Isolation and Structural Characterization of Lupeol from the Stem Bark of *Diospyros ehretioides* Wall.

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ABSTRACT

The stem barks of *Diospyros ehretioides* Wall. (Local name- Auk-chinsa) were selected for this research. The phytochemical screening tests of the stem barks were carried out by standard methods. The preliminary phytochemical investigation revealed that the sample contains flavonoids, terpenoids, steroids, glycosides, reducing sugars, lipophilics, polyphenols, tannins and phenolic compounds. The organic compound (1) could be isolated as colorless crystals by using some sophisticated separation techniques such as thin layer and column chromatography. The complete structure assignment of the isolated compound (1) was accomplished using 1D and 2D Nuclear Magnetic Resonance (NMR) Spectroscopy. The isolated triterpene compound could be identified as Lupeol by the comparison of the experimental NMR spectral data with those of literature values.

Keywords : *Diospyros ehretioides* Wall., NMR spectroscopy, MS spectrometry, lupeol

1 INTRODUCTION

Plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. Since the middle of the 19th century, different bioactive phytoconsitutants have been isolated and characterized. Many of these are used as the active ingredients of the modern medicine, or as the lead compounds for new drugs discovery. [1] Medicinal plants are the plants that possess the properties or virtues that qualify them as drugs and therapeutic agents, and are used for medicinal purposes. Medicinal plants can provide biologically active compounds for the development of modified derivatives with increased activity and reduced toxicity. [2] The uses of medicinal plants are effective, safe due to low side effects, low costs, and ease of availability. [3- 5]

The genus *Dipspyros* is a large genus of trees or shrubs, belonging to the family Ebenaceae, which are distributed in Asia countries such as China, India, Japan, Thailand, Indonesia, Philippines and South Africa. [2, 6, 7] A. Ravikumar and *et al.* reviewed the medicinal uses and chemical constituents of various *Diospyros* species and reported about 300 organic chemicals have been isolated and identified. [2] In traditional medicinal system of the worlds, *Diospyros* species are used for many medicinal applications such as antifungal and for internal hemorrhage and bedwetting, for insomnia and hiccough, anti-hypertention, dysponea, vermicide and vermifuge, sedative, antifebrile, promotes secretions, astringent and bactericidal. All parts of these plants have been used for medicinal purposes. The fruits are carminative, astringent and cure biliousness and have been used as an anti-inflammatory and antipyretic drug in many local traditional medicines. Almost all parts of *Diospyros ehretioides* are used as traditional herbal medicines. The leaves are used for lumbago and the seeds are sedative and the bark is bitter, astringent and febrifuge. The unripe fruit is a more powerful astringent. [1, 6-7]

The genus *Dipspyros* is known to contain many phytochemical constituents, mainly different quinone, flavonoid and triterpenoid compounds. [7-10] Carter and et al reported the naphthoquinones, 7-methyljuglone and its dimer isodiospyrin isolated from the wood of D. *virginiana* possessed termiticidal activity against *Reticulitermes flavipes*. [11] The stem bark of one Myanmar traditional indigenous medicinal plant, Auk-chinsa, (*Diospyros ehretioides*) grown in Yezin, Pyinmanar Township, Mandalay Region was selected for the isolation and structure identification of organic compound.

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2 MATERIALS AND METHODS

2.1 General Procedures

Precoated siligica gel (Merk. Co. Inc, Kiesel gel 60 F_{256}) plate was used to perform thin layer chromatography. The developing agents in column chromatography used in this research were UV lamp (Lambda – 40, Perkin – Elmer Co, England) for UV active compound and Iodine vapor. Commercial grade solvents and reagents were used after distillation. Brucker AVANCE 600, Germany was used to measure 1D NMR such as ^{1}H-and ^{13}C NMR and 2D NMR such as COSY, HSQC, and HMBC (operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C NMR) at 298 K respectively. Chemical shifts values are given in δ-value (ppm) with tetramethylsilane (TMS) as internal standard.

2.2 Plant Materials

The stem barks of *Diospyros ehretioides* Wall. were collected from Yezin, Pyinmanar Township, Mandalay Region, Myanmar. The plant was screened and identified by authorized botanist from Botany Department, Mandalay University, Myanmar. The collected sample was washed properly with water to remove any contaminants.

2.3 Preliminary Phytochemical Screening

The preliminary phytochemical screening of the stem barks of *Diospyros ehretioides* were carried out to know the different kinds of phytochemical constituents at Department of Chemistry, University of Mandalay, Myanmar to detect the different kinds of chemical constituents in the sample. [12- 15]

2.4 Extraction and Isolation of Organic Compound

The air dried stem barks of *Diospyros ehretioides* L. (500 g) was percolated with ethanol (2 L) for two months. The solution was filtered and evaporated to concentrate at room temperature. The filtrate was extracted with petroleum ether and ethyl acetate solvents. The pet-ether crude extract (1.41 g) and ethyl acetate crude (1.87) g were obtained. The pet- ether portion (1.41 g) was fractionated with column chromatography on silica gel as adsorbent and stepwise eluted with a gradient solvent system of increasing polarity (n-hexane only, n-hexane: ethyl acetate, then ethyl acetate only) to give pure compound (15 mg). The structure of the isolated compound was identified by NMR spectroscopic methods such as ^{1}H-NMR, and ^{13}C-NMR, DEPT, COSY, HSQC and HMBC respectively.

3 RESULTS AND DISCUSSION

3.1 Results of Preliminary Phytochemical Tests

According to the phytochemical tests of the crude extracts from the stem barks of *Diospyros ehretioides*, the sample contains many phytochemical constituents such as flavonoids, steroids, glycosides, phenols, polyphenols, tannins, reducing sugars, saponins and terpenes respectively.

3.2 Structural Characterization of Isolated Compound

Different spectroscopic techniques were applied for the structure assignment of the isolated compound including ^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC. The ^{1}H NMR spectrum showed angular methyl protons at 0.76 (s), 0.79 (s), 0.83(s), 0.94 (s), 0.96 (s), 1.03 (s) and 1.67(s) correspond to C-24, C-28, C-25, C-27, C-23, C- 26, and C-30 indicating seven methyl groups in the compound. The proton NMR showed the proton of H-3 appeared as a doublet of doublet at δ 3.2 ppm. It also showed two olefinic protons at 4.57 and 4.69 representing the exocyclic double bond.

The ^{13}C-NMR spectrum of the compound indicated 30 carbon signals [seven methyl, eleven methylene, six methine and six quaternary carbons] for the terpenoid of lupane skeleton which includes a carbon bonded to the OH group at C-3 position appeared at δ 78.996 ppm. The olefinic carbons of the exocyclic double bond appeared at 150.966 ppm (quaternary C) and 109.312 ppm (methylene C) which are assigned as C-20 and C-29 double bond of the lupane type triterpenoid compound. Complete assignment of all protons and carbons was confirmed by ^{1}H-^{1}H COSY and long range signals in HMBC spectra. Thus, the isolated compound from the stem bark of *Diospyros ehretioides* was assigned as lupeol that was consistent to the reported literature values.

The ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H Correlation (COSY) and ^{1}H-^{13}C long range correlation (HMBC) were described in table (1). All 1D and 2D NMR spectra are shown in the figures (2-6).

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Table (1) ^{1}H and ^{13}C NMR Spectral Data of the compound and that of Lupeol from Literature (at 600 and 150 MHz, resp. in $CDCl_3$; 298 K; δ in ppm, J in Hz)

No.	C/H	δ_H ($CDCl_3$)	δ_C ($CDCl_3$)	^{1}H-^{1}H COSY correlation	J correlation (HMBC)	δ_C ($CDCl_3$)[16]
1	CH_2	0.90, 1.65 (o)	38.853	H-2a,2b	H-2a, 3, 5, 9, 25	38.85
2	CH_2	1.52, 1.67(o)	27.439	H-1a,1b,3	H-3, 23	27.40
3	CH	3.2(dd)	78.996	H-2b	H-2a,2b, 5, 23,24	79.01
4	C		38.701		H-3,5,6a,6b,23,24	38.69
5	CH	0.67 (o)	55.291	H-6a,6b	H-1a,1b,6b,7,25	55.28
6	CH_2	1.37, 1.52 (o)	18.311	H-5, 7	H-5, 7	17.99
7	CH_2	1.39 (o)	34.274	H-6a,6b	H-5,6a,6b,9,26	34.26
8	C		40.825		H-6a,6b,7,9,13,26, 27	40.82
9	CH	1.25 (o)	50.431	H-11a,11b	H-1a, 5, 11a, 11b, 25, 26	50.42
10	C		37.163		H-1a,1b,2a,2b,6a,6b,5,9,25	37.16
11	CH_2	1.20, 1.40 (o)	20.922	H-9, 12a, 12b	H-9,12b,13	20.92
12	CH_2	1.06, 1.62 (o)	25.134	H-11a,11b, 13	H-11a, 11b,13	25.12
13	CH	1.66 (o)	38.046	H-12a, 12b, 18	H-11b,12a,19,27	38.04
14	C		42.823		H-12a,13, 15a,16a,18,26,27	42.82
15	CH_2	1.05,1.60 (o)	27.411	H-16a, 16b	H-13, 16a, 16b	27.40
16	CH_2	1.35, 1.45 (o)	35.576	H-15a	H-18, 22a, 22b, 28	35.57
17	C		42.993		H-16a, 16b, 18, 21a, 21b, 22a, 22b, 28	47.98
18	CH	1.36, 1.37 (o)	48.296	H-13,19	H-19, 21b	48.29
19	CH	2.40 (m), 1.45 (o)	47.978	H-21a, 21b	H-21b, 29a,29b	47.98
20	C		150.966		H-19,30	150.99
21	CH_2	1.3(o), 1.91(m)	29.841	H-19,22a,22b	H-19, 22a,	29.83
22	CH_2	1.18, 1.37 (o)	39.995	H-21a,21b	H-18, 21b, 28	39.99
23	CH_3	0.90 (s)	27.981		H-3	27.98
24	CH_3	0.76 (s)	15.364		H-3, 5, 23	15.36
25	CH_3	0.83 (s)	16.109		H-1a, 1b, 9, 11b	16.11
26	CH_3	1.03 (s)	15.968		H-9, 13	15.96
27	CH_3	0.94 (s)	14.602		H-13, 15a	14.54
28	CH_3	0.79 (s)	17.994		H-16b, 22a, 22b	18.31
29	CH_2	4.57, 4.69 (d, J= 1.9 Hz)	109.312	H-30	H-19,30	109.31
30	CH_3	1.67 (s)	19.298	H-29a,29b	H-19,29a,29b	19.29

(s = singlet, d = doublet, o= overlap, m = multiplet)

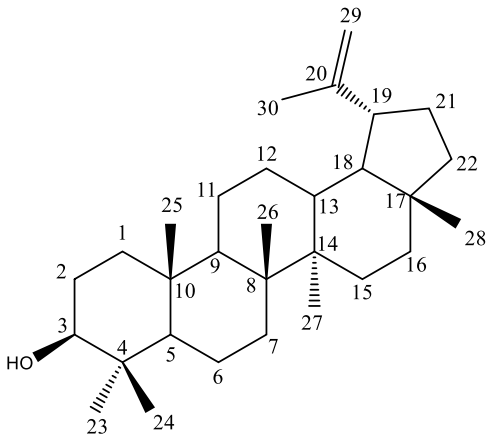

Fig.1 Complete structure of lupeol compound

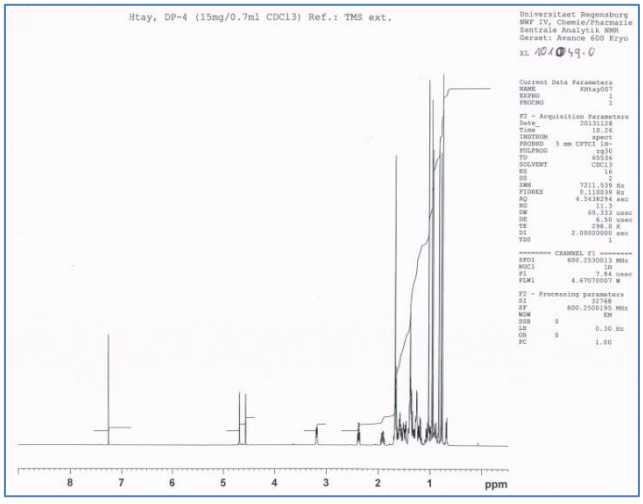

Fig.2 ^{1}H NMR spectrum of lupeol compound

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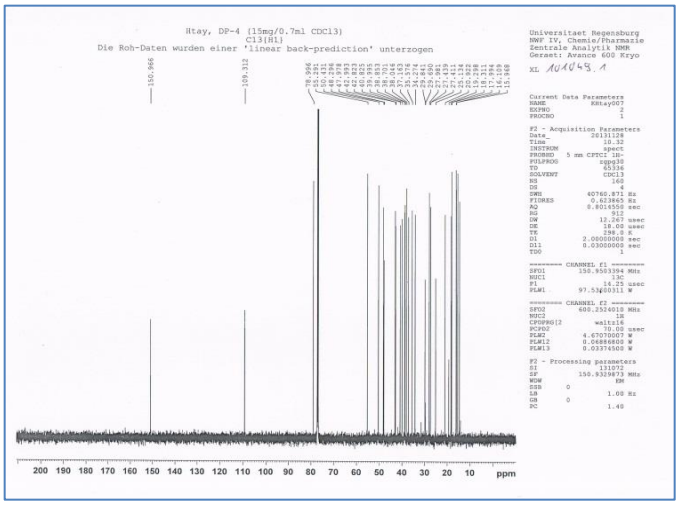

Fig.3 ^{13}C NMR spectrum of lupeol compound

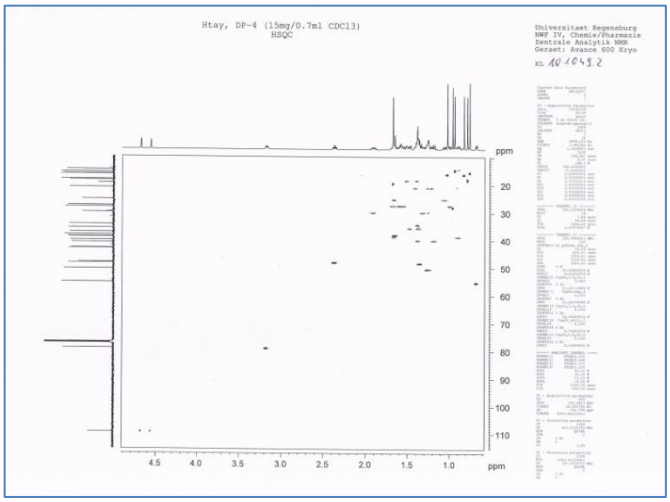

Fig.4 HSQC spectrum of lupeol compound

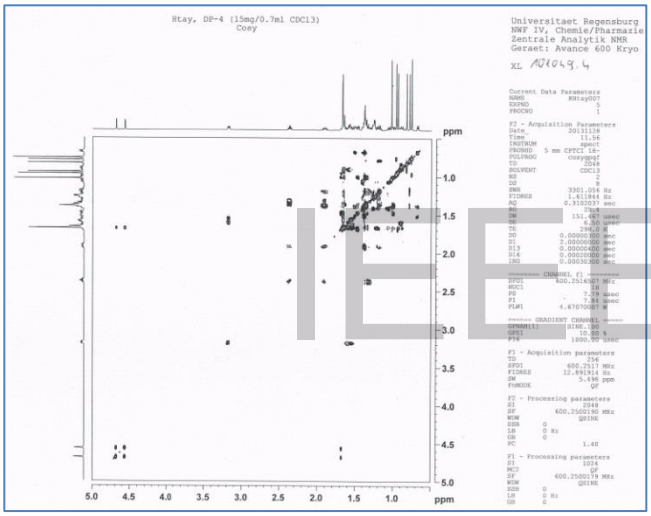

Fig.5 COSY spectrum of lupeol compound

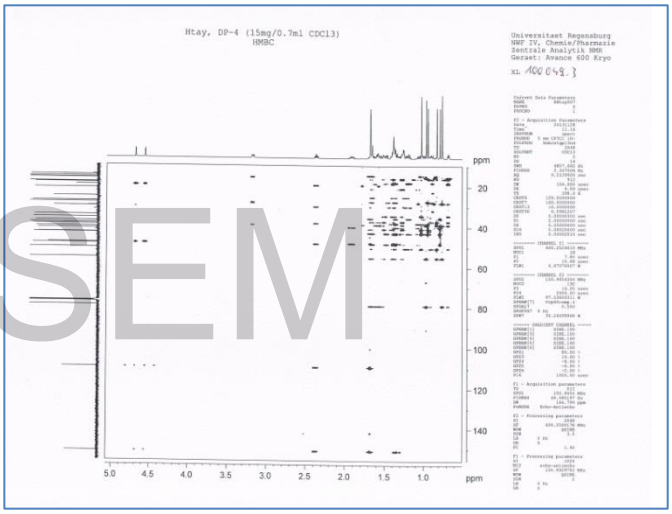

Fig.6 HMBC spectrum of lupeol compound

4 CONCLUSION

The sample contains alkaloid, flavonoid, terpene, steroid, glycoside, polyphenol, sugar, saponin and tannin respectively from the preliminary phytochemical tests of the sample. The isolated compound from the pet ether extract of the sample was obtained as white powder using column and thin layer chromatographic methods. The ^{1}H NMR spectrum showed seven singlet methyl protons at δ_H 0.76, 0.79, 0.83, 0.94, 0.96, 1.03 and 1.67 corresponding to their respective carbons at δ_C 15.364, 17.994, 16.109, 14.602, 27.981, 15.968 and 19.298 ppm. The existence of the triterpene compound could be confirmed by 30 signals of ^{13}C NMR spectrum. The two alkene carbon signals at 150. 966 (quaternary C) and 109.312 (methylene C) indicated the presence of exocyclic double bond providing the structure of lupeol compound. The structure of the compound could be accomplished by the interpretation of ^{1}H- ^{1}H correlations in COSY and ^{1}H- ^{13}C long range correlations in HMBC spectra. Finally, the isolated compound from *Diospyros ehretioides* could be assigned as lupeol compound that was consistent to the reported literature values.

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