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Alhagi maurorum: A convenient source of lupeol

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ABSTRACT

Lupeol, a bioactive triterpenoid, has been isolated from the root barks of *Alhagi maurorum* for the first time in considerable quantity *via* an easy extraction and isolation process. In this study, a new and versatile LC–MS method has also been developed by optimizing various parameters for the rapid determination of lupeol in plant extract. The anti-inflammatory property of *A. maurorum* can be correlated to this compound. The superiority of *A. maurorum* over other plant sources of lupeol is due to its wild nature and ability to grow throughout the year. On the basis of this fact, *A. maurorum* can be used as a cheaper and ever available source for the lupeol.

1. Introduction

Triterpenoid, lupeol (*3β-hydroxylup-20(29)-ene*, Fig. 1), is an immense bioactive compound present in different medicinal plants (Sturm et al., 1996; Fernández et al., 2001; Cammareri et al., 2008). A wide range of bioactivities and bioassays of lupeol are reviewed (Gallo and Sarachine, 2009), which suggest its useful medicinal properties with diversity of action against different diseases. This compound is reported to be antiangiogenic (You et al., 2003), antioxidative and anti-inflammatory in nature (Nguemfo et al., 2009; Nikiéma et al., 2001; Sudhahar et al., 2006). It inhibits early responses of tumor growth induced by benzoyl peroxide (Saleem, 2009). It also plays very important role in normalization of lipid profile (Sudhahar et al., 2007), wound healing activity (Harish et al., 2008), protective effect in hypercholesterolemia associated with renal damage (Sudhahar et al., 2008) and suppression of immune factors (Bani et al., 2006; Vasconcelos et al., 2008; Akihisa et al., 1996). Nevertheless, due to its difficult synthesis, lupeol has mostly been isolated from the plant sources, *e.g.* 3 μg/g from fruits of Olive (*Olea Europe*), 180 μg/g from pulp of mango (*Mangifera indica*), 280 μg/g dry leaf of Aloe (*Aloe vera*), 880 μg/g bark from bark of Elm plant (*Ulmus campestris*), 175 μg/g from twig bark of Japanese pear (shinko) (*Pyrus serotina* Rehd) 15.2 mg/100 g from Ginseng oil (*Panax quinquefolium* L.). Some of previously reported plant sources are summarized in Table 1 (Saleem, 2009).

There is increasing trend of isolating particular compound which is found in considerable quantity in certain plants and its identification to support industry by providing new source for that bio active compound (Memon et al., 2010). In addition to isolation of lupeol from different plants, various methods have been used for its determination in plant extracts. Berridge et al. have reported the quantification and identification of phytosterol contents including lupeol from American Ginseng seed oil by GC and GC–MS through pre-derivatization method. Ferreira and co-workers have determined the lupeol from *Cordia verbenacea* by using GC–MS technique. The separation and identification of lupeol and some common isomeric plant triterpenoids has been made by Vovk et al. through TLC and HPLC from different plant extracts. Consequently, in view of its medicinal importance and particularly in chemical reactions to synthesize its derivatives (Cmoch et al., 2008; Reddy et al., 2009); a rich natural source of lupeol is still needed to be explored.

Alhagi maurorum is a 60–100 cm tall herb (Ahmed et al., 2010) that has been used in folk medicine (Laghari et al., 2010) and is widely distributed in Asia, Middle East, Europe, Russia, and Africa (Nasir and Ali, 1973). Various reports have revealed that a variety of bioactive compounds is present in *A. maurorum*, and certainly those compounds are responsible for its bioactivity (Laghari et al., 2010). In the present study, isolation of particular bioactive compounds *via* an easy procedure has been focused in order to correlate them according to the previously reported bioactivities of *A. mauro-*

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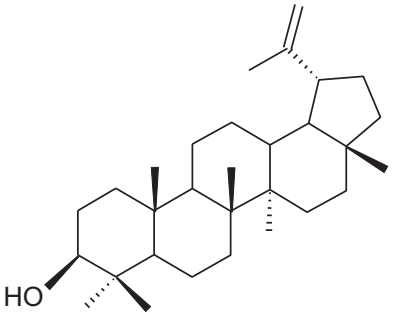

Fig. 1. Molecular structure of lupeol.

rum. Bioactive constitutes isolated from this species showing same activity against certain disease has also been reported (Laghari et al., 2010). Nevertheless, some properties shown by this plant in medicinal system are not still proved by isolation of particular constituents which may be considered as responsible for those activities. For example, the anti-rheumatic property (Brown, 1995) of *A. maurorum* oil is one of those activities which are reported earlier (Laghari et al., 2010). However, no any compound is reported to date in the literature that can correlate to this activity. This is the first report that reveals the presence of lupeol, which is already studied (Nguemfo et al., 2009; Nikiéma et al., 2001; Sudhahar et al., 2006) regarding its anti-inflammatory and dosage study (Yamashita et al., 2002). From these studies, it has also been noticed that anti-inflammatory activity of lupeol is dose dependent too. Consequently, it has been concluded that anti-inflammatory property of *A. maurorum* can be correlated to the presence of lupeol, being reported here. When the plant is used as ingredient in drugs to cure the ailment may be having varied concentrations of active components. Therefore, profiling of particular components to make a proper dosage form and composition is a best way of pharmaceutical practice.

In this study, *A. maurorum* as a convenient and rich source of lupeol is herein reported. The convenient isolation process and its characterization through multiple analytical tools have also been discussed. Besides this, a new and versatile LC–MS method has been developed by optimizing various parameters for the rapid determination of lupeol in plant extract.

2. Material and methods

2.1. Chemicals and materials

Melting points were determined on a Gallenkamp apparatus (UK) in a sealed glass capillary tube. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer in $CDCl_3$ using TMS (tetramethyl silane) as an internal standard at room temperature. IR spectra were recorded on a Thermo Nicollet AVATAR 5700 FTIR spectrometer in the spectral range 4000–400 cm^{-1}. All the reagents used were purchased from Merck (Darmstadt, Germany) and were used as supplied. Thin layer chromatography (TLC) was performed on pre-coated silica gel plates (SiO_2, PF_{254}, Merck). Column chromatography was performed with flash grade silica. All aqueous solutions were prepared with deionized water filtered through a Millipore Milli-Q Plus water purification system.

2.2. Plant material

The root barks (100 g) of *A. maurorum* were collected from vicinity of village Malkani, located in district Badin of Sindh Province in Pakistan during February 2010. Plant species was identified by Prof. Dr Tahir Rajpoot, former Director Institute of Botany, Univesrity of Sindh, Jamshoro. It was found to be single species of genus *Alhagi* present in Pakistan, as per record of flora of Pakistan.

2.3. Extraction and isolation

The air dried root barks of *A. maurorum* were ground and dipped in methanol for 48 h at room temperature and process was repeated twice. Dark brown syrup so obtained was filtered. Combined methanol extract was concentrated under reduce pressure and resulting thick residue was washed exhaustively by hexane to get non polar as well as less polar constituents. When hexane extract stopped to show oily yellow color then hexane fractions were combined and concentrated under vacuum. The crude extract so obtained was flash column chromatographed by eluting column with 100% hexane along with gradual increase in the concentration of gradient ethyl acetate. When mobile phase composition reached at 20% ethyl acetate, a white powder with some impurity was obtained in elute. Adsorbed yellowish impurity was soluble in hexane and, the remaining white soft needles like compound (0.11 g) was soluble in chloroform. That was then subjected to different analytical techniques for its identification.

2.4. Identification of lupeol isolated from A. maurorum

Identification of lupeol was carried out by using different spectroscopic techniques and comparing the results to previously reported literature that elucidated the structure of this compound (Lutta et al., 2008; Imam et al., 2007; Burns et al., 2000; Fotie et al., 2006). The detailed properties of lupeol isolated from *A. maurorum* are summarized in Table 1.

2.5. Liquid chromatography–mass spectrometry (LC–MS) method

A new method has been developed for lupeol determination by single component analysis procedure. Optimized chromatographic conditions are as follows:

Mobile phase was composed of methanol (A) and formic acid (B) running in isocratic flow of 1.0 mL/min. Total run time was 6 min, while retention time (Rt) of lupeol was 4 min. Lupeol sample isolated from *A maurorum* as well as standard reference of the same were dissolved in isopropanol. Concentration range of standard solution, to make calibration curve was 0.3–5.0 mg/L. For validation of method, basic parameters were taken into account: such as selectivity, linearity, precision and accuracy of data obtained by running authentic reference standard of lupeol and sample on LC–MS. MS conditions were as follows, Capillary temperature 150 °C, Source heater temperature 400 °C, Sheath gas flow 37.0 (arbitrary unit), Aux/sweep gas flow 5.00, Source voltage 6.00 kV, Source current 5.00 μA, Tube lens offset 25.0 V.

3. Discussions

3.1. Characterization of lupeol isolated from A. maurorum

3.1.1. Mass spectrometry of lupeol

Lupeol, isolated from *A. maurorum* was subjected to EI-MS and direct control mode of APCI techniques. Diagnostic factor was taken into account in each of the above techniques, *e.g.* some common fragments of the compound in EI-MS (Baas et al., 1992) and parent ion peak in APCI appeared at *m*/*z* 409 (M+H-H_2O) as reported earlier (Baas et al., 1992). In EI-MS formation of the diagnostic fragment [M-111]$^{+}$ of Lup-20(29)-en derivative was observed at *m*/*z* 315. This fragmentation pattern is given in Fig. 2.