4. Hager's Test	
6	**Tests for flavonoids**	
	1. Ferric chloride Test.	+
	2. Shinoda Test.	+
	3. Alkaline Reagent Test.	+
	4. Lead Acetate Test.	+
7	**Tests for tannins**	
	1. Ferric chloride Test.	+-
	2. Gelatin Test	

Table-2: Physical and spectral properties of isolated compound

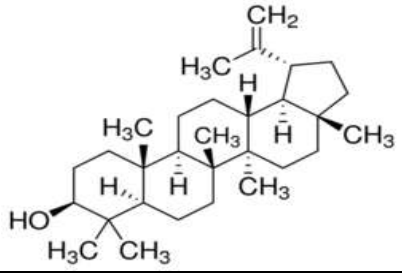
S. No	Description	
1.	**Appearance**	Yellowish amorphous powder
2.	**Solubility**	Chloroform
3.	**Melting point**	218-220^{0}C
4.	**TLC profile**	R_f = 0.7
5.	**m/z**	271.3[M+1]
6.	**Molecular formula**	$C_{30}H_{50}O$
7.	**IR**	Intensely broad band at 3454 cm^{-1}, moderate intense band at 1194 cm^{-1} 1696 cm^{-1} & 1453 cm^{-1} was weakly intense band.
8.	**^{1}H NMR (500 MHz, $CDCl_3$, δ, TMS=0)**	0.67(3H,S), 0.691(3H,S), 0.760(3H,S), 0.787(3H, S),1.014(3H,S),1.178(3H,S),1.199(3H,S), 2.372(1H,S), 3.175(1H, m), 4.562(2H,S), 4.689(2H,S).
9.	**^{13}C NMR (75.4Hz, $CDCl_3$, δ, TMS=0)**	14.56, 15.38, 15.98, 16.13, 18.02, 18.33, 19.32, 20.94, 25.14, 27.41, 27.45, 28.00, 29.72, 29.85, 34.28, 35.59, 37.18, 38.05, 38.71, 38.87, 40.01, 40.83, 42.84, 43.01, 48.00, 48.30, 50.44, 55.30, 76.77 and 79.05.
10.	**Chemical structure**	

Fig-2: TLC chromatogram

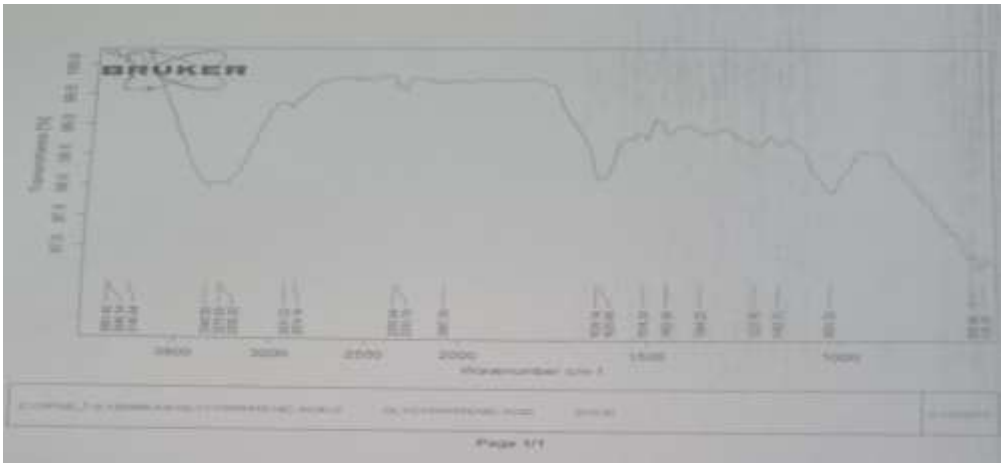


Fig-3: IR Spectra

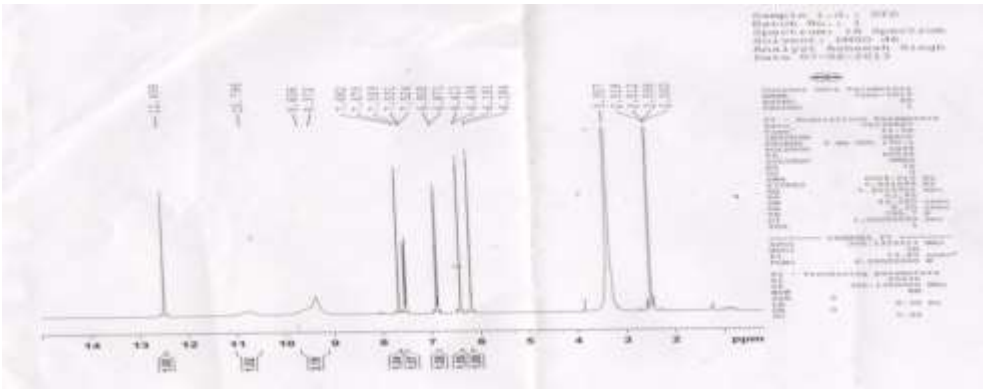


Fig-4: NMR Spectra

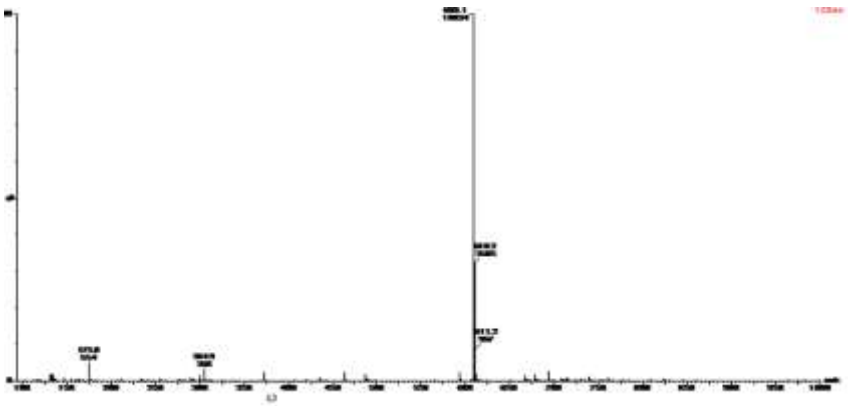


Fig-5: Mass spectra

CONCLUSION

Lupeol is a pentacyclic triterpenoid that is lupine. It is a secondary alcohol and a pentacyclic triterpenoid. Lupeol has been shown to exhibit diverse pharmacological activities. These include its advantageous activity against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity. The results of this study, it is clearly indicate that isolated compound from *E. hirta* is lupeol.

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