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Identification and isolation of methyl gallate as a polar chemical marker for *Labisia pumila* Benth.

(Pengenalpastian dan pemencilan metil galat sebagai kimia penanda berkutub untuk *Labisia pumila* Benth.)

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Abstract

This paper describes for the first time the isolation and purification of a polar chemical marker known as methyl gallate from *Labisia pumila* leaves. This compound was isolated and purified through an open column chromatography. Analytical thin layer chromatography (TLC) was used to monitor the separation of the compound. The pure compound obtained was further analyzed using gas chromatography mass spectrometry direct injection probe (GCMS DI-probe) while identification of the structure was performed using nuclear magnetic resonance (NMR) spectroscope by conducting various analysis such as ^{1}H, ^{13}C and Heteronuclear Multiple Bond Correlation (HMBC).

Introduction

Labisia pumila is a popular herb in Malaysia belonging to the family Myrsinaceae. It is locally known as 'kacip fatimah' and mainly used for the production of herbal products in Malaysia. The increase in knowledge on herbal nutraceuticals and health benefits has driven the growth and demand of the herbal products. Based on the traditional use, *L. pumila* is consumed by Malay women to induce and facilitate childbirth as well as a post-partum medicine (Burkill 1935). It is also being used by the indigenous people of the Malay archipelago for menstrual irregularities.

The plants are usually boiled and the water soluble extract is taken as a drink. Husniza et al. (2002) showed that water extracts of *L. pumila* were able to displace estradiol binding antibodies, making it similar to other estrogens such as estrone and estradiol. Ayida et al. (2007) reported a possible role for *L. pumila* var alata in modulating postmenopausal adiposity through the initiation of the lipolysis process in adipose tissue. In Malaysia, three varieties of *L. pumila* were identified, viz. alata, pumila and lanceolata (Stone 1988).

Most herbal products which contain *L. pumila* do not have the information on the active ingredient presence in the product. Therefore, identification and quantification of the chemical presence in the herbs is essential for standardization

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and verification purposes. Based on European Medicines Agency (EMEA), standardization of herbal product is defined as herbal preparation containing a substance or group of substances with known therapeutic activity which can be used to standardise a biological effect or used as marker compounds (Zhari and Jamshed 2010). Therefore, identification of an active chemical marker from *L. pumila* extract is essential for standardization of *L. pumila* based products.

The objective of this study was to isolate a potential chemical marker for *L. pumila* which can be used for standardization and verification of the herbs. In this study, methyl gallate was isolated from *L. pumila* leaves and its spectroscopy data were analysed to prove the identity of the compound. In a previous study, methyl gallate was demonstrated to have high antioxidant activity and cytotoxic towards cancer cell lines (Yong et al. 2005). Various types of bioactivities were also reported such as antimicrobial, sucrase inhibitor, collagenase inhibitor and used as treatment for enteritis (Chaubal et al. 2005).

Materials and methods

Sample preparation

Three kg of fresh *L. pumila* leaves were collected from MARDI Kluang station and ground to 2.0 mm particle size.

Preparation of methanol extract

The fresh *L. pumila* leaves were ground to 2.0 mm particle size and soaked in 100% methanol (MeOH, which is close to aqueous extract) for 4 days at room temperature after which the extract was decanted (Mohd Nazrul Hisham et al. 2010). The plant material was replenished with fresh MeOH and the same extraction procedure repeated twice. The extracts collected from each soaking were pooled and concentrated to dryness using a rotary evaporator to obtain the crude extract.

Isolation of methyl gallate

The crude methanol extract of *L. pumila* was then resuspended in distilled water and solvent partitioned with hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and butanol (BuOH) (Yong et al. 2005). Ethyl acetate fraction (10 g) was first subjected to normal phase column chromatography (CC). Elution started with 100% chloroform ($CHCl_3$) followed by mixtures of $CHCl_3$:MeOH of increasing polarity to yield a total of ten fractions.

Based on similar TLC patterns, fractions 3 to 5 (4 g) were recombined and subjected to normal phase open column chromatography using 100% chloroform ($CHCl_3$) as mobile phase, followed by mixtures of $CHCl_3$:MeOH of increasing polarity. Ten fractions were collected, and based on similar TLC patterns, fractions 7 to 10 (2.5g) were recombined and subjected to open mini column normal phase chromatography using 100% chloroform ($CHCl_3$) as mobile phase, followed by mixtures of $CHCl_3$:MeOH of increasing polarity to obtain 20 mg of white crystals.

Mass analysis

Mass spectra were recorded on a Shimadzu GC-17A gas chromatography mass spectrometer (GCMS). The GCMS was equipped with a direct injection probe (DI-probe) to analyse pure samples without passing through the capillary column. The pure isolated compound (in white crystal form) was subjected to mass spectroscopy analysis and the mass spectrum elucidated to support the NMR analysis for determination of the chemical structure.

Nuclear Magnetic Resonance (NMR) analysis

The pure white crystals were placed on top of the probe and subjected to ^{1}H, ^{13}C NMR and two dimensional analyses such as Heteronuclear Multiple Bond Correlation (HMBC). The different spectrum of the isolated compound were recorded on a Varian Unity Inova (500 MHz) equipped

with pulsed field gradients (PFG), using an indirect detection probe. Deuterated methanol was used and chemical shifts (δ and δC) were given in parts per million (ppm).

Results and discussion

Characterization of compound isolated as methyl gallate

The compound isolated from repeated normal phase column chromatography as white crystals were subjected to mass analysis using the GCMS DI-probe and NMR spectrometric analysis for confirmation of the chemical structure.

The ^{1}H NMR spectrum of the isolated compound (*Figure 1*) demonstrated a typical signal for aromatic protons at δ 7.0. Based on the integration of the signals, there are two aromatic protons (H-2 and H-6) which are symmetrical to each other. Therefore, both protons are giving the same signal at δ_H 7.0. In the higher field region, there is another signal from aliphatic methoxy protons at δ_H 3.8 and based on the integration of the signal, the presence of these protons are in agreement with the number of protons in the methoxy substituent.

The ^{13}C NMR spectrum of the isolated compound (*Figure 2*) exhibited resonances for seven types of carbons comprising a C=O signal at δc 167.9 (indicating the presence of carbonyl ester), one oxygenated aromatic carbon signal at δc 138.8 (C-4), two symmetrical oxygenated aromatic

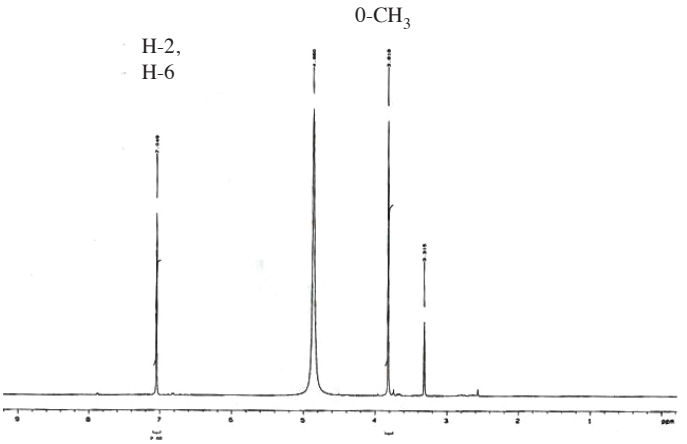

Figure 1. ^{1}H NMR spectrum of methyl gallate

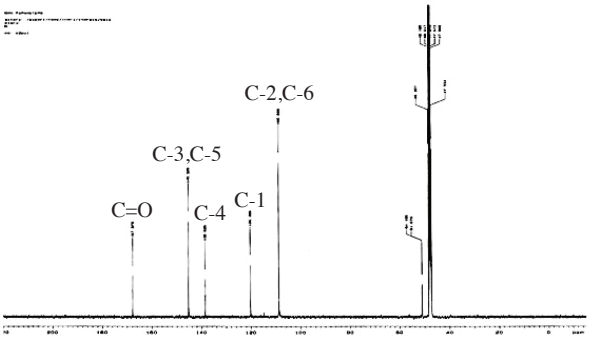

Figure 2. ^{13}C NMR spectrum of methyl gallate

carbon signals at δc 145.3 (C-3 and C-5), one quaternary aromatic carbon signal at δc 120.3(C-1) and two symmetrical aromatic methine carbon signals at δc 108.9 (C-2 and C-6). These signals are similar to all carbon signals from methyl gallate.

Analysis of the HMBC spectra (*Figure 3a-c*) showed the correlation between aromatic proton signal and the carbonyl ester. The spectrum in *Figure 3a* indicates the ^{2}J correlation of methine aromatic protons signal at δ_H 7.0 with two symmetrical oxygenated aromatic carbon signals at δc 145.3 (C-3 and C-5) and also with one quaternary aromatic carbon signal at δc 120.3(C-1). Based on HMBC spectra in *Figures 3a* and *3c*, ^{3}J correlations were observed between methine aromatic protons signal at δ_H 7.0 with the carbonyl ester carbon signal at δc 167.9 and an oxygenated aromatic carbon signal at δc 138.8 (C-4).

The HMBC spectra also demonstrated that the ^{3}J correlations between H-2 proton signal at δ_H 7.0 with aromatic methine carbon signal at δc 108.9 (C-6) and H-6 proton signal correlated to aromatic methine carbon signal at δc 108.9 (C-2). Similar correlations were also observed in gallic acid structure elucidation as reported by Tatsuya et al. (2008). Further analysis of HMBC spectrum in *Figure 3b*, ^{3}J correlation was observed between the methoxy proton signals at δ_H 3.8 with carbonyl ester carbon signal at δc 167.9.

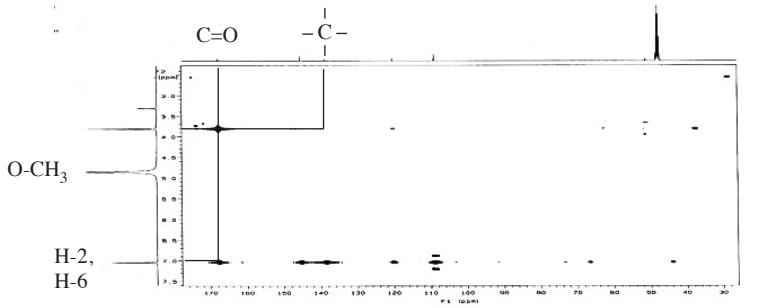

Figure 3a. HMBC correlation of symmetrical aromatic protons (H-2 and H-6) with carbonyl ester and methoxy with aromatic quaternary carbon

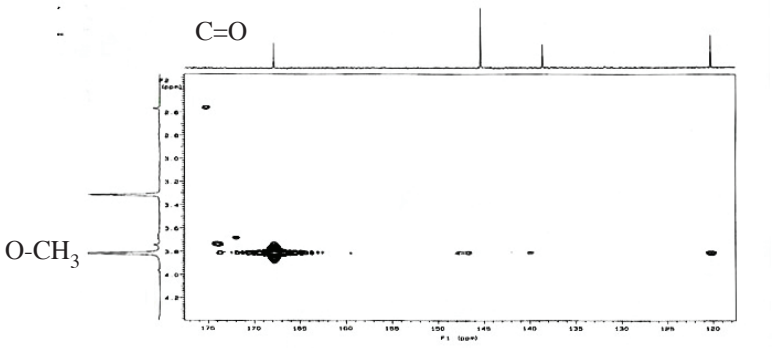

Figure 3b. HMBC correlation of carbonyl with methoxy

The mass spectrum of the electron impact-mass spectroscopy (EI-MS) (*Figure 4*) shows the molecular ion peak at m/z 184 which agrees with the molecular weight of methyl gallate. The base peak at m/z 153 showed the most abundant of ion species which resulted from the loss of the methoxy group from the molecular ion. The presence of signal at m/z 125 is due to the loss of the carbonyl group from the base peak ion. Further fragmentation formed a signal at m/z 107. Based on the NMR data and mass interpretation above, the compound isolated from *L. pumila* is ambiguously identified as methyl gallate (*Figure 5*).

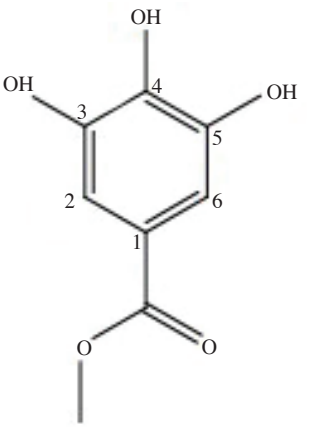

Figure 5. Structure of methyl gallate

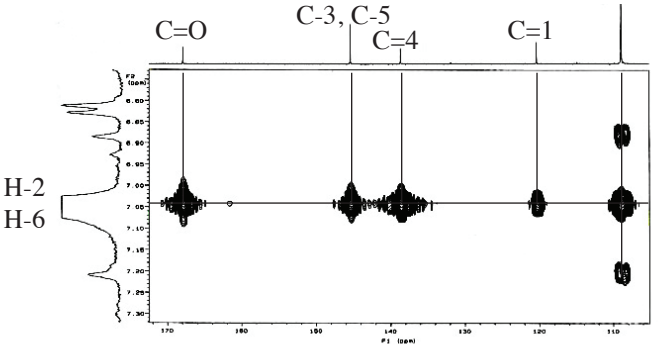

Figure 3c. HMBC correlation of symmetrical aromatic protons (H-2 and H-6) with carbonyl ester and oxygenated aromatic carbon with symmetrical aromatic proton

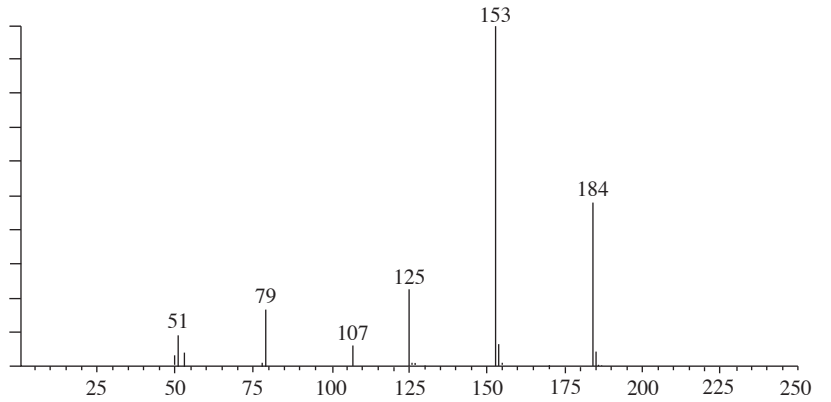

Figure 4. Mass spectrum of methyl gallate isolated from ***Labisia pumila***

Conclusion

Methyl gallate was successfully isolated and identified from *Labisia pumila*. From this discovery, methyl gallate could be a good indicator as the standard compound to standardize *L. pumila* extract. It may be used as a potential biomarker since it has been reported to have various bioactivities such as high antioxidant activity and cytotoxic against tumour cell lines. Using methyl gallate as a chemical marker, analysis of *L. pumila* products can be achieved through normal analytical technique with the High Performance Liquid Chromatography (HPLC).

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References

Ayida, A.W., Wan Nazaimoon, W.M., Farihah, H.S. and Azian, A.L. (2007). Effect of ovariectomy, *Labisia pumila* var alata treatment and estrogen replacement therapy on the morphology of adipose tissue in ovariectomized Sprague Dawley rats. *Journal of Medicine and Biological Sciences* 1: 1–7

Burkill, I.H. (1935). *A dictionary of the economic products of the Malay Peninsular*. London: Crown Agent

Chaubal, R., Deshpande,V.H. and Deshpande, N.R. (2005). Methyl gallate, the medicinally important compound: A review. *EJEAF*Che 4(4): 956–962

Husniza, H., Wan Nazaimoon, W.M., Khozirah, S., Jamia, A.J., Balaspramaniam, A., Paridah, J. and Khalid, B.A.K. (2002). Estrogenic and androgenic activities of *kacip fatimah* (*Labisia pumila*). Abstracts of Research Projects, Institute for Medical Research, Ministry of Health Malaysia, Kuala Lumpur

Mohd Nazrul Hisham, D., Mohd Lip, J., Ariff Zaidi, J. and Normah, A. (2010). Main non-polar chemical constitutent from *Morinda citrifolia* fruits. *J. Trop. Agric. and Fd. Sci*. 38(1): 97–101

Stone, B.C. (1988). Notes on the genus *Labisia* Lindl. (Myrsinaceace). *Malayan Nature Journal* 42(1): 43–51

Tatsuya, H., Fumihide, T., Takanobu, T., Masato, N. and Tomihisa, O. (2008). Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of *Eucalyptus globules*. *Phytochemistry* 69: 747–753

Yong, H.C., Sung, S.H., Hyun, O.L. and Seung, H.B. (2005). Biological activity of bioactive components from *Acer ginnala* Max. *Bulletin Korean Chemistry Society* 26(9): 450–452

Zhari, I. and Jamshed, S. (2010). Standardization of herbal product. Paper presented at International Conference and Talkshow on Medicinal Plant, 39th Meeting of National Working Group on Indonesian Medicinal Plant, 19–20 Oct. 2010, Jakarta

Abstrak

Kertas kajian ini menerangkan buat pertama kali pemencilan dan penulenan sebatian kimia penanda berkutub yang dikenali sebagai metil galat daripada daun kacip fatimah (*Labisia pumila*). Sebatian kimia ini telah dipencilkan dan ditulenkan melalui turus kromatografi terbuka. Kromatografi tipis analitikal telah digunakan bagi memantau pemisahan sebatian kimia tersebut. Sebatian kimia tulen yang diperoleh kemudiannya dianalisis menggunakan DI-prob kromatografi gas dan spektrometeri jisim (GCMS DI-prob), manakala struktur kimia dikenal pasti dengan menggunakan resonan magnetik nuklear (NMR) melalui beberapa analisis seperti ^{1}H, ^{13}C dan Korelasi Multi Ikatan Heteronuklear (HMBC).

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