Research Article

ISOLATION AND CHARACTERIZATION OF LUPEOL, A TRITERPENOID FROM *CALOTROPIS GIGANTEA* LATEX

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ABSTRACT

Natural products and herbal remedies used in traditional folklore medicine have been the source of many medically beneficial drugs because they elicit fewer side effects, relatively cheap, affordable and claimed to be effective. However, in order to make these remedies acceptable to modern medicine, there is a need to scientifically evaluate them to identify the active principles and to understand their mechanism of action. *Calotropis gigantea* R.Br. (Asclepiadaceae), commonly known as milk weed or swallow-wort, is a medicinal plant widely used as a folk medicine in India. It exhibits a wide array of pharmacological activities including wound healing and antimicrobial properties. Lupeol, a pentacyclic triterpenoid was extracted for the first time from the latex of *Calotropis gigantea* and characterized by spectral studies. The presence of lupeol in the latex in appreciable amounts may account for its various biological activities.

Keywords: *Calotropis gigantea,* Triterpenoid, Lupeol, Latex.

INTRODUCTION

Recently, calotropis species received special attention because of distinct relevant activities found to be present in its latex[1]. Calotropis species are non-cultivated, xerophytic shrubs of geographic distribution covering Asia, Africa and northeast of South America. In India two species viz *Calotropis procera* and *Calotropis gigantea* are widely distributed. *Calotropis gigantea* R.Br. (Asclepiadaceae), commonly known as milk weed or swallow-wort, is a medicinal plant widely used as a folk medicine in India as a rich source of biologically active compounds capable of promoting diverse benefits such as control for dermal fungal infections, antimicrobial and pain relief among other useful properties[2].

The latex has been described for exhibiting clinically important pharmacological properties[3, 4]. Nevertheless, its properties are accompanied by toxic effects following injection, oral administration or dermal contact of latex in animals[5, 6]. Further, it is well known that the latex has severe toxicity on digestive and blood circulatory system which greatly limits its clinical application[7]. The therapeutic potential of the latex as a pharmaceutical depends on separating the curative properties from the toxic properties. Fractionation of the latex into its rubber and rubber free fractions prior to analysis, affords better insight into its efficacy and limitation. The rubber free fraction of the latex is rich in soluble proteins and is responsible for most of its medicinal properties[8].

Recently, we have reported that rubber free extract of latex possesses significant antimicrobial as well as wound healing properties[9, 10]. To identify the effective compounds in the latex which are responsible for the pharmacological activity, studies have been performed in recent years[11]. In the present study, we have isolated and characterized lupeol, a pentacyclic triterpene, from the dialyzable fraction of *C. gigantea* latex extract.

Lupeol is reported to exhibit a spectrum of pharmacological activities against various disease conditions. These include conditions such as inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer[12]. The available literature suggests that lupeol is a nontoxic agent and does not cause any systemic toxicity in animals at doses ranging from 30 to 2000 mg/kg[13].

MATERIALS AND METHODS

Plant material and latex collection

The fresh latex of *Calotropis gigantea* was collected from the aerial parts of the healthy plants[14] in plastic tubes containing distilled water with a dilution rate of 1:1 v/v. The plant exsiccate was deposited at the herbarium of the Centre for Advanced Studies in Botany, University of Madras, where the plant was identified by a taxonomist. The latex mixture was gently handled to maintain homogeneity during transport to the laboratory where they were stored overnight at 4˚C[4]. The supernatant was decanted and centrifuged at 12000 x g for 20 min at 25˚C. The clear supernatant devoid of rubber was decanted carefully and subjected to exhaustive dialysis using a membrane of 8000 Da molecular weight cut-off against distilled water at 25˚C[8]. Finally the samples were centrifuged as previously described and the clear soluble supernatant was collected and lyophilized.

Isolation of lupeol

The lyophilized rubber free fraction of the latex extract was subjected to hot extraction with petroleum ether (90%) by soxhlet extractor. The extract obtained was concentrated under reduced pressure using a rotary

evaporator, yielding a dry residue (32.16%). The residue was chromatographed on a silica gel column (70-130 mesh) with gradient elution using n-hexane and ethyl acetate[15]. Fractions that showed identical behaviour in thin layer chromatography (TLC) were combined. Briefly, a mixture of silica gel G in water was degassed and poured on TLC plates. The plates were activated at 110ºC for 2 h. TLC plates developed with n-hexane and ethyl acetate (97.5:2.5 v/v) showed a violet spot at a R_f value of 0.52 when sprayed with 1% Vanillin-sulphuric acid reagent and heated at 110ºC for 5 minutes[16]. Co-TLC with authentic sample and chemical characterization using IR and UV spectral studies indicated that the isolated compound is lupeol.

RESULTS

Chemical structure and analysis of lupeol

The chemical formula of lupeol is $C_{30}H_{50}O$ and its structure is presented in Fig. 1. The melting point of lupeol is 215–216°C and the structural analysis shows that it possesses the exact mass of 426.386166.

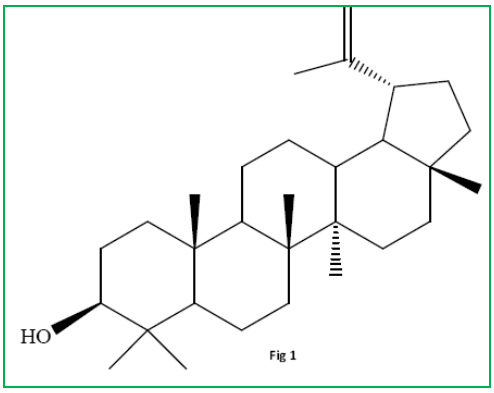

Figure 1: Lupeol structure

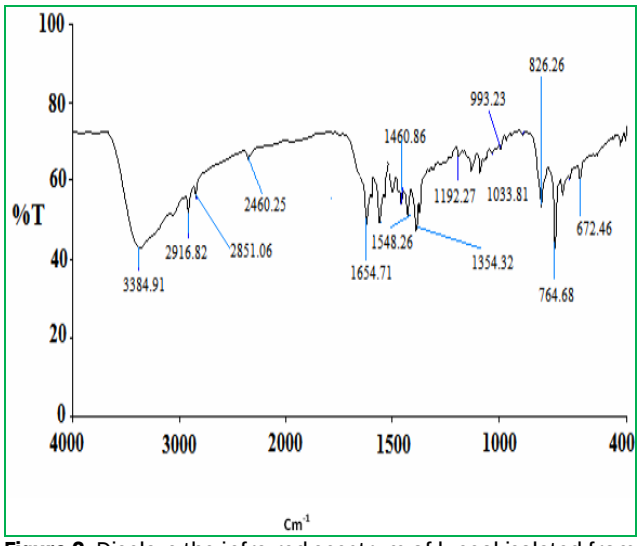

Figure 2: Displays the infra-red spectrum of lupeol isolated from the *Calotropis gigantea* latex. The presence of a hydroxyl function and an olefinic moiety which show their presence in the spectrum at 3384 and 1654 cm^{-1} respectively were identified.

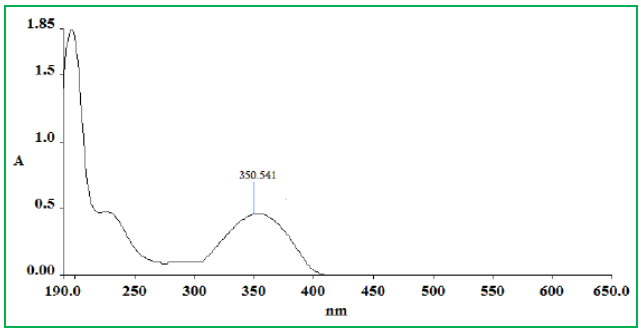

Figure 3: Displays the UV λ_{max} value of lupeol which was found to be 350 nm. The melting point was found to be 215-216ºC. Properties computed from the structure of lupeol show that it has a molecular weight of 426.7174 (g/mol).

DISCUSSION

Triterpenoid, lupeol (3˘-hydroxylup-20(29)-ene), is an immense bioactive compound present in different medicinal plants[17-19]. A wide range of bioactivities and bioassays of lupeol are reviewed[20], which suggest its useful medicinal properties with diversity of action against different diseases. This compound is reported to be antiangiogenic, antioxidative and anti-inflammatory in nature[21]. It inhibits early responses of tumor growth induced by benzoyl peroxide[22]. It also plays very important role in normalization of lipid profile[23], wound healing activity[24], protective effect in hypercholesterolemia associated with renal damage[25] and suppression of immune factors[26, 27].

Triterpenes represent a varied class of natural products. Thousands of structures have been reported till date with hundreds of new derivatives discovered each year. Pentacyclic triterpenes are all based on a 30-carbon skeleton comprising five, six-membered rings (ursanes and lanostanes) or four, six-membered rings and one, five-membered ring (lupanes and hopanes). Pentacyclic triterpenes are produced by arrangement of squalene epoxide molecules.

Lupeol, a pentacyclic triterpene was isolated and characterized for the first time, from the latex of *Calotropis gigantea* by chromatographic techniques. The spectral and physical data generated were found to be in accordance with the earlier literature data of lupeol[28, 29]. Lupeol has been reported to have potent pharmacological properties and it exhibits no toxicity up to 2g/kg body weight in rats and mice[30]. The presence of anti-inflammatory activity of triterpenes seems to be interesting, since they possess hydro-aromatic ring system more or less similar to that of steroids. It is reported that well over 2400 subjects have taken part in clinical studies with different types of triterpenes with dosage up to 25 g or more per day in human with no adverse effect reported[31].

IR spectrum of lupeol, a very intensely broad band at 3384 cm^{-1} and moderately intense band at 1192 cm^{-1} and 672 cm^{-1} were observed for the O-H bond vibration of hydroxyl group. The out of plane C-H vibrations of the

unsaturated part was observed at 826 cm^{-1}. The corresponding C=C vibrations was shown around 1654 cm^{-1} was weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intence band 2916 cm^{-1} and medium intensity band at 1460 cm^{-1}. The vibration of the methylenic part was shown by the band at 2851 cm^{-1} and the medium band at 1548 cm^{-1}. The moderate intense band at 764 cm^{-1} was attributed to the rocking movement of methylenic par. The corresponding C-C vibration was shown as weak intense band at 1033 cm^{-1}.

In this study, the presence of lupeol, a potent bioactive compound, in wild plant *C.gigantea* latex has been reported for the first time. Lupeol is vastly used as an anti-inflammatory compound. The huge quantity of lupeol in the extract makes this plant as future source of lupeol that helps and supports the pharmaceutical industry in any drug formulation. Furthermore, a very simple and reliable chromatographic method for this important component has also been developed that needs short analysis time. Therefore, this method can be used as a routine lupeol assay procedure for the standardization of any drug formulation.

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