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THERMODYNAMIC AND INTERFACIAL STUDIES OF PHARMACEUTICAL ACTIVE NICOTINAMIDE - VANILLIN DRUG SYSTEM

602-609

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Metrics based on Scopus® data as of April 2020

Dr Rashmi Sharma  2 months ago

Excellent.

reply

Melanie Ortiz  1 month ago

Dear Dr. Rashmi, thanks for your participation! Best Regards, SciImago Team

Richardson Brown  2 years ago

It's an enormous pleasure and honor to organize "15th World Congress On Bioavailability and Bioequivalence" scheduled during July 29-30, 2019 at Bangkok, Thailand. The conference is mainly focused on the theme of "Essential Innovation in the field of Pharmaceutical Science for Public and Medical health". BABE Conference 2019 is designed with prudent keynote sessions, session lectures, and poster presentations, presentations from the young researchers, panel discussions, and the B2B meetings with world-renowned speakers from the stream of clinical and pharmaceutical sciences. It provides the best platform for the researchers to the researchers all over globe to introduce themselves to the world with their unique researches. It's an open forum to discuss new researches and the challenges faced during the BA/BE studies, manufacturing the
THE ACTIVE MARKER COMPOUND IDENTIFICATION OF ARTOCARPUS CHAMPEDEN SPRENG. STEMBARK EXTRACT, MORACHALCHONE A AS ANTIMALARIAL

ACHMAD FUAD HAFID1*, NI PUTU ARIANTARI2, LIDYA TUMEWU3, AGRIANA ROSMALINA HIDAYAT1, ATY WIDYAWARUYANTI1

1Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Jalan Drhammawangsa Dalam, Surabaya 60226, Indonesia, 2Department of Pharmacy, Faculty of Mathematics and Natural Science, Udayana University, Bali, Indonesia, 3Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia, 4Department of Pharmacology, Faculty of Medicine, Mataram University, Mataram, Indonesia. Email: achmadjufad@unair.ac.in

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ABSTRACT

Artocarpus champeden Spreng. (Moraceae), commonly known as “cempedak”, has been traditionally used for malarial remedies. Several studies reported that A. champeden Spreng. stembark has in vitro and in vivo antimalarial activity. The main aim of this research was to isolate and identify an active marker compound of A. champeden stembark for quality control purpose of A. champeden stembark extract in the development of this extract as phytopharmaceutical antimalarial product. The isolation method was bioactivity guided column chromatography and preparative TLC techniques. The marker compound that was isolated showed potent antimalarial activity with an IC50 value of 0.18 μg/ml against Plasmodium falciparum 3D7 strain. Based on UV, IR, 1H NMR, 13C NMR, 2D NMR (COSY, HMBC and HMQC) spectral and identification of other references, the active marker compound was identical with known prenylated chalcone compound, Morachalcone A.

Keywords: Artocarpus champeden Spreng., Antimalarial, Morachalcone A. Active marker compound.

INTRODUCTION

Artocarpus champeden Spreng. commonly known as “cempedak”, belongs to Moraceae. It is widely distributed in Indonesia and has been traditionally used for malarial remedies1. Previous studies reported that several prenylated flavonoids isolated from A. champeden Spreng. have potential in vitro antimalarial against P. falciparum 3D7 strain2 and in vivo against P. berghei strains ANKA (unpublished). This indicates that A. champeden Spreng. is prospective to be developed as phytopharmaceutical product for antimalarial.

The pharmaceutical requirements for a herbal product destined for a multi-target therapy are very complex. The constituents of medicinal herbs can vary greatly as a result of genetic factors, climate, soil quality and other external factors3. Therefore, despite the use of authenticated botanical voucher specimens to help assure proper identity, modern concepts and methods relating to the quality (i.e., chemical consistency) of herbal materials and products pertain to phytochemical markers and fingerprint analyses are needed. These markers are the threads that tie together the production and the quality control4. Ideally, chemical markers should be unique compounds that contribute to the therapeutic effects of a herbal medicine5. Markers should be commercially available or able to be isolated in own laboratory as well6.

The standardized extract should have consistent constituent in order to ensure the consistency of quality, safety, and efficacy of the product. The marker approach to ensure consistency is based upon the assumption that the content of other constituents will vary in proportion to the marker compound. If each batch contains the same standardize amount of marker, the content of other constituents will also be relatively consistent6,7,8.

In order to develop A. champeden as antimalarial phytopharmaceutical product with multi-component approach, it is needed to standardize A. champeden extract as raw material. Therefore, the study to obtain standardized extract of A. champeden Spreng. stembark as raw material of antimalarial phytopharmaceutical product using active marker compound was conducted.

The present study aims to isolate and determine marker compound from 80% ethanol extract of A. champeden stembark. This study consists of several steps: marker compound isolation using chromatography techniques, followed by identification of isolate based on UV-Vis, IR, 1H NMR, 13C NMR, 2D NMR (COSY, HMBC and HMQC) and MS spectra. Marker isolation from ethanol extract of A. champeden stembark was done by bioactivity guided fractionation.

MATERIAL AND METHODS

General Experimental Techniques

Silica gel was used for column chromatography (Merck, 0.063-0.200 mm) and preparative thin layer chromatography (Merck). Spots on plates were detected under UV light (A 254 and 366 nm) and by sprayed 10% H2SO4 in water followed by gentle heating. IR spectrum obtained in a Shimadzu spectrometer IR Prestige-21 type. The NMR experiments (both 1D and 2D) were obtained in a Jeol spectrometer ECA 500 type operating at 500 MHz. HPLC analysis was performed with Hewlett Packard Agilent 1100 series, an Agilent 1100 series Degasser G1322A, a Rheodyne 7725 injection valve with a 20-μl loop, Agilent 1100 series Quaternary Pump G1311A, Agilent 1100 series Column Compartment G1316A, Agilent 1100 series diodearray detector (DAD) G1315A. Compounds were separated in a 250×4.6 mm Varian Microsorb MV 100-5 CB column.

Plant material

The stembark of Artocarpus champeden Spreng. was collected from Bogor, West Java, Indonesia, on June 2007 and 2008. A voucher specimen was identified and deposited at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia.

Extraction and Isolation

One kilogram of Artocarpus champeden Spreng. stembark was extracted with 80% ethanol at 60°C, yielded 74,64 g of crude extract. This extract was applied to ODS column chromatography, using MeOH-H2O (4:1 v/v)-MeOH-acetonitril (1:1 v/v) as eluent, resulting in 10 major fractions (fraction 1-10). Fraction 4 (866.9 mg) was applied to silica gel column and eluted with CHCl3 followed by increasing polarity of CHCl3-MeOH 1-10% by gradient elution, yielded 9 major subfraction. Further separation of subfraction 4B (47.1 mg) was conducted by several steps of Preparative TLC techniques using silica RP-18 as stationary phase with MeOH-H2O:0(7:3 v/v) as mobile phase yielded active subfraction 4.8B (18.7 mg). The process was then continued using silica as stationary phase with CHCl3-MeOH (95:5.0 v/v) as mobile phase yielded active subfraction 4.8B.7 (12.2 mg).

Purification of this subfraction was conducted by reverse-phase PLIC with MeOH-H2O:0(4:1 v/v) as mobile phase resulting an active marker compound, Morachalcone A (7.2 mg).
Morachalcone A: orange powder; UV [MeOH] λ max nm 250, 316 and 385; IR (KBr) cm⁻¹: 3,456, 1,697. ¹H and ¹³C NMR data are given in Table 1.

**Antimalarial activity Assay**

The antimalarial activity of fractions and the isolated compound were determined by the procedure described by Budimulya et al. (1997). In brief, each fraction or compound was separately dissolved in DMSO (10⁻² mol L⁻¹) and kept at -20°C until used. The malarial parasite P. falciparum 3D7 strain was propagated in a 24-well culture plate in the presence of a wide range of concentration of each fractions or compound. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Giemsa stain. The antimalarial activity of each fraction or compound was expressed as an IC₅₀ value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control.

**HPLC Conditions**

HPLC analysis was conducted using methanol-water (65:35 v/v) as mobile phase by isocratic elution. IL 250x4.6 mm Varian Microsorb MV 100-5 column at flow rate of 1 ml/minute, column temperature of 30°C, stop time of analysis at 20 minutes and detection wavelength set at 385 nm.

**Sample preparation**

10 mg of ethanol extract of A. champeden Spreng stembark was accurately weighed and dissolved in methanol using vortex for 5 minutes. Sample solution was filtered through a 0.45 μm syringe membrane filter.

**RESULT AND DISCUSSION**

The dried stembark of A. champeden was extracted by 90% ethanol. In a preliminary test of in vitro antimalarial activity against P. falciparum 3D7 clone of this ethanol extract showed significant inhibition. Isolation of active marker compound from ethanol extract of A. champeden stembark was done by bioactivity guided isolation. The ethanol extract was fractionated by open column chromatography using ODS as stationary phase and methanol-water as mobile phase, resulting in 10 major fractions and fraction 4 was active against P. falciparum 3D7 strains. Furthermore, fraction 4 was applied to silica gel column and eluted with CHCl₃ followed by increasing polarity of CHCl₃-MeOH 1-10%, yielded active subfraction 4.8 with an IC₅₀ value of 0.03 μg/mL. Further separation of subfraction 4.8 with several steps of PTLC techniques using RP-18 silica gel as stationary phase with MeOH-H₂O as mobile phase yielded active subfraction 4.8.8 (IC₅₀ value of 0.10 μg/mL), then silica as stationary phase with CHCl₃-MeOH mixtures as mobile phase yielded active subfraction 4.8.8.7 (IC₅₀ value of 0.39 μg/mL). Purification of this subfraction by reverse-phase PTLC with MeOH-H₂O as mobile phase resulting in active marker compound.

The identification of isolated compound was based on UV-Vis, IR, ¹H NMR, ¹³C NMR, 2D NMR (COSY, HMBC and HMQC) spectra and comparison of other references. Marker compound was isolated as orange powder. The UV-Vis absorptions at 250, 316 and 385 nm were suggestive of a chalcone skeleton. The IR spectrum of morachalcone A contained absorption bands at 3,456 cm⁻¹ and 1,697 cm⁻¹ corresponding to hydroxyl and carbonyl groups, respectively. The ¹H NMR spectrum contained characteristic signals ascribable to an isoprenyl group (5H 1.65, 1.77, 5.22, 3.34). 2 proton signals at 8H 7.73 (1H, dd, J=15.25 Hz) and 8.1 (1H, dd, J=15.9 Hz) form an AB system, the large coupling constant indicating the trans geometry of a double bond. The ¹H-NMR spectrum (table 1) also indicated signals for ortho coupled aromatic protons in ring A (6.636 (2H, m) and δ 7.52 (1H, dd, J= 8.55, 3.7 Hz) and two aromatic protons in ring B δ 6.43 (1H, q, J=8.55, 17.75 Hz) dan 6.74 (1H, d, J=8.55 Hz). The presence of proton signals at δ 13.93 and δ 14.48 indicated that the hydroxyl groups are located at C-2 and C-4 in ring A and C-2 and C-4 in ring B. The ¹³C-NMR spectrum contained signals from 20 carbon atoms including that of a ketone carbonyl carbon at δc 194.25 (table 1).

On the basis of HMQC and HMBC spectral analysis, all protons and carbon signals were fully assigned and the positions of the substituents on the aromatic rings were determined. The HMBC correlations for H-1’/C-2’, C-3’, and C-3’ confirmed that the 3,3-dimethylallyl group was located at C-3’.

The structure determination for marker compound also conducted based on the comparison of their spectroscopic data from literature values.²,³ Thus, the structure of marker compound was deduced as known prenylated chalcone, Morachalcone A. Morachalcone A was previously isolated from callus culture of Macha pusifera (Moraceae)² and also from methanol extract of A. champeden stembark (unpublished).

**Table 1. The ¹H NMR and ¹³C NMR (500 MHz) data for marker compound**

<table>
<thead>
<tr>
<th></th>
<th>¹H</th>
<th>¹³C</th>
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<tbody>
<tr>
<td>α</td>
<td>7.73 dd (15.25 Hz)</td>
<td>117.93</td>
</tr>
<tr>
<td>β</td>
<td>8.1 dd (15.9 Hz)</td>
<td>142.15</td>
</tr>
<tr>
<td>C=O</td>
<td>-</td>
<td>194.25</td>
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<tr>
<td>1’</td>
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<tr>
<td>2’</td>
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<td>163.50</td>
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<tr>
<td>3’</td>
<td>-</td>
<td>116.67</td>
</tr>
<tr>
<td>4’</td>
<td>-</td>
<td>165.32</td>
</tr>
<tr>
<td>5’</td>
<td>6.43 q (8.55, 17.75 Hz)</td>
<td>108.26</td>
</tr>
<tr>
<td>6’</td>
<td>7.76 d (8.55 Hz)</td>
<td>130.47</td>
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<td>CH2</td>
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<tr>
<td>CH=</td>
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<td>123.70</td>
</tr>
<tr>
<td>C=</td>
<td>-</td>
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<tr>
<td>E-Me</td>
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<td>26.09</td>
</tr>
<tr>
<td>Z-Me</td>
<td>1.77 s</td>
<td>18.04</td>
</tr>
</tbody>
</table>

*CD₃OD, TMS as internal standard.

Only signals that are significant to the comparison are reported.
Fig. 1: The chemical structure of active marker compound isolated from *Artocarpus champeden* Spreng.

Fig. 2: Chromatogram obtained from ethanol extract of *A. champeden* stem bark (A) and marker standard morachalcone A (B)

The marker compound revealed inhibitory activity against *P. falciparum* 3D7 strain with an IC50 value of 0.18 μg/mL. The inhibitory activity of this compound showed that the isolated compound was an active marker.

According to Ref. [2004], marker compound should be accessible to the quantification of common analytical equipment (e.g., high-performance liquid chromatography [HPLC]) to keep costs of routine analysis moderate. Therefore, preliminary analysis of the existence of Morachalcone A in ethanol extract of *A. champeden* Spreng stem bark was conducted using HPLC.

The chromatogram profile of ethanol extract using the mixture of methanol and water (65:35 v/v) as mobile phase by isocratic elution, C8 250x4.6 mm Varian Microsorb MV 100-5 column at flow rate of 1 mL/minute, column temperature of 30°C, stop time of analysis at 20 minutes and detection wavelength set at 369 nm (Figure 2) showed that peak at time retention shown as Mean ± SD (RSD) of 13.001 minutes ± 0.37 (2.87%), has resolution of 1.42, plate number of 3524, peak width at half height of 0.5200, symmetry factor of 0.82 and selectivity factor of 1.11. The purity factor of this peak was 997.034, indicated that targeted peak fall within acceptable purity. The identity of this peak against marker spectrum was shown as match factor value of 981.261 (>950.000), indicated that targeted peak analyzed in ethanol extract was marker compound that has been detected in ethanol extract of *A. champeden* stem bark.

The result of this study showed that the active marker compound, Morachalcone A, can be used as a marker compound in standardization of ethanol extract of *A. champeden* stem bark as antimalarial phytochemistry product. Further studies are needed to develop an analysis method of Morachalcone A for quality control and standardization purpose of ethanol extract of *A. champeden* Spreng.

ACKNOWLEDGEMENT

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REFERENCES


