The Potency of Plant Dye Extracts for Halal Detection on Consumed Animal Fats

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Abstract. One point of the halal concepts is the food does not contain any lard or any pigs derivatives. UV-vis spectrophotometry using natural dyes, among other methods, can be developed for animal fat analysis. One factor that influence the analysis is the solubility of animal fats and natural dyes in organic solvents. This research was conducted to study the differences of UV-vis spectrum profile in animal fats (chicken, beef, lamb, pork) using various natural dyes (namely Curcuma longa Linn., Curcuma zanthorrhiza, Curcuma heyneana, Zingiber montanum, Uncaria, Caesalpinia sappan, and Areca catechu) in different type of solvents. The research consists of four main steps: 1) screening of plant dyes in several solvents with different polarity, 2) screening of animal fat in several solvents with different polarity, 3) screening of animal fats mixed with plant dyes in selected solvents, and 4) analysis of animal fats mixed with plant dyes in selected solvents by UV-Vis spectrophotometry. The results showed that plant dyes from C. longa Linn., C. zanthorrhiza, A. catechu, and Uncaria, well as all animal fats used in this work, were soluble in ethyl-acetate and isopropanol. UV-vis spectrum profile of the animal fats with turmeric dyes in ethyl-acetate for chicken, beef, pork, and lamb fats, shows peaks at λ of 484, 792, 488.5, and 741 nm with absorbance of 3.913, 0.816, 3.524, and 0.175 respectively. Meanwhile in isopropanol, the profile for chicken, beef, pork, and lamb fats have λ of 751, 787, 712, 499 nm with absorbance of 0.007, 1.012, 0.479, and 3.913, respectively. Therefore, the UV-vis spectrum profile of animal fat with C. longa Linn dyes in ethyl-acetate can distinguish lard from the beef and lamb fats. In isopropanol, the lard cannot be distinguished from the chicken and beef fats, but it can differentiate lard from the lamb fats. The C. zanthorrhiza dyes in ethyl-acetate cannot distinguish lard from the chicken, beef, and lamb fats, but C. zanthorrhiza dyes can distinguish lard from the beef fat using isopropanol as the solvent. For A. catechu and Uncaria dyes in ethyl-acetate and isopropanol, they all cannot distinguish lard from the chicken, beef and lamb fats.

1. Introduction
Exploration of animal fat analysis by UV and FTIR has been developed mainly because of the efficiency and simplicity of the procedure. However, these methods have limitations in which all UV spectrum profiles of animal fats are similar, as is the analysis with FTIR. Those analyses cannot identify the type and composition of each fatty acid component of a sample [1]. Fat consists of several kinds of fatty acids, both types and composition, including saturated fatty acids and unsaturated fatty acids. One type of fatty acid is distinguished by the number of carbon atoms (the length of the short chain of carbon atoms, the number of double bonds, structural configuration). The differences in the structure of these fatty acids cause differences in solubility in organic solvents with different polarity.
Fatty acid analysis can be done qualitatively by adding a fat-soluble diazonium dye, and the mixture of these compounds gives an orange to deep red color. Color intensity that occurs is proportional to the levels of unsaturated fatty acids in fatty acids. The color reaction is caused by the coupling reaction (merging) of diazonium with compounds in the samples that have double bonds. The azo-couple compound that produced is a compound which has an extension of π electron delocalization system and absorbs UV radiation in the visible area [2].

Preliminary research has been carried out for qualitative analysis of fatty acids in various vegetable fats (coconut, palm, soybean, corn, olive oils) using the diazonium reagent, producing an orange to orange-red colour. The increase in the intensity of the orange colour obtained is proportional to the levels of unsaturated fatty acids (oleic, linoleic, linolenic acids) in these vegetable fats, each of which are coconut, palm, soybean, corn and olive fat [3].

This analysis method was developed to identify animal fats that have different compositions in solvents with different polarity. To differentiate the absorption intensity, an analysis is performed by UV-vis spectrophotometry. However, this method has limitations in distinguishing color density with a short range [4]. In addition, the use of diazonium compounds as coloring reagents is carcinogenic, hence their use must be limited and reduced [5].

Plant dyes can be used as natural dyes, an alternative for reducing the use of harmful synthetic dyes. Yellow fat-soluble dyes include curcumin and lutein, orange (zeaxanthin, α and β-carotene) and red (lycopene and fucoxanthin), while green ones are chlorophyll a and b and pheophytin a and b. The solubility of fat-soluble dyes in organic solvents corresponds to their polarity, and the difference in fat polarity is proportional to the levels of unsaturated fatty acids, the higher the levels of unsaturated fatty acids, the higher the polarity, thereby increasing the solubility of the dye in the polar solvent, which is also proportional to the increase in intensity. It is noticed that plant dyes have limitations in its stability. It is influenced by pH, oxidizing agents, light and storage time. In addition, the intensity range of the resulting mixture is relatively less sharp.

Preliminary results of qualitative analysis of fatty acids in vegetable oils, with different levels of saturated fatty acids (coconut and palm oils) using yellow, orange and red dye reagents from plant extracts have been carried out. The results showed a difference in color intensity that was proportional to the levels of unsaturated fatty acids [3]. These results will be applied to the fat of some meat sold in the market, which are known to have different levels of saturated fatty acids. In other words, it is expected to be used for the detection of halal animal meat.

This study aims to develop a new analytical technique for the detection of halal consumption of fatty meat with plant dye reagents using UV-vis spectrophotometry in several stages. Generally speaking, pork fat has relatively higher levels of unsaturated fatty acids than other consumed meat fats. The plant dyes produce sharp intensities and will be used for reagents in the qualitative analysis of consumed animal fats (chicken, beef, goat, pork). Fat extract was obtained from the extraction by the Folch method, using solvents with different polarity. Differences in color intensity from UV-vis spectrophotometry were analyzed. The differences in the profile of UV-vis spectrum (wavelength, intensity) of each selected animal fat in solvents with a certain polarity were used to distinguishing animal fat in a mixed meat fat [6]. In addition, digital image analysis is also performed, in which the color intensity profile of consumed animal fats, RGB values, and Gray Value digital imaging are discussed.

2. Experimental Details

2.1 Materials and Instrumentations

All chemicals are in analytical grade, namely methanol, ethanol, isopropanol, ethyl-acetate, n-hexane, and chloroform. A technical grade of isopropanol was also used as comparison. Natural dyes from several plants (Curcuma longa Linn – code A1, Curcuma zanthorriza – code A2, Curcuma heyneana – code A3, Zingiber montanum – code A4, Uncaria – code A5, Caesalpinia sappan – code A6, Areca...
catechu – code A7) and animal fats (chicken, beef, pork, lamb) were obtained from a local grocery store in Malang, Indonesia.

UV-vis spectrophotometer (Shimadzu 1601), 13 MP digital camera, and Image J 1.50i free software (Wayne Rasband, National Institution Institutions of Heath, USA) were used in this work.

2.2 Screening of natural plant dyes in several solvents with different polarity

The screening was done by dissolving the dyes in nonpolar (chloroform, n-hexane), semi-polar (ethyl-acetate) and polar (methanol, ethanol, isopropanol) solvents. A 2 g of each natural dyes powder (from A1 to A7) was added into Erlenmeyer flask and added with 20 mL of the solvent (chloroform, n-hexane, ethyl-acetate, methanol, ethanol, isopropanol). The solution was then homogenized using a vortex mixer and allowed to stand at room temperature for 1 hour. The solubility of each dye in each solvent was observed. The absorbance of each dye was then measured by UV-vis spectrophotometry to determine its maximum wavelength (λ_max). Next, the color stability of the dyes in several solvents was checked by measuring the absorbance at λ_max of each dye solution after one week, two weeks, and three weeks.

2.3 Screening of animal dyes with plant dyes in selected solvents

The screening was done by dissolving the animal fat in nonpolar (chloroform, n-hexane), semi-polar (ethyl-acetate) and polar (methanol, ethanol, isopropanol) solvents. A 2 mL of each animal fat (chicken, beef, pork, and lamb) was added into reaction tube and added with 4 mL of the solvent (ethyl-acetate and isopropanol) the solution was then homogenized using a vortex mixer and rested for 5 minutes. The solubility of each animal fat in ethyl-acetate and isopropanol solvent was observed. The absorbance of each animal fat was then measured by UV-vis spectrophotometry to determine its maximum wavelength (λ_max).

2.4 Digital image profile of halal detection of plant dyes extracts in various animal fats

Each solution of a mixture of animal fat and each natural dye extract which was dissolved in ethyl-acetate and isopropanol is captured using a digital camera with 13 Megapixel resolution. The results of digital images are stored in computer data and saved in JPEG (Joint Photographic Experts Group) file format. The digital images were analyzed using the free Image J 1.50i software (Wayne Rasband, National Institutional Institutions of Heath, USA). The color parameters obtained from this software are the average value of RGB (Red Green Blue). The RGB value is obtained through plugins> analyze> RGB Measure (Ferreira and Rasband, 2011). The percentage of each value of R, G, and B is calculated using the formula: % R = ((mean R / (mean R + mean G + mean B)) x 100%, percentages G and% B are obtained in the same above. Digital RGB values used to determine the difference in color intensity of a mixture of animal fat solutions and natural dyes extracts based on the range of RGB values.

3. Results and Discussion

3.1 The solubility screening of natural dyes in non-polar, semi-polar and polar solvents

The solubility of natural dyes was carried out using non-polar, semi-polar and polar solvents. The results showed that the non-polar chloroform dissolves more natural dyes compared to that of n-hexane. Solubility test using semi-polar solvents shows that all-natural dyes have good solubility in ethyl-acetate. As for polar solvents, all-natural dyes have good solubility in methanol and ethanol, as well as in isopropanol. However, the A5 natural dye was slightly soluble in isopropanol.

Solubility of natural dyes in various solvents can be identified from the intensity of the color produced. Thus, from the screening results (Table 1), it is found that natural dyes are soluble in several solvents, namely methanol, ethanol, isopropanol and ethyl acetate.
Table 1. Solubility of natural dyes in several solvents.

| Plant Dyes | n-Hexane | Chloroform | Ethyl-acetate | Methanol | Ethanol (technical) | Isopropanol (technical) | Isopropanol (analytical) |
|------------|----------|------------|--------------|----------|---------------------|------------------------|--------------------------|
| 1          | A1       | Slightly soluble | Soluble | Soluble | Soluble | Soluble | Soluble |
|            | A2       | Slightly soluble | Soluble | Soluble | Soluble | Soluble | Soluble |
|            | A3       | Slightly soluble | Soluble | Soluble | Soluble | Soluble | Slightly soluble |
|            | A4       | Insoluble | Soluble | Soluble | Soluble | Slightly soluble | Slightly soluble |
|            | A5       | Insoluble | Slightly soluble | Soluble | Soluble | Slightly soluble | Slightly soluble |
|            | A6       | Slightly soluble | Slightly soluble | Soluble | Soluble | Slightly soluble | Slightly soluble |
|            | A7       | Slightly soluble | Slightly soluble | Soluble | Soluble | Soluble | Soluble |

Note: A1 = C. longa Linn., A2 = C. zanthorriza, A3 = C. heyneana, A4 = Z. montanum, A5 = Uncaria, A6 = C. sappan, A7 = A. catechu.

Analysis of natural dye extracts using UV-vis spectrophotometer produces the maximum wavelength (λ<sub>max</sub>) and absorbance data of each extract of natural dyes using n-hexane and chloroform as the solvent, as presented in Table 2. The natural dyes extract in n-hexane has λ<sub>max</sub> range of 580-670 nm with the highest λ<sub>max</sub> was detected in C. zanthorriza (745 nm with absorbance of 0.011). The natural dyes extract in chloroform has λ<sub>max</sub> range of 665-670 nm. In addition, λ<sub>max</sub> of natural dyes extracts in chloroform has a close difference of λ<sub>max</sub> among each other.

Table 2. Absorbance and λ<sub>max</sub> results of extracts by UV-vis.

| No | Solvent | Sample | λ<sub>max</sub> (nm) | Abs. | No | Solvent | Sample | λ<sub>max</sub> (nm) | Abs. |
|----|---------|--------|----------------------|------|----|---------|--------|----------------------|------|
| 1  | n-Hexane (3) | A1.1 | 748.4 | 0.018 |
|    |         | A2.1 | 746.8 | 0.019 |
|    |         | A3.1 | 722  | 0.038 |
|    |         | A4.1 | 725.6 | 0.012 |
|    |         | A5.1 | 663.8 | 0.109 |
|    |         | A6.1 | 747  | 0.015 |
|    |         | A7.1 | 739  | 0.565 |
| 2  | Chloroform (7) | A1.2 | 748.6 | 0.008 |
|    |         | A2.2 | 745  | 0.011 |
|    |         | A3.2 | 570  | 0.091 |
|    |         | A4.2 | 727.0 | 0.008 |
|    |         | A5.2 | 665.2 | 0.085 |
|    |         | A6.2 | 569.4 | 0.182 |
|    |         | A7.2 | 569.8 | 0.918 |
| 3  | Ethyl-acetate (6) | A1.4 | 747.2 | 0.008 |
|    |         | A2.4 | 746.2 | 0.008 |
|    |         | A3.4 | 741.4 | 0.012 |
|    |         | A4.4 | 744.2 | 0.019 |
|    |         | A5.4 | 665.6 | 0.125 |
|    |         | A6.4 | 547.5 | 0.286 |
|    |         | A7.4 | 741  | 0.083 |

Stability test of natural dyes extract in various solvents was carried out to determine the maximum wavelength (λ<sub>max</sub>) and absorbance values of each natural dye extract in weeks 1, 2 and 3. Table 3 and
Figure 1 showed that the natural dyes were stable in $n$-hexane, chloroform, ethyl-acetate, methanol, ethanol and isopropanol was stable until the 2nd week.

**Table 3.** Stability test result of natural dyes extracts in several solvents.

| Solvent         | Sample Code | Week of observation |            |   |   |  |
|-----------------|-------------|---------------------|------------|---|---|---|
|                 |             |                     | 1          | 2 | 3 | Abs |
|                 |             | $\lambda_{\text{max}}$ (nm) | Abs | $\lambda_{\text{max}}$ (nm) | Abs | $\lambda_{\text{max}}$ (nm) | Abs |
| $n$-Hexane      | A1.3        | 389                 | 1.698      | 389.4 | 0.895 | 406 | 2.555 |
|                 | A2.3        | 745                 | 0.011      | 388   | 1.553 | 491 | 0.365 |
|                 | A3.3        | 581.6               | 0.009      | 286.4 | 2.698 | 681.5 | 0.025 |
|                 | A4.3        | 324.8               | 3.104      | 315   | 3.740 | 315.6 | 3.559 |
|                 | A5.3        | 669                 | 0.020      | 222   | 2.507 | 408 | 0.234 |
|                 | A6.3        | 593.8               | 0.004      | 229.8 | 3.025 | 404 | 0.104 |
|                 | A7.3        | 653.4               | 0.020      | 350.2 | 2.619 | 548 | 1.673 |
| Chloroform      | A1.7        | 670.2               | 0.079      | 669.5 | 0.110 | 508 | 1.704 |
|                 | A2.7        | 666.0               | 0.113      | 744.5 | 0.046 | 487.5 | 1.285 |
|                 | A3.7        | 669.0               | 0.048      | 669   | 0.059 | 463 | 0.158 |
|                 | A4.7        | 667.8               | 0.068      | 773   | 0.038 | 470.5 | 0.609 |
|                 | A5.7        | 667.0               | 0.119      | 667.5 | 0.210 | 478 | 0.485 |
|                 | A6.7        | 670.2               | 0.042      | 795   | 0.055 | 477.5 | 0.729 |
|                 | A7.7        | 665.0               | 0.304      | 666.5 | 0.446 | 549 | 1.814 |
| Ethyl-acetate   | A1.6        | 465.2               | 3.436      | 464.5 | 3.436 | 470 | 3.374 |
|                 | A2.6        | 473.8               | 0.012      | 474.5 | 3.612 | 471 | 3.436 |
|                 | A3.6        | 570                 | 0.091      | 406.5 | 2.960 | 410 | 2.872 |
|                 | A4.6        | 451.6               | 0.018      | 406   | 3.175 | 406 | 3.135 |
|                 | A5.6        | 665.2               | 0.085      | 664   | 0.725 | 665.5 | 0.308 |
|                 | A6.6        | 569.4               | 0.182      | 426   | 2.069 | 426 | 2.094 |
|                 | A7.6        | 569.8               | 0.918      | 396   | 3.763 | 529 | 1.860 |
| Methanol        | A1.1        | 748.4               | 0.018      | 507.5 | 3.913 | 506 | 3.957 |
|                 | A2.1        | 746.8               | 0.019      | 455   | 3.311 | 457 | 3.175 |
|                 | A3.1        | 722                 | 0.038      | 491.5 | 3.612 | 457.5 | 3.135 |
|                 | A4.1        | 725.6               | 0.012      | 453   | 3.311 | 663.5 | 0.124 |
|                 | A5.1        | 663.8               | 0.109      | 664   | 0.148 | 491 | 3.436 |
|                 | A6.1        | 747                 | 0.015      | 484   | 3.612 | 489 | 3.436 |
|                 | A7.1        | 739                 | 0.565      | 496   | 4.000 | 499 | 3.913 |
| Ethanol         | A1.2        | 748.6               | 0.008      | 507   | 3.913 | 508 | 3.763 |
|                 | A2.2        | 745                 | 0.011      | 486.5 | 3.524 | 484 | 3.524 |
|                 | A3.2        | 570                 | 0.091      | 380.6 | 2.659 | 350.8 | 2.972 |
|                 | A4.2        | 727.0               | 0.008      | 436.5 | 3.315 | 447 | 3.135 |
|                 | A5.2        | 665.2               | 0.085      | 665.5 | 0.043 | 665.5 | 0.028 |
|                 | A6.2        | 569.4               | 0.182      | 467.5 | 3.612 | 471.5 | 3.524 |
|                 | A7.2        | 569.8               | 0.918      | 443.5 | 3.215 | 444 | 3.101 |
| Isopropanol     | A1.4        | 747.2               | 0.008      | 481   | 3.612 | 493 | 3.612 |
|                 | A2.4        | 746.2               | 0.008      | 478   | 3.436 | 469.5 | 3.436 |
|                 | A3.4        | 741.4               | 0.012      | 752   | 0.001 | 404.5 | 2.422 |
|                 | A4.4        | 744.2               | 0.019      | 429   | 2.960 | 440.5 | 3.010 |
|                 | A5.4        | 665.6               | 0.125      | 666.5 | 0.062 | 666 | 0.058 |
|                 | A6.4        | 547.5               | 0.286      | 547.5 | 0.453 | 547 | 0.2898 |
|                 | A7.4        | 741                 | 0.083      | 420   | 1.542 | 419.5 | 1.527 |
Figure 1. Stability test of natural dyes in several solvents a. n-hexane; b. chloroform; c. ethyl-acetate; d. methanol; e. ethanol; f. isopropanol.

The solubility screening test of natural dye extracts were also conducted in various solvents (non-polar, semi-polar and polar). Based on the result (Table 4), it can be concluded that ethyl-acetate and isopropanol are suitable solvents to be used in the solubility of animal fats – natural dye extracts mixture due to its good solubility.

Table 4 shows that the C. longa Linn., C. heyneana, Uncaria, and A. catechu dye extracts have the best solubility in animal fat using ethyl-acetate, as observed from the fully dissolving of each natural dye extract and color changes in response to animal fats. Meanwhile, in isopropanol, the C. longa Linn, C. heyneana, C. sappan and A. catechu dye extracts are slightly soluble in animal fat and form 2 layers with a color change in the lower layer of each animal fat.

3.2 Analysis of animal fats with plant dyes in selected solvents by UV-Vis spectrophotometry

The ethyl-acetate and isopropanol solvents which has good solubility in natural dye and fat extracts mixture were further used in the next stage. The extract of natural dyes – animal fat mixture in ethyl-acetate has $\lambda_{\text{max}}$ range of 792-367 nm. Meanwhile in isopropanol, the $\lambda_{\text{max}}$ was obtained in a range of 798-414 nm. The lowest $\lambda_{\text{max}}$ was detected for A. catechu dyes which was 367.8 nm with an absorbance of 2.647. These results are presented in Table 5.
Table 4. Solubility test of animal fat in different dye extracts and solvents.

| Solvent       | Extracts | Solubility and Color of Animal Fat |Chicken | Beef | Pork | Lamb |
|---------------|----------|-----------------------------------|--------|------|------|------|
|               |          |                                   |        |      |      |      |
| Ethyl-acetate | A1       | soluble, orange                   | soluble, orange | slightly soluble, pale yellow | slightly soluble, pale yellow |
|               | A2       | soluble, orange                   | soluble, orange | slightly soluble, orange (2 layers) | slightly soluble, orange (2 layers) |
|               | A3       | soluble, yellow                   | soluble, yellow | soluble, yellow | soluble, yellow |
|               | A4       | soluble, yellow                   | soluble, yellow | soluble, yellow | soluble, yellow |
|               | A5       | slightly soluble, pale yellow     | soluble, pale yellow | slightly soluble, pale yellow | soluble, pale yellow |
|               | A6       | soluble, yellow                   | soluble, yellow | soluble, yellow | soluble, yellow |
|               | A7       | soluble, yellow                   | soluble, yellow | soluble, yellow | soluble, yellow |

| Isopropanol   | A1       | soluble, orange                   | soluble, orange | slightly soluble, orange (2 layers) | soluble, orange |
|               | A2       | soluble, orange                   | soluble, orange | slightly soluble, orange (2 layers) | soluble, orange |
|               | A3       | slightly soluble, orange (2 layers) | light orange | slightly soluble, orange (2 layers) | soluble, orange |
|               | A4       | soluble, yellow                   | soluble, yellow | soluble, yellow | soluble, yellow |
|               | A5       | slightly soluble, pale yellow     | soluble, pale yellow | slightly soluble, pale yellow | soluble, pale yellow |
|               | A6       | soluble, yellow                   | soluble, yellow | slightly soluble, orange (2 layers) | slightly soluble, orange (2 layers) |
|               | A7       | slightly soluble, yellow (2 layers) | slightly soluble, orange (2 layers) | slightly soluble, orange (2 layers) | slightly soluble, orange (2 layers) |

Note: A1 = C. longa Linn., A2 = C. zanthorrhiza, A3 = C. heyneana, A4 = Z. montanum, A5 = Uncaria, A6 = C. sappan, A7 = A. catechu.

Table 5. UV-Vis results of animal fat mixed with natural dyes extracts in selected solvents

| Solvent       | Dyes      | Animal Fat          |        |        |        |        |
|---------------|-----------|---------------------|--------|--------|--------|--------|
|               |           |                     | Chicken | Beef | Pork | Lamb |
|               |           | λ<sub>max</sub> (nm) | Abs.    | λ<sub>max</sub> (nm) | Abs. | λ<sub>max</sub> (nm) | Abs. | λ<sub>max</sub> (nm) | Abs. |
| Ethyl-acetate | A1        | 484                 | 3.913   | 792    | 0.816 | 488.5  | 3.524 | 741    | 0.1755 |
|               | A2        | 742.5               | 0.056   | 487.5  | 4.000 | 782.5  | 1.489 | 780.5  | 2.613  |
|               | A3        | 484                 | 3.763   | 445    | 3.436 | 436.5  | 3.135 | 454    | 3.436  |
|               | A4        | 411.5               | 3.311   | 789    | 0.067 | 440    | 3.135 | 789.5  | 0.384  |
|               | A5        | 667.5               | 0.109   | 666.5  | 0.309 | 662.5  | 0.085 | 665.5  | 0.734  |
|               | A6        | 639                 | 0.026   | 753.5  | 0.071 | 745.5  | 0.046 | 464.5  | 3.612  |
|               | A7        | 376.8               | 3.101   | 387.2  | 3.311 | 367.8  | 2.647 | 394    | 3.763  |
| Isopropanol   | A1        | 751                 | 0.007   | 787    | 1.012 | 712    | 0.479 | 499    | 3.913  |
|               | A2        | 754                 | 0.048   | 471    | 4.000 | 752.5  | 0.063 | 771.5  | 0.042  |
|               | A3        | 797                 | 0.796   | 460.5  | 3.913 | 760    | 0.270 | 762    | 0.170  |
|               | A4        | 783.5               | 0.692   | 749    | 0.024 | 783.5  | 1.459 | 750    | 0.310  |
|               | A5        | 794                 | 0.602   | 766.5  | 2.237 | 777    | 0.471 | 661    | 1.086  |
|               | A6        | 793.5               | 0.815   | 476    | 4.000 | 784.5  | 0.663 | 791    | 0.138  |
|               | A7        | 794                 | 1.043   | 460.5  | 3.913 | 788    | 0.276 | 774    | 0.319  |

Note: A1 = C. longa Linn., A2 = C. zanthorrhiza, A3 = C. heyneana, A4 = Z. montanum, A5 = Uncaria, A6 = C. sappan, A7 = A. catechu.
3.3 Digital image profile of halal detection of plant dye extracts in various animal fats
Digital image profiles were used to quantify and differentiate the % RGB values (red, green, blue) of animal fat mixed with plant dye extracts in ethyl-acetate and isopropanol solvents. Thus, it is expected to be able to determine the profile differences of digital image of animal fat mixed with plant dye extracts. The results of the % RGB values are presented in Table 6.

| Solvent       | Extract | RGB   | RGB Mean Values of Animal Fat |
|---------------|---------|-------|--------------------------------|
|               |         |       | Chicken | Beef | Pork | Lamb |
| Ethyl-acetate | A1      | Red   | 144.958 | 170.304 | 166.824 | 166.419 |
|               |         | Green | 80.881 | 107.123 | 102.184 | 98.486 |
|               |         | Blue  | 31.273 | 31.053 | 36.838 | 41.636 |
|               |         | % Red | 56.38 | 55.21 | 54.55 | 54.29 |
|               |         | % Green | 31.46 | 34.73 | 33.41 | 32.13 |
|               |         | % Blue | 12.16 | 10.07 | 10.07 | 13.58 |
|               | A3      | Red   | 146.510 | 148.386 | 140.660 | 140.794 |
|               |         | Green | 90.595 | 82.817 | 75.025 | 77.078 |
|               |         | Blue  | 5.448 | 4.671 | 5.656 | 4.860 |
|               |         | % Red | 60.40 | 62.91 | 63.55 | 63.21 |
|               |         | % Green | 37.35 | 35.11 | 33.90 | 34.61 |
|               |         | % Blue | 2.25 | 1.98 | 2.56 | 2.18 |
| Isopropanol   | A1      | Red   | 171.853 | 184.926 | 149.552 | 162.023 |
|               |         | Green | 81.853 | 122.917 | 52.456 | 72.023 |
|               |         | Blue  | 26.043 | 20.840 | 4.945 | 19.371 |
|               |         | % Red | 62.78 | 56.32 | 72.26 | 63.94 |
|               |         | % Green | 29.90 | 37.44 | 25.35 | 28.42 |
|               |         | % Blue | 7.32 | 6.24 | 2.39 | 7.64 |
|               | A3      | Red   | 170.304 | 176.042 | 143.217 | 157.533 |
|               |         | Green | 81.199 | 94.542 | 44.049 | 71.616 |
|               |         | Blue  | 15.083 | 21.458 | 5.236 | 17.074 |
|               |         | % Red | 63.88 | 60.28 | 74.40 | 63.98 |
|               |         | % Green | 30.46 | 32.37 | 22.88 | 29.09 |
|               |         | % Blue | 5.66 | 7.35 | 2.72 | 6.93 |

Table 6 showed that the %RGB mean values of each animal fat – plant dye extract in ethyl-acetate and isopropanol are distinguishable. The percentage of the RGB value showed the intensity of the color produced after the addition of extracts of natural dyes on each animal fat. However, not all plant extracts have the potential to be halal detectors for animal fat due to the difference of each extract dye. From the result, C. longa Linn and C. heyneana extracts have the potential as halal detectors for consumed animal fats in isopropanol solvent. However, consumed animal fats were less soluble in isopropanol. Lard (fat of pork) has the highest %RGB value as shown by the digital image of C. longa Linn and C. heyneana extracts in isopropanol. Meanwhile, the highest %RGB was shown only on C. heyneana in ethyl-acetate.

4. Conclusion
This research result showed that the extract of plant dyes from A1 (C. longa Linn) and A3 (C. heyneana) in ethyl-acetate and isopropanol solvents, by combination of UV-vis and digital image spectrophotometer methods, can be developed to distinguish halal fats (chicken, beef, lamb) from a non-halal fats (lard or fat of pork).
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