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IDENTIFICATION AND QUANTIFICATION OF LUPEOL IN *DIPTERACANTHUS PATULUS* (JACQ.) NEES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY- PHOTO DIODE ARRAY METHOD

MRS.N.KANNIKAPARAMESWARI*[1] AND DR.P.CHINNASWAMY[2]

[1]PG and Research department of Biochemistry Dr. N.G.P.Arts and Science College, Coimbatore.
[2]Institute of Laboratory Medicine, Kovai Medical Centre and Hospital, Coimbatore.

ABSTRACT

Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. Hence the study focuses on the identification and quantification Lupeol from the methanolic leaves extract of *Dipteracanthus paulus* (Jacq.) Nees. High Performance Liquid Chromatography with Photodiode array detector (HPLC-PDA) was used to analyse the compound of interest. The quantity of lupeol was calculated from the respective peak areas according to individual standard curves. The content of the compound in the extract was 0.04mg/g dry weight (0.004%). The results of present study confirm the presence of good percentage of Lupeol which supports the biological potency of the plant.

KEYWORDS: **HPLC-PDA, Dipteracanthus patulus and Lupeol**

MRS.N.KANNIKAPARAMESWARI
PG and Research department of Biochemistry Dr. N.G.P.Arts and Science College, Coimbatore.

Corresponding author

INTRODUCTION

Plant cells contain many compounds produced by the basic metabolism. The term "basic metabolism" includes all processes necessary for cell survival, while secondary plant metabolites are synthesized usually only in special, differentiated cells. The roles of these natural products or secondary metabolites played in plants have only recently come to be appreciated in an analytical context. Natural products appear to function primarily in defense against predators and pathogens and in providing reproductive advantage as attractants of pollinators and seed dispersers.They may also act to create competitive advantage as poisons of rival species. Infact, many secondary metabolites are highly toxic and are often stored in specific vesicles or in the vacuole.These secondary metabolites are produced from universally present precursors (mostoftenacetyl-CoA,amino acidsorshikimate) by specific enzymes that probably arose by the duplication and divergence of genes originally coding for primary metabolism. Many plant compounds have had an outstanding role in medicine for centuries[1]. Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. For example, some studies have reported that extracts from natural products, such as fruits, vegetables and medicinal herbs, have positive effects against cancer, compared with chemotherapy or recent hormonal treatments[2]. Naturally occurring phytochemicals of dietary and nondietary origin have been the focus of intense research and development activities in the recent past due to their ability to modulate degenerative diseases like cancer, cardiovascular diseases (CVD), diabetes, arthritis, cataract, aging etc. The resurgence of interest in the bioactive phytochemicals could mainly be attributed to the large body of scientific evidence gathered from well designed epidemiological and experimental studies conducted during the last two decades[3]. Most species (~2500) of the relatively large acanthaceae family grow primarily in tropical areas as shrubs or herbs among 250 genera of considerable biological variety. The families of acanthaceae found application in African and indian primitive medicine for problems to a treatment for cancer, heart disease, gonorrhoea, and snake-bite[4]. *Dipteracanthus patulus* (Jacq.) Nees. (Syn. *Ruellia patula* Jacq). (Acanthaceae) is a medicinal herb traditionally used in the treatment of wounds in the rural areas. The leaves are used for treating itches, insect bites, paranychia, venereal diseases, sores, tumours, rheumatic complaints and eye diseases. It is cardiotonic and single drug remedy for against the deadly poison of kaduva chilanthi(Tiger Spider) by kani tribes in agasthiarmalai[5,6]. The methanolic extract of *Dipteracanthus patulus* (Jacq.) Nees has shown promising antimicrobial and hepatoprotective activity. Leaves of this plant are used to cure liver complaints by the peoples of Sholapur region (MS), India[7]**.** Hence the study focuses on the identification and quantification of Lupeol from the leaves of *Dipteracanthus patulus.*

MATERIALS AND METHODS

Chemicals and Reagents

Lupeol (purity 95%), were purchased from Sigma Alrich.The solvent acetonitrile with HPLC grade were were procured from E. Merck Mumbai,India. All water was ultra-pure (distilled and de-ionised).

Preparation of Standard Lupeol

Accurately weighed 2.3mg of Lupeol standard was dissolved in 2ml of HPLC grade Methanol. 5µl was injected for analysis (5.75µg/5ul).

Extraction of Sample

Accurately weighed 72mg of the extract and dissolved in 2ml of HPLC grade methanol. The dissolved material was centrifuged for 5 min at 10000rpm. The supernatant was injected into HPLC system (360µg/10µl)

Apparatus and Estimation

A HPLC unit comprising of two LC-8A preparative pumps connected with a SPD-M20A PDA detector (Photo Diode Array detector) which has ability to scan from 200-800nm and a system controller CBM-20A. The system is equipped with LC solution software version 1.2, which also manages the evaluation of datas collected. C18(250 X 4.6mm SS, 5µ particle size) column was used for the study. The solvent system was based on the solubility of the compound present in the extract. Commercial standard of Lupeol was used for running HPLC with methanolic leaves extract. Injected 5µl of the standard Lupeol and 10µl of the sample solution respectively to get area reproducibility for two consecutive injections. The area of two consecutive injections should not vary more than 2 percent. Acetonitrile : Water (80:20 v/v) was used[8]. The flow rate was 1 ml/min and a maximum peak was obtained at a wavelength of 210 nm. From the HPLC chromatogram the percentage of Lupeol was calculated.

Calculation

$$\text{Lupeol content} = \frac{A2}{A1} \times \frac{M1}{M2} \times P$$

A1 – Peak area of the Standard
A2 – Peak area of the sample
M1 – mass in ug of the standard
M2 – mass in ug of the sample
P – purity of the standard

RESULTS AND DISCUSSION

HPLC has preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs because of its simplicity, sensitivity, accuracy, suitability for thorough screening etc[9]. HPLC analysis was conducted to quantitatively estimate the content of Lupeol in the methanolic leaves extract of *Dipteracanthus patulus* at a detection wavelength of 210 nm. The quantity of Lupeol was calculated from the respective peak areas according to individual standard curves. Figure 1 and Table 1 shows the retention times and peak area of the standard. Figure 2 and Table 2 indicates the retention times and peak area of the sample and The content of the compound the content was 0.04mg/g dry weight (0.004%)

Figure 1
Chromatogram of Lupeol Standard

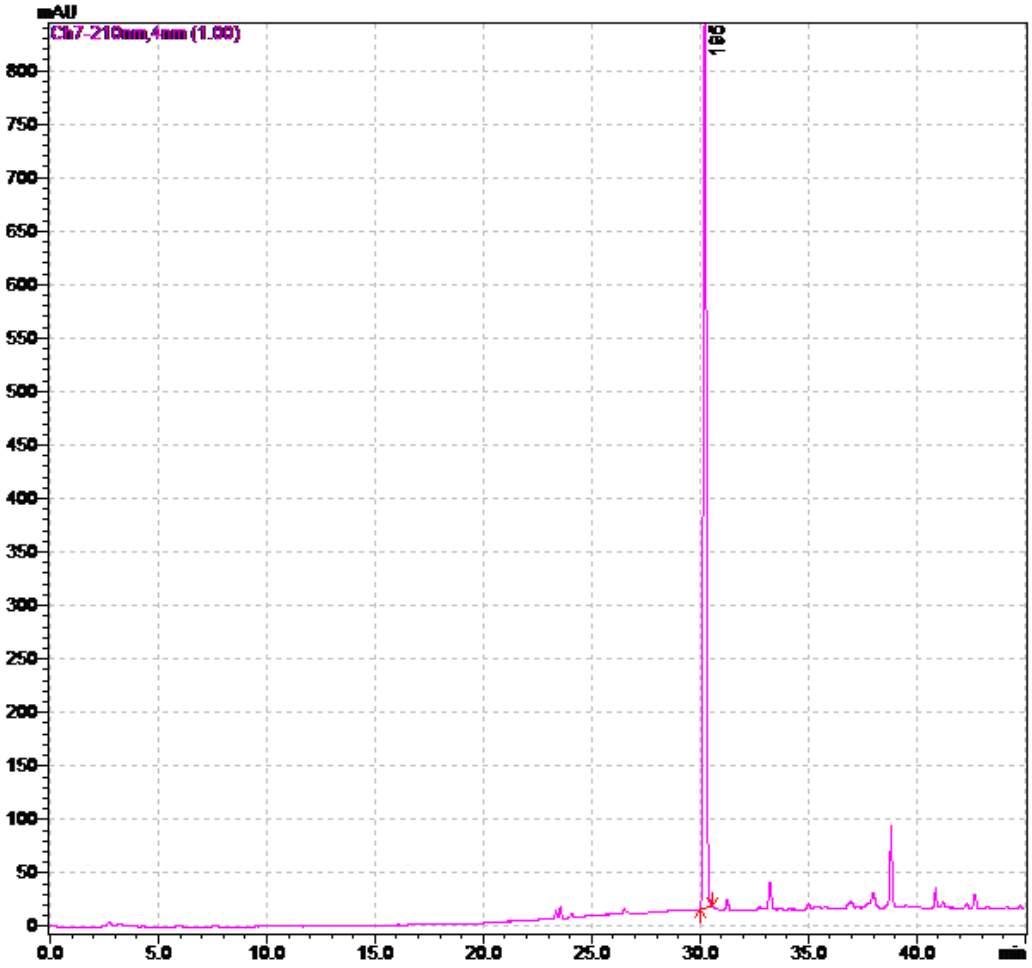


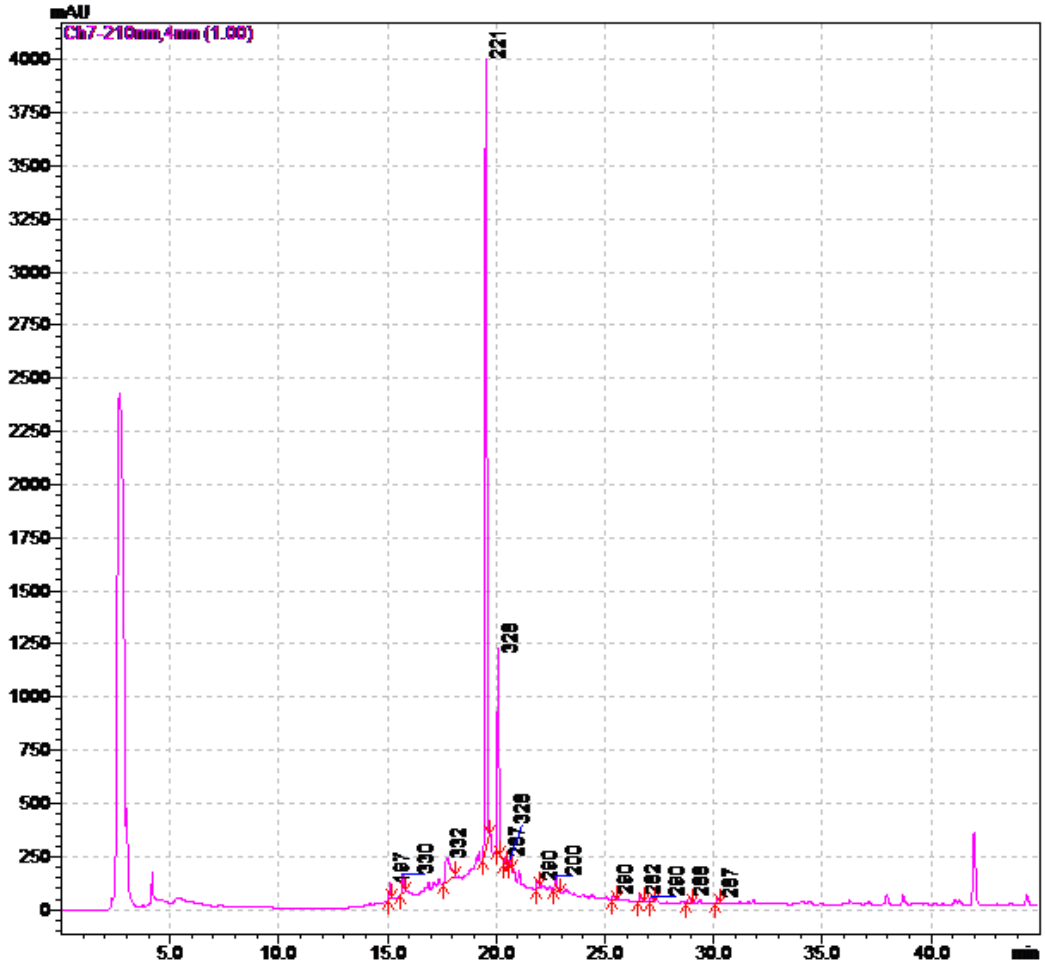
Table 1
Retention Time and Peak area of Standard Lupeol

Retention time (minutes)	Peak area(mAU)
30.223	8856303

Table 2
Retention Time and Peak area of Sample Lupeol

Retention time (minutes)	Peak area(mAU)
30.213	176629

Figure 2
Chromatogram of Lupeol Sample



Almost all terpenes have some biological activities in animals including man and also play a meaningful role in human medicine. From this point of view the most important group of terpenes are triterpenes, triterpene glycosides, (also known as saponines), and other triterpenoids, representing one of numerous classes of natural compounds. They serve as secondary metabolites of mixed biosynthesis, like alkaloids, flavonoids, oligosaccharides etc. There is a growing interest in natural triterpenoids caused as much by the scientific aspects of extraction and structural analysis of these compounds, as by the fact of their wide spectrum of biological activities;they are bactericidal, fungicidal, antiviral, cytotoxic, analgetic, anticancer, spermicidal, cardiovascular, antiallergic and so on. In recent years, a considerable number of studies conducted in many scientific centres have been devoted to the three compounds of this group, especially: lupeol, betulin and betulinic acid[11]. Lupeol, a triterpene, is the principal constituent of common fruit plants such as olive, mango, fig and medicinal herbs that have been used to treat skin aliments. Lupeol has been reported to possess a wide range of medicinal properties that include strong antioxidant, antimutagenic, anti-inflammatory and antiarthritic effects. In the present study, we show that Lupeol possesses antitumor-promoting effects in a mouse skin tumorigenesis model file[12].

CONCLUSION

The results of present study confirm the presence of good percentage of lupeol in *Dipteracanthus patulus*. The medicinal property of this plant may be related to their bioactive compounds. Lupeol may be one of the bioactive compounds which is responsible for the biological activities of the plant. These features make this plant a promising candidate for the further studies on isolation and pharmacological studies of this compound from *Dipteracanthus patulus*.

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