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COMPARATIVE STUDY OF SINENSETIN COMPOUND IN THE LEAVES OF *ORTHOSIPHON STAMINEUS* BENTH. FROM DIFFERENT REGIONS OF INDONESIA

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ABSTRACT

The optimal growth of plant can be influenced by various factors, including the growing place. Because it could influenced the metabolites biosynthesis. This aimed study is to comparison the metabolite level, such as sinensetin of *O. stamineus* leaves from three regions in Indonesia (Tawangmangu, Batu and Trenggalek). The determination of sinensetin using TLC-densitometry with silica gel as solid phase and Chloroform-ethylaceate (60:40) as solvent system. The result showed the percentage of sinensetin in samples Tawangmangu, Batu and Trenggalek region were 0.04 ± 0.01; 0.07± 0.01 and 0.14 ± 0.01 respectively. It can be concluded that the best planting area that can produce fertile *Orthosiphon stamineus* with the highest content of sinensetin was in Trenggalek region.

KEYWORD: *Orthsiphon stamineus*, Sinensetin, planting, regions

INTRODUCTIONS

Orthosiphon stamineus Benth of Lamiaceae family is a medicinal plant that widely grown in tropical areas. The plant can be identified by its white or purple colored flowers that resembles cat whiskers. It is also commonly referred as "Kumis Kucing" which means cat whiskers. The herb is popularly known as Java tea and is used widely in the form of herbal tea in Asia.[1]

The leaves of this plant are used in traditional medicine as a diuretics, rheumatisme, urinary tract infection, kronic and acute kidney infection and billiary lithiasis.[2] There are many

chemicals found in the O. Stamineus plant. Such as caffeic acid derivatives (rosmarinic acid and 2,3-dicaffeoyltartaric acid), cichoric acid, lipophylic flavones such as eupatorine (6-hydroxy-5,7,4-trimethoxyflavone), 3'-hydroxy-5, 6, 7, 4' tetramethoxyflavone (TMF), and sinensetin (5, 6, 7, 3', 4'-pentamethoxyflavone) as marker compound of this plant.[3]

Plant secretes metabolites that are used for growth, reproduction, defense from other organisme attacks and for survival in place of growth that often experience environmental changes. Environmental factors are very influential in the regulation of plant metabolite biosynthesis.[4] Cultivation areas can significantly affect the quality of the herb. Therefore, in this research, we evaluate the comparison study of sinensetin compound in the leaves of *O. stamineus* that cultivated in different regions in Indonesia, such as Tawangmangu, Trenggalek and Batu City. Through this research is expected to know the best planting area that can produce fertile plants with the highest content of sinensetin.

MATERIAL AND METHODS

Plant material

O. stamineus Benth leaves were collected from Batu, East Java; Trenggalek, East Java and Tawangmangu, Central Java, Indonesia. The plant was identified and the voucher spesimen was deposit in the herbarium of the faculty of Pharmacy, Universitas Airlangga. It was dried at cool temperature (20° C). The dried leaves were pulverized by using mesh size 500 μm.

Material and reagents

Methanol p.a (Mecrk,Germany), ethyl acetate p.a (Mecrk,Germany), Chloroform p.a (Mecrk, Germany) and ethanol p.a (Mecrk,Germany). Sinensetin standard (Sigma), silica gel GF254 for thin layer chromatography preparations was purchased from Merck, Germany.

Instrumentation

The CAMAG linomat 5, an automatic TLC sampler, TLC visualizer; and The CAMAG analyzer, a reflectance spectrometer equipped by monitoring range 250 - 400 nm. All of the instruments were controlled by win CATS manager software.

Preparation of samples

Approximately 1 g of the pulverized dried leaves of O. stamineus from 3 district were accurately weighed and added to ethanol (25mL). After ultrasonication for 1 hour in ultrasonic cleaning bath, it were filtered. Standard solution (sinensetin) was prepared for the

construction of caliberation curves and quality control of the samples. Sinensetin was prepared in methanol at a consentration of 15; 20; 30 and 60 µg/mL, respectively.

Chromatography conditions

Standards and samples were applied to the plates by means of linomat (camag) equipped with syringe size 100 µl, band length of the spot is 4.0 mm, the application volume of standards and samples were 1 µL respectively. Spot per plate were applied 8 mm from the bottom edge, 14 mm from left edge was first application and 11.4 mm apart distance between spot. The plate was developed at room temperature in an saturated gkass twin through a chamber in solvent system chloroform-ethyl acetate (60:40). After separations, the plate was dried in a warm air. The plate was removed, dried and the spots were visualized by means of TLC visualizer under UV lamp 366 nm. The chromatograms scanned with the spectrodensitometer at the maximum absorbance of sinensetin.

RESULT AND DISCUSSION

The chromatograms of the samples were visualized in UV light 366 nm by TLC visualizer. Then, scanned the plate by densitometer camag scanner 3. It showed the presences of the spots with the same color and the same line of Rf values as of the standard (Fig 1 & 2). While Fig 3. Shows the UV spectra profil of the samples and the standard, from this data we can observed that each samples gived the same profil of spectrum in each samples, and same maximum wavelength with the sinensetin standard (334 nm).

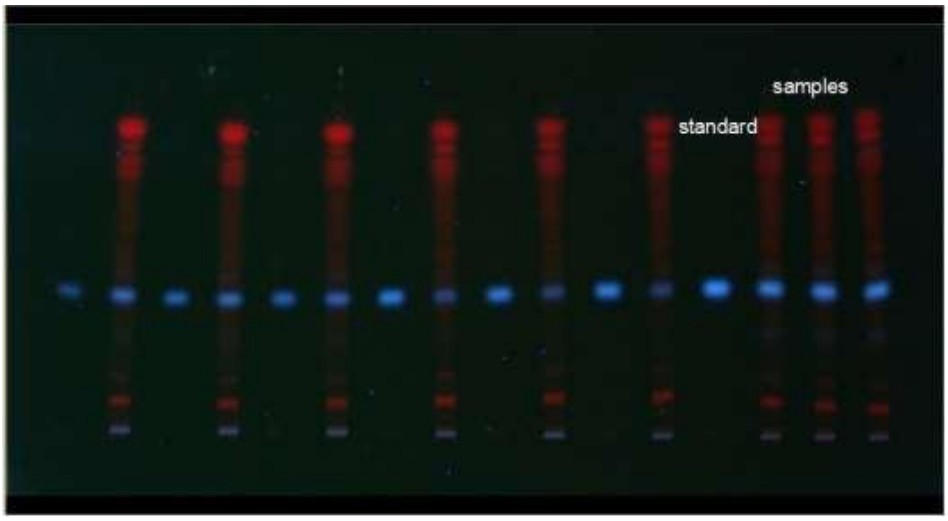

Fig 1: The chromatograms imaging of the samples and sinensetin standards in UV light 366 nm.

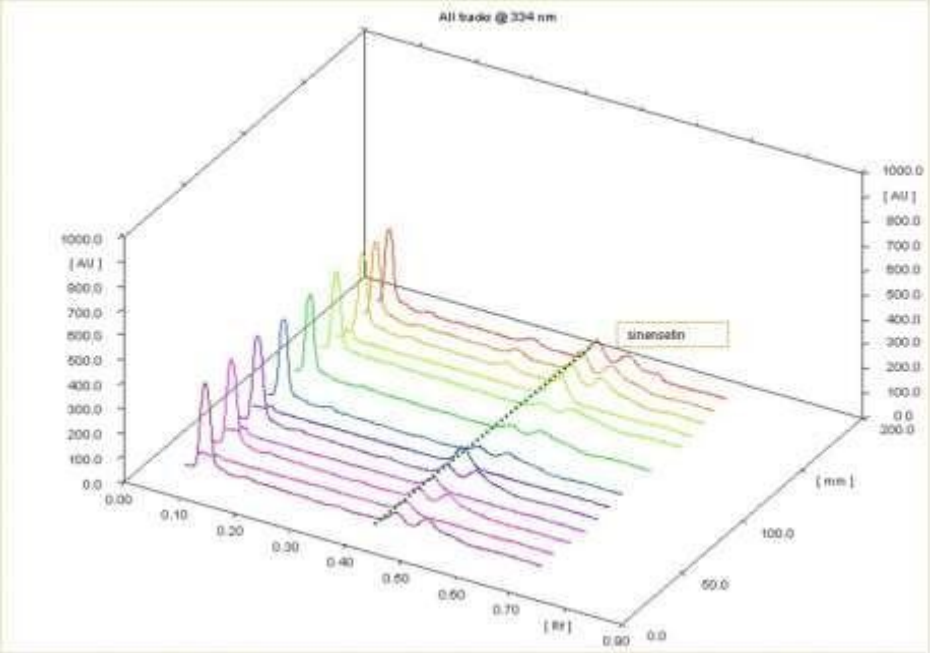

Fig 2: 3D Chromatograms of the samples and sinensetin standard observed at 334 nm.

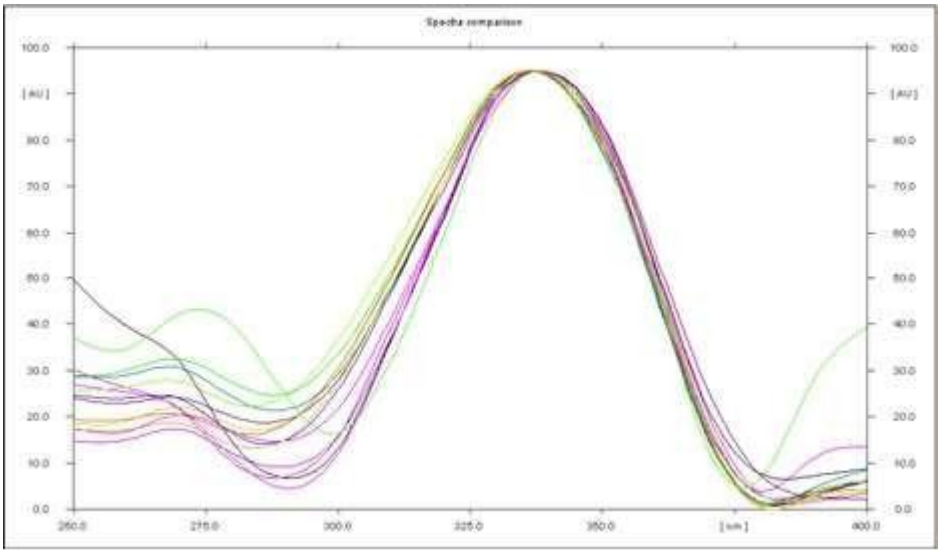

Fig 3: Spectra profil of sinensetin in standard and samples *O. stamineus* leaves with scanned range 250-400 nm. It showed maximum wavelength at 334 nm.

The quantitative determination of sinensetin was done by TLC Densitometry using calibration curve method. The calibration curve already prepared with known consentrations of sinensetin, was read using the winCATS software programme (Fig 4).

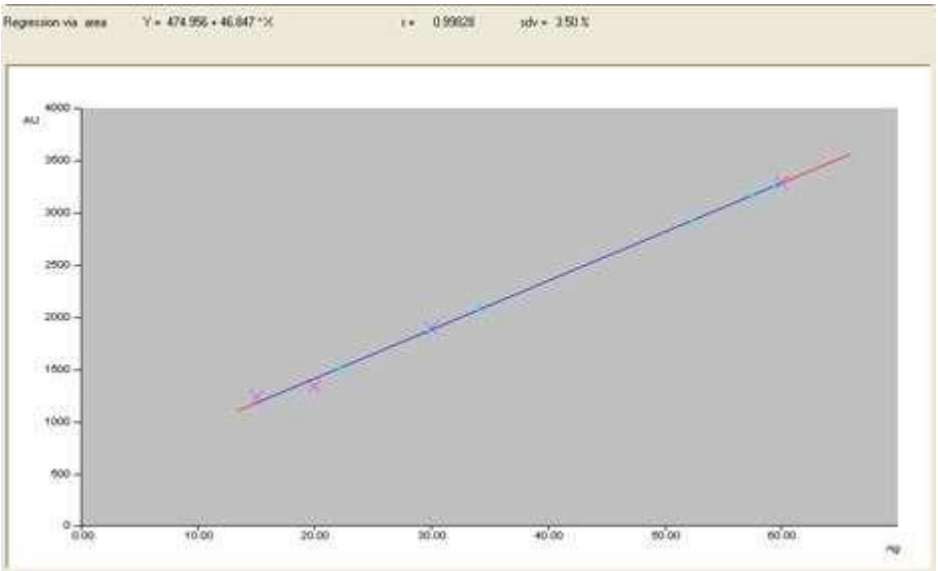

Fig 4: Calibration curve for sinensetin determination in each samples.

Table 1: The calculated % sinensetin for the standard and for the separated spot from samples.

Samples/standard (sinensetin)	Consentration	Rf values	%of sinensetin ± SD
Standard 1	15 ppm (1 µl)	0.43	-
Standard 2	20 ppm (1 µl)	0.43	-
Standard 3	30 ppm (1 µl)	0.43	-
Standard 4	60 ppm (1 µl)	0.43	-
O. stamineus leaves Batu city area (3 rep)	1µl	0.43	0.07 ± 0.01
O. stamineus leaves Tawangamngu area (3 rep)	1 µl	0.43	0.04 ± 0.01
O. stamineus leaves Trenggalek area (3 rep)	1 µl	0.43	0.14 ± 0.01

From the result of sinensetin percentage in the leaves of *O. Stamineus* (Table 1).It can be showed that *O. stamineus* leaves from Trenggalek area is highest than Tawangmangu and Batu City region. It proved that the growth of *O. stamineus* can be influenced by various factors, including the height of the growing place and the intensity of light.[5] From the data based it informed that *O.stamineus* grows optimally in the area with an altitude between 500-900 above sea level and the ideal rainfall more than 3000mm/year, with full sun shade without shade. Because the presence of shade will lower levels of chemical content in the leaves. The state of the air temperature is good for the growth of the cat's whiskers plant is hot to medium.[6]

This result study is in line and appropriate with topography condition of each region. Trenggalek is located on the south coast and has the northern boundary district ponorogo, east with tulungagung district, the west with pacitan districts and the south with the Indian

Ocean. So in this region get full sun shade more than others regions (Tawangmangu and Batu). Trenggalek is located at an altitude of 0-690 m above sea level and annual rainfall 3000 mm/year. It was optimal condition to growth well.

While Batu city is located on East Java, Indoensia. It has a topography of mountains and hills with the above sea level of 600-3000 m.The minimum temperature is 18-24 and the maximum temperature is 28-32. The humidity of the air is about 75-98% and the average rainfall is 875-3000 mm/year.[6]

And Tawangmangu, the highest area in Karang anyar, Central Java, located at an altitude of 2000 m above sea level. Tawangmangu is a national strategic road connecting two district in two provinces, Karang anyar in Central Java and Magestan in East Java.

The topography of the area is relatively high due to stiff hill and valey.[7]

CONCLUSION

Based on comparative study in three different region, it can be said that the best planting area that can produce fertile *Orthosiphon stamineus* with the highest content of sinensetin was in Trenggalek.

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