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การแยกและการวิเคราะห์ลักษณะของสาร 3β-lup-20(29)-en-3-ol (Lupeol) จากส่วนใบและกิ่งของต้น *Diospyros phuketensis* Phengklai

Isolation and Characterization of 3β-lup-20(29)-en-3-ol (Lupeol) from the Leaves and Twigs of *Diospyros phuketensis* Phengklai

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บทคัดย่อ

Diospyros เป็นสกุลหนึ่งในวงศ์ *Ebenceae* ซึ่งพบว่ามีการรายงานการออกฤทธิ์ทางเภสัชวิทยา การตรวจสอบทางชีวภาพและการหาองค์ประกอบทางเคมี จากการสกัดส่วนต่าง ๆของ *Diospyros* เป็นจำนวนมาก สาร lupeol จัดเป็นสารในกลุ่ม pentacyclic lupane พบมากถึง 90 เปอร์เซนต์ในสกุล *Diospyros* และมีรายงานการออกฤทธิ์ทางชีวภาพอย่างหลากหลายเช่น ฤทธิ์ต้านการอักเสบ ป้องกันข้อต่ออักเสบและต้านการก่อกลายพันธุ์โดยมีการศึกษาแล้วทั้งในหลอดทดลองและในสัตว์ทดลอง ทำการแยกและวิเคราะห์ลักษณะหาสารจากส่วนใบและกิ่งของต้น *Diospyros Phuketensis* Phengkai โดยวิธีคอลัมน์โครมาโทกราฟีพบสาร lupeol โดยอาศัยการวิเคราะห์ข้อมูลจากวิธีแมสสเปกโทรสโกปีอินฟราเรดสเปกโทรโฟโตเมทีและข้อมูลทาง NMR สเปกโทรสโกปี งานวิจัยนี้เป็นการพบสาร lupeol ใน *Diospyros Phuketensis* Phengkai เป็นครั้งแรก

คำสำคัญ: *Diospyros phuketensis* Phengklai/ lupeol / 3β-lup-20(29)-en-3-ol

Abstract

Diospyros is one of most important genus of the *Ebenceae* family. The pharmacological activities, bio-assays and chemical constituent of various *Diospyros* extracts have been reviewed by various research groups. Lupeol, a pentacyclic lupane-type, has been discovered in over 90% of the *Diospyros* species. Its biological activities have been reported as being anti-inflammatory, anti-arthritic and anti-mutagenic, both in vitro and in vivo systems. Herein, lupeol is found for the first time from the leaves and twigs of *Diospyros Phuketensis* Phengkai which have never been reported. Column chromatography of the extract gave lupeol confirmed structure by mass spectrometry IR and NMR data.

Keywords: *Diospyros phuketensis* Phengklai/ lupeol/ 3β-lup-20(29)-en-3-ol

1. Introduction

The *Ebenaceae* family consists of about 500 species and is widespread throughout the tropical and subtropical regions of the world. It consists of 7 genera; *Diospyros*, *Euclea*, *Maba*, *Onotheca*, *Rhaphidanthe*, *Royena* and *Tetraclis*. The genus *Diospyros* (Syn: Persimmon, Ebony), which includes more than 350 species, plays a critical role in economy and medication.[1] Sixty-four species of *Diospyros* have been reported in Thailand.[2] According to previously reported literature, [1] the biological activities of compounds isolated from the genus *Diospyros* mostly originate from flavonoids, coumarins, terpenoids and naphthoquinones. [1] Triterpenoids are widely distributed and found in more than 90% of screened *Diospyros* plants. While they can be found in most parts of plants, they are found most abundantly in the leaves and the heartwood. Almost all isolated triterpenoids are in a pentacyclic core structure and belong to a group of lupeol, lupane, ursane, oleanane, taratexane and friedelane. [3], [4]

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Previously, many studies showed the bioactive compounds in *Diospyros* species. Triterpenoides, such as lupeols, have been identified in over 90% of the *Diospyros* species. [1] Due to their anti-inflammatory [5], [6], [7] anti-oxidant, [8], [9] and anti-diabetic activities,[10] we are therefore interested in the isolation and characterization of lupeol in leaves and twigs of *D. Phuketensis* Phengklai.

2. Materials and method

General experimental procedures: attenuated total reflection (ATR)-FTIR spectra were recorded on a Bruker. Low resolution EI mass spectra were recorded on a Thermo Finnigan Polaris Q mass spectrometer at 70 eV (probe). The high resolution mass spectra were recorded employing the time of flight (TOF) mode on a MicroTOF model. The high resolution nuclear magnetic resonance spectra were mainly recorded on Bruker AV-500 spectrometers. The NMR spectra were recorded in deuterochloroform solution, unless otherwise stated; and reported as δ values in ppm down field from TMS (internal standard δ = 0.00). TLC aluminum sheets of silica gel 60 PF_{254} were used for analytical purposes and the bands were visualized with ultraviolet light (either at λ_{max} 254 or λ_{max} 366 nm). Column chromatography was performed by using silica gel 60 H (70-230 mesh ASTM, cat. No. 7734 E. Merck).

Plant material: the dried leaves and twigs of *D. Phuketensis* Phengklai were collected from Phuket province in the southern part of Thailand. A voucher specimen (BKF no. 068682) was deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand. Extraction and Isolation. The air-dried and powdered leaves and twigs (3.6 kg) of *D. phuketensis* Phengklai were percolated five times in methanol (five days for each time) at room temperature and followed by filtration. The filtrate was evaporated to dryness under reduced pressure to produce a crude MeOH extract (73.68 g). The MeOH extract was first subjected to a course separation on a silica gel column chromatography using EtOAc-MeOH (9:1), EtOAc-MeOH-H_2O (8:2:0.1), and EtOAc-MeOH-H_2O (7:3:0.3) as solvent systems to afford four fractions (A-D). Fraction B (2.1g) was applied to column chromategraphy over silica gel. Elution was initially conducted with hexanes, gradually enriched with ethyl acetate, followed by increasing amounts of methanol in ethyl acetate and finally with methanol. The solvents were evaporated to dryness to afford six fractions (B1-B6). Fraction B4 (EtOAc-MeOH, 30:70) was recrystallized in dichloro methane-methanol to afford lupeol (50.5 mg)

Lupeol : a white amorphous solid m.p. 209-211 ^{o}C; IR ν_{max} (ATP) cm^{-1} : 3368, 1639, 1454, 1380, 1190, 1140, 1106, 1083, 1043, 1014, 983, 943, 917, 880, 547. EI-MS *m/z* (% rel. int.): 426 [M^+] (7), 411 (8), 383 (5), 207 (34), 189 (100); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ (ppm) 0.68 (1H, *d*, *J* = 9.2 Hz, H-5), 0.76 (3H, *s*, 24-CH_3), 0.78 (3H, *s*, 28-CH_3), 0.82 (3H, *s*, 25-CH_3), 0.92 (3H, *s*, 27-CH_3), 0.96 (3H, *s*, 23-CH_3), 1.02 (3H, *s*, 26-CH_3) and 1.67 (3H, *s*, 30-CH_3), 2.36 (1H, *td*, *J* = 12.0 Hz, 6.0, H-19), 3.17 (1H, *dd*, *J* = 9.6, 4.8 Hz, H-3), 4.56 (1H, *d*, *J* = 2.4 Hz, H-29a), 4.68 (1H, *m*, H-29b); ^{13}C NMR ($CDCl_3$, 500 MHz): δ (ppm) 14.55 (C-27), 15.37 (C-24), 15.98 (C-26), 16.12 (C-25), 18.00 (C-28), 18.33 (C-6), 19.31 (C-30), 20.94 (C-11), 25.16 (C-12), 27.43 (C-15), 27.45 (C-2), 27.99 (C-23), 29.86 (C-21), 34.29 (C-7), 35.59 (C-16), 37.19 (C-10), 38.07 (C-13), 38.72 (C-1), 38.87 (C-4), 40.00 (C-22), 40.86 (C-8), 42.85 (C-14), 43.00 (C-17), 47.99 (C-19), 48.32 (C-18), 50.45 (C-9), 55.31 (C-5), 79.02 (C-3), 109.31 (C-29), 150.97 (C-20) .

3. Results and discussion

Lupeol was obtained as a white amorphous solid. The molecular formula $C_{30}H_{50}O$ was deduced from HR-ESI-MS at *m/z* 449.3761 $[M+Na]^+$ (calcd. for $C_{30}H_{50}ONa$: 449.3759). The IR(ATR) spectrum showed the presence of an O-H absorption band of alcohol at 3368 cm^{-1} and C=C stretching band of olefinic double bond at 1639 cm^{-1}.

The 500 MHz ^{1}H-NMR spectrum of this compound in $CDCl_3$ displayed the presence of seven tertiary methyls at δ 0.76 (*s*, 24-CH_3), 0.78 (*s*, 28-CH_3), 0.82 (*s*, 25-CH_3), 0.92 (*s*, 27-CH_3), 0.96 (*s*, 23-CH_3), 1.02 (*s*, 26-CH_3), and 1.67 (*s*, 30-CH_3). These results suggested that the isolated compound was a pentacyclictriterpene. The

signal at δ 3.17 (1H, *dd*, *J* = 9.6, 4.8 Hz, H-3) indicated the presence of a hydroxymethine proton. The olefinic methylene protons were observed at δ 4.68 (1H, *m*, H-29a) and 4.56 (1H, *d*, *J* = 2.4 Hz, H-29b)

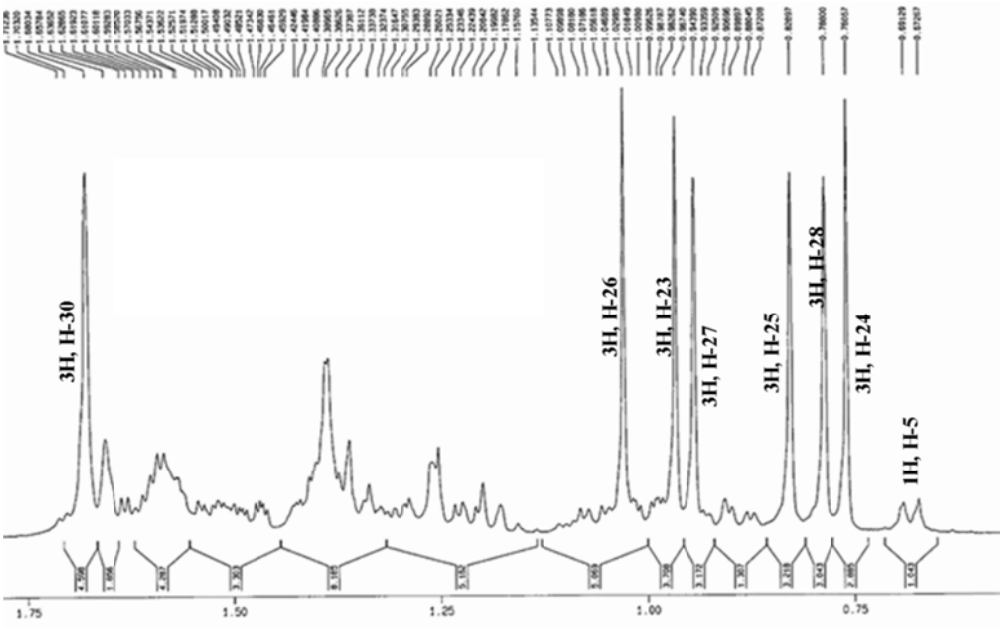

Figure 1 ^{1}H-NMR (500 MHz, $CDCl_3$) Spectra of isolated compound lupeol

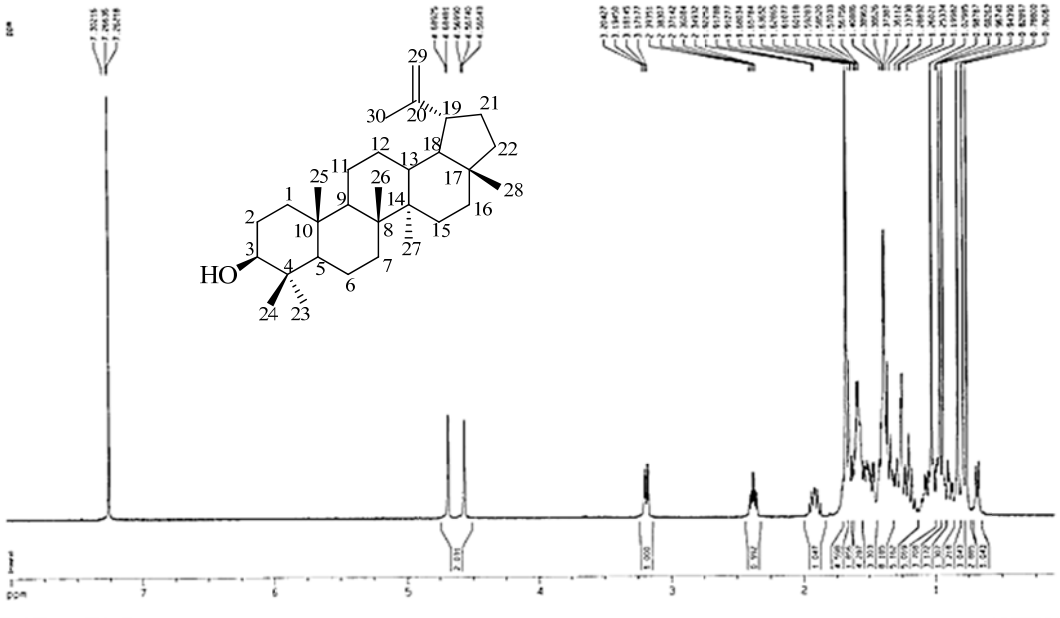

Figure 1 (*Continued*)

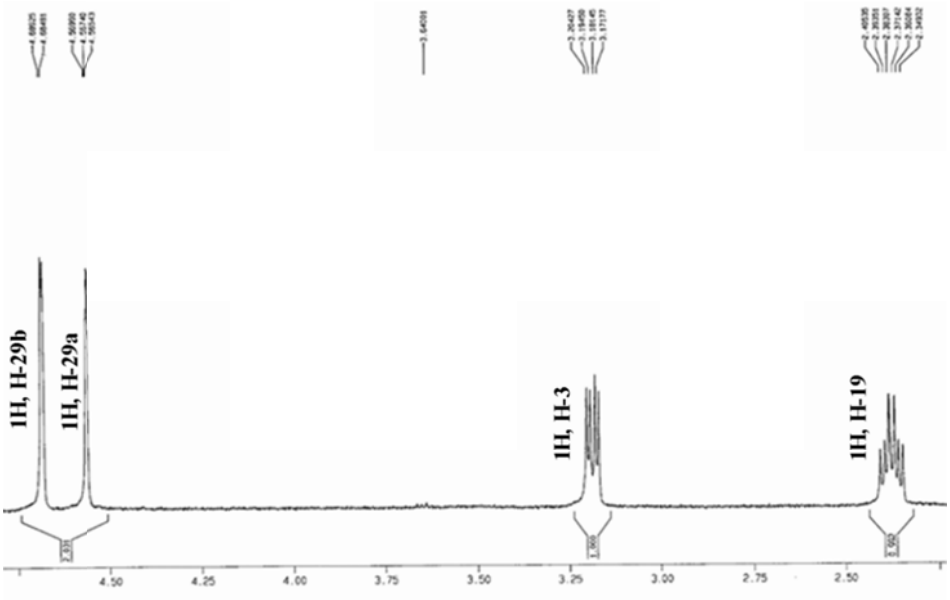

Figure 1 (*Continued*)

The 125 MHz ^{13}C-NMR spectrum of the isolated compound in $CDCl_3$ showed thirty signals for thirty carbon atoms. The results from the DEPT-135 spectra suggested that presence of seven methyl carbons, eleven methylene carbons, six methine carbons and six quaternary carbons.

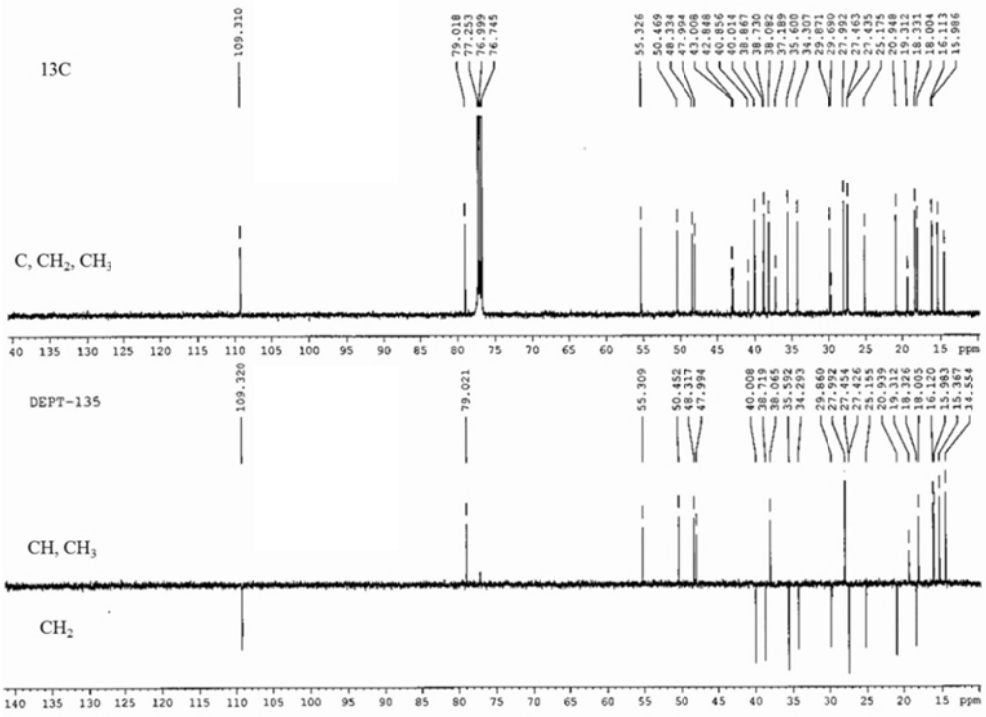

Figure 2 ^{13}C-NMR (125 MHz, $CDCl_3$) and DEPT-135 Spectra of isolated compound lupeol Based on the spectroscopic data described above, this compound was identified as lupeol, which has been previously reported from *Tamarindus indica* Linn. [11]

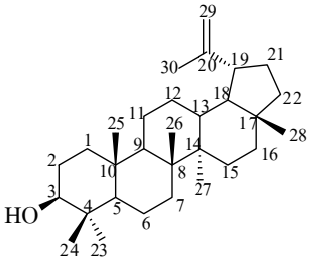

Figure 3 Chemical structure of lupeol

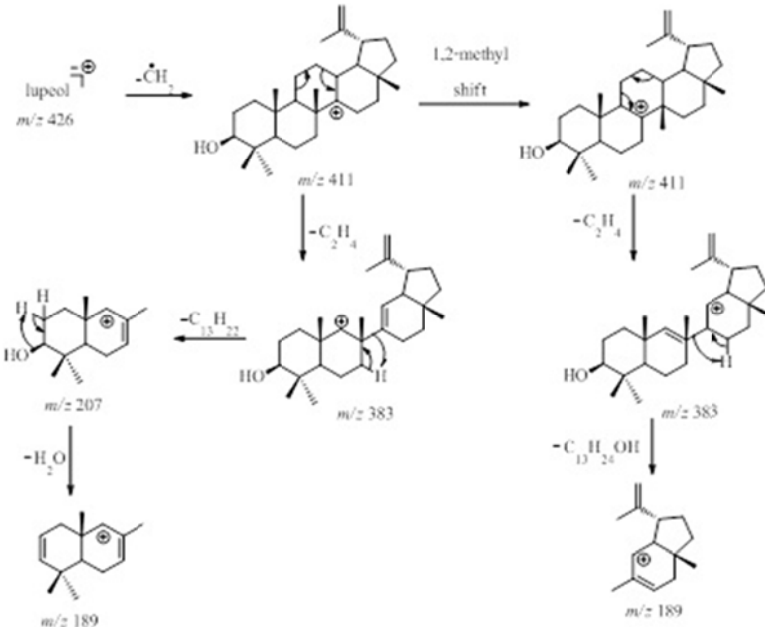

Figure 4 The fragmentation pathway of lupeol using electron ionization mass spectrometry(EI-MS) [12]

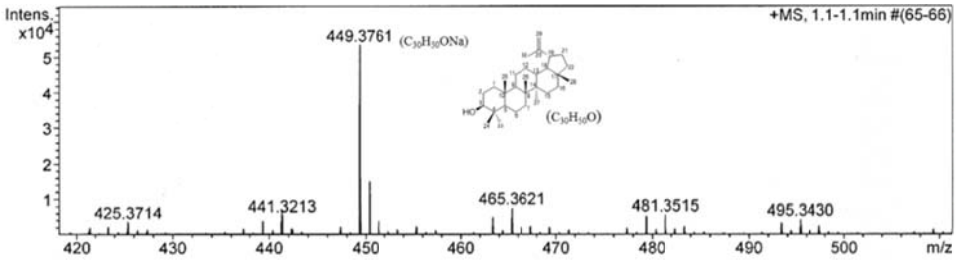

Figure 5 Mass spectrum of Lupeol. HRMS (ESI): *m/z* calculated ($C_{30}H_{50}ONa$) 499.3759, found 499.3761

Conclusion

Lupeol was extracted for the first time from the leaves and twigs of *D. Phuketensis* Phengkai. On the basis of characterization studies, the isolated compound has physical properties, ^{1}H-NMR, ^{13}C-NMR IR and Mass spectral data, which shows that the isolated compound matches the reported data of lupeol. So, the isolated compound was identified as lupeol. Furthermore, in continuation of this investigation, bioactive and crucial compounds in *D. phuketensis* Phengkai will be studied to search for new potentially useful lead compounds for antitumor, anti-HIV and anti-inflammatory drug development.

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