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Quantification of Lupeol: An Active Marker from Kanchnar Products by HPTLC

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ABSTRACT: Kanchnar (*Bauhinia variegate*) is tree growing in different parts of India. It is known to have analgesic and anti-inflammatory properties. Kanchnar's anti-inflammatory and antioxidant characteristics encourage the growth of new skin cells, which aids in wound healing. Kanchnar is available in the market as a variety of dosage forms, including churna, kashay, ointment, pills, topical gel etc. Prior to administration, standardization of herbal products is crucial for ensuring their quality. In light of this, the current study involved quantification of lupeol, an active marker found in kanchnar (*Bauhinia variegate*) bark and its two commercially available formulations. Bark extraction was done using maceration. The mobile phase was optimized and locating agent was used to visualize lupeol.

KEYWORDS: *Bauhinia variegate*, standardization, HPTLC, Lupeol.

INTRODUCTION

Since our rushi-munies discovered the herbal medicines many years ago, the Indian subcontinent has been using them. Herbal remedies are primarily used to treat many illnesses and to maintain health. However, the use of traditional treatments rises when modern medicine fails to effectively treat an illness, as is the case with advanced cancer and emerging infectious diseases. The most frequent justifications for using traditional medicines are their affordability, conformity to patients ideologies that local drugs are safer and more effective, along with concerns about the side effects of chemical(synthetic) medicines, satisfaction of a desire for more individualized health care and greater public access to health information. The need of standardization of herbal medicine is realized now. In order to ensure the quality control of AYSH (Ayurveda, Yoga, Naturopathy, Unani, Siddha, and Homeopathy) medications in the nation, the Ministry of AYUSH has taken a number of actions. Ayurveda and other Indian medical systems are also being developed to meet international standards. A Memorandum of Understanding for the creation of the "One Herb One Standard" was also signed by Pharmacopoeia Commission for Indian Medicine and Homoeopathy (PCIM&H) and the Indian Pharmacopoeia Commission (IPC).

The plant that we chose, kanchnar, is an ayurvedic remedy being used by practitioners for long. About 150 species of trees, shrubs, and climbers belong to this genus, of which 20 are found on the Indian subcontinent.

Some images of flowers are shown in figure 1. They are grown for their lovely foliage and alluring blossoms in Indian gardens. The Caesalpiniaceae family member Bauhinia variegate Linn is one of the species we have chosen[1]. It is a lovely medium-sized tree that can reach a height of 10 metres. In winter it shades its leaves, and from February to April, beautiful pink blooms bloom. It can be found in dry deciduous woods in sub-Himalayan regions from Punjab eastward to Burma and China and are currently grown all over India. Both therapeutic and commercial benefit can be found in it.

Among the different chemical constituents, one of the major chemical constituent is lupeol. Lupeol is a pharmacologically active pentacyclic triterpenoid. Structure is shown in figure 2. Lupeol is also found in variety of plants, including mangoes, Acacia visco and dandelion coffee. Kanchnar has several potential medicinal properties, like management of hypothyroidism, anticancer, antimicrobial, antitumor, anti-protozoal and anti-inflammatory activity etc[2-3]. Kanchnar leaves have anthelmintic activity[4,5]. There is a report of quantification of Quercetin as a biomarker from formulation[6]

The aim of current study was to check whether active marker lupeol is present in the bark of kanchnar found locally in Pune and two marketed formulations.We have separated the different chemical constituents present in them using a new, economic, environmentally friendly and rapid High Performance Thin Layer Chromatography [HPTLC] Method. The developed method was validated for quantitative determination of lupeol in the kanchnar bark. HPTLC is a more efficient version of thin layer chromatography [TLC]. The method was validated for various parameters as per the international conference on Harmonization guidelines ICH Q2 (R1) [7]. A major advantage of HPTLC is its ability to analyse several samples simultaneously using a small quantity of mobile phase; thus reducing the time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. Method was successfully employed for the estimation of lupeol the active marker in the bark of kanchnar and the two marketed formulations. Principle of HPTLC is adsorption. The mobile phase solvents travel up the plate in ascending manner because of capillary action. The components of sample move according to their affinities towards the adsorbent and we can get resolved components on TLC plate. The plate is scanned using scanner to determine the peak area densitometrically [8].

MATERIALS AND METHOD

The active marker lupeol was purchased from Yucca Enterprizes, Mumbai. It was dissolved in methanol and then its dilutions were scanned using UV-Visible spectrometer to find the wavelength of maximum absorption. The samples were weighed by using digital balance make-Shimadazu (AY 120). Maceration procedure was used to extract components from the bark of kanchnar which was dried and powdered before use. The powder was soaked in HPLC grade methanol for 24hrs.The solution was then filtered using whatmann filter paper and residue was subjected to 3 more cycles of maceration with the same procedure. In the beginning spotting was done by manual method i.e. capillary method. But this manual method had some disadvantages like spotting was uneven. So later linomat applicator was used for application of bands.Using this applicator , different microlitre volumes of Lupeol solution were spotted on each track on TLC plate. And the distance between adjoining tracks was uniform. The spots of Lupeol were not visible on TLC plate due to lack of chromophore. So locating reagent (vanillin in sulphuric acid) was used. The reagent was used by dipping method. In this method TLC plate was dipped in the locating reagent. Dipping method requires a large volume of locating reagent. So to reduce the amount of locating reagent, pouring method was tried. In this method locating reagent was poured form one side of TLC plate. Two marketed formulations were purchased, extracted using methanol. Spotted along with the linearity of standard marker on Merck TLC plate. Plate was warmed after development at 50 degree for 10 minutes and scanned using TLC plate scanner.

RESULT

The two densitograms shown above are for the assay of marketed products kashay and churna respectively. The first densitogram (Fig. 3) has the sample tracks as follows: first 5 tracks are of standard lupeol linearity, sixth and seventh are of raw bark extract, eighth and ninth are of marketed product kashay and last five are again are of standard lupeol linearity. Where as in second densitogram (Fig. 4) eighth and ninth track are of marketed product churna. Lupeol peak is not observed on tracks of kashay.Where as in Fig. 4 for churna, peaks for Lupeol are visible. This shows that there is no presence of lupeol content in kashay but it is present in churna. Lupeol content in marketed churna sample was found to be 0.0135%.

Result and Discussion:

This study can be employed for standardization of marketed products of kanchnar by determination of lupeol content. This method can be further extended for stability studies, to support the proposed expiry date.

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FIGURES:

Fig.1- Different species of kanchnar plant

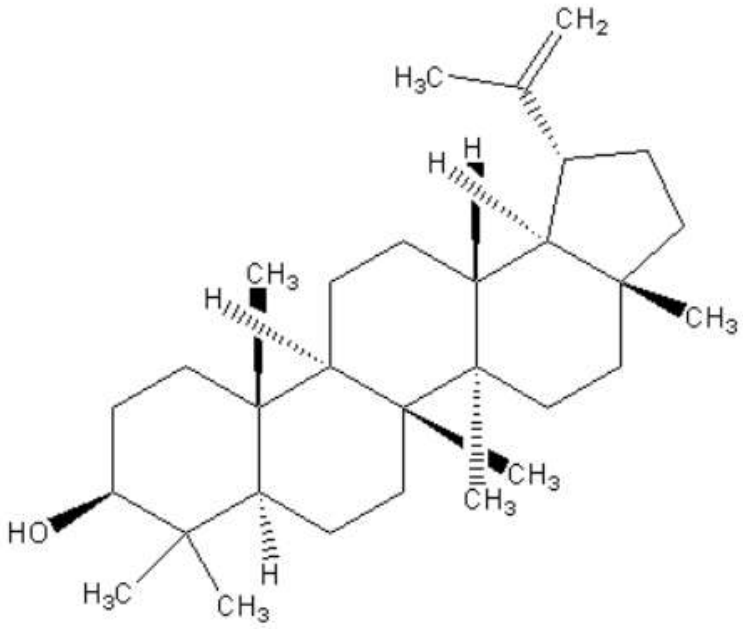

Fig.2-Structure of lupeol

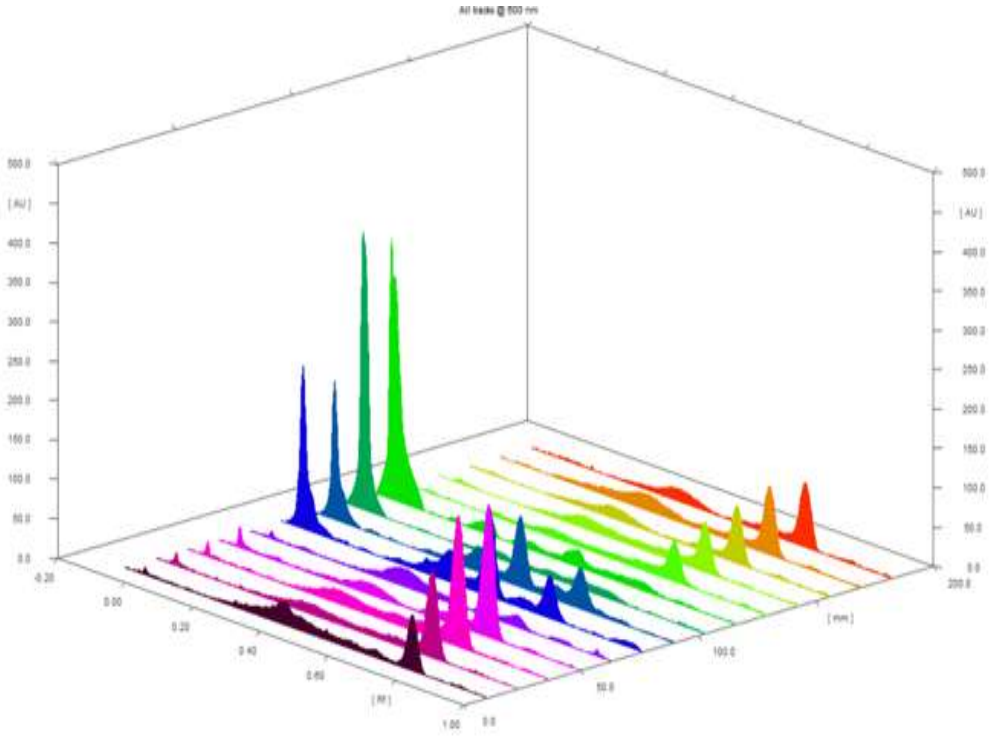

Fig.3- Track 1-5 and 10-14 linearity of standard lupeol : Track 6,7 of bark extract, Track 8,9 of marketed product (Kashay)

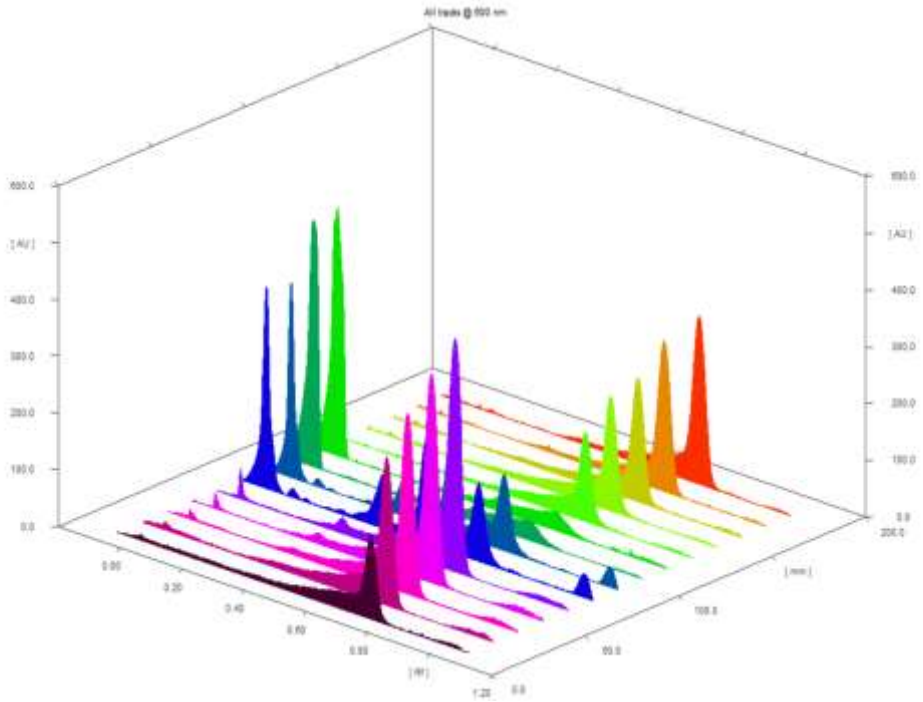

Fig.4- Track 1-5 & 10-14 linearity of standard lupeol : Track 6,7 of bark extract; Track 8,9 of marketed product (Churna)