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'Validation of UV-Vis Spectrophotometric Method for Determination of Bio oil Total Phenolic Content from Pyrolisis of Cashew Nut Shell.

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ABSTRACT

The bio oil of cashew nut shell (CNS) is a source of natural phenolic which can be obtained by pyrolisis. Cashew nut shell liquid (CNSL) has many benefits, both in industry and the medical field. Phenolic a class of chemical compounds that can be used as a natural pesticide. The purpose of this study to validate the method and determine the total phenolics content (TPC) of bio oil from CNS pyrolisis by UV-Vis spectrophotometer. Bio oil production are done with CNS pyrolysis at temperature 400, 500, 600 and 700 °C, respectively. Determination of TPC using UV-Vis spectrophotometric method using the Folin-Ciocalteu (FC) reagent. In this research, maximum wavelength absorption was obtained at 765 nm and working range of concentration from 0.05 to 9 mg/L with R^2 = 0.9997. Limits of detection (LoD) and limits of quantitation (LoQ) are 0.1852 ppm and 1.0579 ppm, respectively. The total content of phenols in a sample of the CNSL are 1.2300, 2.0575, 2.1781 and 1.8374 g/L, respectively. The specificity test showed similarities gallic acid standard curve with the FC reagent with a correlation value of r = 0.9930. Additionally, in this study, accuracy and precision analysis, this methods can be used accurately and have good precision with value of recovery tested is 103.15 % and RSD is 0.43%. The validation has been carried out proved linear, specific, accurate and reproducible.

Keywords: Validation, total phenolic, cashew nut shell, bio oil, pyrolisis.

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INTRODUCTION

Cashew nut shell (CNS) bio oil is the oil of cashew nut shell and one source to produce natural phenolic, which can be used as a natural pesticide. Cashew nut shell liquid (CNSL) has many benefits, both in industry and the medical field. Indonesia is one of the cashew (*Anacardium occidentale Linn*.) producing countries in the world after India, Vietnam, Nigeria, and Brazil [1].

Cashew nut shell liquid (CNSL) mainly consists of anacardic acid, cardol, cardanol and small amount of other phenols and less polar substances. The composition percentage varies with many parameters like, nature of origin, climatic condition and method of extraction. Cashew nut shell consists of kernel 20-25%, kernel liquid 20-30%, testa 3% and other being the shell by the weight of nut. The shell and kernel consists of honeycomb structure containing dark colored, high viscous phenolic material known as CNSL [2,3].

Plant phenolics, derived from a wide range of plant secondary metabolites, have attracted increasing attention for their antioxidant properties and marked effects in the prevention of various oxidative stress associated diseases such as cancer. Therefore, in the last few years, the extraction and identification of phenolic compounds from different plants has become a major area of health and medical-related research [4].

Phenolics include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignans, and lignins. Polyphenol quantification methods are well established in terms of time, amount of samples, reagents and data analysis [5]. Colorimetric reactions are widely used in the UV-Vis spectrophotometric method, which is easy to perform, rapid and applicable in routine laboratory use, and low-cost [6,7].

Polyphenols in plant extracts react with specific redox reagents from FC reagent to form a blue complex that can be quantified by visible-light spectrophotometry. The Folin-Ciocalteu method is described in several pharmacopoeias. The reaction forms a blue chromophore constituted by a phosphotungstic phosphomolybdenum complex where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds. Many studies have discussed the use of the Folin-Ciocalteau reagent to determine polyphenols, and the general or specific value of the method, because some specific details may be modified [8,9].

Pyrolysis is the decomposition of an organic material by heating in the absence of oxygen, but with inert gases or less reactive gases (argon and nitrogen) producing a viscous dark liquid (oil), gases and leaving a charring material composed mainly of carbon [11]. It is endothermic cracking process requires large energy supply at higher temperature with short residence time of the cracked products [11]. Pyrolysis process is of importance key as this thermal degradation of biomass is present in both combustion and gasification. It has a key influence over the quality of the char that is either gasified or burned [12]. The pyrolisis temperature is the most important parameter affecting the pyrolisis product. Therefore, in this study, bio oil from pyrolysis of CNS is validated and it determined the TPC using UV-Vis spectrophotometer.

MATERIALS AND METHODS

Materials

The materials used in this study are CNS waste collected from cashew nut processing in Southeast Sulawesi province of Indonesia, gallic acid (GA) was used as standard compound for the validation of the method, ethanol, Na_2CO_3, FC reagent. All the chemicals used in this study were of analytical grade from Merck.

Methods

Pyrolisis

The pyrolysis reaction was performed in a reactor with a temperature range between 400–700 °C at a heating rate of 60°C/min. The gases through a condenser where bio-oil collected (procedure modification of Mashuni et al.) [13].

Determination of Total Phenolics Content (TPC)

Bio oil from pyrolisis of CNS were determinated the TPC using the Folin-Ciocalteu method (modification of the method of Grujic N. et al.) [14] and UV-Vis spectrophotometer (Jasco V-380), equipped 1-cm path length quartz cell were used for UV-Vis spectra and rotary vacum evaporator (Butchi) with vacuum pump (V-700). The bio oil sample from pyrolisis of CNS with each temperature of 400, 500, 600 and 700 °C were dissolved in ethanol : water (1:1), then taken as 0.2 mL and diluted with 10 mL of distilled water till 0.2% (v/v). The concentration of the phenolics in the sample solution was determined using the FC assay. The each sample solution or a standard solution of GA (5, 10, 30, 50, 70 and 90 mg/L) 0.3 mL is pipetted and FC reagent 1.5 mL was added into a test tube. and shaken. Allowed to stand for 3 minutes, added 7.5% Na_2CO_3 solution 1.2 mL and allowed to stand on a range of operating time at room temperature (27 ± 0.5) °C. The absorbance of the sample solution was measured by UV-Vis spectrophotometer at the wavelength of maximum absorbance. A control sample was prepared at the same time using distilled water (0.3 mL), FC reagent (1.5 mL) and Na_2CO_3 solution (1.2 mL) and then it was well mixed and left in a dark place for 60 minutes. All measurements were performed in triplicate 3.

UV-Vis Spectrophotometric Method

There has been a modification of the Prussian Blue method [15] adapting and validating it to UV-Vis spectrophotometry. The total phenols were determinate at the maximum wavelength using UV-Vis spectrophotometer. In the modern analytical chemistry, chemical suitability of quantitative methods used for certain analysis is often assessed through a validation method [16]. The validation of the method, we have followed the criteria of Regression coefficient (R^2), limit of detection (LoD), limit of quantification (LoQ), precision (repeatability), specificity (matrices interference), and accuracy the quantification of total phenols of CNSL [17,18].

RESULTS AND DISCUSSION

Validation of the method

The method was validated according to international guidelines. Analytical methods must be shown to give reliable data, free from refraction and suitable for the intended use. A common approach is to start with the final measurement stage, using calibration standards of known high purity for each analyte to establish the performance characteristics of the detection system (i.e. range, quantitative response (linearity), sensitivity, stability, reproducibility or repeatability and specificity) [19,20]. This methodology complies with the requirements for analytical application and to ensure the reliability of the results.

Determining Maximum Wavelength

In this study, the determination of the maximum wavelength done using UV-VIS spectrophotometer after the solution has reached the operation time for 60 minutes with GA concentration of 0.3 ppm. Under these conditions, validation by UV-Vis spectrophotometry to prove the method to be linear, reproducible, specific and accurate. Results showed that the wavelength is 765 nm achieved by absorbansi 0.341. Results of the determination of the maximum wavelength can be seen in Fig. 1.

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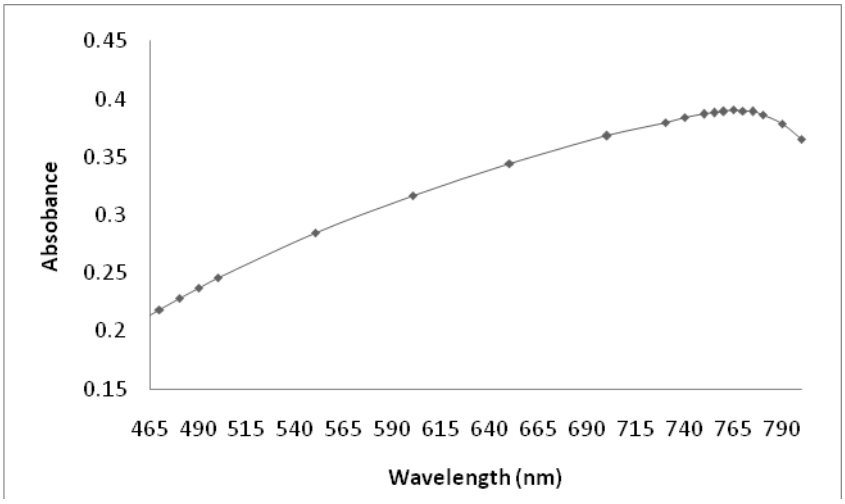

Figure 1: Absorption spectra of maximum wavelength.

Linearity of Calibration, Limits of Detection (LoD) and Limits of Quantitation (LoQ)

The method of analysis is usually based on the existing literature by using the same instrument or nearly the same. Therefore, it is necessary for the validation of the method begins with a calibration curve and analyze linearity. In this study, a calibration curve obtained by making various concentrations of GA standard solutions with concentration 0.5, 1.0, 3.0, 5.0, 7.0 and 9.0 mg/L, then each measured absorbance at the maximum wavelength. The data of linearity curve analysis was shown at Table 1.

Table 1: Data of linearity curve analysis of gallic acid standard solution

Concentration (mg/L)	Absorbance						Mean of Absorbance
	n1	n2	n3	n4	n5	n6	
0.5	0.069	0.070	0.071	0.072	0.07	0.069	0.070
1	0.123	0.123	0.124	0.125	0.124	0.124	0.123
3	0.336	0.335	0.339	0.337	0.339	0.338	0.337
5	0.534	0.533	0.535	0.533	0.534	0.535	0.534
7	0.735	0.733	0.738	0.736	0.739	0.738	0.736
9	0.957	0.962	0.960	0.961	0.965	0.954	0.959
The regression equation	y = 0.1035x + 0.0189	y = 0.1038x + 0.0182	y = 0.1037x + 0.0205	y = 0.1036x + 0.0205	y = 0.1042x + 0.0189	y = 0.1033x + 0.0204	y= 0.1037x +0.0196
R^2	0.9997	0.9995	0.9997	0.9996	0.9996	0.9998	0.9997

Linearity of data was obtained by regression equation which plotting the absorbance of GA standard solution. Value of slope is 0.1037 ± 0.0005 and intercept value is 0.0196 ± 0.0007 in order to obtain the regression equation y = 0.1037 x ± 0.0196 with R^2= 0.9997 (Fig. 2). Based on literature, linearity with correlation coefficient r > 0.995 is having a good linearity qualify [21].

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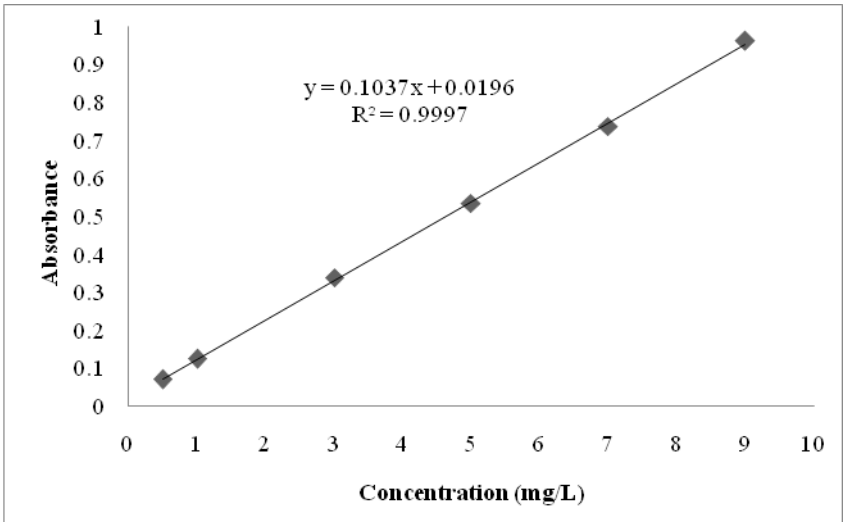

Figure 2: Calibration curve of gallic acid standard solution

Limits of detection (LOD) is the smallest concentration of analytes that can be detected by statistical measurements and its still can be reliable while limits of quantitation (LOQ) is the smallest concentration of analyte that can be measured. Limits of detection (LOD) and limit of quantification (LOQ) were 0.1852 and 1.0579 mg/L, respectively, and there are estimated from the mean of the blank [17].

Precision

The precision of analytical data is the degree of mutual agreement among data that have been obtained in the same way. Instrument performance criteria that can be used to decide whether a given instrumental method is suitable for attacking an analytical problem. The standard deviation of the mean is sometimes referred to as the *standard error*. The standard deviation is sometimes expressed as the relative standard deviation (RSD), which is just the standard deviation expressed as a fraction of the mean; usually it is given as the *percentage* of the mean (% RSD), which is often called the coefficient of variation [19,20].

The repeatability refers to the precision of the method carried out in the same conditions (the same sample, analyst, laboratory, equipments, reagents, etc.) and the same series of analysis in a short interval of time [22]. There test using standard solution of GA in five of concentrations (1, 3, 5, 7 and 9 mg/L) and were analyzed six times in the same day. Relative Standard Deviation (RSD) were 0.73, 0.52, 0.17, 0.32 and 0.41 % for each concentration and mean of RSD 0.43 % (Table 2).

Table 2: Precision Analysis

Measurement	Concentration (mg/L)				
	1	3	5	7	9
1	0.9932	3.0551	4.9719	6.9177	9.0667
2	0.9932	3.0454	4.9622	6.8983	9.1151
3	1.0029	3.0842	4.9816	6.9467	9.0958
4	1.0125	3.0648	4.9622	6.9273	9.1055
5	1.0029	3.0842	4.9719	6.9564	9.1442
6	1.0029	3.0745	4.9816	6.9467	9.0377
SD	0.0073	0.0158	0.0087	0.0219	0.0375
mean	1.0013	3.0681	4.9719	6.9322	9.0942
RSD (%)	0.73	0.52	0.17	0.32	0.41
Mean of RSD	0.43 %				

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Total Phenolics Content (TPC) Analysis of Bio oil from Cashew Nut Shell Pyrolisis

The Folin–Ciocalteu (FC) method is widely applied for the determination of the total phenolic contents in natural products. Phenolic compounds react with FCR and change colour through an electron transfer mechanism only under an alkaline environment [23]. Gallic acid is a tri-hydroxyl-benzoic acid. The carboxyl group of gallic acid is reactive to the hydroxyl groups from FC reagent treatment (prior basification). Esterification condensations were observed in the assays with prior basification for gallic acid used as quantitative standards. The phenolic contents obtained in the samples differed depending on when basification occurred compared with the gallic acid calibration [24].

In this study, TPC of bio oil from pyrolysis of CNS at different temperatures (400, 500, 600 and 700 °C) are 1.2300, 2.0575, 2.1780 and 1.8373 g/L, respectively. Table 3 shown that the result of the analysis of TPC of CNSL.

Table 3: Total Phenolics Content (TPC) of CNSL

Pyrolisis Temperature	CNSL Concentration (mg/L) in 0.2% solvent	Mean of Phenolic concentration (mg/L) in 0.2% solvent	Mean of Phenolic concentration (g/L) in CNSL
400 °C	2.0192	2.4660	1.2300
	2.7039		
	2.6750		
500 °C	4.1022	4.1150	2.0575
	4.1022		
	4.1407		
600 °C	4.3529	4.3561	2.1780
	4.3432		
	4.3722		
700 °C	3.7164	3.6747	1.8373
	3.6586		
	3.6489		

Accuracy

Accuracy analysis is done by using the recovery of spiked sample or standard additions means. Sample to be analyzed with added a certain amount of analyte concentration. This method was carried out to determine of % recovery by percentage of analyte that was added previously to be found [17,21]. The percent recovery was calculated by using the formula was given below :

$$\%\ recovery = \frac{A.100}{A_T}$$

where A = absorbance of sample after addition of the standard; A_T = theoretical absorbance calculated for the sum of the absorbance of bio oil from pyrolisis of CNS and the expected absorbance of gallic acid, based on the calibration curve for each level. The average percent recovery and RSD values were found to be 103.15 % and 1.51 %, respectively. The method is considered accurate if the recovery percentages are between 85% and 115% [9]. Therefore, overall of the accuracy test requirements are acceptable due to fulfill the specified requirements. Accuracy test data by recovery method can be seen in Table 4.

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Table 4: Analysis of % Recovery

Sample Concentration (mg/L)	Analyte Concentration added (mg/L)	Total measured concentration (mg/L)	Recovery (%)
4.3529	1	5.4040	105.11
		5.3847	103.18
		5.3369	99.70
	3	7.4291	102.53
		7.4869	104.46
		7.4676	103.82
	5	9.5120	103.18
		9.4734	102.41
		9.5506	103.95
Mean of recovery SD RSD			103.15 % 1.56 1.51 %

Specificity

Analysis of the results of the specificity test indicated that the conditions were satisfactor. In the case of complex matrices, if the matrix without the analyte is not available, the effects of the matrix system can be tested by comparing the slopes of linearity and specificity [25–27]. Figure 3 shows the comparison of the linearity of the standard curve with standard solutions that have been added to the sample so that there is a matrix effects (specificity). Test specificity in the determination of TPC from CNSL using FC method produces a correlation coefficient 0.9996 (standard solution) and 0.9913 (specificity).

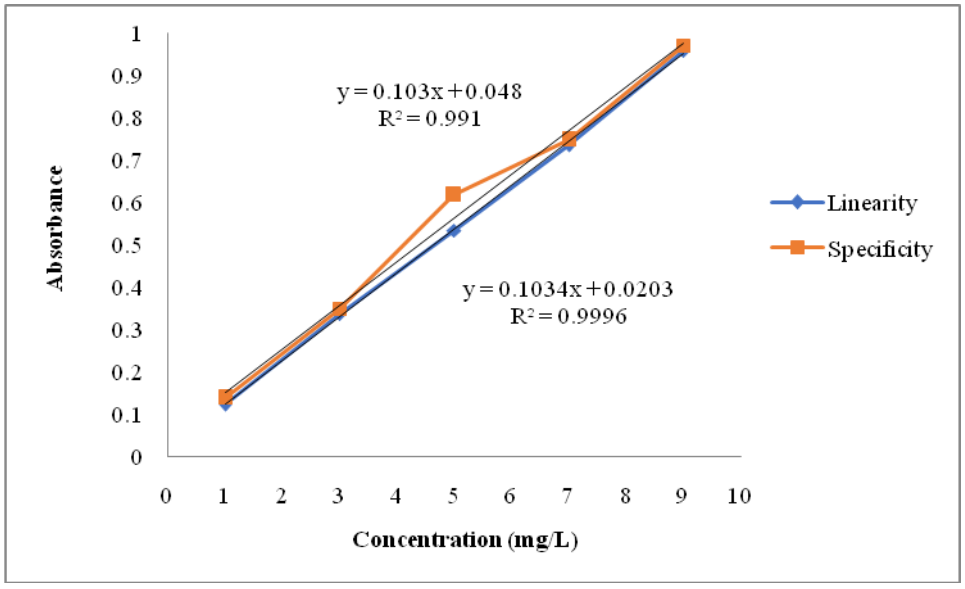

Figure 3: Linearity and specificity curve, with linear equation.

CONCLUSION

The UV-Vis spectrophotometric method described here was successfully validated as suitable for the determination of TPC of bio oil from pyrolisis of CNS. The total content of phenols in a sample of the CNSL are 1.2300, 2.0575, 2.1781 and 1.8374 g/L, respectively. In this study, accuracy and precision analysis, this

methods can be used accurately and have good precision with value of recovery tested is 103.15 % and RSD is 0.43%.

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