

From Knowledge to Wisdom

UCMS

ISSN 1548-6648 (Print)

ISSN 1930-2088 (Online)

Journal of US-China Medical Science

Volume 8, Number 10, October 2011



Publication Information:

Journal of US-China Medical Science (ISSN 1548-6648) is published monthly in print and online by David Publishing Company located at 9460 Telstar Ave Suite 5, EL Monte, CA 91713, USA.

Editorial Board Members:

Dr. George Kweifio-Okai (Australia), Prof. Andrew Paul Zbar (Australia), Prof. Xiaodong Tan (China), Prof. Qiy Gong (China), Prof. Huixiong Xu (China), Prof. Nargis Albert Labib (Egypt), Dr. Mohamed Ebrahim Abd EL-Ha Abou El-Ghar (Egypt), Dr. Itzik Harosh (France), Prof. Géza. László. Lukács (Hungary), Dr. M Ibrahim Masoodi (India), Dr. Mari Cheraghi (India), Prof. Thamer A. Hamdan (Iraq), Prof. Mohammad Esra Akbari (Iran), Prof. Michele Spinelli (Italy), Khalid M. Bofares (Libya), Assi.Prof. Oana-Cristina Arghir (Romania), Assi.Prof. G Chandramohan (Saudi Arabia), Prof. Esther Uña Cidón (Spain), Assi.Prof. Chun-Chieh Tseng (Taiwan), Prof. Kivilcim Gucuyener (Turkey), Dr. Ghulam Sarwar Pirkani (Pakistan), Prof. Ram B Singh (Poland), Dr. Douglas Wilson (UK), Prof. Robert Eduard Suter (USA), Assi.Prof. Yang Xia (USA), Prof. El-Sheikh Enas (Yemen).

Manuscripts and correspondence are invited for publication. You can submit your papers via Web Submission E-mail to doctors@davidpublishing.org. Submission guidelines and Web Submission system are available at <http://www.davidpublishing.org>

Editorial Office:

9460 Telstar Ave Suite 5, EL Monte, CA 91713, USA

Tel: 1-323-984-7526 Fax: 1-323-984-7374

E-mail: order@davidpublishing.org, shelly@davidpublishing.org

Copyright©2011 by David Publishing Company and individual contributors. All rights reserved. David Publishing Company holds the exclusive copyright of all the contents of this journal. In accordance with the international convention, no part of this journal may be reproduced or transmitted by any media or publishing organs (including various websites) without the written permission of the copyright holder. Otherwise, any conduct would be considered as the violation of the copyright. The contents of this journal are available for any citation; however, the citations should be clearly indicated with the title of this journal, serial number and the name of the author.

Abstracted / Indexed in:

Database of EBSCO, Massachusetts, USA

Chinese Database of CEPS, Airiti Inc. & OCLC

Chinese Scientific Journals Database, VIP Corporation, Chongqing, P. R. China

Ulrich's Periodicals Directory

Cambridge Science Abstracts (CSA)

Subscription Information:

Price: US\$420 (print), US\$320 (online), US\$560 (print and online)

David Publishing Company

9460 Telstar Ave Suite 5, EL Monte, CA 91713, USA

Tel: 1-323-984-7526 Fax: 1-323-984-7374

E-mail: order@davidpublishing.org



David Publishing Company
www.davidpublishing.org

Journal of US-China Medical Science

Volume 8, Number 10, October 2011 (Serial Number 83)

Contents

Technical Papers

- 577 **Low Sensitivity of Pooled Chlamydia Testing in a Sample of the Young German General Population**
Karin Haar, Thomas Meyer, Sarika Desai, Michael Thamm, Matthias an der Heiden, Viviane Bremer and Osamah Hamouda
- 581 **Effect of Physical Conditioning with Lifestyle Intervention on A Community-Based Hyperglycemic-Overweight Adults**
João Felipe Mota, Fernando Moreto, Franz Homero Paganini Burini, Wilson Luvizotto Medina, Eric B. Rimm and Roberto Carlos Burini
- 588 **Health Impact Assessment and the Role of Accredited Clinical Laboratory on ISO 15189:2007 International Standard**
Rafael Ríos Tamayo, Francisco Javier Pérez Zenni, Almudena García Rutz, Aurora Bueno Cavanillas, José Juan Jiménez-Moleón, Juan Saluz Pérez, María José Sánchez Pérez and Manuel Jurado Chacón
- 596 **Non-linear Models Applied on Mortality Time Series in Greece from 1932-2009**
Maniadhakis Mihael, Sifaki-Pistolla Dimitra and Pistolla Georgia
- 603 **Autogenous Training in the Treatment of Burning Mouth Syndrome**
Josipa Sanja Gruden Pokupec, Vladimir Gruden and Lajosz Szirowica
- 617 **Development of Standardized Ethanol Extract of Fraxinus Griffithii as CNS Depressant**
Kartini, Sutarjadi, Aguslina Kirtishanti, Dini Kesuma, Soediatmoko Soediman and Kusuma Hendrajaya
- 626 **Parascapular Pedicle Fasciocutaneous Flaps for Regional Reconstruction, Evaluation Study**
Mahdi Hameed Abood and Dana A. Abdilkarim
- 638 **Ageism In View of Paramedical Healthworkers at Departments of Gerontopsychiatry**
Andrea Pokorná

Case Report

- 642 **Vertical Transmission of the Visceral Leishmaniasis: A Case Report**
Arben Pilaca, Zef Delia, Arben Pepa, Edmond Puca, Dhimiter Kraja

Development of Standardized Ethanol Extract of *Fraxinus Griffithii* as CNS Depressant

Kartini¹, Sutarjadi², Aguslina Kirtishanti¹, Dini Kesuma¹, Soediatmoko Soediman¹ and Kusuma Hendrajaya¹

1. Faculty of Pharmacy, University of Surabaya, Indonesia

2. Center for Information and Development of Traditional Medicine, University of Surabaya, Indonesia

Abstract: **Background:** *Fraxinus griffithii* has been widely used as CNS depressant. Its activity based on both empirical and preclinical data. However, standardization on raw material and process of extraction have not been conducted. **Methods:** Extraction of *Fraxinus griffithii* was conducted on different part of plants, as well as different solvents and extraction methods. Each extract was standardized both on specific and nonspecific parameters. Additionally, phenobarbital induced sleeping time test was performed on each extract. **Results:** Leaves of *F. griffithii* extracted with 70% ethanol by kinetic maceration yielded the highest extract. CNS depressant activity of 70% ethanol extract obtained from *F. griffithii* leaves by kinetic maceration was the highest compared to the others. **Conclusion:** All of the extracts have CNS depressant activity, but extract from the leaves, produced by 70% ethanol and kinetic maceration had the optimal activity and quality.

Key words: *Fraxinus griffithii*, standardized, phenobarbital induced sleeping time test, CNS depressant.

1. Introduction

CNS (central nervous system) depressant is a class of drugs used to decrease the brain activity [1]. Based on the pharmacological effect, CNS depressant is divided into five, namely: systemic anesthetics, sedatives and hypnotics, central relaxants, antipsychotics and anti-seizure [2]. In the clinical treatment, CNS depressant is used to treat anxiety, muscle tension, pain, insomnia, acute stress reaction and seizure. Side effects of CNS depressant ranging from confusion, dizziness, memory damage, impaired motor coordination, cognitive function as well as physical and mental dependence. These are problems faced by most groups of CNS depressant [3]. CNS depressants commonly used in the treatment are group of barbiturates and benzodiazepines [4]. Based on these problems, it is necessary to search for other drugs with less side effects. This can be conducted

through two approaches: modification of the chemical structure of existing drugs (1) or development of plant-based CNS depressant (2). The latter alternative is an approach that is likely to be carried out in Indonesia, considering that this country is rich in medicinal plants, has the herbal medicine industry with high growth and there is promising national and international market interest on herbal medicine [5].

Through various policies, the Indonesian government has encouraged the utilization and development of Indonesian medicinal plants. In order for the herbal medicines to be used in formal health services, the government directed the development of herbal medicine from "jamu" to become Standardized Herbal Medicine (SHM) and Phytopharmaca. Jamu is group of herbal medicine that its safety and efficacy are based on empirical data, both of its raw material and product have not been standardized. SHM and phytopharmaca have to fulfill requirement on preclinical and clinical trials, respectively, also both raw material and product have been standardized. It is expected that these two classes of traditional drug can

Corresponding author: Kartini, S.Si., M.Si., Apt., research fields: phytochemistry; standardization of herbal medicine. E-mail: kartini240777@gmail.com.

be used by the medical community in the formal health services. Researchers need to provide the scientific data such as: testing the efficacy and safety, standardization of materials and formulation, as well as dosage form development. Thus, side effects resulting from the use of herbal medicines can be detected as early as possible so that people can choose and use materials that had been proven efficacious and safe.

Fraxinus griffithii grow in various regions in Indonesia. In Java, this plant known by various names, i.e., "tiken", "bedali gombong" and "orang-arang" [6]. Extracts from barks, leaves and twigs of this plant were used as opium adulterant in some areas in East Java [7]. It was reported that the makers of bark extracts experienced dizziness and drowsiness. Early exploration in mice proved that the extract had an ability in reducing attention and motoric activity of animal test as well as effect on CNS [8,9,10]. Many compounds have been reported from this plant, especially iridoids [11].

Requirement of raw materials for manufacturing of SHM and phytopharmaca is a standardized extract. Meanwhile, the quality of the extract itself is influenced by various factors including: extracted material (parts of plant, age and time of collection, environment of plants, drying, storage, etc.) and extraction process (solvent, extraction methods, duration and temperature of extraction, evaporation and drying process, etc.) [12, 13].

Based on the description above, it is necessary to perform a research for the manufacturing of standardized extracts from *F. griffithii* as CNS depressant. To prepare the standardized extract, various extraction variables, such as parts of plant (leaves, barks, twigs), methods (percolation, kinetic maceration, reflux) and concentration of solvent (50%, 70% and 96% ethanol) need to be optimized. Subsequent to extraction, each extract was determined for its specific and non-specific parameters. Activity standardization was conducted by testing CNS depressant activity of each extract.

2. Materials and Methods

2.1 Chemicals

Ethanol, methanol, acetonitrile, chloroform, ethyl acetate and plate of Silica Gel 60 GF254 were purchased from Merck Co. (Germany). Bidestilated water was from PT. Ikapharmindo Putramas (Indonesia), while demineralised water was obtained from Faculty of Pharmacy, University of Surabaya (Indonesia). CMC-Na and Pentothal[®] were purchased from Hospira (USA).

2.2 Plant Materials

The plant materials are leaves, twigs and barks of *Fraxinus griffithii*, obtained from the Pancur Angkrek Garden (PTPN XII). They were identified by the Center for Information & Development of Traditional Medicines, University of Surabaya (Indonesia). Plant materials were dried under indirect sunlight and pulverized using a grinding machine.

2.3 Test Animals

Test animals were male white mice (Balb C strain, 2-3 months, 20-30 g) obtained from the laboratory of experimental animals, Airlangga University, Surabaya (Indonesia). Prior to treatment, mice were kept in fasting for ± 12 hours. 60 test animals used, 20 mice for TPE (*Time Peak Effects*) determination and 40 mice for determination of potentiation effect of extract against thiopental Na. In TPE determination, test animals were divided into four groups (each group consisted of five mice) whereas in potentiation effect, they were divided into four groups (each group consisted of 10 mice).

2.4 Optimization of Extraction

Optimization of extraction conditions was conducted. Variables include: parts of plant (leaves, twigs, cortex or barks), extraction methods (kinetic maceration, reflux, percolation) and concentration of solvent (96%, 70%, 50% ethanol). Each extract was

standardized through determination of non-specific (loss on drying, total ash content and microbial content) and specific parameters (TLC-densitometry and HPLC profiles) as well as CNS depressant activity test in mice.

2.5 Determination of TPE (Time Peak Effects)

20 mice were divided randomly into four groups, each group consisted of five mice. All groups were given *F. griffithii* extract, 3000 mg/kg BW orally. Thiopental Na 60 mg/kg BW was injected i.p and after 30, 45, 60 and 90 minutes, respectively, sleeping time of each mouse was recorded. The measurement started with righting reflex negative (time it got to sleep) until

righting reflex positive (time it wake up). The longest sleeping time of mice is TPE of *F. griffithii* extract.

2.6 Potentiation Test of *F. griffithii* Extract against Thiopental Na

40 mice were divided randomly into four groups, each group consisted of 10 mice. Each group was given 0.5% CMC Na suspension (thiopental Na group) or different amount of *F. griffithii* orally (2500, 3000, 3500 mg/kg BW). A few minutes later (in accordance with TPE of each group), thiopental Na (60 mg/kg BW) was injected i.p. Sleeping time was calculated based on righting reflex (-) to (+). The extension of mice sleeping time can be calculated using the following formula:

$$\frac{\text{Time to sleep of mice (receiving extract)} - \text{Time to sleep of mice (receiving thiopental Na)}}{\text{Time to sleep of mice (receiving thiopental Na)}} \times 100\%$$

2.7 Statistical Analysis

The data from specific and nonspecific parameters determination were analyzed statistically (ANOVA, $\alpha = 0.05$) to see if any significantly differences between various *F. griffithii* extracts. TPE and extension of mice sleeping time were also analyzed using similar statistic test.

3. Results and Discussion

3.1 Determination of Specific and Non Specific Parameters of *F. griffithii* Extract

Result of extraction and determination of loss on drying (LOD), as well as total ash content of extract from three parts of *F. griffithii* using different solvent and method of extraction can be seen in Figs. 1-2, respectively.

Leaves extracted by kinetic maceration using 70% ethanol yielded the highest recovery (Fig. 1). Therefore, such condition can be considered as optimal condition for production of extract in production scale. LOD represents the water and other volatile substances content in an extract, including the residual solvent.

Previous research found that extract of leaf and cortex of *F. griffithii* contain mannitol and predicted to contain saponins, tannins, glycosides and steroids [7]. Because these compounds do not easily evaporate at 105°C, LOD value indicates the water and residual solvent content only. LOD of the extract should not be greater than 10% to avoid rapid growth of microbes [8]. In this research, LOD of all extracts are greater than the standard, so the extract should be placed in an airtight container and stored in a cool, dry place.

Fig. 2 showed that total ash content varied from 0.64-6.53%. This indicates that the remaining inorganic compound contained in extract is 0.64-6.53%. Ash content of each extract varies greatly due to differences in species, parts of plant, post harvesting and extraction processes. So, there is no fixed standard for this parameter [13]. Determination of heavy metal was performed using ICPS. Results showed that all of extract have no content of heavy metal (Hg, Pb, Cd, As), except 50 and 70% ethanol extract of *F. griffithii* leaves resulted from kinetic maceration. They contained Pb, but in not quantified concentration. WHO mentioned that maximum limit of

Pb and Cd allowed in crude drug are 10 and 0.3 mg/kg, respectively [15]. Microbial contaminant in *F. griffithii* extracts were counted by the TPN method. Results showed that seven kinds of *F. griffithii* extract have ALT $<1 \times 10^3$ CFU. This means that the extract does not contain any microbe. Acceptable standard for ALT values and pathogenic bacteria in general extract are 10^3 and 10^6 /g or ml, respectively [16]. In this study ALT value is quite satisfactory, because it shows a very low number of bacteria. The number of microbes must be restricted because its negative effect on the stability of extract and dangerous (toxic) for human health [13]. TLC-spectrophotodensitometry

and HPLC profile of *F. griffithii* extract are presented in Figs. 3-4, respectively.

Profile of all extracts are relatively similar, except 96% ethanol extract of *F. griffithii* leaves resulted from kinetic maceration (Fig. 3f). Recently, the marker compound for *F. griffithii* has not been determined. Compound with the highest Rf can be proposed as marker compound (analytical marker), because its concentration is relatively higher than the others and has good separation. So, the quantitative analysis will be easier. The highest concentration of marker resulted from 96% ethanol extract of *F. griffithii* leaves obtained from reflux (Fig. 3b).

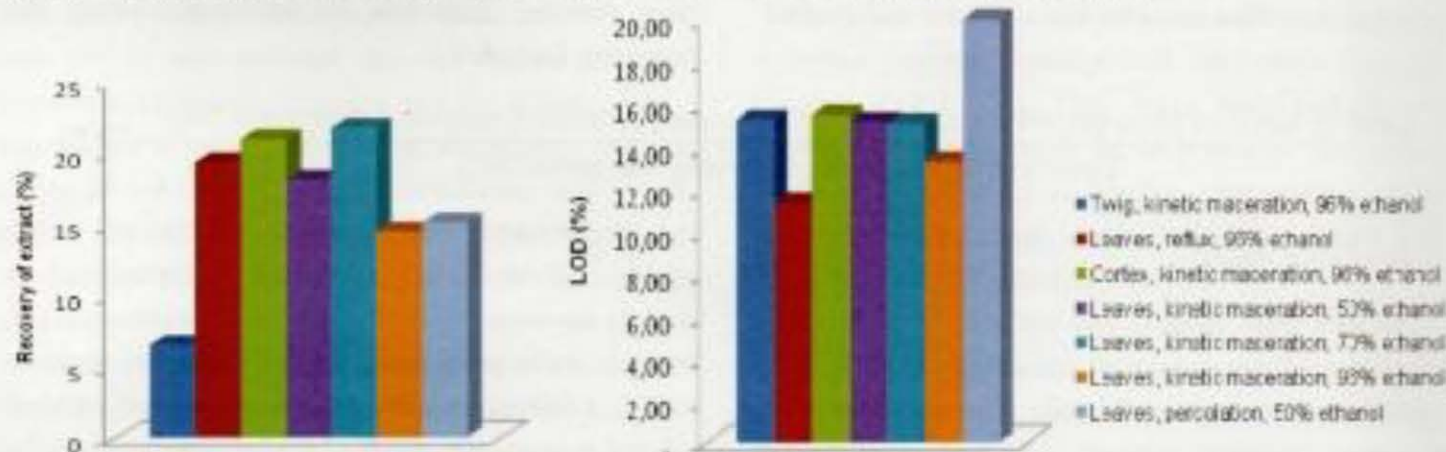


Fig. 1 Recovery of extract (%) and LOD of *Fraxinus griffithii* Clarke extract.

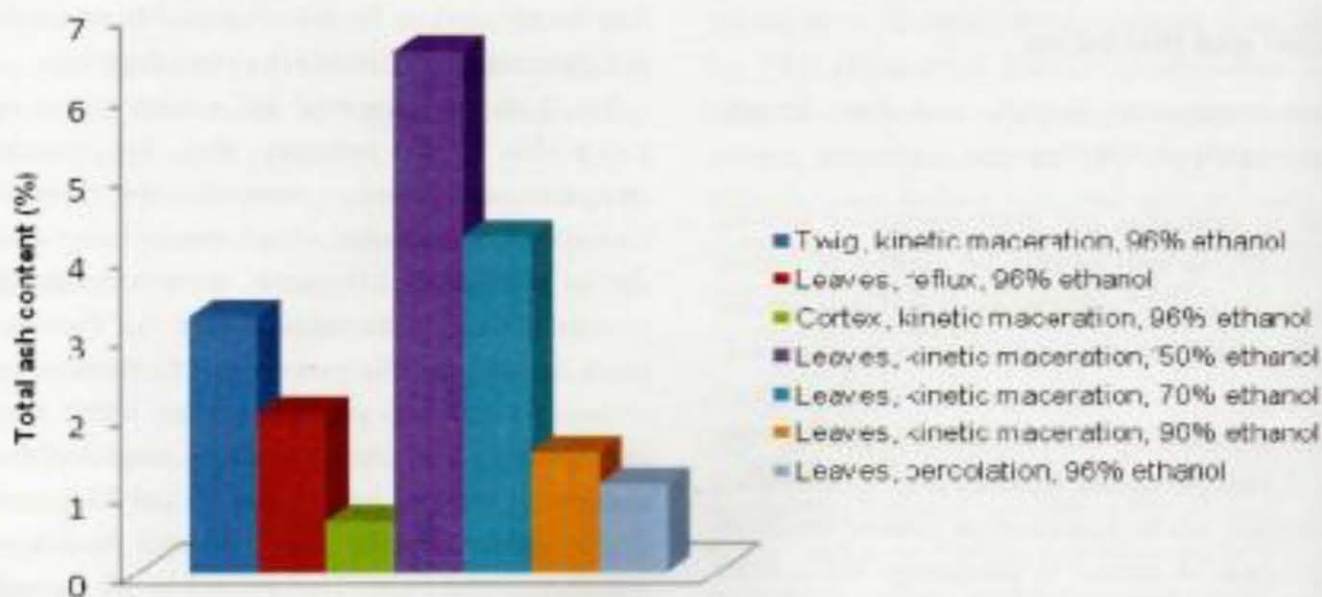


Fig. 2 Total ash content of *Fraxinus griffithii* extract.

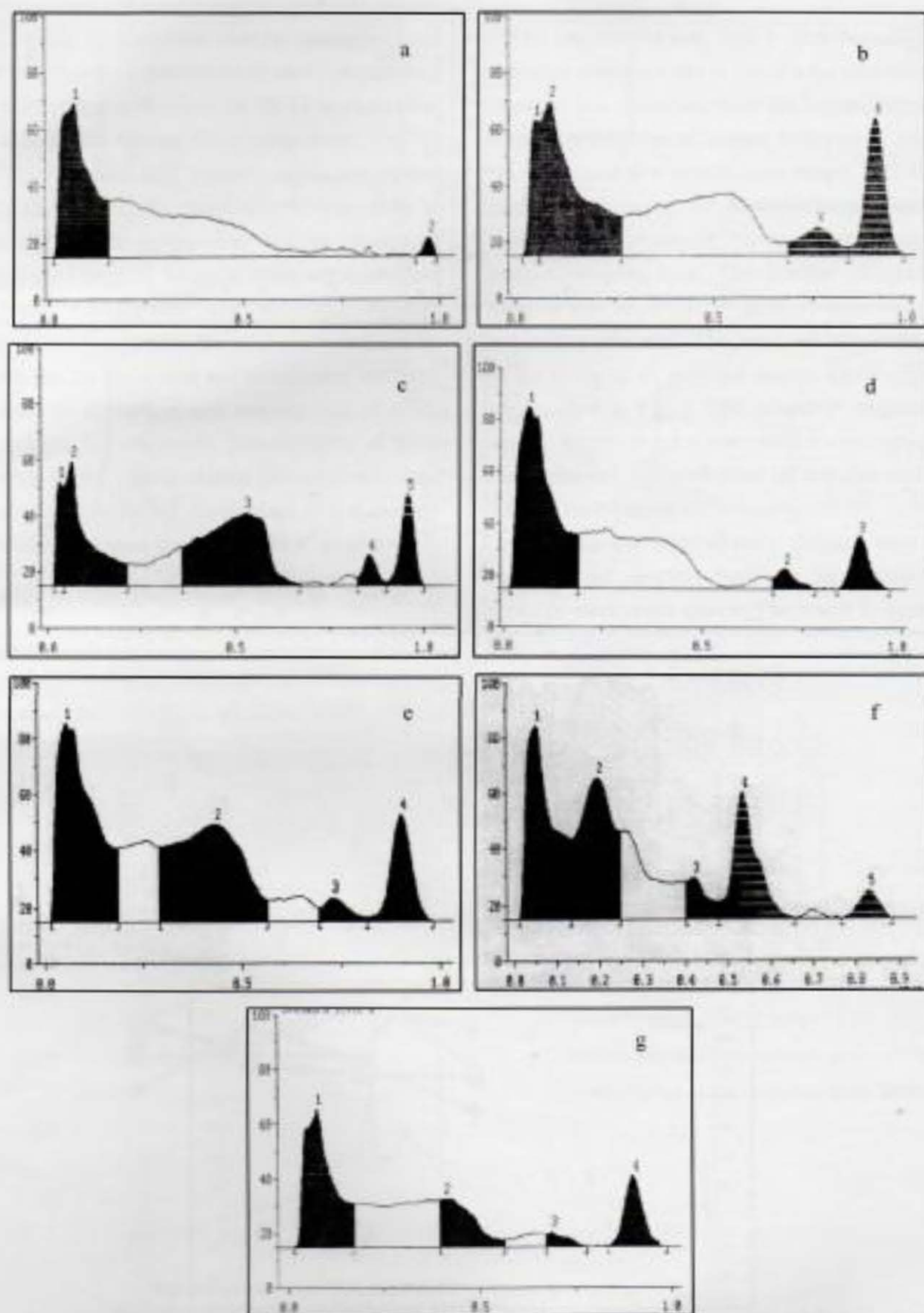


Fig. 3 TLC-spectrophotodensitometry profile of *F. griffithii* extract on Silica Gel 60 GF254, chloroform-methanol (5:2), detected in 230 nm (a: Twigs, kinetic maceration, 96% ethanol; b: Leaves, reflux, 96% ethanol; c: Cortex, kinetic maceration, 96% ethanol; d: Leaves, kinetic maceration, 50% ethanol; e: Leaves, kinetic maceration, 70% ethanol; f: Leaves, kinetic maceration, 96% ethanol; g: Leaves, percolation, 96% ethanol)

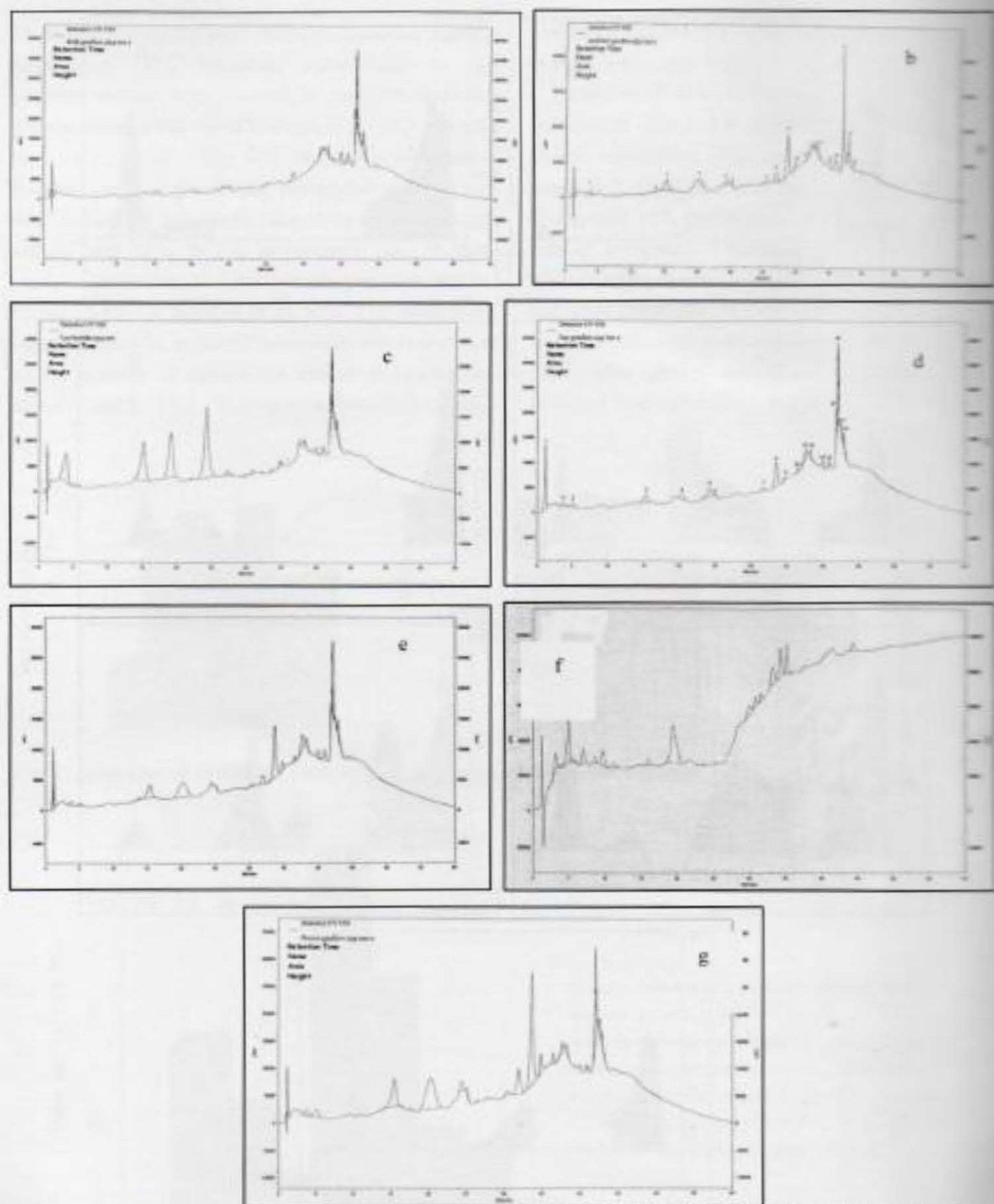


Fig. 4 HPLC profile of *F. griffithii* extract on RP-18, eluted gradiently in acetic acid-water (0.15%) and acetic acid-methanol (0.15%), detected at 254 nm (a. Twig, kinetic maceration, 96% ethanol; b. Leaf, reflux, 96% ethanol; c. Cortex, kinetic maceration, 96% ethanol; d. Leaf, kinetic maceration, 50% ethanol; e. Leaf, kinetic maceration, 70% ethanol; f. Leaf, kinetic maceration, 96% ethanol; g. Leaf, percolation, 96% ethanol).

HPLC profile all of extracts vary on amount of peak and area (represent concentration of each component). Each extract contains between 16 till 21 components. Fig. 4c showed the highest three components, i.e., on Rt of 15.2, 19.1 and 24.2 minute, concentration are 21.15, 21.49, and 35.53%, respectively. According to Li et al. [17], such components can be chosen as marker because they are found in abundant quantities. These main components are not characteristic components and bioactivity are not known, but can be used as marker for qualitative and quantitative analysis, for adulteration evaluation and stability test of crude drug or extract. Unfortunately, concentration of these components in six others extract are relatively low, even undetected. In further experiment, it is necessary to search and determine the marker for *F. griffithii*.

3.2 Results of CNS Depressant Activity Test of *F. griffithii* Extract

Prior to conducting potentiation test extract against Na thiopental, determination of Time Peak Effects

(TPE) was carried out. TPE is time required to reach maximal concentration in blood after administration of extracts. It is calculated from the longest sleeping time after administration of extract followed by injection of Na thiopental at a certain time range. TPE is used as time range between the administration of extract and injection of thiopental Na in order to obtain the longest sleeping time. The amount of extract from twigs is very small (32.59 g), so its bioactivity test has not been performed. The result of TPE determination of six kinds of *F. griffithii* extract (3000 mg/kg BW) can be seen in Fig. 5. TPE of each *F. griffithii* extract varies. So, subsequent potentiation test extract against Na thiopental was performed on the time according to TPE of each extract.

Based on the mean of mice sleeping time (Fig. 6), extension of sleeping time can be calculated using formula mentioned above. The result is presented in Fig. 7.

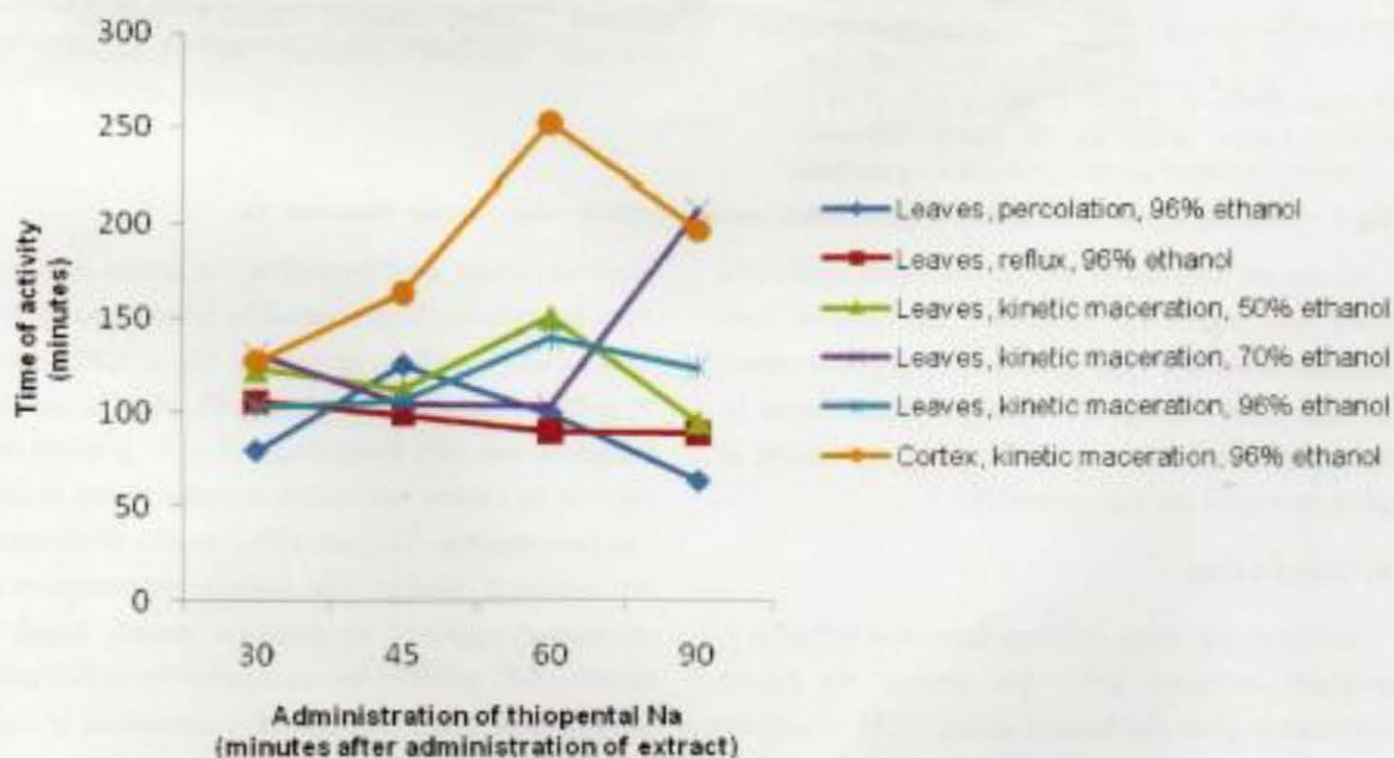


Fig. 5 TPE of *F. griffithii* extract.

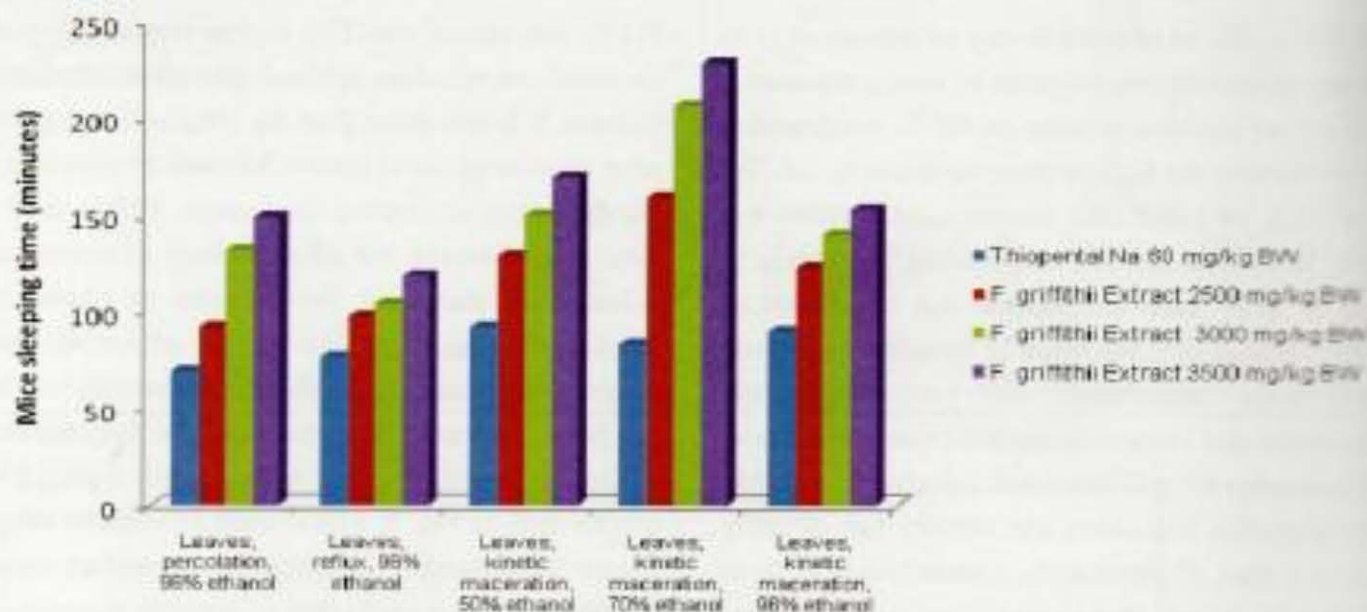


Fig. 6 Sleeping time of mice on potentiation test *F. griffithii* extract against Na thiopental.

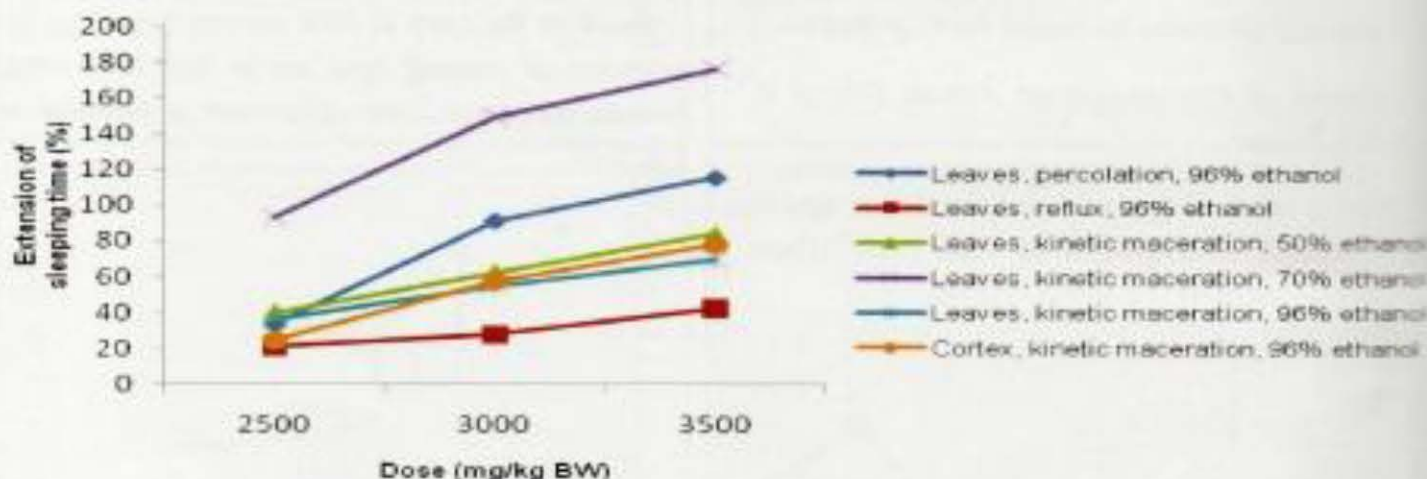


Fig. 7 Extension of mice sleeping time in potentiation test of *F. griffithii* extract against thiopental Na.

Based on Analysis of Variance continued with LSD, there was significant difference on sleeping time between doses and type of extract. *F. griffithii* extract at dose 3500 mg/kg BW obtained from leaves by kinetic maceration, using 70% ethanol as solvent of extraction gave the highest activity.

4. Conclusion

Based on this study, it is concluded that leaves of *F. griffithii* extracted with 70% ethanol by kinetic maceration gives the highest result. LOD of extracts vary from 11.35-16.00%. The lowest of LOD is resulted from *F. griffithii* leaves extracted with 96% ethanol by reflux. Total ash content from all of *F.*

griffithii extract are 0.64-4.23%, the lowest is extract from leaves using 96% ethanol by kinetic maceration. TPN of all *F. griffithii* extract are $<1 \times 10^{-1}$ CFU. All of *F. griffithii* extract do not contain Pb, Hg, Cd, and As, except 50 and 70% ethanol extract of *F. griffithii* leaf resulted by kinetic maceration contains Pb but in very low concentration. TLC and HPLC profile all of extract are relatively similar. The highest concentration of compound proposed as analytical marker found in extract of *F. griffithii* leaves resulted by reflux using 96% ethanol, while the highest concentration of main components found in extract of *F. griffithii* cortex resulted by kinetic maceration using 96% ethanol. Depressant activity of 70% ethanol extract obtained

from *F. griffithii* leaves by kinetic maceration is the highest compared to other extracts.

Acknowledgement

The authors greatly appreciate Institute for Research and Community Services, University of Surabaya (Indonesia) for financial support, contract No. 12/Lit/LPPM/FF/VII/2009 and students for technical assistance.

References

- [1] G. A. Goodman and W. Rall, Goodman and Gilman's: The Pharmacological Basis of Therapeutics (11th ed.), Mc Graw-Hill: New York, 2006.
- [2] Siswandono and B. Soekardjo, Kimia Medisinal 2, Airlangga University Press: Surabaya, 2000, p. 236.
- [3] Fontanarosa, Alternative Medicine: An Objective Assessment, American Medical Association, 2000.
- [4] NIDA (National Institute on Drug Abuse), CNS Depressants, available online at: <http://www.nida.nih.gov/Researchreports/Prescription/prescription3.html>.
- [5] W. Sumaryono, Peluang, Tantangan dan Strategi Pengembangan Produk Bahan Alami untuk Penanganan Kanker, Prosiding Simposium Penelitian Bahan Obat Alami XIV, Jakarta, 2009, pp. 1-12.
- [6] Sutarjadi, *Fraxinus Griffithii* Clarke, Penelitian Taksonomi dan Fitokimia, Desertasi, Universitas Airlangga, 1980.
- [7] Sutarjadi and N. C. Zaini, *Fraxinus eedenii* Boerl. Et Kds (Studi Pendahuluan Farmakognosi dan Fitokimia), Research Report, Airlangga University, 1973.
- [8] S. A. Ahaditomo and H. D. Ma'rifin, Penelitian pendahuluan beberapa khasiat tiken: *Fraxinus griffithii* clarke, Prosiding Simposium Penelitian Tanaman Obat I, Bogor, 1975, pp. 267-398.
- [9] A. Basori, Isolation and pharmacodynamic screening of ligustroside-A CNS Active Substances-Isolated from *Fraxinus griffithii* Clarke, *Folia Medica Indonesiana* 4 (1999) 4-8.
- [10] A. Basori, Pharmacodynamic studies of central nervous system depressant effects of ligustroside: A CNS Active Substances-Isolated from *Fraxinus griffithii* Clarke, *Folia Medica Indonesiana* 1 (2000) 39-46.
- [11] R.A. Macahig, L. Harinantenaina, K. Matsunami, H. Otsuka, Y. Takeda and T. Shinzato, Secoiridoid and Iridoid Glucosides from the Leaves of *Fraxinus griffithii*, *J Nat Med* 64 (2010) 1-8.
- [12] P. H. List and P. C. Schmidt, *Phytopharmaceutical Technology*, CRC Press: Boca Raton, 1989.
- [13] R. I. Depkes, Parameter Standar Umum Ekstrak Tumbuhan Obat, Dirjen POM: Jakarta, 2000.
- [14] S. Soetarno and I. S. Soediro, Standardisasi Mutu Simplisia dan Ekstrak Bahan Obat Tradisional, Prosiding Temu Ilmiah Nasional Bidang Farmasi, 1997.
- [15] WHO, Quality Control Methods for Medicinal Plant Materials, WHO: Geneva, 1998.
- [16] R. O. B. Wijesekera, *The Medicinal Plant Industry*, CRC Press: Boca Raton, 1991.
- [17] S. Li, Q. Han, C. Qiao, J. Song, C. L. Cheng and H. Xu, Chemical Markers for the quality control of herbal medicines: An overview, *Chinese Medicine* 3 (2008).