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DETERMINATION OF MARKER COMPOND OF
ARTOCARPUS CHAMPEDEN SPRENG. STEMBAK EXTRACT
AND VALIDATION OF THE ANALYSIS METHOD USING
HPLC

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Abstract: Artocarpus champeden SPRENG. (Moraceae), commonly known as "cempedak", has
been traditionally used for malarial remedies. Several studies also reported in vitro and in vivo
antimalarial activity of this plant. In order to develop A. champeden stem bark as antimalarial
phytopharmaceutical product, a marker approach was chosen for quality control purpose of
the extract as raw material.

The aims of this study are to determine marker compound of A. champeden stem bark
extract and develop an analysis method for quantification of marker compound in ethanol extract
of A. champeden SPRENG. stem bark using HPLC.

Isolation of marker isolate was carried out by column chromatography and preparative
TLC techniques. Based on UV, IR, ¹H NMR, ¹³C NMR, 2D NMR (COSY, HMQC and HMBC)
and MS spectra and comparison with other references, confirmed that marker isolate was identical
with known prenylated chalcone, Morachalcone A. The HPLC profile showed that marker
compound has time retention shown as Mean ± SD (RSD) of 12.547 minutes ± 0.39 (3.08%).
Validation of analysis method for quantification of marker in ethanol extract was conducted. The
analyte were chromatographed on CB 250x4.6 mm column, methanol-water as mobile phase
(65.35 v/v) by isocratic elution at flow rate of 1 mL/minute, column temperature of 30°C, stop
time of analysis at 20 minutes and detection wavelength at 385 nm. The method was validated in
terms of selectivity, specificity, linearity, accuracy and precision. The calibration curve was linear
within a high range concentration of 25 - 50 µg/mL and low range concentration of 3.5 - 25
µg/mL. The accuracy and intra-day precision were found to be well within acceptable limits. The
marker content in ethanol extract of A. champeden SPRENG. stem bark was shown as Mean ± SD
(RSD) of 0.3361% ± 0.03 (8.45%).

Keywords: A. champeden SPRENG., antimalarial activity, ethanol extract, marker compound,
morachalcone A, HPLC analysis

INTRODUCTION
Artocarpus champeden SPRENG. belongs to Moraceae, commonly known as "cempedak", is widely distributed in Indonesia and has been traditionally used for
malarial remedies [Hakim et al., 2006]. Previous studies reported that several prenylated
flavonoids isolated from A. champeden SPRENG. has potent antimalarial activity in vitro
against P. falciparum strains 3D7 [13] and in vivo against P. berghei strains ANKA
(unpublished). This indicates that A. champeden is prospective to be developed as
phytomedicine product for antimalarial.

The pharmaceutical requirements for a herbal product intended for a multi-target
therapy are very complex [Kroll and Cordes, 2006]. The constituents of medicinal herbs
can vary greatly as a result of genetic factors, climate, soil quality and other external...
factors [Schulz et al., 2004]. 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. These markers are the threads that tie together production and quality control [Reif et al., 2004]. Ideally, chemical markers should be unique components that contribute to the therapeutic effects of a herbal medicine [Li et al., 2008].

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, that if each batch contains the same standardize amount of marker, the content of other constituents will also be relatively consistent [Lazarowych and Pekus, 1998; McCutcheon, 2002].

In order to develop A. champeden as antimalarial phytomedicine 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 phytomedicine product using active marker compound was conducted.

The preliminary study showed that 80% ethanol extract of A. champeden stembark was active extract that inhibit parasit growth both in vitro against Plasmodium falciparum strains 3D7 and in vivo against Plasmodium berghei strains ANKA. Then, from this ethanol extract has been identified an isolate belongs to chalcone group, based on UV spectra, as characteristic orange spot, detected in extract, fraction and subfracton using TLC-Densitometer and HPLC. This isolate also showed in vitro antimalarial activity against P. falciparum strains 3D7 with an IC50 value of 0.69 μg/mL, therefore this isolate can be used as marker candidate from 80% ethanol extract of A. champeden stembark.

The present study aims to determine and isolate marker compound from 80% ethanol extract of A. champeden stembark, then develop an analysis method for quantification of marker content in ethanol extract of A. champeden stembark. Therefore, in the beginning we isolate and identify the stucture of an isolate that we used as marker candidate for ethanol extract of A. champeden stembark. This study consist of several steps, are begun with isolation of marker compound using conventional column chromatography and preparative TLC techniques, followed by identification of isolate based on UV-Vis, IR, 1H NMR, 13C NMR, 2D NMR (COSY, HMBC and HMQC) and MS spectra. Further step is development of analysis method for quantification of marker content in ethanol extract of A. champeden stembark using HPLC that conducted by validation of an analysis method.

MATERIALS AND METHODS

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

**Chemicals and Reagents**
All reagents were analytical grade, otherwise reagents for HPLC analysis. Methanol for HPLC analysis was purchased from Mallinckrodt, Inc. (USA). Samples for HPLC were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA). Reference standard, marker compound Morachalcone A, was isolated from the stembark of *Artocarpus champeden* SPRENG. In our laboratory, and mass spectrometry, NMR spectrometry and infrared spectrometry were applied to determine compound characteristics.
General Experimental Techniques and Instruments
Silica gel was used for column chromatography (Merck, 0.063-0.200 mm) and silica gel for preparative thin layer chromatography (Merck). Spots on plates were detected under UV light (λ 254 and 366 nm) and by spraying with 10% H₂SO₄ in water followed by gentle heating. IR spectrum obtained on a Shimadzu spectrometer IR Prestige-21 type. The NMR experiments (both 1D and 2D) were obtained on a Jeol spectrometer ECS 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 on a 250x4.6 mm Varian Microsorb MV 100-5 C8 column.

Extraction and Isolation
One kilogram of Artocarpus champeden SPRENG stem bark was extracted with 80% ethanol at 60°C in rotavapor, yielded 74,636 g of crude extract. This extract was applied to ODS column chromatography, using methanol-water (4:1 v/v)-methanol-acetone-titrit (1:1 v/v) and acetone-titrit as eluent, resulting in 10 major fractions (fraction 1-10). Fraction 4 (686.9 mg) was applied to silica gel column and eluted with CHCl₃-H₂O mixture by increasing polarity of CHCl₃-MeOH 1-10% by gradient elution, yielded 9 major subfraction. Further separation of subfraction 4.8 (47.1 mg) was conducted with several steps of Preparative TLC techniques using silica RP-C₁₈ as stationary phase with MeOH-H₂O (7:3 v/v) mixtures as mobile phase yielded active subfraction 4.8.8 (18.7 mg). This was then continued using silica as stationary phase with CHCl₃-MeOH (9.5:0.5 v/v) mixtures as mobile phase yielded active subfraction 4.8.8.7 (12.2 mg). Purification of this subfraction was conducted by reverse-phase PTLC with MeOH-H₂O (4:1 v/v) mixtures as mobile phase resulting in active marker isolate, 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 Testing
The antimalarial activity of fractions and the isolated compound was determined by the procedure described by Budimulya et al. (1997). In brief, each fraction and compound was separately dissolved in DMSO (10⁻² mol L⁻¹) and kept at -20°C until used. The malarial parasite P. falciparum 3D7 clone 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 fractions 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 with methanol-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 385 nm.

Preparation of standard solution
The standard stock solution (1 mg/mL) was prepared by dissolving 5 mg of standard to 5 mL of methanol. The stock solution was stored at 4°C.
Sample preparation
Ten mg of ethanol extract of A. champeden SPRENG stem bark was accurately weighed and dissolved in methanol using vortex for 5 minutes. Sample solution then filtered through a 0.45 μm syringe membrane filter.

Calibration
The standard stock solutions of marker substance was diluted by methanol to give serial concentrations at: 25; 30; 35; 40; 45 dan 50 μg/mL (high range concentration) and at 25; 20; 10; 7; 5.6 dan 3.5 μg/mL (low range concentration). After filtering through a 0.45 μm membrane filter, 20 μL of each concentration was injected into the HPLC column for analysis. Linear regression model was used to describe the relationship between peak area and concentration of standard solution.

Method validation
Method validation for quantification of marker content in ethanol extract of A. champeden stem bark using HPLC was carried out in terms of selectivity, specificity, linearity, limit of detection, limit of quantitation, accuracy and precision.

Selectivity and Specificity
Selectivity and specificity was checked by using an extract of Artocarpus champeden SPRENG stem bark and marker compound Merachaione A and optimizing separation and detection. The purity of the peaks was checked by acquisition of spectra (λ = 200–400 nm) by use of the DAD detector. Spectra were acquired at the upslope, apex, and downslope of each peak, computer normalized, and superimposed. Peaks were considered pure when there was coincidence between the three spectra (match factor >950.000). Selectivity study resulting in methanol-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, step time of analysis at 20 minutes and detection wavelength set at 385 nm.

Limits of Detection and Limit of Quantification
Limits of detection and quantification were determined as the amounts for which the calculated signal-to-noise ratios were approximately 3:1 and 10:1, respectively.

Precision
Standard stock solution was diluted in methanol for intra-day test (injecting certain concentration of standard six times within 24 hours). The standard deviation (SD) and relative standard deviation (RSD) were calculated.

Accuracy
Accuracy study was carried out by spiking certain concentration of marker to sample. Recovery rate (%) was calculated.

RESULT AND DISCUSSION
The dried stem bark of A. champeden was extracted with 80% ethanol. In a preliminary test of in vitro antimalarial activity against P. falciparum 3D7 clone of this ethanol extract showed significant inhibition (unpublished). Marker isolation from ethanol extract of A. champeden stem bark was done by bioactivity guided fractionation. The ethanol extract was fractionated with open column chromatography using ODS as stationary phase and methanol-water as mobile phase, resulting in 10 major fractions and fraction 4 was proved to be active against P. falciparum strain 3D7 in vitro. Fraction 4 was applied to silica gel column and eluted with CHCl₃ followed by increasing polarity of CHCl₃-MeOH (1:0%) by gradient elution, yielded active subfraction 4B with an IC₅₀ value of 0.03 μg/mL. Further separation of subfraction 4B with several steps of PTLC
techniques using silica RP-C18 as stationary phase with MeOH-H2O mixtures 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 mixtures as mobile phase resulting in active marker isolate.

Identification of isolate based on UV-Vis, IR, 1H NMR, 13C NMR, 2D NMR (COSY, HMBC and HMQC) spectra and comparison with 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 [Mabry, et al., 1970]. 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 [Silverstein et al., 1991]. The 1H NMR spectrum contained characteristic signals ascribable to an isoprenyl group (δH 1.65, 1.77, 5.22, 3.34). Two proton signals at δH 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 (δ 8.55, 6.36, 2H, m) and δ 7.52 (1H, dd, J=8.55, 3.7 Hz) and two aromatic protons in ring B δ 8.43 (1H, q, J=8.55, 17.75 Hz) and δ 7.76 (1H, d, J=7.75 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 HMBC and HMQC 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 is located at C-3'. Structure determination for marker compound also conducted based on comparison with their spectroscopic data from literature values [Abegaz et al., 1998; Monache et al., 1995]. Thus, the structure of marker compound was deduced as known prenylated chalcone, Morachalcone A. Morachalcone A was previously isolated from callus culture of *Maclura pomifera* (Moraceae) [Monache et al., 1995] and also from methanol extract of *A. champeden* stem bark (unpublished).

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<td>6.43 q (8.55, 17.75 Hz)</td>
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Table 1. The 1H NMR and 13C NMR (500 MHz) data for marker compound.
CD,OD, TMS as internal standard. Only signals that significant for the comparison are reported.

The marker compound revealed inhibitory activity against *P. falciparum* strain 3D7 with an IC₅₀ value of 0.18 μg/ml. The inhibitory activity of this compound showed that the isolated compound was an active marker. The result of this study showed that active marker compound, Morachalcone A, can be used to standardize ethanol extract of *A. champeden* stem bark as antimalarial phytomedicine product. Morachalcone A, obtained from this study was then used as reference standard for analysis of marker content in ethanol extract of *A. champeden* stem bark using HPLC. According to Reif [2004], marker compound can be isolated in own laboratory if it is not commercially available and used as reference standard with defined identification, purity and content.

The development of analysis method for quantification of marker content in ethanol extract of *A. champeden* stem bark using HPLC was conducted by validation of an
analysis method. Validation of the method was done in terms of selectivity, specificity, linearity, accuracy, precision, limit of detection and limit of quantitation.

The chromatographic condition was optimized through comparison of different solvents and solvent ratio. Compared with other solvents, the mixture of methanol and water (65:35 v/v) as mobile phase by isocratic elution, C8 250×4.6 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 showed the best separation and minimize peak tailing.

Under this condition, the chromatogram profile of ethanol extract (Figure 1) 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. Linearity study showed that analysis within the high range concentration of 50 - 25 µgml⁻¹ and low range concentration of 3.5 - 25 µgml⁻¹ resulting in response proportional against concentration with correlation coefficient (r) of 0.9989 and 0.9997, *Vₙₐ* value of 1.26% and 7.11% respectively, indicated linear correlation. Limit of detection was 1.57 µg/ml⁻¹ and limit of quantitation was 4.71 µg/ml⁻¹. The compound analysis were obtained with the recovery rate shown as Mean ± SD (RSD) of 102.599 (5.48%) with intra-day for instrument precision shown as Mean ± SD (RSD) of 31.066 (6.06 %) and method precision shown as RSD of 8.45%. Based on parameter values obtained from validation study, the analysis method can be assumed to be valid for quantification of marker content in ethanol extract of *A. champeden* stem bark using HPLC. Amount of Morachalcone A as marker in ethanol extract of *A. champeden* stem bark shown as Mean ± SD (RSD) was 0.3361% ± 0.03 (8.45%).

**CONCLUSION**

Results of this study showed that prenylated chalcone, Morachalcone A, can be used as marker for ethanol extract of *Artocarpus champeden* SPRENG. stem bark. HPLC method also has been developed for quantitation of Morachalcone A in ethanol extract of *A. champeden* SPRENG. stem bark. Results indicate that the present method was valid in controlling the quality of ethanol extract of *A. champeden* SPRENG. stem bark. The quantitative analysis showed that the content of marker Morachalcone A in ethanol extract of *A. champeden* was shown as Mean ± SD (RSD) of 0.3361% ± 0.03 (8.45%).

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**REFERENCES**


Kroll U., Cordes C., 2006, Pharmaceutical prerequisites for a multi-target therapy, Phytomedicine, 13, 12-19
Li S., Han Q., Qiao C., Song J., Chen, C.L., Xu H., 2008, Chemical Markers for The Quality Control of Herbal Medicine: An Overview, Chinese Medicine, 3(7), 1-16
SURAT TUGAS
Nomor: /788 /H.3.1.5/KP/2010

Sehubungan dengan adanya International Conference On Medicinal Plants The Future of Medicinal Plants: Form Plant to Medicine pada tanggal 21 – 22 Juli 2010 di Fakultas Farmasi Universitas Widya Mandala, dengan ini Wakil Dekan II Fakultas Farmasi Universitas Airlangga menugaskan:

- Dr. Aty Widyawaruyanti, MSi NIP. 19620426 199002 2 001
- Dr. Achmad Fuad H. MS NIP. 19521212 198103 1 009

Untuk menghadiri acara tersebut.

Demikian Surat Penugasan ini untuk dilaksanakan dengan baik dan penuh tanggung jawab.

Surabaya, 20 Juli 2010

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[Signature]

[Stamp]

DR. H. Umi Athiyah, MS
NIP. 19560407 198103 2 001
CERTIFICATE

This is to certify that

Achmad Fuad Hafid

has participated in

38th Meeting of National Working Group on Indonesian Medicinal Plants

INTERNATIONAL CONFERENCE ON MEDICINAL PLANTS

Surabaya, July 21 - 22, 2010

as

ORAL PRESENTER

The National Working Group on Indonesian Medicinal Plants

Chairman

Scientific and Organizing Committee

Chairman