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Application of Non-Destructive Devices for Determination of Alkaloid Level in Dioscorea Hispida

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Abstract The potential of microwave and imaging application in the determination of alkaloid in *Dioscorea hispida* tubers were explored. The microwave device is used to measure the coefficient permittivity of microwave level at tubers surface using mechanical probe. With the same tubers, 40 grams was selected for chemical analysis at a laboratory for the programme of East Coastal Economic Regions – Kementerian Pengajian Tinggi, Universiti Sultan Zainal Abidin, Malaysia (ECER-KPT UniSZA). The samples were selected from 12 portions of 2 plants (I and B) collected from Kampung Kudat, Ajil, Terengganu, Malaysia. The result show that the correlation between microwave level and weight of alkaloid with regression, R^2 is >0.8 is acceptable. For imaging application, the images of variety harvested of *Dioscorea hispida* will be captured, and then the images will be analysed using the developed color histogram module to determine the maximum Hue image value of *Dioscorea hispida* at different stages of viscosity. The prediction was also made on the statistical relationship between Hue and dioscorine viscosity in the fruit. The development on both studies is grouped as nondestructive method to determine the dioscorine content which is one of the alkaloid components in the tubers of *Dioscorea hispida*.

Keywords Alkaloid Of Dioscorea, Rapid Detection Device, Alkaloid Detection, *Dioscorea Hispida*, Microwave Sensor

1. Introduction

Dioscorea hispida is a poisonous plant where scientific study has shown that its tubers contain toxic alkaloid constituents, dioscorine. The tubers can only be consumed after the poisonous dioscorine is removed. This plant is commonly found in secondary forest and grows under shaded areas or near streams. *D.hispida* is one of the most economically important yam species, which serves as a staple food for millions of people in tropical and subtropical countries[1] and[2] and the only species of which most of the leaves have 3 leaflets and have no aerial tubers[3]. This plant is classified as a wild creeping and climbing plant which can grow up to 20 meters in height[4]. Traditionally, the stem of *D.hispida* has been used to treat sinus. The tubers has been reported to be rich in essential dietary nutrients[5]. This plant is also known by the local with names such as Tuba Ubi, Ubi Nasi, Ubi Cerok, Ubi Kendudok and Ubi Kipas[6]. Figure 1 show during collecting the tubers of *D.hispida* at Ajil, Terengganu, Malaysia.[7];[8] mentioned that the advancement of technology should be introduced for agriculture research and for *D.hispida* the area should focus are harvesting process, dioscorine removal and dioscorine detection devices.

The essence of agriculture is shown within the righteous book of Al-Quran which is there are about eighty three sentences mentioning about agriculture as indicated as benefit of mankind[9]

Figure 1. Collecting the tubers of *D.hispida*

[10];[11] study on relationship of microwave frequency with fruits moisture content and the method used is a non-destructive practice which is possible to be use for determination of alkaloid level for this project. Advantages of using non-destructive method for sensing are that it can be fairly accurate, and yields consistent results thereby reducing costs and making agricultural operations and processing safer for farmers and processing-line workers[12]. It holds great potential and benefits for the agricultural industry because of its simplicity, rapid inspection rate, and broad range of applications.

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2. Objective

The objectives of this study are to investigate the alkaloid level from the different variety of *D.hispida* using the microwave sensor and color sensor. The relationship between the levels of alkaloid with the microwave signal and with the colorimetry of *D.hispida* also will investigate and lastly this study is to generate the simulation model based on alkaloid content in *D.hispida*.

3. Materials and Methods

Two plants of *D.hispida* named as plant I and plant B, were collected from Kampung Kudat, Ajil, Terengganu, Malaysia. There were three levels for each plant. Three tubers were chosen from each level.

3.1. Measurement of microwave of *D.hispida*

The experimental set up for measuring the magnitude of reflection coefficient, (|S11|) of a monopole antenna includes a monopole antenna as a mechanical sensor probe and an Agilent FieldFox network analyzer as shown in Figure 2. The operating frequency to measure the reflection coefficient was set from 2MHz to 4GHz. The antenna was inserted into the flesh of the *D.hispida* tubers and the measurement was made for each tubers sample. Post-processing of the measured data was completed at the Department of Physics, Faculty of Science, Universiti Putra Malaysia.

Figure 2. FieldFox RF Analyzer used in this study

3.2. Measurement of image value of *D.hispida*

The experimental set up to measure the color of *D.hispida* was easier using the Chroma Meter CR-400 made by Konica Minolta as shown in Figure 3. The output of CR-400 is tristimulus value, whether XYZ, L*a*b* or other color space value. So, using this equipment we can measure the color of an object easily. Simply put it on the object, press the button and we get numerical value that represents that color. This measurement is independent of illumination around the object since CR-400 is pressed against the object which blocks other light to interfere.

Figure 3. Chroma Meter CR-400 used at laboratory of Food Technology, UniSZA

3.3. Chemical Experiment for Alkaloid Determination

The experimental method was adopted from the chemical analysis method[5].

After the tuber was peeled, they were weighed (40 grams) and sliced. Each sample was blended with 200mL of 0.5426 M hydrogen chloride (HCl), (specific molarity of HCl obtained from Titrant/Acid Standardisation) using an electric blender. For HCl preparation, measure the 42mL of HCl in measuring cylinder to make 0.5 M HCl, add this to about two-thirds of the final volume of distilled water in separate beaker. Stir the solution properly. Transfer them into a larger measuring cylinder and add distilled water to the required level and mix them thoroughly. The mixtures of sample were transferred into a conical flask covered with parafilm and left at room temperature for 2 days. After that, the samples were filtered using muslin cloth followed by filter paper; (Whatman Cat No 1001 150). The pH of the samples was checked using pH meter. The mixtures were made alkaline (pH 10-11) by adding K_2CO_3 and extracted with 3 portion (600-200mLl x 3) of ether using the separating funnel. All of the extracts were combined and dried overnight with Na_2SO_4. The dried extract was filtered and concentrated under reduced pressure using a rotavapor. The concentrated extract was spotted on a 20 x 20 cm TLC plate (Silica gel G, 60 F_{254}, 0.5 mm thickness, Merck). The compounds were separated with a solvent mixture of chloroform:ethanol:ammonia (100:10:0.5). The plates were air-dried and were sprayed with Dragendorff reagent. The calculated R_f value was compared with literature R_f value[13].

4. Results and Calculation

With an assumption that the alkaloid content in the whole tubers is homogeneous, results in table 1 and 2 were used to calculate weight percentage of alkaloid content versus magnitude of reflection coefficient and image pixel value. Table 3 show the percentage of alkaloid content and magnitude of reflection coefficient |S11| at 561.7 MHz of every sample. The sample I and b indicated 2 plants of *D.hispida*. The symbols of 'a,b,c' indicated the different tubers of tubers while '1,2,3' indicated the level of tuber from stem, 1 is the nearest with stem.

Table 1. weight of alkaloid in tubers

SAMPLE	WT EMPTY FLASK(g)	WT FLASK+ALKALOID(g)
I 1a	103.966	104.016
I 1b	89.700	89.783
I 2a	93.818	93.860
I 2b	98.058	98.071
I 2c	93.943	94.035
B 1a	160.951	161.023
B 1b	92.726	92.843
B 1c	31.228	31.271
B 2a	88.777	88.826
B 2b	88.860	88.945
B 3a	88.495	88.619
B 3b	159.602	159.695

4.1. Determination of Relationship between Image Pixel Value and Percentage of Dioscorine Content

Table 2 show the percentage of alkaloid content and reading of L a b color space of every sample. The, I and B indicated 2 plants of *D.hispida*. The symbols of 'a,b,c' indicated the different tubers of tubers while '1,2,3' indicated the level of tuber from stem, 1 is the nearest with stem.

4.2. Determination of Relationship between Magnitude of the Reflection Coefficient, |S11| and Percentage of Dioscorine Content

Table 1 show the weight parameters of alkaloid in 40 grams of tubers. The tubers from two *D.hispida* plants were harvested and each layer of the tubers was sliced for this experiment.

Table 2. the percentage of alkaloid content and Color pixel Lab

Tuber No.	Color pixel	Sample no. 1	Sample no. 2	Sample no. 3	WT ALKALOID (g)
I1a	L	82.82	78.90	83.06	0.05
	a	-4.81	-4.31	-4.57	
	b	31.76	29.72	35.12	
I2a	L	81.30	80.06	81.23	0.04
	a	-4.20	-4.29	-4.08	
	b	31.72	30.94	31.20	
I2b	L	80.60	79.95	79.71	0.01
	a	-4.34	-3.94	-4.18	
	b	27.73	25.61	26.82	
I3a	L	83.21	83.21	83.01	0.06
	a	-5.01	-4.52	-3.72	
	b	31.54	32.96	29.70	
I3c	L	81.52	82.27	81.63	0.02
	a	-4.93	-4.63	-5.01	
	b	26.92	25.15	28.31	
B3c	L	80.61	81.80	80.43	0.03
	a	-4.28	-3.92	-4.07	
	b	27.77	26.22	27.22	

A large number of data was measured in 2MHz to 4GHz frequency range. A statistical approach was used to determine the optimal frequency which has the best correlation between percentage alkaloid content and magnitude of the reflection coefficient, |S11|. The optimal frequency was found to be 0.9076 at frequency 561.7 MHz.

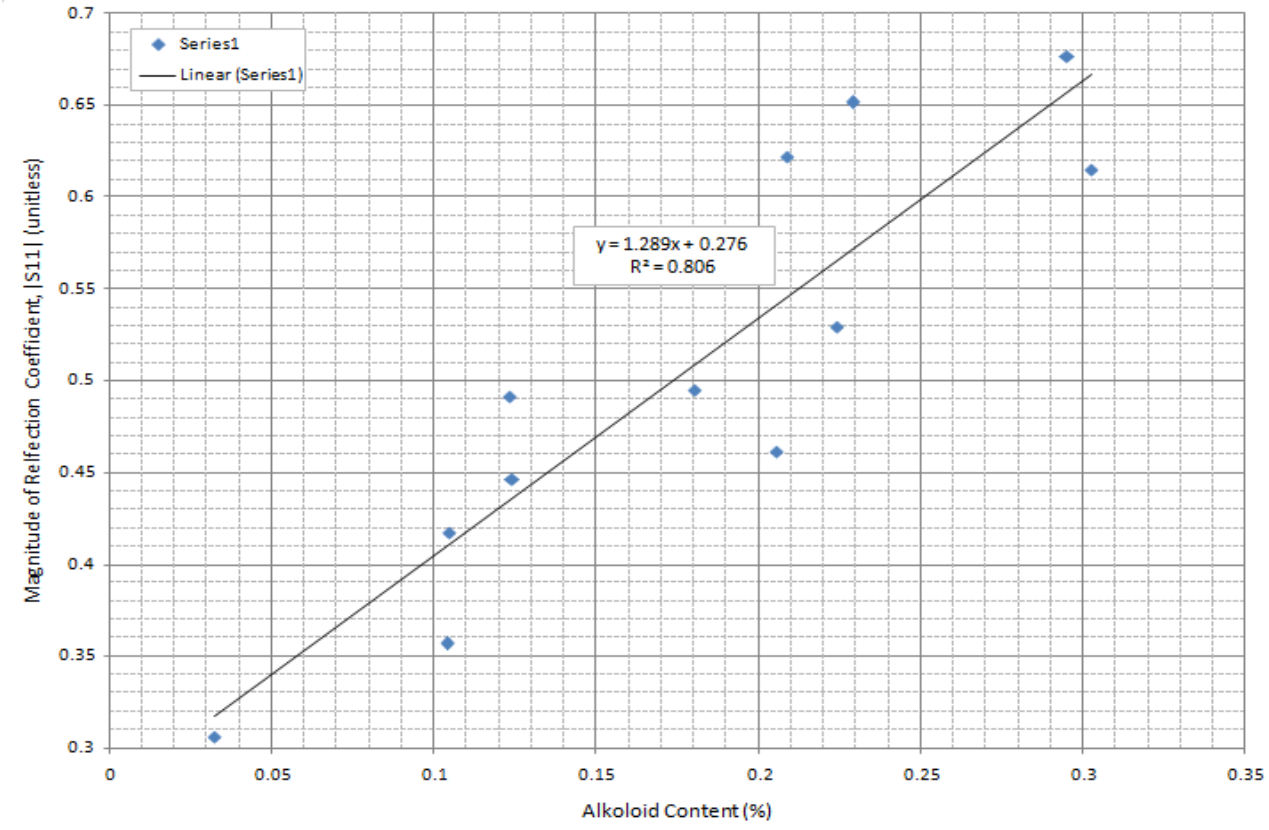

Figure 4. correlation between percentage alkaloid content and magnitude of reflection coefficient |S11|

Table 3. Percentage of alkaloid content and |S11| at 561.7 MHz of every sample

SAMPLE	magnitude of the reflection coefficient |S11|	WT ALKALOID (g)
I 1a	0.45	0.05
I 1b	0.47	0.08
I 2a	0.37	0.04
I 2b	0.31	0.01
I 2c	0.53	0.09
B 1a	0.49	0.07
B 1b	0.68	0.1
B 1c	0.42	0.04
B 2a	0.49	0.05
B 2b	0.62	0.09
B 3a	0.61	0.12
B 3b	0.65	0.09

A graph is plotted in order to obtain the mathematical correlation between equation analyses.

From the linear analysis of trendline regression graph, the correlation square, R^2 indicated 0.806 which mean that the magnitude of the reflection coefficient had strongly linear correlate with the alkaloid level of *D.hispida*. The developed mathematical model is, y=1.289x+0.276, where x=alkaloid content (%) and y= magnitude of the reflection coefficient (unitless) to find the amount of the alkaloid in the tested tubers.

5. Discussion

The correlation between the magnitude of the reflection coefficient of the sensor antenna probe and alkaloid content in the *D.hispida* tubers samples is high, with R^2=0.806. This result means we can use this method and proceed to determine the relation between dioscorine content and the magnitude of the microwave sensor. The pixel value of surface *D.hispida* tubers also had potential to further analysis on getting similar correlation of alkaloid level of dioscorine. Both works are considered nondestructive method, where the users only need to insert the sensor antenna probe and imaging sensor to the tubers.

6. Conclusions

The model using the mathematical equation to predict the level of alkaloid of the *D.hispida* tubers will be developing. Therefore, the amount of water needed to remove the toxic compound and the time consuming in the process can be determined. InshaAllah the sensory device for determine the alkaloid level for *D.hispida* will comprehend.

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