

ISOLATION AND CHARACTERIZATION OF LUPEOL FROM *ECHINOPS ECHINATUS* ROXB. ROOTAmish J. Patel*¹¹Shri B M Shah College of Pharmaceutical Education & Research, Modasa, Gujarat.

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ABSTRACT

Echinops echinatus Roxb, (Asteraceae) is a pubescent annual herb of 1-3 ft height with branches widely spreading from the base. The Plant is bitter, increases the appetite and stimulates liver; used in diseases of the brain, pains in the joints, inflammations, etc. Roots and root bark of the plant are used in various indigenous systems of medicine for treating different ailments. The present work deals with isolation and characterization of Lupeol from the dried roots of *Echinops echinatus* Roxb. The petroleum ether extract of the root of *Echinops echinatus* Roxb was subjected to further fractionation followed by pilot thin layer chromatographic (TLC) experiments and column chromatography. This yielded a pure, white, crystalline solid which resolved at R_f 0.65 upon TLC of chloroform fraction of the petroleum ether extract using the mobile phase toluene:methanol (9:1). This compound was subjected to IR spectroscopy. Results showed that peak obtained at various wave lengths due to various groups, moiety and arrangements of atoms in compounds are identical with standard Lupeol confirming the isolated compound is Lupeol.

KEYWORDS: *Echinops echinatus* Roxb, Roots, Lupeol, IR spectroscopy, Thin layer chromatographic (TLC).**INTRODUCTION**

Echinops echinatus Roxb, (Asteraceae) is a pubescent annual herb of 1-3 ft height with branches widely spreading from the base. The species is found practically throughout India, Pakistan, Afghanistan, etc.^[1] The Plant is bitter, increases the appetite and stimulates liver; used in diseases of the brain, pains in the joints, inflammations, etc. Roots and root bark of the plant are used in various indigenous systems of medicine for treating different ailments. The root is used as abortifacient and aphrodisiac^[2], infusion of the root is given in seminal debility, impotence, hysteria and its decoction is given in dyspepsia, scrofula, syphilis and fevers.^[3]

According to an ethnomedicinal survey carried out by Kakrani et al.^[4], the rural population of Kutch region in Gujarat state, India, uses the suspension of root bark powder in milk (100g/ 250ml) for the treatment of diabetes. The traditional healers of Chhattisgarh in India use this herb in different ways both internally and externally for the treatment of sexual disorders. An aqueous paste of the root is applied in the lower abdominal region to hasten the process of delivery; also the patients are advised to take the paste internally for quick and safe delivery. In case of patients having poor sexual vitality, aqueous paste of the root bark powder is applied externally on the male genitals one hour before intercourse; pure honey can be used in place of water for

better results. A paste prepared by mixing the root bark powder with the juice of *Datura stramonium* and *Blumea lacera* leaves is used to avoid premature ejaculation. The patients suffering from respiratory troubles, particularly asthma, are advised to inhale the fumes obtained by burning the leaves & roots of *E. echinatus* in order to get quick and permanent relief.^[5] Though the plant has been reported for many biological activities like anti-inflammatory^[6], hypoglycemic and diuretic^[7], antibacterial and antifungal^[8], antispasmodic^[9] etc. This work deals with the isolation and characterisation of lupeol and establishes a link between lupeol and its pharmacological activity.

MATERIALS AND METHODS**Isolation of Lupeol****Extraction and fractionation**

Extraction of 1 kg root powder was carried out in a round-bottom flask at a temperature <50°C using petroleum ether (Spectrochem Pvt. Ltd., Mumbai, India). Petroleum ether extract was further fractionated in the order with *n*-hexane and water, carbon tetrachloride and water, toluene and water, diethyl ether and water, dichloromethane and water, *n*-butanol and water, chloroform and water, ethyl acetate and water and finally 80% methanol and water.

Chromatography

A series of pilot thin layer chromatographic (TLC) was carried out with each of the above fractions of the petroleum ether extract using different proportions (0:10 to 10:0) of various solvents like petroleum ether, carbon tetrachloride, toluene, diethyl ether, dichloromethane, *n*-butanol, chloroform, ethyl acetate, acetone and methanol as the mobile phase.

Considering the TLC results, 1g dried chloroform fraction from the petroleum ether extract was subjected to column chromatography and loaded on a glass column (60×3 cm) packed with silica gel G (40 g, 60–120#, Spectrochem Pvt. Ltd.) as the stationary phase. Gradient elution was performed using toluene: methanol (10:0,

9.5:0.5, 9:1 up to 0:10) as the mobile phase. Nearly 200 fractions were collected. Evaporate the solvent from toluene: methanol (9:1) fraction, white crystals of a compound were obtained. Confirm the purity of compound by TLC using the mobile phase toluene: methanol (9:1) and marker compound.

Characterization of isolated Lupeol

Boiling point

Determine the Boiling point of isolated compound by capillary method.

Chemical test for Sterols and Triterpenoids

Libermann-Burchard test and Salkowski's test.

TLC identification test

Sample solution	Prepared solutions of isolated and standard compound in alcohol
Stationary phase	TLC silica gel plate
Mobile phase	toluene: methanol (9:1)
Detection	UV Chamber
Evaluation	Observed R _f

IR spectrum of isolated compound

Isolated compound was dissolved in *n*-hexane and IR spectrum was taken in FTIR instrument (Shimadzu corp., 8400s) taking *n*-hexane background in ATR cell. Interpret the observed spectrum and peaks with Lupeol.

RESULTS AND DISCUSSION

Physical characters

Lupeol was isolated from the root of *E. echinatus* Roxb according to method prescribed. Physical characters like Colour, State, Melting point and Solubility were studied and results are shown in Table 1.

Table 1 Physical characters of isolated Lupeol

Color	State	Melting Point	Solubility
White	Solid Crystalline	215-219°C	Soluble in Petroleum ether, Benzene, Chloroform and Alcohol

Isolated compound was White, solid crystalline, having melting point 215-219°C and soluble in Petroleum ether, Benzene, Chloroform and Alcohol.

Chemical test

Salkowski and Liebermann–Burchard test was positive confirming the presence of sterol and triterpenoid compound.

TLC identification test

Results of TLC study for isolated compound and Lupeol are shown in Fig. 1.



Fig 1 TLC Plate of Isolated compound and Standard Lupeol

Results of TLC plate showed that isolated compound and Lupeol both had shown same R_f value at 0.65.

IR spectrum of isolated Lupeol

IR spectrum of isolated compound from root of *E. echinatus* Roxb is shown in fig. 2 and various peak and its identification is given in Table 2.

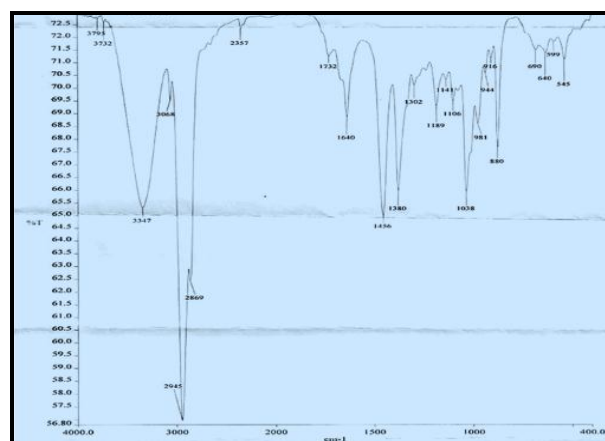
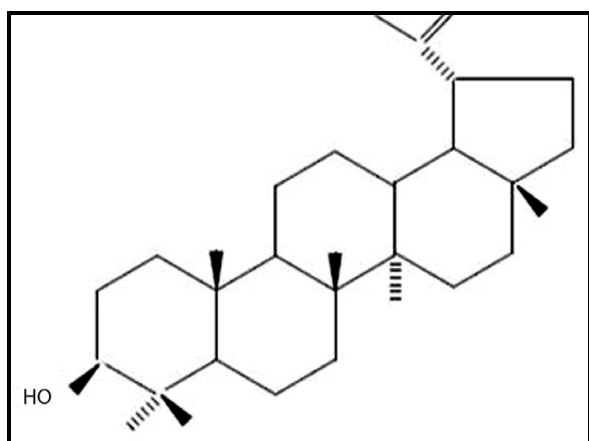


Fig 2 IR spectrum of isolated compound from root of *E. echinatus*

Table 2 Peak and Peak identification of Lupeol

Peak	Peak identification
3347	Presence of a hydroxyl function (OH)
2940	The stretching and bending of methyl group
2869	Presence of a methylenic group
1640	Presence of a olefinic moiety
1456	A medium intensity band
1189	Presence of a hydroxyl function (OH)
1038	C–C vibrations
880	Out of plane C–H vibrations of the unsaturated part

Results showed that peak obtained at various wave lengths (3347, 3068, 2945, 2869, 1640, 1456, 1380, 1189, 1106, 1038, 880, 690, 640, 599 and 545 cm^{-1}) due to various groups, moiety and arrangements of atoms in compounds are identical with standard Lupeol confirming the isolated compound is Lupeol. Molecular structure of Lupeol with IR peaks showing groups is given in Figure 3.

**Fig 3 Molecular Structure of Lupeol**

CONCLUSION

Lupeol was isolated from the roots of *E. echinatus*. It was a white crystalline solid with 215-219°C melting point. It was further identified by Solubility test, Chemical test, TLC identification test and IR Spectroscopy method.

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