Tyrosinase Activity of *Piper betle* and *Piper crocatum* Essential Oil

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Abstract

Piper betle (Local name Sirih Hijau) and Piper crocatum (local name Sirih Merah) are two type of Piper genus which famous in Indonesia. This research is related to application of chemistry in health. The aims of this research were to find the essential oil component in Sirih Hijau and Sirih Merah and to find the potency of the essential oil against tyrosinase. The essential oils were obtained from leaves of Sirih Hijau and Sirih Merah by steam distillation. The components in the essential oils were analyzed by GC-MS. The activities of essential oils against tyrosinase were analyzed with spectrophotometry methods. The results showed that sirih hijau and sirih merah essential oils consisted of alpha thujene, alpha pinene, (+)-sabinene, beta-myrcene, alpha terpinene, beta terpinene, beta phellandrene, gamma terpinene, caryophyllene, alpha caryophyllene, and garmacrene. Camphene, chavicol and eugenol only existed in Sirih Hijau essential oil and not existed in Sirih Merah essential oil. The tyrosinase activities of these essential oils were also different. Sirih Hijau essential oil was an inhibitor for tyrosinase for monophenolase and diphenolase (inhibit about 50% activity compared to control at concentration 7mg/ml), while sirih merah essential oil was an activator for tyrosinase for monophenolase (16x more active compared to control at 7mg/ml) and diphenolase (2.5 x more active compared to control at 7mg/ml). In conclusion, camphene, beta phelandrene, chavicol and eugenol from sirih hijau essential oil can be used for whitening agent due to its inhibitory activity for tyrosinase.

***Keywords**: Piper bettle, Piper crocatum, essential oil, tyrosinase activity*

1. Introduction

Tyrosinase (EC1.14.18.1) is a multicopper monooxygenese enzyme with wide distrubution including in humans [1]. In humans, tyrosinase is responsible for melanogenesis or hyperpigmentation [2]. Tyrosinase also has been reported might contribute to the dopamine neurotoxicity and neurodegeneration associated with Parkinson's disease and Alzeimer diseases [3].

Indonesia has many traditional medicines. One of the famous genus is *Piper* genus. Many species belong to this genus, for instance *P. betle*, local name in Indonesia sirih hijau and *P. crocatum*, local name in Indonesia is sirih merah. *P. betle* and *P. crocatum* have essential oils which have many activities, such as anti-microbe [4]. To give added value to the two species of *Piper*, the research aims to find potency of essential oil from *P. betle* and *P. crocatum* agains tyrosinase and to find the chemical constituent in *P. betle* and *P, crocatum*.

2. Experimental Details

Samples material and preparation

P. betle and *P. crocatum* leaves was collected from Biopharmaca Research Center Field Station, Darmaga, Bogor, Indonesia in 2010. Essential oil of *P. betle* and *P. crocatum* were separated with steam distillation. The chemical component consists in the essential oils were separated and analyzed with GC-MS.

GC-MS condition

Essential oils were separated with column DB-5 MS (0.25 mm x 30 m) with helium gas (flow rate 42 ml/min). The injector temperature was 80ºC and detector temperature was 250ºC. Components separation was performed with condition temperature programme started in 80ºC for 5 minutes and increasing the temperature with

rate 5 C/min until 250ºC, the temperature then constant for 45 minutes. The MS analyses were performed in 70 eV, EI, and split ratio 25.0. MS data was collected in ranged 40 – 500 m/z. The MS spectrum then compared with the data at library.

Tyrosinase activity test

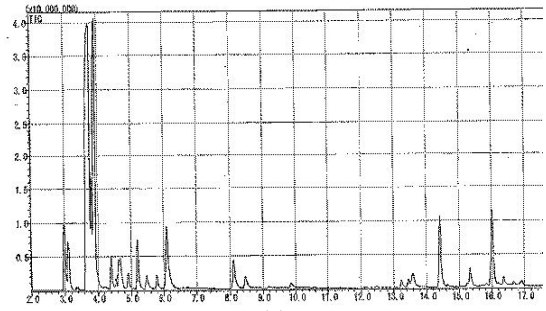
This assay was performed using methods as described earlier [5,6,7]. Essential oils were dissolved in DMSO (dimethyl sulphoxide) to a final concentration of 20 mg/ml.

The essential oils were tested at the concentrations ranged from 0.11 to 7.00 mg/ml (dissolved in 50 mM potassium phosphate buffer (pH 6.5)). In 96-well plate, 70 µl of each extract dilution was combined with 30 µl of tyrosinase (333 Units/ml in phosphate buffer) in triplicate. After incubation at room temperature for 5 min, 110 µl of substrates (2 mM L-tyrosine or 12 mM L-DOPA) was added to each well. Incubation commenced for 30 min at room temperature. Optical densities of the wells were then determined at 510 nm with multi-well plate reader. The tyrosinase activities (monophenolase and diphenolase activities) were analyzed in the present essential oils compared to control.

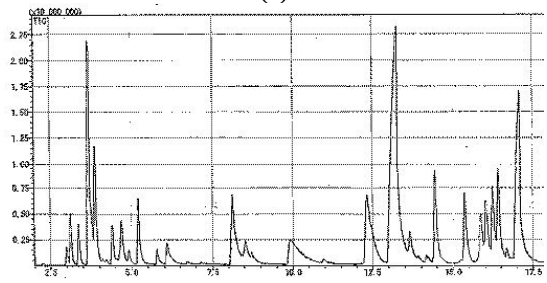
3. Result and Discussion

The essential oils of sirih merah and sirih hijau were obtained by steam distillation method (yielded 0.21% v/w and 0.14% v/w, respectively). The essential oils of sirih merah and sirih hijau gave different smell and different color. The different between the two essential oils for *Piper* genus were analyzed with GC-MS. The chromatograms of sirih merah essential oil and sirih hijau essential oil are shown in Fig 1a and b.

The component analyzes of each peak from chromatograms were shown in Table 1. The components in essential oils classified into two major groups, monoterpene and sesquiterpene. Monoterpene appeared first before sesquiterpene (retention time before 14 minutes for monoterpene). Major components in sirih hijau and sirih merah essential oils were the same. Some structures of essential oils consists in the two essential oils were shown in Fig 2. Sirih hijau and sirih merah essential oils were different in peak with retention time 3.3, 12.4 and 13.1 minutes, the component which only belong to sirih hijau essential oil were shown in Fig 3.



(a)



(b)

Fig 1. Chromatogram of sirih merah (a) and sirih hijau (b) essential oils separated with GC-MS.

Some reports about essential oils activities from sirih merah and sirih hijau had been published. Ngaisah (2009) reported that essential oil of sirih merah had antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Escheria coli,* and *Pseudomonas aeruginosa* [8]. The other report said that essential oil from sirih hijau had antioxidant activity [9].

In this research, we search the activity of essential oil from sirih merah and sirih hijau against tyrosinase, monophenolase and diphenolase activities. The results showed that sirih merah and sirih hijau gave different activity. The activity of sirih merah and sirih hijau essential oils against tyrosinase in monophenolase and diphenolase were shown in Fig 4 and 5, respectively.

Table 1. Components of Sirih Merah and Sirih Hijau Essential oils

Retention time (min)	Major Components	
	Sirih Merah	Sirih Hijau
2.9	Alpha thujene	Alpha thujene
3.1	Alpha pinene	Alpha pinene
3.3		Camphene
3.7	Sabinene	Sabinene
3.9	Beta myrcene	Beta myrcene
4.4	Alpha terpinene	Alpha terpinene
4.7	Beta phellandrene	Beta phellandrene
5.2	Gamma terpinene	Gamma terpinene
5.4	Beta terpineol	Beta terpineol

5.8	Terpinolen	Terpinolen
8.5	Alpha terpineol	Alpha terpineol
12.4		Chavicol
13.1		Eugenol
13.2	Copaene	
14.4	Caryophyllene	Caryophyllene
15.3	Alpha Caryophyllene	Alpha Caryophyllene
16.0	Germacrene D	Germacrene D

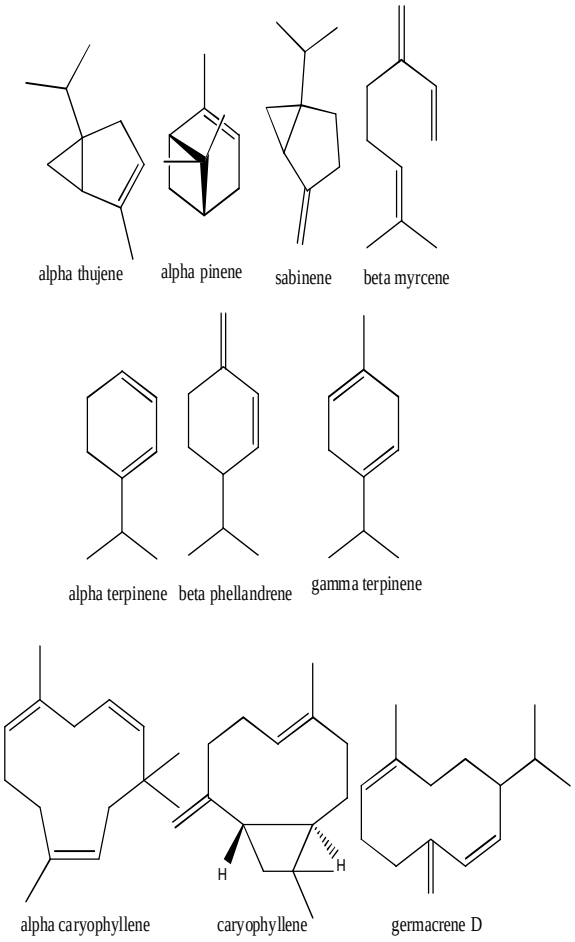


Fig 2. Major component found in *P. crocatum* and *P. bettle.*

On monophenolase activity (Fig 4), sirih merah increased the activity of monophenolase of tyrosinase. By increasing the concentration of sirih merah essential oil, the activity of monophenolase was increasing too. The increasing monophenolase activity in the present of sirih merah essential oil was up to 16 times (at concentration 7.00mg/ml) compared to control (normal activity). Different with sirih merah, sirih hijau essential oil inhibited the activity of monophenolase. The inhibition of sirih hijau essential oil was increasing by increasing the concentration (almost 50% inhibition at concentration 7.00 mg/ml).

The same tendency was found on diphenolase activity. Sirih merah essential oil had activity to increase diphenolase activity up to 2.5 times at concentration 7 mg/ml, while sirih hijau inhibited diphenolase activities of tyrosinase.

The differences between sirih merah and sirih hijau essential oils were only on phenylpropanoid (such as chavicol and eugenol) which found on sirih hijau and not found in sirih merah. It concluded that prenulated phenol can inhibit the tyrosinase activity while monoterpene and sesquiterpene can accelerated the tyrosinase activity.

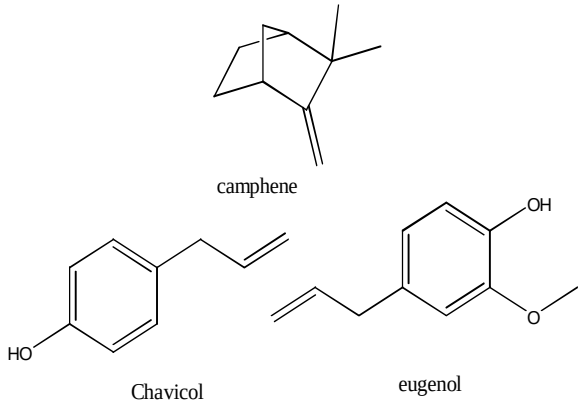


Fig 3. Major component which only found in *P. bettle*

Tyrosinase activity is important because it synthesized tyrosine by oxidation of phenol became melanin. Melanin is a pigment widely distributed in body surface, retina, nigra of brain, adrenal medullae, and so on. Moreover, it is thought to play an important role in skin cancer prevention by protection of cells from ultraviolent rays. While, it is said that melanin is a reason of sunburn and mottle. Therefore, compounds inhibiting melanin are expected to application of cosmetic as whitening agent.

On the other hand, tyrosinase produces DOPA. Martorana et al (2008) reported that L-DOPA modulates motor cortex excitability in Alzheimer's diseases patients [10]. In addition, L-DOPA might represent a reliable tool to study new therapeutic perspective and strategies of Alzheimer's diseases.

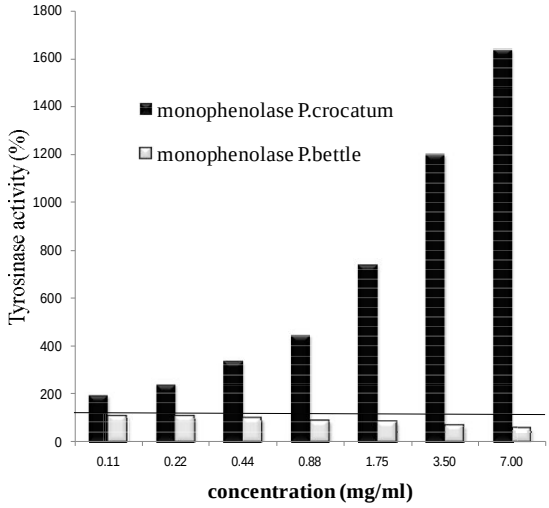


Fig 4. Monophenolase activity in the present of *P. crocatum* and *P. betle* essential oil

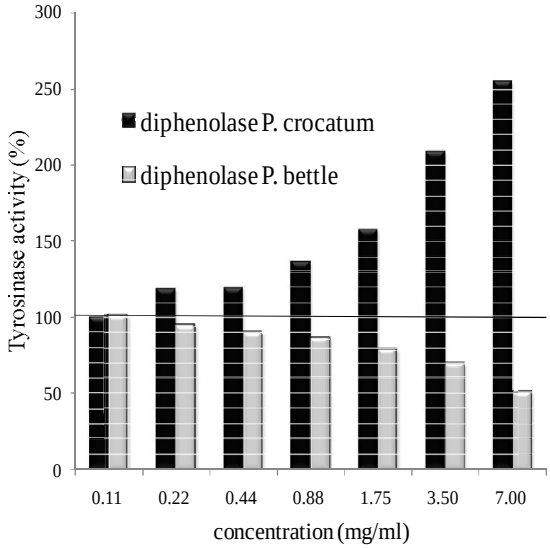


Fig 5. Monophenolase activity in the present of *P. crocatum* and *P. betle* essential oil

Based on our results, phenylpropanoid from sirih hijau essential oil can be used as whitening agent. Monoterpene and sesquiterpene from sirih merah and sirih hijau can be used for Alzheimer diseases.

4. Conclusion

Sirih merah essential oils consisted of monoterpene and sesquiterpene compounds, while sirih hijau essential oils consisted of monoterpene, sesquiterpene, and phenylpropanoid compounds. Monoterpene and sesquiterpene from sirih merah and sirih hijau essential oils can be used for Alzheimer diseases, while phenylpropanoid from sirih hijau can be used in cosmetics for whitening agents.

5. Acknowledgements

We express our gratitude to the Chemistry of Natural Products Laboratory, Gifu University, Japan for GC-MS analyses and Biopharmaca Research Center, Bogor Agricultural University for research funding.

6. References

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