



Title	Structure Elucidation of pure Glycosidic Diterpene compound Isolated from the Root of <i>Launaea secunda</i> Hook.(Dauk-khwa)
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STRUCTURE ELUCIDATION OF PURE GLYCOSIDIC DITERPENE COMPOUND ISOLATED FROM THE ROOT OF

Launaea secunda Hook.
(Dauk-khwa)

Dr Myint Myint Khaing

ABSTRACT

In this PhD research work, one Myanmar indigenous medicinal plant, namely *Launaea secunda* Hook. (Dauk-khwa) was selected for chemical analysis. Firstly, preliminary phytochemical screening of selected plant was performed. According to the phytochemical test, the root of Dauk-khwa contains alkaloid, steroid, terpene, sugar, glycoside, phenolic and polyphenol respectively. Moreover, antimicrobial activities of the crude extract of root of this plant in various solvent systems were tested by agar well diffusion method on six organisms, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumalis*, *Pseudomonas aeruginosa*, *Candida albican* and *Mycobacterium* species. The ethyl acetate extract of the root of Dauk-khwa responds high antimicrobial activities on all selected organisms. A bioactive organic compound (MMK-1) was isolated from the root of *Launaea secunda* Hook. (Dauk-khwa) by using Thin Layer and Column Chromatographic methods. Pure yellowish brown oily form compound (22.0 mg) was obtained and the yield percent was found to be (1.162%) based upon the ethylacetate crude extract. This pure compound gives positive for terpene and glycoside tests. The antimicrobial activities of isolated pure compound were rechecked by agar well diffusion method on six selected organisms and this pure compound gave medium activities on five tested organisms except *Bacillus subtilis*. In addition, the molecular formula ($C_{26}H_{44}O_6$) and the complete planar structure of compound (MMK-1) were elucidated by spectroscopic methods such as FT-IR, 1H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, HMQC, HMBC, DQF-COSY and FAB mass spectral data. Finally, conformational structure and absolute configuration containing (10) chiral carbons of compound (MMK-1) was determined by using 1H NMR, splitting patterns, coupling constant (J-values), NOESY spectral data and model studies. The IUPAC name of elucidated pure compound is (2R, 3S, 4S, 5S, 6S)-2-((2S, 4aS, 4bS, 7S, 10aR)-7-ethyl-1, 1, 4a, 4b-tetramethyl-1, 2, 3, 4, 4a, 4b, 5, 6, 7, 9, 10, 10a-dodecahydrophenanthren-2-yloxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol.

INTRODUCTION

In Myanmar, traditional Myanmar medicinal plants have been used for many years. Even after the introduction and wide spread of modern western medicines, traditional medicinal plants are still widely used for prevention and cure. However, there is a few limited scientific knowledge on Myanmar medicines. Hence, it is necessary to look into Myanmar medicinal plants in scientific ways so that people can get access to safe and reliable on Myanmar medicines. Traditional medication involves the uses of herbal medicines, animals, plants and minerals. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products, that contain as active ingredients present in parts of plants, or other plant materials or combinations. Medicinal plants are important sources for pharmaceutical manufacturing. Medicinal plants and herbal medicines account for a significant percentage of the pharmaceutical market. Research in natural products aimed for drug discovery may serve as leads for the development of new pharmaceuticals that address unmet therapeutic needs.

Traditional medicine is a truly inherited profession whose development has interrelations with the natural and climate conditions, thoughts and convictions and the social system of Myanmar. Traditional medicine in Myanmar is widely practiced by the majority of population, partly as an alternate to modern medicine.

Medicinal plants have played a significant role in many ancient traditional systems of medication. These plants may save many lives if they are used correctly. The use of plants based products for disease prevention and treatment has become increasingly popular in ASEAN countries.

Myanmar traditional medicine is a broad, deep and delicate branch of science covering various basic medicinal knowledge, different treaties, a diverse array of therapies and potent medicine. Local people in Yenangyaung used this species for dysentery and diabetic. Dauk-khwa is found in Meiktila District and Yenangyaung Township, Magway Division. The root of Dauk-khwa was used for the treatment of dysentery and diabetic.

In this research work, *Launaea secunda* Hook. (Dauk-khwa) was selected for chemical analysis. A pure bio-active organic compound (MMK-1) was isolated from the root of Dauk-khwa by using Thin Layer and Column Chromatographic methods.

The conformational structure of pure organic compound (MMK-1) could be determined by modern spectroscopic methods such as 1H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, DQF-COSY, HMQC, HMBC, NOESY and FAB-Mass spectroscopy, respectively.

Botanical Description of Selected Plant



Family : Asteraceae

Botanical name : *Launaea secunda* Hook.

Myanmar name : Dauk-khwa

Medicinal Uses : Dysentery, leucoderma and diabetic

Aim and Objectives of the Present Work

The aim and objectives of the present research work are as follows.

- (1) To investigate the antimicrobial activity of a bioactive organic compound isolated from medicinal plants.
- (2) To isolate and elucidate the structure of the bio active compound using the application of chromatographic and spectroscopic methods.
- (3) To discover new and more effective biologically active compound and to contribute the quality of pharmaceutical preparations from plants.

Materials and Methods

Commercial grade reagents and solvents were used with further purification. Analytical preparative thin layer chromatography was performed by using precoated silica gel (Merk. Co. Inc, Kieselgel 60 F₂₅₄) and silica gel 70 to 230 mesh ASTM was used for column chromatography.

Common laboratory tools were used in the isolation and purification of compound (MMK-1). The advanced instruments which are used in the characterization of samples and elucidation of pure compound are shown below.

1. UV lamp (Lambda-40, Perkin-Elmes Co.England)
2. FT-IR spectrometer (Shimadzu, Japan)
3. ¹H NMR spectrometer (500 MHz)
4. ¹³C NMR spectrometer (125 MHz)
5. EI- Mass spectrometer
6. UV spectrometer (PD-303 UV)

Sample Collection

One Myanmar indigenous medicinal plant, Dauk-khwa was collected from Yenangyaung Township, Magwe Region. The collected sample were cut into small pieces and were dried in the shade. Then the raw materials were stored in the well-stopped glass bottle and used throughout the experiment.

Phytochemical Constituents

In order to know the type of chemical constituents consisting in selected plant, phytochemical tests were carried out. Dauk-khwa (root), gave rise to the positive for alkaloid, glycoside, phenolic, polyphenol, steroid terpene and reducing sugar respectively.

Antimicrobial Activities of Myanmar Traditional Indigenous Medicinal Plant (Dauk-khwa)

The antimicrobial activities of the selected Myanmar indigenous medicinal plant (Dauk-khwa) were tested in various solvent systems by using agar well diffusion method. This plant extract was sent to Development Centre for Pharmaceutical Technology (DCPT), Insein, Yangon. The applying organisms are *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Mycobacterium* species. The ethylacetate extract of the root of Dauk-khwa responds high activities on all tested organisms. Hence, it was selected for chemical analysis.

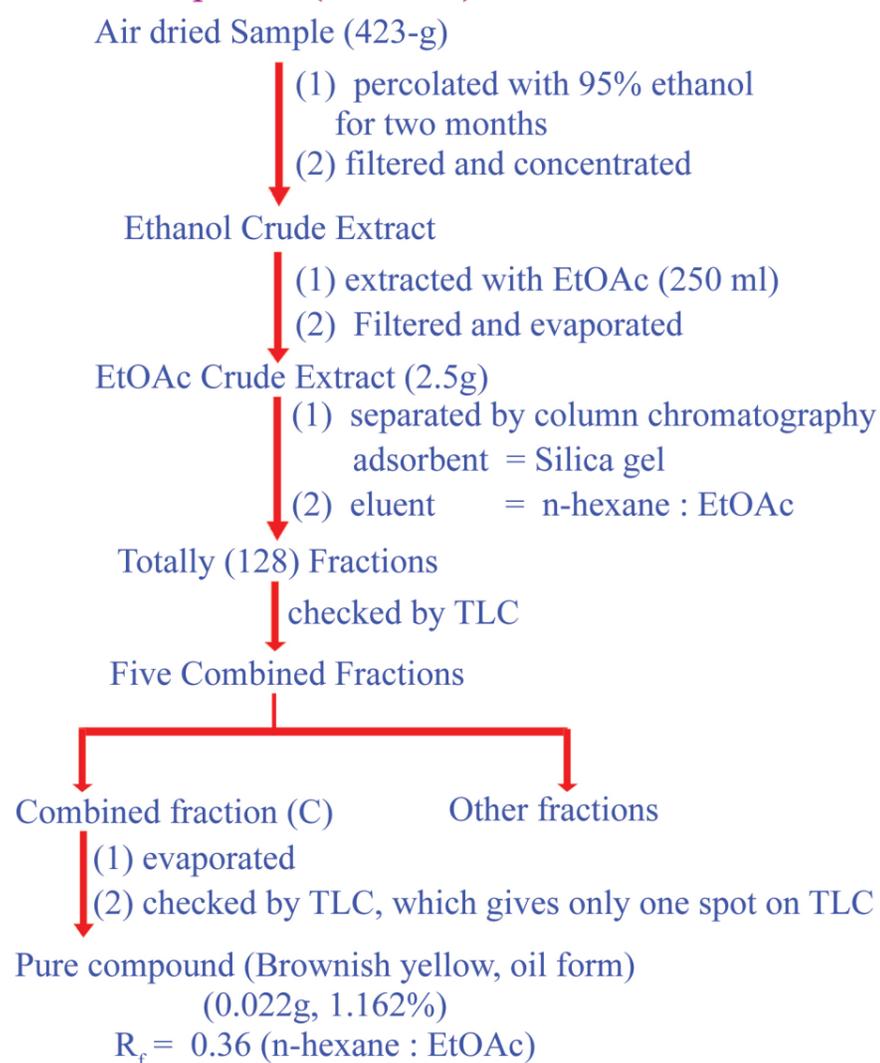
Extraction and Isolation of Pure Compound (MMK-1) from the Root of *Launaea secunda* Hook. (f)

The air dried sample (423 g) was percolated with ethanol (3000 ml) for two months. The ethanol extract was filtered and evaporated at room temperature. The residue was dissolved in 250 ml of EtOAc. When EtOAc extract was filtered and concentrated, the crude sample (2.5 g) was obtained.

Then it was chromatographed on a silica gel column, using n-hexane and ethyl acetate as eluent with various ratios from nonpolar to polar. Totally (150) fractions were obtained. Each and every fraction were checked by TLC and the same R_f values were combined. Five combined fractions were collected.

The major combined fraction (C) gives only one spot on TLC. Pure yellowish brown, oily form compound (2.2 mg) was obtained. Total yield percent of pure compound (MMK-1) was found to be 1.162% based upon the crude EtOAc extract.

Flow sheet for Extraction and Isolation of Pure Compound (MMK-1) from Dauk-khwa



The Antimicrobial Activities of Pure Compound (MMK-1)

The antimicrobial activities of pure compound (MMK-1) were tested by using agar well diffusion method on six selected organisms.

According to above results, this pure compound responds medium activities on five tested organisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican*, *E-coli* species and no activity on remaining organism.

Test for Terpene and Glycoside

Terpene Test

The sample was added by two or three drops of acetic anhydride and pet-ether. Then added by two drops of concentrated H₂SO₄, the solution become red colour.

Glycoside Test

The water extract sample solution was heated for 10 minutes and 10 percent acetate was added. The white precipitate was obtained.

Spectroscopic Studies of the Pure Organic Compound (MMK-1)

Pure organic compound (MMK-1) was done to analyze by FT-IR spectrometer (Hyper-IR, SHIMADZU) at the Department of Chemistry, University of Mandalay, Mass Spectrometer, ¹H Nuclear Magnetic Resonance (¹H NMR, 500 MHz), ¹³C Nuclear Magnetic Resonance (¹³C NMR, 125 MHz), Distortionless Enhancement by Polarization Transfer (DEPT), Double-Quantum Filtered Correlation Spectroscopy (DQF-COSY), Heteronuclear Multiple Quantum Coherence (HMQC), and Heteronuclear Multiple Bond Coherence (HMBC), and Nuclear Overhauser Effect Spectroscopy (NOESY), spectral data were measured at the Department of Natural Resource Chemistry, Faculty of Pharmacy, Meijo University, Japan.

Molecular Formula Determination of Pure Compound (MMK-1)

Table (1) The Results Given by DEPT Spectrum and FT-IR Spectrum

Assignment	No: of carbon	No: of proton	No: of oxygen
Five sp ³ methyl carbon	5	15	-
Seven sp ³ methylene carbon	7	14	-
One sp ³ carbinol methylene carbon (δ 62.15 ppm)	1	3	1
Five sp ³ methine carbon	5	5	-
Three sp ³ carbinol methine carbon (δ 70.14, 76.34; 74.19 ppm)	3	6	3
Three sp ³ quaternary carbon	3	-	-
One sp ² methine carbon	1	1	-
One sp ² quaternary carbon	1	-	-
One ether oxygen (from FT-IR)	-	-	1
Partial Molecular Formula	C ₂₆	H ₄₄	O ₅

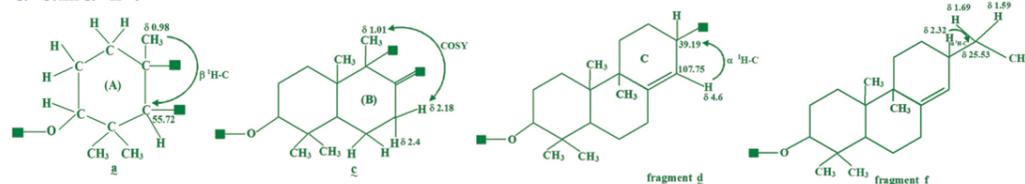
∴ The partial molecular formula = C₂₆H₄₄O₅
 ∴ The remaining partial molecular mass = 452 - 436 = 16

It must be one ether oxygen atom.

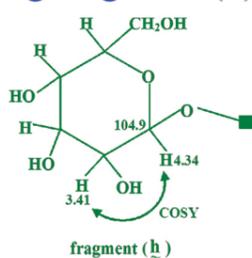
∴ Real molecular formula = C₂₆H₄₄O₆

Structure Elucidation of Pure Organic Compound (MMK-1)

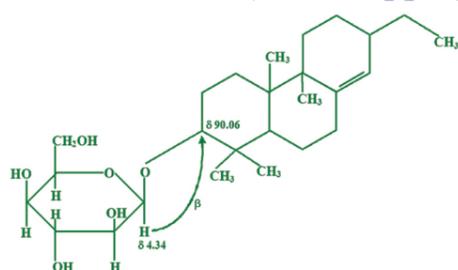
In the structural assignment of this isolated compound, DQF-COSY and HMQC spectra display the following fragments a, c, d and f.



Furthermore, in DQF-COSY spectrum, the existence of medium graphic area of carbinol methine proton (δ 3.41 ppm) with acetal methine proton (δ 4.34 ppm) gives rise to the following glycoside ring fragment (h).



Finally, the complete planar structure of glycosidic diterpene compound (MMK-1) could be established by using the above HMBC spectrum in which acetal methine proton (δ 4.34 ppm) in glycoside fragment (h) responds δ ¹H-C long range coupling with ether bearing methine carbon (δ 90.06 ppm) in fragment (f).



SUMMARY

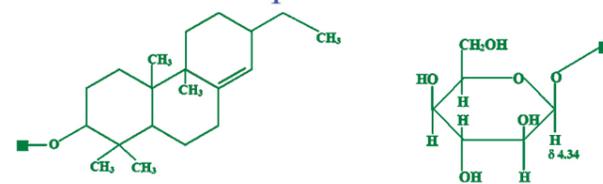
In this PhD thesis, one Myanmar indigenous medicinal plant *Launaea secunda* Hook., Myanmar name, Dauk-khwa (root) was collected from Meiktila Township, Mandalay Division and Yenangyaung Township, Magway Division.

Phytochemical screening and antimicrobial activities tests were done. In the result of phytochemical tests, the root of Dauk-khwa contains alkaloid, steroid, terpene, sugar, glycoside, phenolic and polyphenol compounds. Moreover, the antimicrobial activities of various solvent extracts of selected sample were tested by agar well diffusion method on six tested organisms. The ethyl acetate extract of Dauk-khwa (root) responds high antimicrobial activities on all tested organism such as, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albican* species.

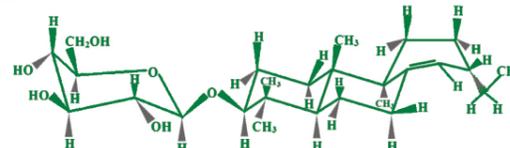
Hence, it was selected for chemical analysis. A pure compound (MMK-1) was isolated from the root of Dauk-khwa by applying Thin Layer and Column Chromatographic methods. The yellowish brown, oily form compound (0.022 g, 1.162 % yield) were collected from the column separation. This pure compound gave positive for terpene and glycoside tests. The antimicrobial activities of this pure compound has medium on five tested organisms except *Bacillus subtilis*.

The molecular formula (C₂₆H₄₄O₆) of isolated compound was determined by advanced spectroscopic methods such as FT-IR, ¹H NMR (500 MHz), ¹³C NMR (125 MHz), DEPT, HMQC, DQF-COSY and FAB mass spectral data, respectively. Moreover, the complete planar structure of this isolated compound was assigned by DQF-COSY, HMQC and HMBC spectroscopic studies.

Furthermore, the following diterpenoid fragment and glycosidic fragment were also elucidated by DQF-COSY and HMBC spectral data as shown below.



In addition, the conformational structure of glycosidic diterpene compound could be assigned by the splitting patterns, coupling constant (J-values) of some prominent protons NOESY spectrum and model studies.



Finally, the absolute configuration of five chiral carbons in diterpene fragment could be assigned as C₂(S), C_{4a}(S), C_{4b}(S), C₇(S), C_{10a}(R) and the absolute configuration of another five chiral carbons in glycosidic fragment could be determined as C₂(R), C₃(S), C₄(S), C₅(S) and C₆(S) respectively.

The IUPAC name of this isolated compound (MMK-1) is (2R, 3S, 4S, 5S, 6S)-2-((2S, 4aS, 4bS, 7S, 10aR)-7-ethyl-1, 1, 4a, 4b-tetramethyl-1, 2, 3, 4, 4a, 4b, 5, 6, 7, 9, 10, 10a-dodecahydrophenanthren-2-yloxy)-6- (hydroxymethyl) tetrahydro-2H-pyran-3, 4, 5-triol.

Since there is no research report that indicates the presence of glycosidic diterpene compound as a constituent in the root of Dauk-khwa, the finding of glycosidic diterpene compound in this plant is the pioneer result of this research.

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