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ORIGINAL ARTICLE

HPTLC densitometric quantification of stigmasterol and lupeol from *Ficus religiosa*

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Abstract This study presents the first report of TLC densitometric method, which has been developed and validated for simultaneous quantification of the two marker compounds (stigmasterol and lupeol) from methanolic extract using the solvent system of toluene:methanol (9:1, v/v). The method employed TLC aluminum plates precoated with silica gel 60 F_{254} as the stationary phase. Densitometric analysis of stigmasterol and lupeol was carried out in the reflectance mode at 525 nm. The system was found to give compact spots for stigmasterol and lupeol (R_f value of 0.37 and 0.60, respectively). The method was validated using ICH guidelines in terms of precision, repeatability and accuracy. Linearity range for stigmasterol and lupeol was 80–480 ng/spot and 150–900 ng/spot and the contents were found to be 0.06 ± 0.005% w/w and 0.12 ± 0.02% w/w, respectively. The limit of detection (LOD) value for stigmasterol and lupeol were found to be 20 and 50 ng, and limit of quantification (LOQ) value were 60 and 100 ng, respectively. This simple, precise and accurate method gave good resolution from other constituents present in the extract. The method has been successfully applied in the analysis and routine quality control of herbal material and formulations containing *Ficus religiosa*.

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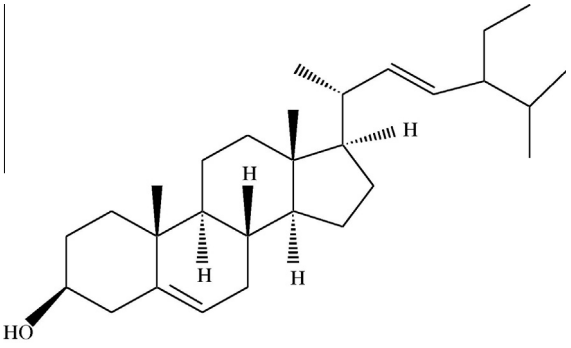
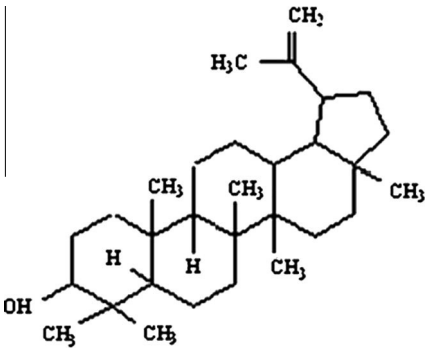
1. Introduction

Ficus religiosa Linn a perennial plant belonging to the Amaryllidaceae family grows in Sub-Himalayan tracts, commonly known as the Bodhi tree, have traditionally been used in Indian folk medicines for respiratory disorders and some skin diseases (Mousa et al., 1994). It is recommended for throat diseases, kidney stones, blindness, otitis, rheumatism, bone dislocations, sprains and fractures, mastitis, jaundice, bloody dysentery, diarrhoea, glossitis, haematuria, miscarriage, indigestion, hernia (http://www.divineremedies). The bark and leaves are taken for diarrhoea and dysentery. The powdered fruit is taken for asthma and the latex is used to treat warts. The bark is astringent, cooling, haemostatic and laxative, also used in diabetes, diarrhoea, leucorrhoea, menorrhagia and nervous disorders, for vaginal and other urinogenital disorders (Kirtikar, 1975). Medicated oil made from the root bark is applied externally to skin diseases such as eczema, leprosy and is also used in rheumatism. Recently, the methanol extract of *F. religiosa* has been reported to have neurotrophic effects and acetylcholinesterase inhibitory activity (Vinutha et al., 2007). It also exhibits antiinflammatory properties (Jung et al., 2008) and several studies have focused mainly on its antitumor, antibacterial (Nair and Chanda, 2007), anthelmintic activity (Iqbal et al., 2001), antifungal activity (Khan et al., 2007; Aqil and Ahmad, 2003), kidney and urinary disorders (Ballabh et al., 2008). The plant is reported to contain beta-sitosteryl-D-glucoside, Vitamin K, *n*-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupeol, campestrol, 28-isofucosterol, a-amyrin, β-amyrin. Bergapten and bergaptol have been isolated from the bark. Asparagine and tyrosine have been isolated from the fruit (http://www.divineremedies). Polyphenols and sterols are also reported to be present in the fruits. Stigmasterol (Scheme 1a) is reported to have antioxidant, thyroid inhibitory, antiperoxidative, hypoglycemic (Panda et al., 2009; Jamaluddin et al., 1994) hypocholesteromic (Battaab et al., 2006) and anti-inflammatory activity (Gabay et al., 2008) and lupeol (Scheme 1b) is reported to have anti-inflammatory (Geetha and Varalakshmi, 2001), hepatoprotective (Sunitha et al., 2001) and anticancer activity (Chaturvedi et al., 2008; Saleem et al., 2001; Nigam et al., 2009; Saleem, 2009).

Nowadays, HPTLC has become a routine analytical technique due to its advantages of reliability in quantitation of analytes at micro and even in nanogram levels and cost effectiveness (Rathee et al., 2010). It has proved a very useful technique because of its low operating cost, high sample throughput and need for minimum sample clean-up. The major advantage of HPTLC is in reducing analysis time and cost per analysis (Rathee et al., 2010) TLC has been known as the fast tool for the detection of compounds. Another advantage of TLC is the capability to detect more compounds than HPLC, although the resolution is poorer. In this regard, the compounds which cannot be eluted still can be detected. Moreover, the compounds having no UV absorption, e.g., sugar, still can be detected by reagent spraying. The TLC chromatogram pattern comparison seems to be promising for fingerprinting the active compounds in plant extracts. Thus, it can be used as a tool in the quality control in order to warranty that the active compounds are extracted. By means of data analysis system and optimized experimental conditions, HPTLC is also feasible for development of chromatographic fingerprint methods to determine and identify complex herbal extracts just like HPLC and GC (Chen et al., 2006). Furthermore, the colorful picture like HPTLC image provides extra intuitive parameters of visible color and or fluorescence and, unlike HPLC and GC, HPTLC can simultaneously determine different samples on the same plate. Such an approach causes the HPTLC method to maintain its innate advantage as well as get over the limitations of developing distance and plate efficiency. Some of the analytical methods reported for the qualitative and quantitative analysis of stigmasterol and lupeol are discussed herewith. Kpoviéssia et al. (2008) developed the method for determination of sterols and triterpenes using capillary gas chromatography. Haliński et al. (2009) studied the chromatographic fractionation and analysis of the main constituent's sterols and triterpenes by HPTLC. Qualitative and quantitative standardization of stigmasterol and lupeol was performed using HPTLC but not from this plant by Singh et al. (2009). Lupeol alone was also quantified by Shrishallappa et al. (2002), Anandjiwala et al. (2007), Darekar et al. (2008), Padashetty and Mishra (2007), Shrishallappa et al. (2002), and Rahul et al. from plants and polyherbal formulation. A rapid quantification of free and esterified phytosterols using APPI-LC-MS/MS was done by Lembcke et al. (2005). Determination of stigmasterol in dietary supplements by gas chromatography was performed by Sorenson and Sullivan (2006), and HPTLC determination of Stigmasterol was done by Hamrapurkar and Karishma (2007). Literature survey revealed that no method has been reported for the simultaneous quantitation of stigmasterol and lupeol from *F. religiosa* fruits extract. So, in the present study, a HPTLC method for the simultaneous quantification of stigmasterol & lupeol has been developed.



Scheme 1a Structure of stigmasterol.



Scheme 1b Structure of lupeol.