Detection of Lupeol in Calendula Officinalis Grown in Iraq by GC-MS Analysis

Noor Ali Kadhim *, Widad M K Al-Ani **, Ibrahem S. Al-Joboury ***
****National Center for Drug Control and Research (NCDCR)***
*****Ashur University College***
****** Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, Al-Mustansiriya University, Baghdad, Iraq..***

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Corresponding Author email:
dr.ibrahim@uomustansiriyah.edu.iq
orcid: https://orcid.org/0000-0002-5345-1011

Abstract:

Triterpenes are abundant group of natural compounds with important structural components of plant's cell membranes. Free triterpenes stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membranes. Lupeol is a pentacyclic triterpene reported to have important physiological and therapeutic effects in human health issues, therefore its extraction from Calendula officinalis flowers and detection by GC-MS is of significant importance.

Key words: Triterpenes, lupeol.

تشخيص مركب اللوبيول في نبات الاقحوان النامي في العراق بتقنية الجي سي ماس

نور علي كاظم*،وداد مصفى العاني،ابراهيم صالح عباس الجبوري *****
***المركز الوطني للرقابة و البحوث الدوائية**
****كلية اشور الجامعة**
*****فرع العقاقير والنباتات الطبية /كلية الصيدلة الجامعة المستنصرية**

الخلاصة:

تعد التربينات من المركبات الطبيعية واسعة الانتشار حيث تكمن اهميتها في المحافظة على تكامل وظيفة الجدار الخلوي للنبات. ان التربينات الحرة تساعد على استقرار الفوسفولبد ثنائي الطبقة في جدار الخلية النباتية كوظيفة الكوليسترول في الخلايا الحيوانية. اللوبيول هو مركب خماسي الحلقة و اظهرت العديد من الدراسات الحديثة التي تبين اهمية اللوبيول فسلجيا و علاجيا للانسان مما جعل استخلاصه من النبات ذو اهمية. ان الهدف من هذه الدراسة هو استخلاص و تشخيص اللوبيول في نبات الاقحوان النامي في العراق باستخدام تقنية مطياف الكتلة.

الكلمات المفتاحية: التربينات , لوبيول.

Introduction

Terpenes are one of the biggest classes of plant's secondary metabolites which are mainly consists of five carbon isoprene units which are gathered by different ways to produce monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes and phytosterol (C30-derived) and the tetraterpenes (carotenoids, C (40). While terpenoids are modified class of terpenes with different functional groups and oxidized methyl group moved or removed at different positions [1].

A great attention has been directed to natural triterpenoids, phytosterols owing to their wide range of biological activities [2]. Triterpenes are abundant group of natural compounds with important structural components of plant's cell membranes, free triterpenes stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membranes [3]. The cholesterol – lowering effect of phytosterols is interesting since they compete for absorption with cholesterol in the digestive tract [4]. *Calendula officinalis* flowers (as shown in Figure 1) is native to South Europe, USA and west of Asia [5]. *Calendula* flowers have been used to treat many health issues as measles and jaundice [6]. The plant is also reported to have anti-inflammatory, cytotoxic and antitumor activities [7,8]. Thus, it is important to diagnose the active constituents with pharmacological effects.

The pharmacological effects are attributed tovarious terpenoids that extracted from *Calendula officinalis* flowers such as stigmasterol [9] and lupeol [10].

Figure 1: An image of *Calendula officinalis* flowers.

Lupeol is a pentacyclic triterpene $C_{30}H_{50}O$ present in plants but not fungi and animals, with molecular weight 426.7 g/mol and melting point 215-216°C. When lupeol administered to rats, it showed good anti-inflammatory action but it did not have any ulcerogenic or antipyretic actions in comparisons with non-steroidal anti-inflammatory drugs [11]. It was reported that lupeol involved in apoptosis [12]. This phytosterol, is widely found in fruits, and vegetables. Extensive studies during last three decades have shown several important pharmacological activities of lupeol. Various preclinical animal studies suggested that lupeol has a potential to act as an anti-microbial, anti-protozoon, anti-proliferative, anti-invasive and cholesterol lowering agent. Employing various *in vitro* and *in vivo* models, lupeol has also been tested for its therapeutic efficiency against conditions including wound healing, diabetes, cardiovascular disease, kidney disease, and arthritis. It has been found to be pharmacologically effective in treating various diseases under preclinical settings (in animal models) irrespective of varying routes of administration. Such as, topical, oral, intra-peritoneal and intravenous. It is noteworthy that lupeol has been reported to selectively cure unhealthy human cells, while sparing normal and healthy cells [13].

Many methods were used previously to detect Lupeol Thin Layer Chromatography (TLC) was used as simple detection, it reveals violet spot of same R_f value of the standard [14].

Another two important methods used to detect this valuable compound in medicinal plants were Gas Chromatography (GC) and High-Performance Thin Layer Chromatography (HPTLC) techniques [15].

The most powerful and highly accurate method is Gas Chromatography-Mass spectrometry (GC-MS) which give ion species fully resolved by comparison with the previous data bases [16].

Materials and Methods

Collection of the plant

Calendula officinalis flowers were collected from the garden of medicinal plants at the College of Pharmacy/ Mustansiriyah University during March. Whole plant was authenticated by the National herbarium in AbuGraib Baghdad. Flowers were dried at room temperature in the shade. Then grinded to powder, weighed and kept in clean dry jars until use.

Extraction of terpenoids

Shade-dried pulverized plant material (90 g) of thedried flowers was extracted by Soxhlet apparatus with hexane (700 millilitres) [17]. The solvent was evaporated under reduced pressure using rotary evaporator. Hexane extract was analysed for the presence of terpenes by a chemical test [17] as well as by thin-layer chromatography (TLC) with spray reagent and confirmed by gas chromatography-mass spectrometry (GC-MS).

Detection of terpenoids by chemical test

Four millilitres of extract were treated with 0.5 millilitre of acetic anhydride and 0.5 millilitres of chloroform. Then, a concentrated solution of sulphuric acid was added drop by drop slowly until red violet color was observed for terpenoid [18].

Detection of lupeol by analytical TLC

A TLC plate of 0.25 mm thickness was used as a stationary phase, and the following mobile phases were used as shown in Table1.

Table 1: mobile phases for lupeol.

Mobile phase	Solvent system content
S1	Chloroform: Acetone (9:1)
S2	Hexane: Ethyl Acetate (7:2)
S3	Toluene: Ethyl Acetate: Chloroform (5:1:4)

Lupeol standard was applied as a spot and concentrated hexane extract was applied as band on the baseline of TLC plate and developed with the mobile phase. TLC plate was dried and the side of the plate was sprayed by vanillin-sulphuric acid reagent. The reagent was prepared as the following: vanillin (1g) was dissolved in ethanol (100 mL) to prepare solution I. Concentrated H_2SO_4 (10 mL) was added drop wise to ethanol (90 mL) to prepare solution II. The TLC plate was sprayed with solution I, followed directly by solution II. Then the plate was heated for five to ten minutes at 110°C [19].

Detection of the lupeol by GC-MS

The GC-MS detection was carried out to identify th presence of terpenes in the plant extract. It was performed at the Ministry of Sciences and Researches/ Enviroment research centre in Iraq. One microliter of the sample was injected in the instrument at 250°C. Helium was used as carrier gas and the column temperature was rose up from 80°C to 310°Cat rate time of 10°C/minute [20].

Results and Discussion

Chemical test

Red violet color was observed at the interface of test tube for terpenoids (as shown in Figure 2).

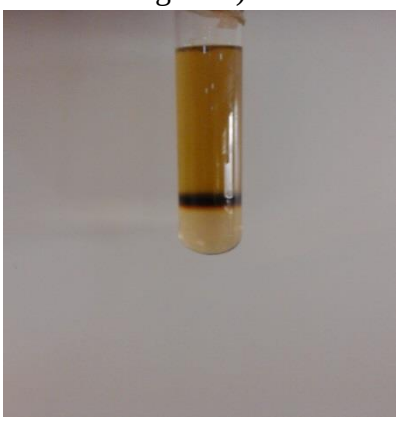


Figure 2: chemical test for terpenoids.

Preparative TLC

Hexane extract of *Calendula officinalis* was developed in three solvent systems S1, S2 and S3 as shown in Table 1. Lupeol compound gave different R_f values

depending on the used solvent systems. These values were comparable to the R_f values of the standard lupeol as shown in Table 2. The lupeol band in mobile phase S1 (which illustrated in Figure 3) is very matching with standard spot and it confirm the identity of the compound.

Table 2: R_f values of lupeol reference standard and lupeol in hexane extract of flowers using three developing solvent system in TLC.

Mobile phase	R_f value for lupeol in extract	R_f value for standard
S1: Chloroform: Acetone (9:1)	0.78	0.782
S2: Hexane: Ethyl Acetate (7:2)	0.53	0.531
S3: Toluene: Ethyl Acetate: Chloroform (5:1:4)	0.45	0.45

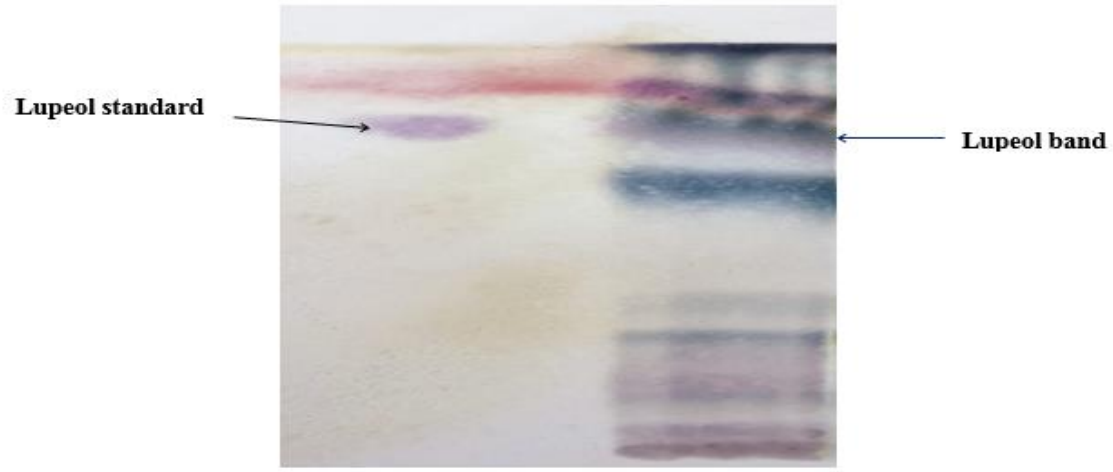
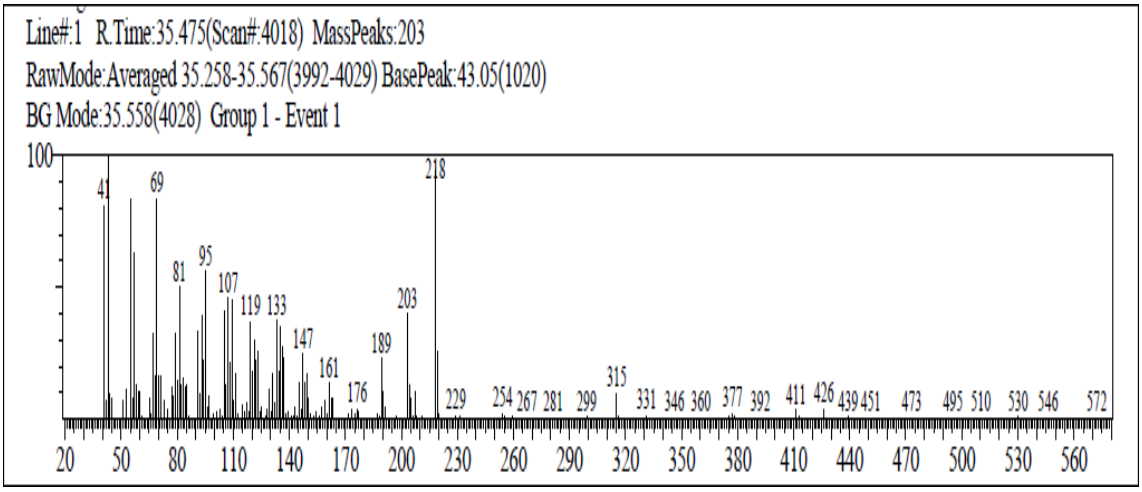


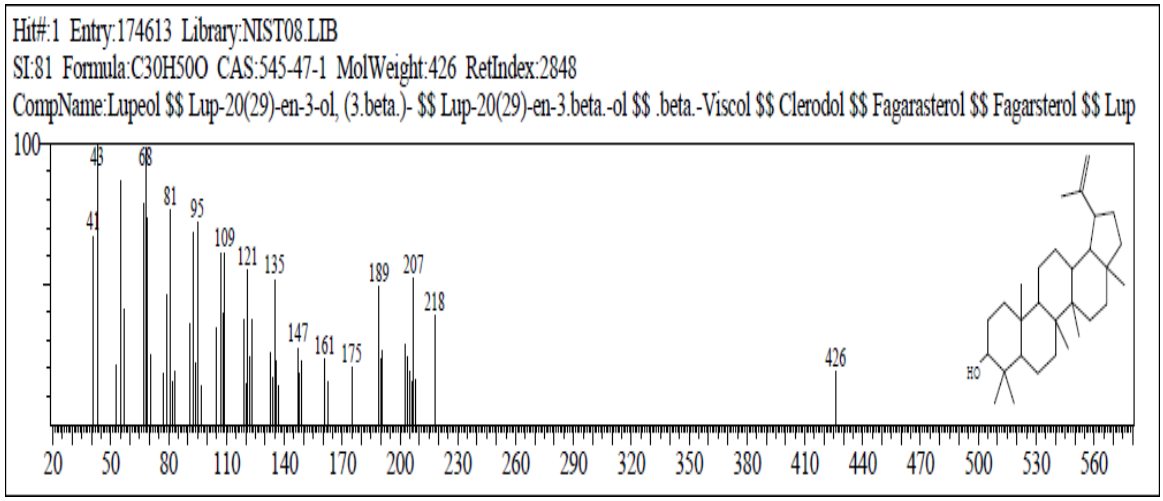
Figure 3: TLC detection of Lupeol in hexane extract of *C.officinalis* flowers in S1 mobile phase.

GC-MS analysis

The GC-MS analysis for the stusied compound was performed to confirm its chemical structure .The molecular ion peak of lupeol was identified and it was found to be equal to the calculated molecular weight of the compound at *m/z* 426.this molecular ion underwent another cleavage to fragment methyl moiety to produce fragment of *m/z* 411. Researches suggest that lupeol fragmentation starts at cleavage at C14-C27 (shown in Figure 5) followed by subsequent $^{\cdot}CH_3$ removal. Fragmentation peaks at *m/z* 411 and 383 are not easily to be recognized because they readily decompose into fragment ions at *m/z* 189 that is suggested to be produced from two ways (as shown in Figure 6) [21].



A



B

Figure 4: Mass spectrum of lupeol (A:lupeol in hexane extract, B:standard lupeol).

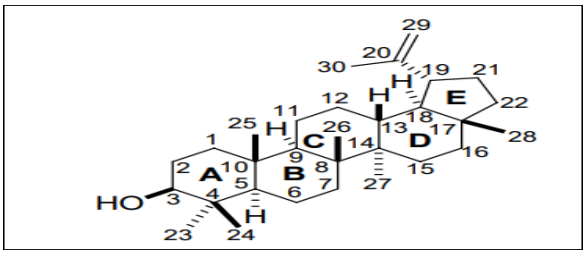


Figure 5:Chemical structure of lupeol.

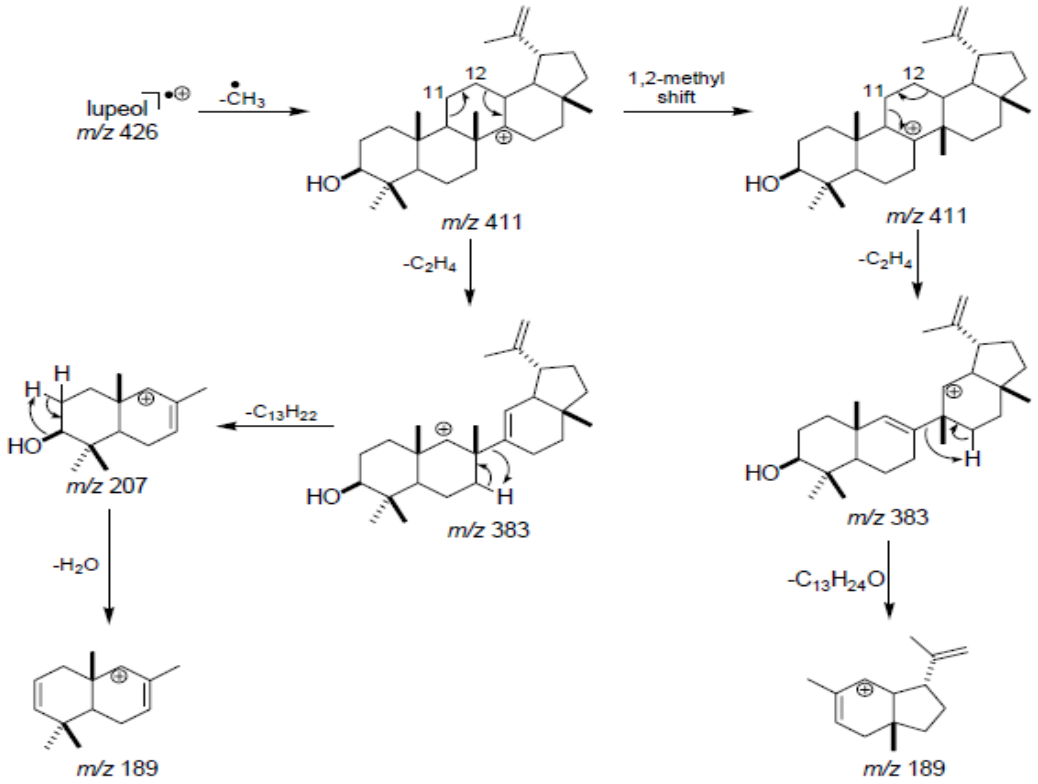


Figure 6: Fragmentation pattern of lupeol.

Another fragmentation pattern explained in Figure 7 showing *m/z* 218 and *m/z* 203 peaks in mass spectrum of lupeol [22]

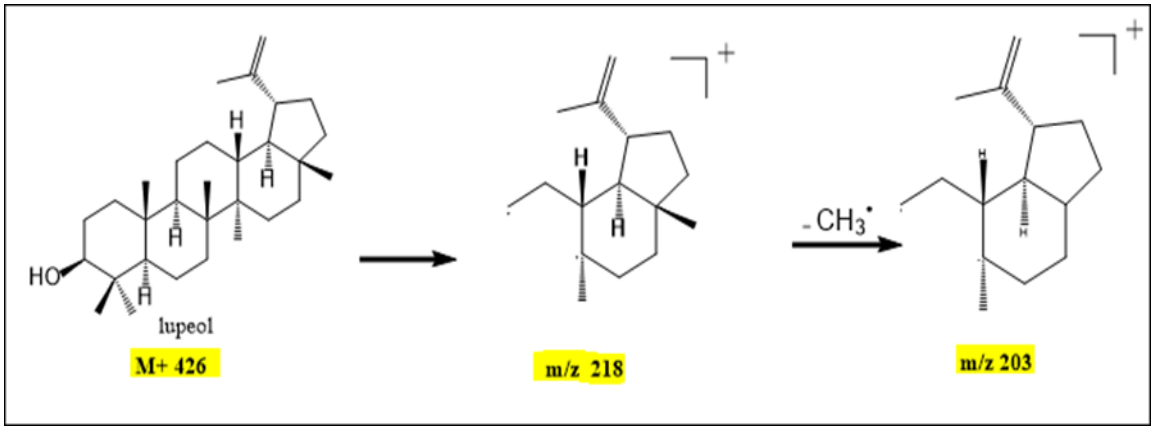


Figure 7: Second fragmentation pattern of lupeol.

Conclusion

From the study results, it could be concluded that *Calendula officinalis* flowers grown in Iraq contain Lupeol compound, that was detected by chemical test, Thin Layer Chromatography (TLC) and confirmed by Gas Chromatography-Mass spectroscopy (GC-MS). The most powerful, and highly accurate method among the used methods for detection is Gas Chromatography-Mass spectroscopy (GC-MS).

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