ผลการประเมินคุณภาพวารสารที่อยู่ในฐานข้อมูล TCI

โปรดระบุหมายเลข ISSN หรือชื่อของวารสารที่ต้องการทราบผลประเมิน : ค้นหา

ลำดับ	ชื่อวารสาร	ISSN	เจ้าของ	จัดอยู่ในวารสารกลุ่มที่	สาขา
1	วารสารวิชาการมหาวิทยาลัยอีสเทิร์นเอเชีย ฉบับวิทยาศาสตร์และเทคโนโลยี	2286-6175	มหาวิทยาลัยอีสเทิร์นเอเชีย	1	วิทยาศาสตร์และเทคโนโลยี

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ที่ สหก.ว. 019 /2562

แบบตอบรับการตีพิมพ์บทความ
ลงวารสารวิชาการ มหาวิทยาลัยอีสเทิร์นเอเชีย (EAU Heritage Journal)

วันที่ 19 เมษายน 2562

เรียน อาจารย์รุจิรา คุ้มทรัพย์
อาจารย์นันทรักษ์ รอดเกตุ

ตามที่ท่านได้ส่งบทความเรื่อง "Establishment of Methyl Anthranilate Quantification by UV-Spectroscopy and Identification Using FTIR (การพัฒนาวิธีการวิเคราะห์หาปริมาณเมทธิลแอนทรานิเลตในพืชสมุนไพรด้วยวิธี UV-Spectroscopy และพิสูจน์เอกลักษณ์ด้วย FTIR)" เพื่อเสนอตีพิมพ์ลงในวารสารวิชาการมหาวิทยาลัยอีสเทิร์นเอเชีย โดยผ่านการกลั่นกรองจากผู้ทรงคุณวุฒิ (Peer Reviewers) และกองบรรณาธิการได้พิจารณาดังนี้

(✓) มีความยินดี ตีพิมพ์บทความลงในวารสารวิชาการ มหาวิทยาลัยอีสเทิร์นเอเชีย
ฉบับวิทยาศาสตร์และเทคโนโลยี
ปีที่ 13 ฉบับที่ 3 เดือน กันยายน – ธันวาคม พ.ศ. 2562

( ) ไม่สามารถตีพิมพ์บทความลงในวารสารวิชาการ มหาวิทยาลัยอีสเทิร์นเอเชีย
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ขอแสดงความนับถือ

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ฉบับวิทยาศาสตร์และเทคโนโลยี

กองบรรณาธิการวารสาร EAU Heritage
โทรศัพท์ 0-2577-1028 ถึง 31 ต่อ 377, 378
โทรสาร 0-2577-1053

Establishment of Methyl Anthranilate Quantification by UV-Spectroscopy and Identification Using FTIR
การพัฒนาวิธีการวิเคราะห์หาปริมาณเมทธิลแอนทรานิเลตในพืชสมุนไพรด้วยวิธี UV-Spectroscopy และพิสูจน์เอกลักษณ์ด้วย FTIR

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Received: January 7, 2019
Revised: April 17, 2019
Accepted: April 19, 2019

Abstract

The objective of this research was to develop the extraction method of methyl anthranilate from herbal (medicinal) plants ginger, galangal, and lemongrass, with 2 types of solvents, 1% (v/v) sulfuric acid in methanol and 1 M sulfuric acid in water. The absorbtion of the extracts were measured and compared to the standard curve of methyl anthranilate with a wavelength of 273 nanometers. The study found the extractions with the two methods yielded parallel results. And the highest amount of methyl anthranilate of galangal, lemon grass and ginger with 1% (v/v) sulfuric acid in methanol were 6.813, 1.349 and 0.606% w/w fresh plant, respectively. The accuracy resulted in the average percentage of recovery from between 97.2-104.3% and the precision result in the percentage of the relative standard deviation (%RSD) with the concentration 30 mgL^{-1} were 3.6, 3.3 and 0.8% of ginger, galangal and lemongrass, respectively. The results of the percentage yield analysis of the methyl anthranilate with the diazotization reaction showed that the standard methyl anthranilate substance was 58.97% and was sufficient to prove the identity of the diazotization reaction with the FTIR technique.

Keywords: extraction, herbal plant, methyl anthranilate, UV-spectroscopy, Fourier transform infrared spectroscopy

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาวิธีการสกัดสารเมธิลแอนทรานิเลทจากพืชสมุนไพรจาก ขิง ข่า และตะไคร้ ด้วยตัวทำละลาย 2 ชนิดคือ 1 % (v/v) ซัลฟิวริกในเมทานอล และ 1 M ซัลฟิวริกในน้ำ สารสกัดที่ได้นำไปวัดค่าการดูดกลืนแสงเทียบกับกราฟมาตรฐานเมธิลแอนทรานิเลทที่ความยาวคลื่น 273 นาโนเมตร พบว่าการสกัดทั้งสองวิธีให้ผลสอดคล้องกัน และสารสกัดที่มีปริมาณสารเมธิลแอนทรานิเลทมากที่สุดคือ ข่า เท่ากับ 6.813 %w/w พืชสด รองลงมาคือ ตะไคร้ และขิง เท่ากับ 1.349 และ 0.606 %w/w พืชสด ที่สกัดด้วย 1% (v/v) ซัลฟิวริกในเมทานอล ตามลำดับ ผลจากการวิเคราะห์ความถูกต้องของการวัดได้ค่าเปอร์เซ็นต์การกลับคืนอยู่ระหว่าง 97.2-104.3% และความเที่ยงในการวัดได้ค่าส่วนเบี่ยงเบนมาตรฐานสัมพัทธ์ที่ความเข้มข้น 30 mgL^{-1} ในขิง ข่าและตะไคร้ เท่ากับ 3.6, 3.3 and 0.8% ตามลำดับ และจากการศึกษาการวิเคราะห์สารเมธิลแอนทรานิเลทด้วยปฏิกิริยาไดอะโซไทเซชัน ผลของการวิเคราะห์ค่าร้อยละผลผลิตสารมาตรฐานเมธิลแอนทรานิเลทเท่ากับ 58.97 เปอร์เซ็นต์ และสามารถพิสูจน์เอกลักษณ์ของปฏิกิริยาไดอะโซไทเซชันด้วยเทคนิค FTIR

คำสำคัญ: การสกัด, พืชสมุนไพร, เมทิลแอนทรานิเลท, ยูวีสเปกโทรสโกปี,ฟูเรียร์ทรานฟอร์มอินฟราเรดสเปกโทรสโกปี

Introduction

Methyl anthranilate (MA) is an ester of anthranilic acid. Its chemical formula is $C_8H_9NO_2$. It has a melting point of 24 °C, a boiling point of 256 °C, and a density of 1.17 g/mL^{-1} (Lanzafame et al., 2017). Methyl anthranilate is a phytochemical in plants that is useful both for medicinal properties and as a food additive found in concord grapes, jasmine, bergamot, lemon, orange, strawberries (Lanzafame et al., 2017), rice (Primus, et al., 1995), honey (Nozal et al., 2001; Sesta et al., 2008), and Japanese tea (Sawai et al., 2004). In many countries, methyl anthranilate is used as a catalyst in the paper industry, as a substrate in many colors, pigments and bird repellent (Yadav & Krishnan, 1998).

Moreover, methyl anthranilate also has a grape-like odor, used for flavoring candy and soft drinks such as grape soda, chewing gum, drugs and nicotine products (Avery, 2002). Methyl anthranilate is both a component of many natural essential oils and synthetic chemical odors. It is widely used for bird repellent (Clark, 1998; Clark et al., 2000; Homan & Linz, 2014). Most synthetic materials identified to date that deter birds have unacceptable toxic and environmental properties (Clark et al., 1993; Clark & Aronov, 2000).

The function of methyl anthranilate is registered as an avian repellent and the compound is mainly used in the United States to protect crops (Esther et al., 2012). It is a food and can be used to prevent bird damage to sunflowers, rice, fruit and golf courses (Avery, 1992; Avery et al., 1996). Early analytical methods for methyl anthranilate, reported by Hesse and Zeitschel and Erdmann in 1901 applied to essential oils and used gravimetry and titrimetry (Thompson & Quaife, 2001). Gas Chromatographic--GC methods for methyl anthranilate in wine in 1976 and Concord grape essence in 1967 have been reported, and fluorometry has been used for the analysis of Concord grape juice in 1976 (Thompson & Quaife, 2001). During

the development of a fluorimetric detector for Liquid Chromatography--LC. Colorimetric analysis involving an adaptation of the AOAC method based on diazo coupling of the primary amine function of methyl anthranilate was applied to several grape juice products and essences and compared with gas chromatographic analysis in 1990 and analyzing methyl anthranilate for High Performance Liquid Chromatography--HPLC have been reported by in 2008 (Sesta et al., 2008). This research was to develop a simpler and more rapid means of determining methyl anthranilate by using UV-spectroscopy method for some herb plant such as lemongrass ginger and galangal oils which have been reported as insect repellent compositions (Abdullah et al., 2015; Shukla et al., 2018).

Objectives

The objectives of this study were to develop the extraction method of methyl anthranilate from herbal plants such as ginger, galangal and lemongrass by using UV-spectroscopy method and diazotization reaction identification using FTIR.

Materials and Methods

1. Raw materials and chemicals

Herbal plants (ginger rhizome, galangal rhizome and lemongrass) from Phetchabun market area, Methyl Anthranilate (MA), (C8H9NO2), 99%, ACROS Organics™, USA.

2. Raw Materials Preparation

The ginger, galangal and lemongrass were washed with tap water and then dried at room temperature. Sample plants were cut into medium pieces, followed by spinning the pieces to fine particles. The sample plants were stored in a beaker at 4°C.

3. Determination of Methyl Anthranilate

3.1 Determination of Optimal Wavelength

In determination of the optimal wavelength for the methyl anthranilate analysis by using 20 mgL^{-1} methyl anthranilate standard in 1% (v/v) sulfuric acid in methanol and 5 mgL^{-1} in 1 M sulfuric acid, and then the samples were measured at 190-450 nm with a Shimadzu UV-Vis Spectrophotometer.

3.2 Calibration Curve

The first method involves the preparation of standard solutions of methyl anthranilate with 15, 20, 25, 30, 50, 100, 120 and 150 mgL^{-1} and then dissolution in 1% (v/v) sulfuric acid in methanol. The second method involves preparation of standard solutions of methyl anthranilate with 5, 10, 15, 20, 30, 50, 60 and 100 mgL^{-1} 1 in 1 M sulfuric acid and then measurement by UV-Vis Spectrophotometer.

3.3. Extraction of plant samples

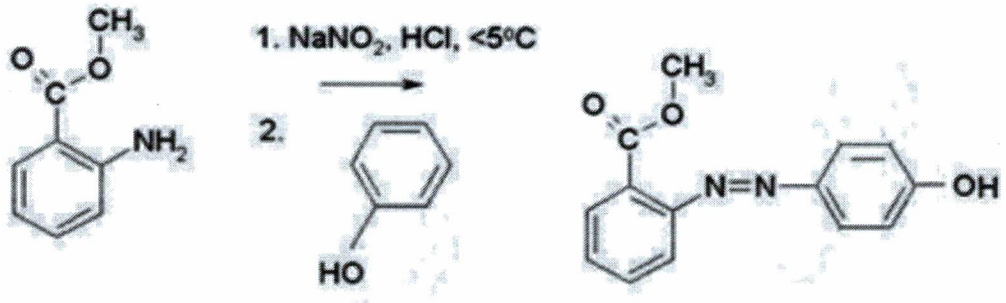
Three herbal plants were used: ginger, galangal and lemongrass. Two solvents were used: 1% (v/v) H_2SO_4 in methanol and 1 M H_2SO_4. Place 2 g of blended sample plants in a test tube. Add 10 mL of solvent and shake with vortex for 10 s. Extract in an ultrasonic bath for 20 minutes and isolate the solution with centrifuge at 1500 rpm for 30 min. Keep the plant extracts in the test tube and close the stopper. Finally, measure the samples with a UV-Vis Spectrophotometer and calculate the amount of methyl anthranilate by comparisons with the dilution factor.

4. Determination of Methyl Anthranilate by Diazotization

4.1 Diazonium Salt Preparation Place a mass of 0.5 g methyl anthranilate standard in a 125 mL conical flask. Prepare the Hydrochloric acid--HCl (3x of the number of methyl anthranilate mole). Pipette 0.3 mL to dissolve in 15 ml distilled water and add the solution to the methyl anthra-

nilate soaked in an ice bath at 0-5°C. Stir the sodium nitrite ($NaNO_2$) solution for 30 minutes, and then, prepare the sodium nitrite solution (1x of the number of methyl anthranilate mole) with 0.2277 g in a few drops of distilled water. Drop the sodium nitrite solution into a methyl anthranilate solution, and finally, stir the solution slowly for 30 minutes at 0-5 °C.

4.2 Coupling Reaction Prepare the phenol solution (0.5x of the number of methyl anthranilate mole) with 0.1553 g in 10 mL distilled water, add it to the prepared solution, and then stir for 10 minutes. Adjust the pH to approximately 5 with a 0.1 M sodium carbonate solution and then stir for another 10 minutes. After that, adjust the pH to approximately 8 with a 0.1 M sodium carbonate solution and then stir for another 10 minutes. Add sodium chloride (NaCl) to get the precipitated azo dye. Then, filter the color with filter paper No. 42 and steam at 100°C for 3 hours. Next, weigh and calculate the %yield of the reaction as shown in Figure 1.



Methyl anthranilate Azo dye *(Orange)*

Figure 1 Diazotization in the synthesis of azo dyes from a methyl anthranilate standard

5. Identification Validation of Methyl Anthranilate and Azo Dye by the Fourier Transform

Infrared Spectrometer--FTIR Technique Add drops of methyl anthranilate standard and azo powder to the sample base, and then, analyze by FTIR version Spectrum TWO.

Results and Discussion

1. The study result of Optimal Wavelength for Methyl Anthranilate Determination

The results of the optimal wavelength for the determination of methyl anthranilate that was scanned at 190-450 nm with a UV-Vis Spectrophotometer are shown in Figures 2 and 3.

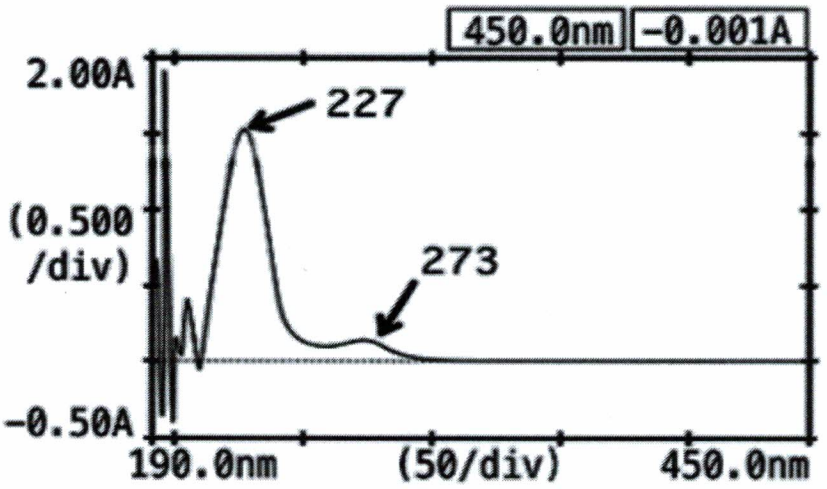


Figure 2 UV-Vis absorption spectra of 20 mgL^{-1} MA in 1% (v/v) H_2SO_4 in methanol

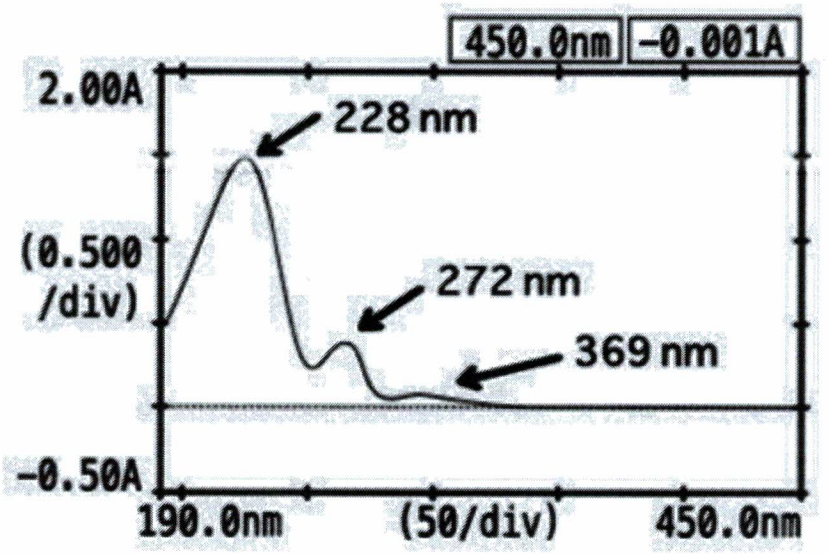


Figure 3 UV-Vis absorption spectra of 5 mgL^{-1} MA in 1 M H_2SO_4

From Figure 2, the spectra of method 1 at a concentration of 20 mgL^{-1} in 1% (v/v) H_2SO_4 in optimal methanol has the highest absorbance at two wavelengths: 227 nm and 273 nm. From Figure 3, the spectra of method 2 at a concentration of 5 mgL^{-1} has the highest absorbance at 3 wavelengths: 228 nm, 272 nm and 369 nm. Primus et al., (1995) reported the UV spectrum of methyl anthranilate, found the highest absorbance at 3 wavelengths: 220, 248 and 336 nm which are close to the values discovered in our research. Askham (1992) determined methyl anthranilate Ultraviolet--UV

range (200-320 nm) in a light cabinet maintained at 29.5°C. However, in the selection of absorbance, it was found that the 227 and 228 nm wavelength had the best absorption, but with disturbance. On the other hand, the wavelength of 369 nm had no disturbance but low the sensitivity to measure for absorbance. Therefore, a wavelength of 273 nm was selected as the optimal wavelength for the determination of the methyl anthranilate in this study.

2. The Results of Methyl Anthranilate Determination

The measured absorbance of the methyl anthranilate in ginger, galangal and lemongrass extracted by 1% (v/v) H_2SO_4 in methanol and 1 M H_2SO_4 with the methyl anthranilate standard at a 273 nm wavelength.

Table 1

Value obtain in the validation parameter of the method of the determining of Methyl Anthranilate

Solvents	Calibration range (mgL^{-1})	Calibration curve	Coefficient of determination (r^2)	LOD (mgL^{-1})	LOQ (mgL^{-1})
1%(v/v) H_2SO_4 in Methanol	15-150	y=0.0070x+0.0142	0.9960	4.89	16.30
1M H_2SO_4 in Water	5-100	y=0.0086x+0.0774	0.9927	2.98	4.98

Table 1, calibration curve of the methyl anthranilate concentration vs. absorbance using 1% (v/v) H_2SO_4 in methanol as a solvent reveals that the linear equation is y=0.0070x+0.0142 and r^2=0.9960, and with 1 M H2SO4 the linear equation is y=0.0086x+0.0774 and r^2=0.9927. A comparison of the solvent used by linear regression analysis shows that the extracts with the 1% solvent (v/v) of H_2SO_4 in methanol have more linearity.

The value comparisons of the linear regression data of the methyl anthranilate concentration and absorbance. We found that a good slope value should be high if it has good measurement sensitivity. The slope of 1 M H_2SO_4 (0.0086) is steeper than the 1% (v/v) H_2SO_4 solvent in methanol (0.007). In addition, the closer the y-intercept is to 0, the more linear the graph will be. From the table, the y-intercept value of the solvent 1% (v/v) H_2SO_4 in methanol is the closest to zero, so it is a good trend for determination.

In addition, r^2, or the coefficient of determination, is a value that represents the degree of correlationbetween the two variables close to one. The coefficient of determination shows that both variables are significantly correlated,

and in r^2, the determination should not be less than 0.98. The data of calibration curve, the LOD and LOQ can be detected and quantified at 4.89 mgL^{-1} and 16.30 mgL^{-1}, respectively for 1% (v/v) H_2SO_4 solvent in methanol and for 1 M H_2SO_4, 2.98 and 4.98 mgL^{-1} respectively.

3. Method Validation

We determined the validity and accuracy of the methods by taking standard samples that were analyzed in triplicate. The %recovery and %RSD were analyzed by the spiking 30 $mgL^{-1}$of methyl anthranilate standard into sample blank in 1% (v/v) H_2SO_4 solvent in methanol.

Table 2

Recovery Validation for Determination of Methyl Anthranilate

Herbal Plants	Replications	Determined (mgL^{-1})	Recovery (%)	Average Recovery (%)	%RSD
Ginger	replicate 1	28.01	93.4	97.2	3.6
	replicate 2	29.32	97.7		
	replicate 3	30.12	100.4		
Galangal	replicate 1	31.54	105.1	104.3	3.3
	replicate 2	32.13	107.1		
	replicate 3	30.18	100.6		
Lemongrass	replicate 1	31.21	104.0	103.1	0.8
	replicate 2	30.83	102.8		
	replicate 3	30.72	102.4		

The validation results of the method by the spiking of the standard methyl anthranilate at a concentration 30 mgL^{-1} in the sample are shown in Table 2, which shows that the accuracy result in the average% recovery was between 97.2-104.3% and the precision results in the percentage of the relative standard deviation (%RSD) with the concentration 30 mgL^{-1} were 3.6, 3.3 and 0.8% of Ginger, Galangal and Lemongrass, respectively. It was found that the %RSD was <5 which can be acceptance criteria.

Our study produced the values of the methyl anthranilate extracted from ginger, galangal and lemongrass that were extracted with 1% H_2SO_4 and 1 M H_2SO_4 dissolved in different amounts of solvent, which were used to measure the absorbance at a wavelength of 273 nm. After that, the absorbance values were compared with the methyl anthranilate standard curve to calculate the concentration of the methyl anthranilate. The results are shown in Table 3.

Table 3

Values of Methyl Anthranilate in herbal plants in % w/w.

Solvents	Herbal Plants	% w/w (MA in plants per fresh herb) n=3
1%(v/v) H_2SO_4 in Methanol	Ginger	0.605±0.018
	Galangal	6.813±0.021
	Lemongrass	1.349±0.012
1M H_2SO_4 in Water	Ginger	0.192±0.007
	Galangal	0.700±0.006
	Lemongrass	0.426±0.005

Table 3 presents the values of the methyl anthranilate extracted from the herbal plants in %w/w for two different solvents. From the calculation of 2 g of fresh herbs, the highest values of methyl anthranilate were found in galangal, followed by lemongrass and ginger, for both solvents. The determination of methyl anthranilate from ginger, galangal and lemongrass extracted with 1% (v/v) H_2SO_4 in methanol shows that fresh galangal has the highest value of methyl anthranilate with 6.813 %w/w, followed by fresh lemongrass and fresh ginger with 1.349 and 0.605 %w/w,respectively. On the other hand, the 1 M H_2SO_4 solvent extraction method shows that fresh galangal has the highest value of methyl anthranilate with 0.700% w/w, followed by fresh lemongrass and fresh ginger with 0.426 and 0.192% w/w, respectively.

This research is preliminary study which apply the extract obtained from the herbs will be used for an experiment with birds to test the aversive behavior and exposures to methyl anthranilate. Therefore, galangal will be selected to test as bird repent in next project. Consequently, Galangal is a plant in the ginger family, may repel mosquitos and other insects (Misni et al., 2016).

4. The Results of Methyl Anthranilate Determination by Diazotization.

The synthesis of azo dye from the methyl anthranilate standard is shown in Figure 4. The weight gain was calculated to find the % yield of the azo dye synthesized from the methyl anthranilate with 3 replications as shown in Table 4.

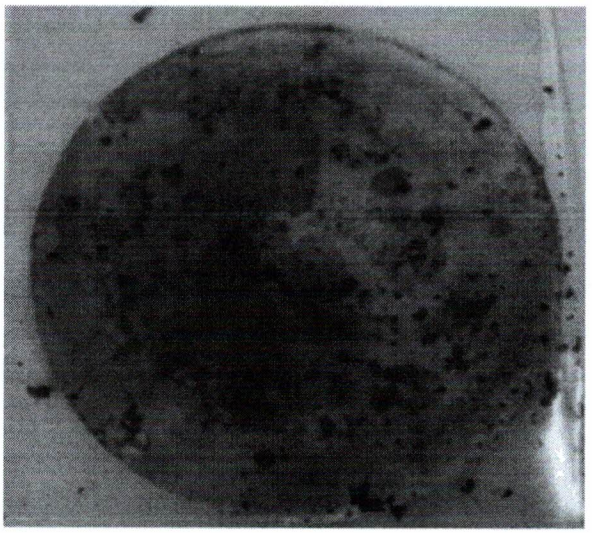


Figure 4 Azo Dye from Diazotization.

Table 4

The calculation to find the % yield of the azo dye synthesized from methyl anthranilate

Number of Times	% Yield
1	55.15
2	57.90
3	62.38
4	60.59
5	58.81
Average	58.97±2.74

The calculation to find the % yield of the azo dye synthesized from methyl anthranilate with 3 replications (Table 4) shows average 58.97% for azo dye product. Consequently, the azo dye product was synthesized by incompletion of diazotization.

Therefore, the involved parameters affect to reaction such as temperature, number of mole, and stirring, so the experiment should explore the optimal condition. Additionally, the methyl anthranilate in the extract is insufficient for the synthesis of azo dyes (Trusova, et al., 2011).

Qualitative and approximate quantitative presence of methyl anthranilate in plants can be detected by the formation of azo dye in diazotization. The relative quantity of azo dye formed reveals the relative amount of methyl anthranilate contents of plants (Athula et al., 1990)

. Diazotization was performed to identify

and compare the presence of methyl anthranilate in some fruits, vegetables and plants. The mass of azo dye formed was directly proportional to methyl anthranilate content in a sample. Therefore, the mass of azo dye formed, the relative quantitative presence (in percentage content by mass) of methyl anthranilate can be compared.

5. The Results of Identification Validation of Methyl Anthranilate and Azo Dye by Fourier Transform Infrared Spectrometer--FTIR Technique

Identification validation of the synthetic azo dye by comparison to the methyl anthranilate and azo dye standard spectrums via observation of the functional group with the FTIR technique is shown in Figures 5 and 6

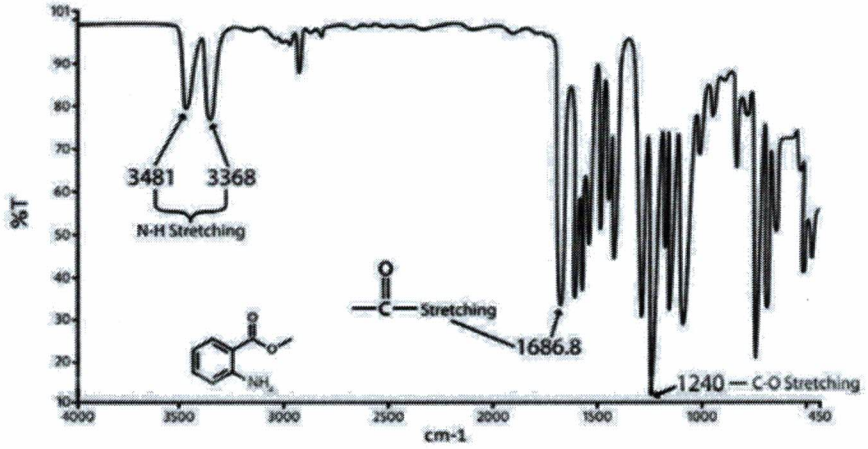


Figure 5 Spectrum of methyl anthranilate standard by FTIR

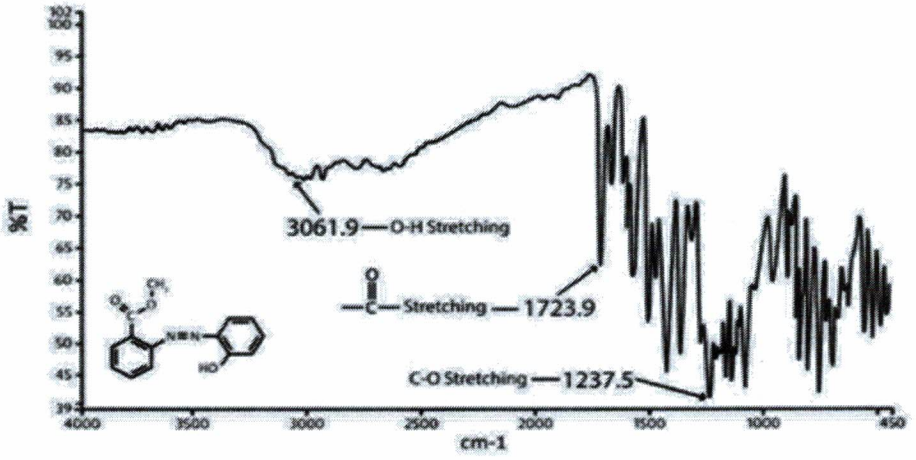


Figure 6 Spectrum of azo dye by FTIR

The absorbance spectrum of the methyl anthracene standard in Figure 5 shows the amine at frequencies of 3,481 and 3,368 cm^{-1}, the ketone at 1686.8 cm^{-1}, and the carboxylic acid at 1,240 cm^{-1}. In Figure 6, the absorbance spectrum of azo dye phenol was found at 3,061.9 cm^{-1}, and instead of amine, aldehyde at 1,723.9 cm^{-1}, and carboxylic acid at 1,237.5 cm^{-1}. Both figures show that the FTIR can be used to validate the identity of methyl anthracene and azo dye.

The FTIR can be used to validate the identity of the synthetic azo dyes by observing the group of functions in which amine disappears. For the determination of methyl anthranilate has suggested a procedure based on the diazotization of methyl anthranilate, a primary aromatic amine. The ester is washed out of the oil with dilute sul furic or hydrochloric acid, and the acid solution is then titrated with an alkaline solution of β-naphthol (Scott, 1923).

Conclusion

This study found that the extraction from galangal produces the greatest amount of methyl anthranilate, followed by lemongrass and ginger, respectively, as determined by UV spectroscopy. Moreover, from the result of the diazotization reaction, the azo dye from methyl anthranilate resulted in an orange color, which indicates that the amount of methyl anthranilate in plants can be obtained by dyeing azo (diazotization) in correspondence with the amount of azo dyes that occur in plants.

Acknowledgements

This study was financially supported by the National Research Council of Thailand and the Research and Development Institute, Phetchabun Rajabhat University.

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หนังสือยินยอม
(ให้นำบทความไปตีพิมพ์เผยแพร่ในวารสาร)

ทำที่คณะวิทยาศาสตร์และเทคโนโลยี
มหาวิทยาลัยราชภัฏเพชรบูรณ์
วันที่ ๑๓ เดือน...กุมภาพันธ์...พ.ศ...๒๕๖๒.....

ข้าพเจ้า ผู้ช่วยศาสตราจารย์ ดร.กาญจน์ คุ้มทรัพย์ สังกัดหลักสูตรสาขาวิชาวิทยาศาสตร์ศึกษา คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏเพชรบูรณ์ ที่อยู่ ๘๓ หมู่ ๑๑ ต.สะเดียง อ.เมือง จ.เพชรบูรณ์ เจ้าของบทความวิจัย เรื่อง Preliminary Determination of Methyl Anthranilate from Ginger, Galangal and Lemongrass ซึ่งเป็นผู้แต่งชื่อที่ ๒ ซึ่งมีส่วนร่วมในผลงานร้อยละ ๕๐ ของบทความวิจัย ยินยอมให้นางสาวรุจิรา คุ้มทรัพย์ ซึ่งเป็นผู้แต่ง ชื่อที่ ๑ ของบทความวิจัย สามารถนำบทความวิจัยเรื่องดังกล่าวข้างต้น นำไปตีพิมพ์เผยแพร่ในวารสารวิชาการมหาวิทยาลัยอีสเทิร์นเอเชีย ฉบับวิทยาศาสตร์และเทคโนโลยี และนำไปขอรับการสนับสนุนงบประมาณค่าตอบแทนการตีพิมพ์บทความวิจัย ในวารสารระดับชาติ ตามประกาศมหาวิทยาลัยราชภัฏเพชรบูรณ์ เรื่อง หลักเกณฑ์และอัตราการจ่ายเงินกองทุนสนับสนุนการวิจัย พ.ศ. ๒๕๕๗ ได้ จึงลงลายมือชื่อไว้เป็นหลักฐานต่อหน้าพยาน

ลงชื่อ.....(ผู้ยินยอม)
(ผู้ช่วยศาสตราจารย์ ดร.กาญจน์ คุ้มทรัพย์)

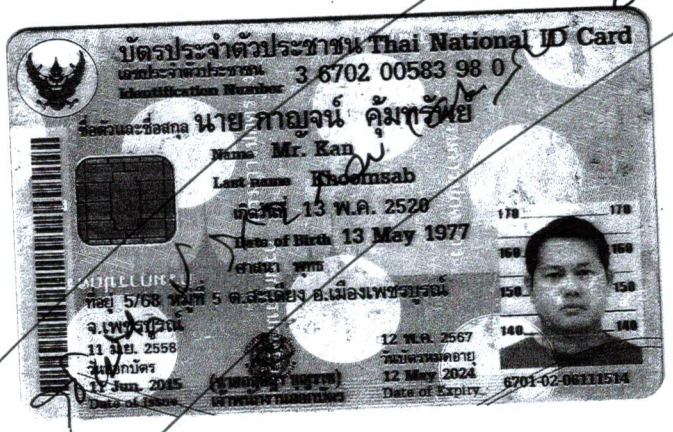
ลงชื่อ.....(ผู้ขอใช้บทความวิจัย)
(นางสาวรุจิรา คุ้มทรัพย์)

ขอรับรองเป็นลายมือชื่อของผู้ยินยอมและผู้ขอใช้บทความวิจัยจริง

ลงชื่อ.....(พยาน)
(.....)

ลงชื่อ.....(พยาน)
(.....)

หมายเหตุ *: แนบสำเนาบัตรประชาชนหรือบัตรข้าราชการหรือบัตรพนักงานมหาวิทยาลัยของผู้ยินยอม จำนวน ๑ ชุด*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วันที่บัตรหมดอายุ
12 May 2024
Date of Expiry
6701-02-06111514