INTERNATIONAL SEMINAR ON
MEDICINAL CHEMISTRY
AND TIMMERMAN AWARD 2011

PROGRAM AND ABSTRACT BOOK

SURABAYA, OCTOBER, 15, 2011
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International Seminar on Medicinal Chemistry and Timmerman Award
Surabaya 2011
SEMINAR PROGRAM

08.30 – 09.00: Registration
09.00 – 09.10: Opening Ceremony
09.10 – 09.30: Coffee Break

08.30 – 15.00: POSTER DISPLAY

Session I
Moderator: Prof. Dr. Umar Anggara Jenie (UGM)

09.30 – 10.10: Plenary Lecture I
Prof. Dr. Henk Timmerman (Vrije Universiteit, Amsterdam)
“Receptor, from Concepts to Reality and Beyond”

10.10 – 10.50: Plenary Lecture II
Prof. Dr. Masahiro Toyota (Osaka Prefecture University)
“Development of Two Different Types of Palladium-Catalyzed Cycloalkenylation and Application to Bioactive Natural Product Synthesis”

10.50 – 11.30: Plenary Lecture III
Prof. Dr. Siswadono (PERAKMI and Airlangga University)
“Molecular Modeling and QSAR of Benzoylurea Derivates as CNS Depressant”

11.30 – 12.00: Discussion
12.00 – 13.00: Break for prayer and lunch

Poster Session
13.00 – 14.00: Discussion.
Presenter should be on poster site for discussion.

Session II
Moderator: Prof. Dr. Daryono Hadi Tjahjono (ITB)

14.00 – 14.40: Plenary Lecture IV
Prof. Dr. L. Broto S. Kardono (Indonesian Institute of Science)
“Search on Lead Compounds from Indonesian Natural Products and Their Prospect for Further Development”

14.40 – 15.20: Plenary Lecture V
Dr. Raghu Rangaswamy (Senior Director, Schrodinger Inc.)
“Combinatorial Library Building and Lead Optimization”

15.20 – 16.00: Plenary Lecture VI
Prof. Dr. Madhavi Shastri (Senior Application Scientist, Schrodinger Inc.)
“Prediction of Cytochrome P-450 Sites of Metabolism using Induced Fit Docking”

16.00 – 16.30: Discussion
16.30 – 17.00: Coffee break and prayer
17.00 – 17.15: Timmerman Award Presentation.
17.15 – 17.30: Closing Ceremony.

International Seminar on Medicinal Chemistry
and Timmerman Award
Surabaya 2011
Isolation of Iridoide Glycoside from *Fraxinus griffithii* Clarke Leaves

**Kartini**, Sutarjadi

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**ABSTRACT**

Finding new and has relatively less side-effect anti-seizure is important for pharmacist. To answer this issue, research to isolate iridoide glycoside from ethanol extract of the cortex and leaves of *Fraxinus griffithii* Clarke (tiken) has been conducted. Pharmacological screening test showed that those extract have anti-seizure activity on mice. It was conducted with electroshock method. Quantitative analysis with Trim-Hill reagent showed that the concentration of total iridoide of tiken leaves is higher than its cortex, 2.12% and 0.55% AE (Aucubin Equivalent), respectively. Isolation of iridoide glycosides from tiken cortex and leaves was done on an open column using silica gel as stationary phase and CHCL3-Methanol (47:3) as mobile phase. Identification of isolate from cortex (isolate X) and leaves (isolate Z) was done with three different TLC systems and used anisaldehid-H2SO4 (vanillin-H2SO4) as spray reagent. Each isolate showed a red-brown and red-purple spot. Spectrum of isolate X in methanol showed a maximum wavelength at 277.8 and 223.4 nm, whereas isolate Z at 278 and 223 nm.

Keywords: iridoide glycoside, *Fraxinus griffithii*, anti-seizure
Isolation of Iridoid Glycoside from *Fraxinus griffithii* Clarke Leaves

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2Centre of Information & Development of Traditional Medicine, University of Surabaya
3Under graduate student, Faculty of Pharmacy, University of Surabaya

*Corresponding author, email: kartini@ubaya.ac.id or kartini240777@gmail.com

**Abstract.** Development of new and has relatively less side-effect anti-seizure is important for pharmacist. To answer this issue, research to isolate iridoide glycoside from ethanol extract of the cortex and leaves of *Fraxinus griffithii* Clarke (tiken) has been conducted. Pharmacological screening test showed that those extract have anti-seizure activity on mice. It was conducted with electroshock method. Quantitative analysis with Trim-Hill reagent showed that the concentration of total iridoid of tiken leaves is higher than its cortex, 2.12% and 0.55% AE (Aucubin Equivalent), respectively. Isolation of iridoid glycosides from tiken cortex and leaves was done on an open column using silica gel as stationary phase and CHCl3-Methanol (47:3) as mobile phase. Identification of isolate from cortex (isolate X) and leaves (isolate Z) was done with three different TLC systems and used anisaldehid-H2SO4 (vanillin-H2SO4) as spray reagent. Each isolate showed a red-brown and red-purple spot. Spectrum of isolate X in methanol showed a maximum wavelength at 277.8 and 223.4 nm, whereas isolate Z at 278 and 223 nm.

**Keywords:** iridoid glycoside, *Fraxinus griffithii*, anti-seizure

**INTRODUCTION**

*Fraxinus griffithii* Clarke (“Tiken” or “orang-aring”) is a plant that grows wild on the slopes of the mountains, like in the Besuki (Probolinggo) and Lumajang (Sutarjadi, 1980; Ali, 1988). In East Java, this plant has become famous again related with the circulation of tiken extract as adulterant for opium in 1961-1969 (Ali, 1988).

Various pharmacological studies have been conducted to investigate the efficacy of tiken extract. Tiken leaf extract has sedative and analgesic effects in mice, as well as reduce the blood pressure in dog (Ahaditomo, 1972; Ahaditomo et al., 1975). It was reported that the tiken bark extract may extend the mice sleeping time due to barbiturate (Ali, 1988). According to Ahaditomo et al. (1975), tiken leaf extract may improve the heart tone and lowering the heart rate in frog. Tiken also thought to have cardiotonic properties (Sutarjadi and Zaini, 1973; Sutarjadi, 1980; Ali, 1988). Tiken bark extract has CNS depressant effects, i.e.: mild sedation, does not cause paralysis of muscle, and does not cause sleep when tested on mice (Basori and Purwaningsih, 2004). Recent study states that tiken extracts have anti-seizure effects in mice (Purwaningsih, 2005).

Tiken bark contains a variety of compounds including ligustrosid glucoside, and glucoside of siringin and sinapaldehyde. It is expected that sedation effects of tiken bark extract caused by ligustrosid glucoside (Sutarjadi, 1980). Part of tiken which has been widely researched is the bark. However, there are many things to be considered in the development of plant-based drugs (fitoterapi). One of them is the raw material should be easily obtained and processed (Depkes RI, 1985). Leaves are an abundant part of this plant, so it is important to study how its potency as an anti-seizure.

Anti-seizure activity of ethanol extract from tiken leaves will be conducted in this study. The comparative study of total iridoid concentration between leaves and stem bark extracts of tiken also will be conducted in this experiment. In order to develop anti-seizure compounds, iridoid glycoside compounds will be isolated from extracts of tiken leaf and stem bark.

**METHODS**

**Plant materials, chemicals and Equipment**
The plant material is leaves and stem barks of *Fraxinus griffithii* Clarke, obtained from the Pancur Angkrek Garden (PTPN XII). They were identified by the Center of Information & Development of Traditional Medicines (PIPOT), Faculty of Pharmacy, UBAYA. Plant materials were dried
under indirect sunlight and pulverized using a grinding machine. Ethanol, methanol, chloroform, ethyl acetate, ether, Silica gel 60, anisaldehyde, vanillin and sulfuric acid were purchased from Merck Co. (Germany); bi-distillate water (PT. Ikapharmaindo Putramas); de-mineralized water (Faculty of Pharmacy, UBAYA); CMC-Na and Phenobarbital Na. The equipment used include: electric stirrer (Janke & Kunkel IKA-Werk RE16), chromatography chamber (Camag), column chromatography, rotary evaporator (Buchi R-114), analytical balance (OHAUS), water-bath (Memmert), oven (Ecocell, MMM), UV spectrophotometer (Cintra 101), MES (Maximum electroshock seizure) and laboratory glassware.

Test animals
Test animals were male, white mice (Balb C strain, 2-3 months, 20-30 g), obtained from the Pusvetma (Pusat Veterinaria Farma), Surabaya. Prior to treatment, mice were kept in fasting ± 12 hours. Fifty test animals were grouped into five groups, each group consists of ten mice.

Preparation of extract
One kg of powdered leaves of *F. griffithii* was extracted by kinetic maceration (400 rpm) using 96% ethanol. The extract then evaporated at low pressure (60°C), followed by using a water-bath (60°C) until viscous extract. Thick extract then fractionated with ether and chloroform, respectively. Remaining water fraction is then concentrated and continued into the purification process.

Anti-seizure activity test
Mice were randomly divided into five groups, each group consisted of 10 mice. Five groups namely control group (1% Na CMC), test group I (tiken leaf extract, 4000 mg/kg), test group II (tiken leaf extract, 5000 mg/kg), test group III (tiken leaf extract, 6000 mg/kg), and the standard group (phenobarbital sodium, 26 mg/kg). 60 minutes after administration of oral dosage form, induction of seizure is performed using MES. The duration of seizure of each mouse is then documented.

Total iridoid determination
The content of iridoid was determined according to a colorimetric method based on the Trim and Hill reaction (Galvez at al., 2005). Briefly, 400 μL of each extract was mixed with 4.0 mL Trim-Hill reagent (acetic acid/0.2% CuSO4/HCl concentration 10:1:0.5). After the samples had been heated (100°C, 5 min), the absorption was read at 609 nm, a blue color indicating the presence of iridoid. The concentration of total iridoid was calculated on the basis of the standard absorption of aucubin.

Purification and identification of iridoid
Purification of residual water fraction of *F. griffithii* was performed using column chromatography, with silica gel as stationary phase and chloroform-methanol (47:3) as mobile phase. Then, isolates are identified by TLC and spectrophotometer (200-300 nm).

RESULT AND DISCUSSION

Anti-seizure activity
Anti-seizure activity was measured from the reduction of seizure duration of mice, either tonic seizures, clonic or tonic-clonic seizures (table 1).
Table 1. Anti-seizure activity of *F. griffithii* leaves extract in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of seizure (second)</th>
<th>Tonic ± SD</th>
<th>Clonic ± SD</th>
<th>Tonic-clonic ± SD</th>
<th>Total ± SD</th>
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<td>25.71±0.59</td>
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Table 1 has shown that the duration of seizure of mice in the test groups are smaller than control. This difference is significant (ANAVA & LSD, α = 0.05). It indicated that tiken leaf extract has anti-seizure activity. Thus, *F. griffithii* leaf has possibility to be developed as an anti-seizure drug, even though its activity is smaller than standard. Extract is a mixture of many compounds, both active and non active compounds. So, that’s why the activity of extracts is smaller than standard. In the next step, isolation of anti-seizure lead compounds from *F. griffithii* leaf extracts is substantial to be conducted.
Total iridoids concentration
Visible Spectrum resulted from aucubin reaction and Trim-Hill reagent can be seen in Figure 1, while the concentration of iridoid total of tiken leaf extract and cortex are shown in Table 2.

Table 2. Total iridoid content in cortex and leaves extract of *F. griffithii*

<table>
<thead>
<tr>
<th>Replication</th>
<th>Cortex extract (total iridoid (%AE))</th>
<th>Leaves extract (total iridoid (%AE))</th>
</tr>
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<td>1</td>
<td>0.39</td>
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<td>6</td>
<td>0.43</td>
<td>2.26</td>
</tr>
</tbody>
</table>

Table 2 shows that the concentration of total iridoid in *F. griffithii* leaf extract is greater than its cortex extracts. Anti-seizure potency of *F. griffithii* leaves is predictably higher than its cortex because iridoid glycosides is expected responsible for such activities.

Extraction, purification and identification of iridoid glycoside
1 kg of leaf powder and 1 kg of *F. griffithii* cortex powder yielded 84.12 g (8.41%) and 80.468 g (8.05%) viscous extract, respectively. From the column chromatography of the residual water fraction of ethanol extract of *F. griffithii* leaves and cortex obtained sub-fractions that are divided into 45 vials. To view the results of purification, TLC was performed with results as shown in Figure 2.
TLC profile (fig.2) shows that only one sub fraction of tiken leaves extract has shown one spot (test number 21) while the cortex extract has 3 pure sub-fractions (test number 14, 15, 16). Further, sub-fraction 21 (leaves) and 14-16 (cortex) called as X and Y isolates, respectively. Purity test results of each isolate can be seen in Figure 3.

![TLC profile of isolate X and Y](image)

Figure 3 has shown that isolates X and Y are pure in TLC. UV spectrum of the two isolates could be seen in Figure 4.

![UV-vis Spectrum of isolate X and Y](image)

Figure 4 shows that isolates X and Y have two peaks, at 278; 223 nm and 223.4; 227.8 nm, respectively. Based on Sutarjadi et al. (1978), to identify iridoid compounds as ligustroside, solution of compound in ethanol have to show maximum at wavelengths of 227 and 208 nm. Maximum at 227 nm with large enough extinction indicate the presence of iridoid group. In alkaline conditions, the maximum at 290 nm indicate the presence of phenol group, and a maximum at 227 nm is not shifted show that R’ is not H but CH3. The results of the identification of isolates Y in methanol showed a maximum at a wavelength of 277.80 and 223.40 nm as shown in Figure 4 (b), so it can be said that isolates Y is the class of iridoid compounds. Under alkaline conditions after the addition of NaOH, the maximum at 292 nm, it can be concluded that the presence of phenol groups as said by Sutarjadi et al. (1978). Isolate X is not expected as iridoid ligustroside because it has λ max 278 nm and 223 nm. Further identification (MS, NMR, and IR) is required to ensure the structure of these isolates.
CONCLUSION
From this research can be concluded that ethanol extract of *F. griffithii* leaves has anti-seizure activity in mice. Ethanol extract of *F. griffithii* leaves contain iridoid total greater than its cortex extracts. From *F. griffithii* leaves and cortex extracts successfully isolated a compound which is estimated as iridoid.

ACKNOWLEDGEMENT
The authors greatly appreciate students (Rizka, Shenita & Indah) for technical assistance.

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