

Original Article

Identification and authentication of *Eurycoma longifolia* root extract from *Zingiber officinale* rhizome using FTIR spectroscopy and chemometrics

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Abstract: Pasak bumi (*Eurycoma longifolia*) is one of the plants with androgenic effect having high price in the herbal medicine market. This study was aimed to apply FTIR spectroscopy in combination with chemometrics for identification and authentication of *Eurycoma longifolia* root extract (ELRE) from *Zingiber officinale* rhizome (ZOR). Identification of ELRE was performed by determining the functional groups responsible for infrared absorption, while the authentication of ELRE was carried out by measuring a mixture of ELRE and ZOR with different concentrations (0-100% of ELRE) using FTIR spectroscopy combined with principal component analysis (PCA) for classification between ELRE and ELRE adulterated with ZOR and partial least square regression (PLSR) for quantification of adulterants. The results indicated that ELRE could be identified at selected fingerprint wavenumbers of 1500 -1300 cm^{-1} , by the presence of several functional groups including O-H, C=O, C=C, C-O, and C-H. PCA could successfully ELRE and ELRE adulterated with ZOR with a total of 99% variations could be described by two principle components. In addition, PLSR using second derivative spectra successfully predict ZOR levels with coefficient of determination (R^2) of 0.993, root mean square error of calibration of 2.563% and root mean square error of cross validation (RMSECV) of 5.057%. The combination of FTIR spectroscopy and chemometrics was an ideal method for identification and authentication of ELRE adulterated with ZOR..

Keywords: authentication, *Eurycoma longifolia*, *Zingiber officinale*, FTIR spectroscopy, PLS, PCA

1. INTRODUCTION

Pasak bumi (*Eurycoma longifolia*) is one of the plants found in Kalimantan which has an androgenic effect [1]. The efficacy of *E. longifolia* root as an aphrodisiac is well known to public so that its uses as a traditional medicine is growing [2]. In the market, *E. longifolia* powder is relatively expensive so that it tends to be falsified with lower priced of herbal plants. Falsification can occur by mixing *E. longifolia* with other ingredients such as ginger (*Zingiber officinale*). The product named with "Pasak Bumi Plus Toga" revealed that *Z. officinale* extract is used as a mixture because it has synergistic effect and relatively cheap. Red ginger (*Z. officinale* var. Rubrum) extract is less potential

as an aphrodisiac effect compared to *E. Longifolia* root extract [3]. Because of the expensive price of *E. longifolia* roots in the market of herbal industries, manufacturers may substitute intentionally or unintentionally with raw herbal materials with cheaper components [4].

Adulteration of herbal medicines is a common practice to gain economical profits. Adulteration poses a serious problem because it is related to the efficacy, safety, and quality of products [5]. Therefore, it is very important to develop fast and reliable method to detect the adulteration of herbal medicines. Several methods have been reported for authentication of *E. longifolia* roots. The various conventional analytical techniques such as organoleptic, microscopic and macroscopic analyses with their own advantages and disadvantages have been reported for authentication of herbal components [6]. Instrumental methods used are High Resolution Melting (HRM) analysis combined with DNA barcode [7], high performance liquid chromatography [8].

FTIR spectroscopy, due to its property as fingerprint technique, can be an ideal method for authentication of *E. longifolia* roots [9]. Combined with chemometrics, FTIR spectroscopy have been used for the authentication of *Sedum sarmentosum bunge*, *S. lineare* Thunb., *S. erythrostictum migo*, and *S. aizoon* L [10], distinguishing of American and Asian ginsengs from two morphological fakes [11], authentication of *Radix astragali* and authentication Greek pennyroyal (*Mentha punigeum* L) samples according to their origin [12] and authentication of *Mentha pulegium* L. (Pennyroyal) [13]. In this study, FTIR spectroscopy combined with chemometrics for authentication of *Eurycoma longifolia* root extract (ELRE) from *Zingiber officinale* rhizome.

2. MATERIALS AND METHODS

The samples of *E. longifolia* root used were obtained from 3 different places in Kalimantan, namely Mandiangan Village in South Kalimantan Province, Sabuai Village in Central Kalimantan Province and Condong Village in West Kalimantan Province. The rhizome samples of *Z. officinale* variant Rubrum were obtained from Pengaron Village, South Kalimantan Province. The other chemicals and solvents used were of pro-analytical grade.

2.1. Preparation of extract

The samples was sorted wet and washed, then they was chopped and oven at temperature 60 °C for *E. longifolia* root and 50 °C for the *Z. officinale* rhizome until their constant weight was obtained. The dried samples was sorted dry and then smoothed and sieved through 25 mesh for *E. longifolia* root and 40 mesh for *Z. officinale* rhizome [3,14-16]

2.2. Extract *E Longifolia* and *Z officinale*

A-150 grams of *E. longifolia* root powder of and 100 grams of *Z. officinale* rhizome powder were macerated by 70% ethanol, their ratio 1:25 and 1:15 respectively for 3x24 hours. Then, macerate was filtered by filter paper and concentrated by vacuum rotary evaporator at 60 °C and then evaporated by water bath at 60 °C [17-18]. The thick extract was dried by aerosil with ratio (1: 0.8 and 1: 0.9) for *E. longifolia* root and (1: 0.5) for *Z. officinale* rhizome. Homogeneous extract mixture and aerosil was oven at 50 °C for 1 hour until the extract was dried [19].

2.3. FTIR-ATR spectroscopic analysis

A-50.0 mg of each extract mixture model was mixed with 950.0 mg of KBr powder for IR crushed until homogeneous. The sample was analyzed by FTIR-ATR spectrophotometry at

wavenumber 4000-400 cm^{-1} with resolution of 2 cm^{-1} and 32 scans [20]. The quantification model based on multilevel concentrations of 20,40,60,80 and 100%.

2.4. Data analysis

Qualitative analysis based on spectra data interpretation, it observed absorption peaks in spectra that referred the particular functional groups. Besides that, it also used Principal Component Analysis (PCA) for data classification. [21-22]. The quantitative analysis used Partial Least Square Regression (PLSR). The Validation data whereas the accuracy of the prediction model for determined total flavonoid extract of Pasak Bumi root using PLSR was by the correlation value and the RMSE value obtained. The total flavonoid prediction model can be used if the RMSEC and RMSECV values are close to 0 and the R^2 value is close to 1.

3. RESULTS AND DISCUSSION

3.1. Extraction of *E.longifolia* Root and *Z. Officinale* Rhizome

Table 1 is the result of the extraction of *E. longifolia* from 3 regions namely Mandiangin, Sabuai and Condong. This regional selection is based on a study of where to grow and empirically the community uses it for traditional medicine.

Table 1. The extraction result of *E. longifolia* root

Sample	Powder (g)	Extract (g)	Rendement (%)
Mandiangin Village	150.0	10.24	6.82
Sabuai Village	150.0	7.83	5.22
Condong Village	150.0	7.88	5.25

Extraction of 100 gram of *Z. officinale* rhizome powder obtained 3.8 gram thick extract with yield of 3.8%. The extract was dried by aerosil as an adsorbent. Aerosil was used because it only had absorption at wavenumber region 970 cm^{-1} so that it would not interfere the absorptions in other wavenumber regions [23]. The results of dry extract can be seen in Table 2.

Table 2. The results of *E. Longifolia* dried root extract

Sample	Thick extract (g)	Aerosil (g)	Dry extract (g)	Water Content (%)
Mandiangin Village	6.50	5.80	11.14	0
Sabuai Village	6.38	5.10	10.90	0
Condong Village	6.16	4.93	10.87	0

The *Z. officinale* dried rhizome extract was obtained 5.062 gram. The dried extract produced in the form of fine powder with brown color that is brighter, smells typical and tastes bitter.

3.2. Analysis of Extracts by FTIR Spectrophotometry

FTIR spectra were scanned at the mid-infrared region corresponding to wavenumber range of 4000-400 cm^{-1} . The results of FTIR spectra of *E. longifolia* root and *Z. officinale* rhizome extract were shown in Figure 1.

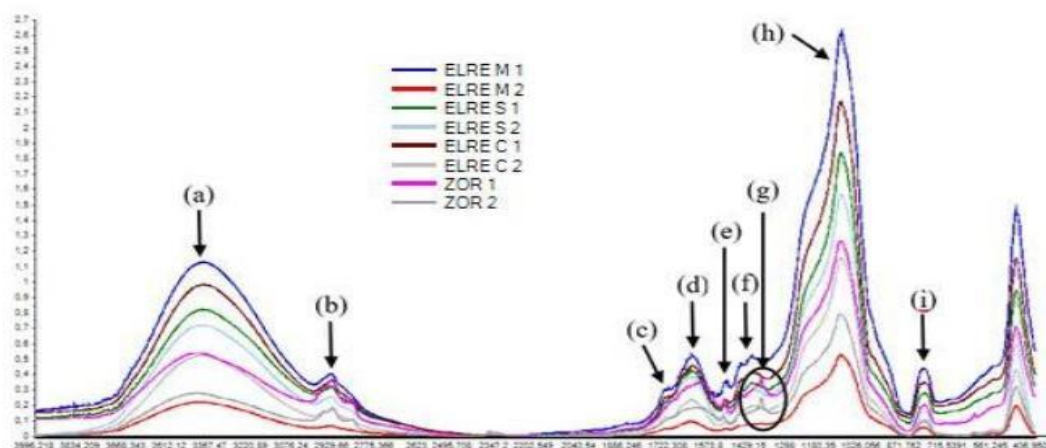


Figure 1. FTIR spectra of *E. longifolia* root and *Z. officinale* rhizome extracts (a) at wavenumber 4000-400 cm^{-1} *E. longifolia* (ELRE) from Mandiangin Village 1st and 2nd replications, 1 and 2: *E. longifolia* (ELRE) from Sabuai Village 1st and 2nd replications, 1 and 2: *E. longifolia* (ELRE) from Condong Village 1st and 2nd replications, and J 1 and 2 : *Z. officinale* (ZOR) 1st and 2nd replications.

The absorption peaks corresponding to functional groups responsible for the presence of specific components at certain wavenumbers can be seen in Table 3.

Table 3. The functional groups from FTIR spectra of *E. longifolia* root and *Z. officinale* rhizome extract [24]

Codes	<i>E. longifolia</i>		<i>Z. officinale</i>		Vibration type
	Frequency (cm^{-1})	Functional group	Frequency (cm^{-1})	Functional group	
(a)	3392	O-H	3419	O-H	Free
(b)	2931	C-H ₂	2928	C-H ₂	Stretch
(c)	1733	C=O	1710	C=O	Stretch
(d)	1635	C=C	1603	C=C	Stretch
(e) & (f)	1418 & 1518	C-H ₂	1518	C-H ₂	Bend
(g)	1397	C-H ₃	1385	C-H ₃	Bend
(h)	1097	C-O	1099	C-O	Stretch
(i)	798	C-H	798	C-H	Bend

The difference between the *E. longifolia* root and *Z. officinale* rhizome extracts could be observed at peak (g) due to CH₃ bending vibration. The absorption intensity of the *Z. officinale* rhizome spectra was stronger than the *E. longifolia* root spectra. Both extracts had the same

functional groups but their absorption peaks had different intensities and exact wavenumbers. This difference was influenced by atomic masses and the bond between atoms according to Hooke's law [24]. The wavenumber regions of 1500-1300 cm^{-1} was included in the fingerprint region. Each compound had different pattern in this region, therefore it was difficult to analyze the bond types because its absorption was usually very complex. This wavenumber region also had a lot of bending vibration of methyl groups (-CH₃) and methylene groups (-CH₂) [25]. Based on the chemical structure of eurycomanone, an active compound present in *E. Longifolia* had more methyl and methylene groups than the other components. It might also affect the absorption intensity result as intensity is directly proportional with the concentration of functional groups.

3.3. Principal component analysis of FTIR spectra of extracts

The combined spectra of series concentrations of evaluated samples at wavenumber range of 4000-400 cm^{-1} using 1st and 2nd were shown in Figure 2. Optimization of wavenumber regions in PCA analysis using 1st and 2nd derivatives could be seen in Table 4. The selected segment optimization was R2 segmentation spectra data with preliminary process because there was distinctly difference between the spectra of *E. longifolia* root and *Z. officinale* rhizome extracts [23]. The result of score plot in this segment could also clearly classify among pure of *E. longifolia* root and *Z. officinale* rhizome extracts and their mixed extracts. Their plot score results can be seen in Figure 3.

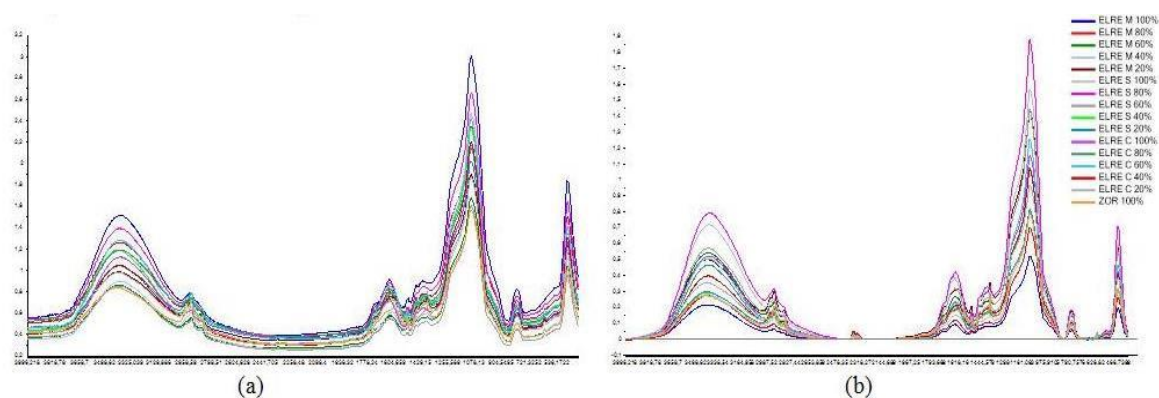


Figure 2. FTIR spectra mixed between *E. longifolia* root and *Z. officinale* rhizome extracts with level concentration (0-100%) at wavenumber range 400-4000 cm^{-1} (a) 1st replication (b) 2nd replication

3.4. Analysis using partial least square calibration

Determination of the contents of *E. longifolia* root and *Z. officinale* rhizome extracts in the mixture based on the wavenumber range which had best separation and high total variation from the PCA analysis result, it was R² segmentation spectral data with preliminary process [26]. The combined spectra of the concentration series in the characteristic wavenumber (1500-1300 cm^{-1}) can be seen in Figure 4.

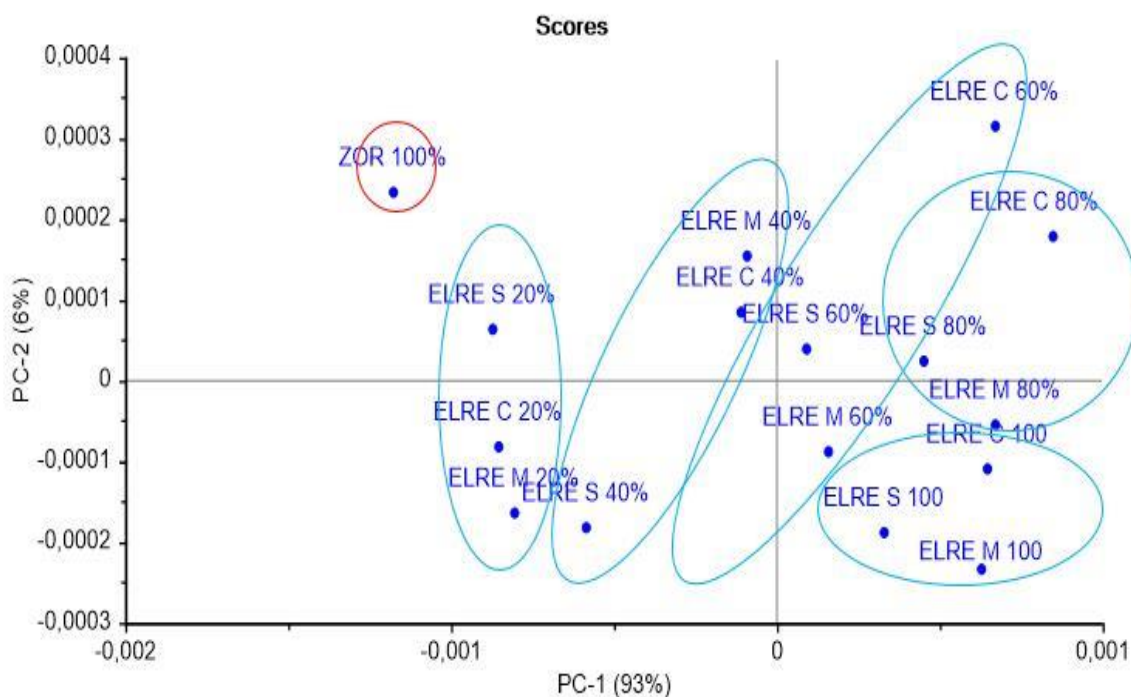


Figure 3. Score plot PCA R2 on level concentration (0-100%) at wavenumber 1500-1300 cm^{-1} with preliminary process

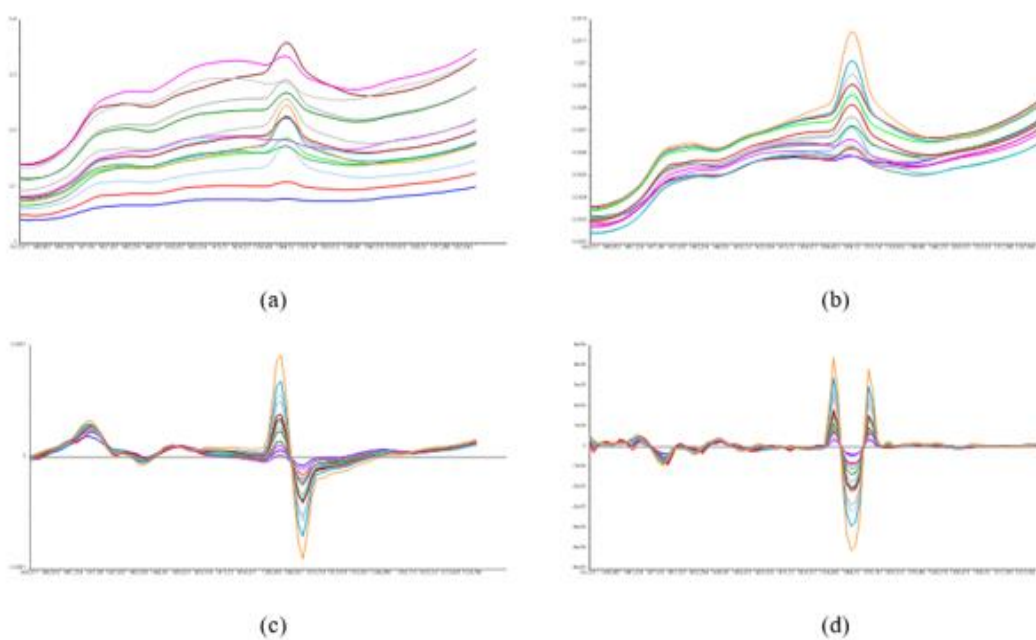


Figure 4. FTIR spectra mixed extract of *E. longifolia* root with *Z. officinale* rhizome with level concentration (0-100%) at wavenumber region 1500-1300 cm^{-1} . (a) normal spectra, (b) spectra with preliminary process (B and N), (c) spectra derivative 1, (d) spectra of derivative 2 (B dan N)

The results of the PLSR calibration and validation model between normal spectra, with the preliminary process, and derivative spectra can be seen in Table 5. The model selected in this study

was derivative spectra 2 because it had higher R^2 value calibration and lower RMSEC and RMSECV values compared to other spectral models [21,27]. The relationship graph between the reference value (x-axis) and predicted value (y-axis) extract of *E. longifolia* root and *Z. officinale* rhizome based on calibration and validation analysis sample can be seen in Figure 5.

The model obtained in this study was the calibration model $y = 0.993x + 0.384$ with R^2 of 0.993 and the RMSEC of 2.563. Validation based on the calibration model was obtained by cross validation method. It result was obtained $y = 0.996x - 0.272$ with R^2 of 0.977 and the RMSECV of 5.057. According to [28-29] calibration model can be accepted if R^2 was greater than 0.99. The smaller the RMSEC and RMSECV values obtained from model, indicated the less error.

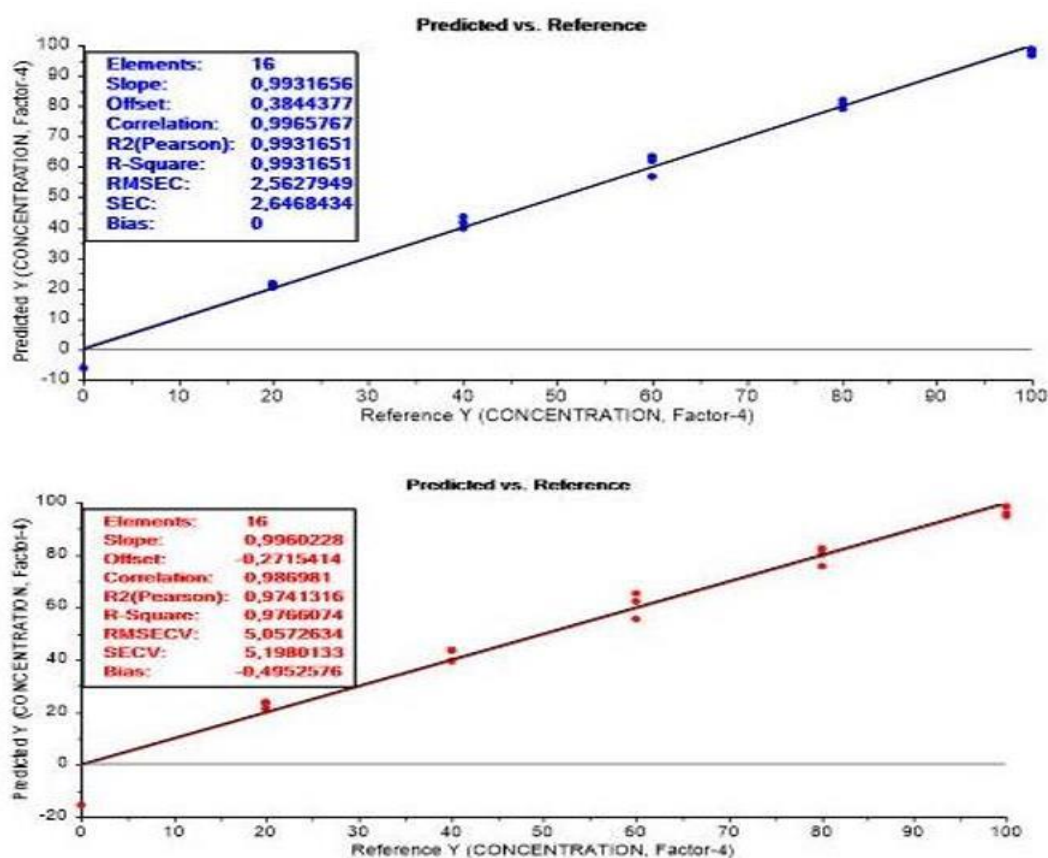


Figure 5. The partial least square regression (PLSR) which model correlates between reference with predicted value in mixture *E. longifolia* root extract with *Z. officinale* rhizome extracts using derivative spectra 2 at wavenumber range ($1500-1300\text{ cm}^{-1}$) (a) calibration (b) validation.

4. CONCLUSION

Identification of infrared spectrum pattern of *E. longifolia* root extract obtained functional groups O-H (3392 cm^{-1}), C=O (1733 cm^{-1}), C=C (1635 cm^{-1}), C-O (1097 cm^{-1}), C-H stretch (2931 cm^{-1}) and C-H bend (1418 cm^{-1} dan 798 cm^{-1}). The FTIR spectrophotometry combined with chemometrics at wavenumber region $1500-1300\text{ cm}^{-1}$ is able to classify the mixture of *E. longifolia* and *Z. officinale* root extracts with total score of 99% PC and can be used for their mixture authentication. The model is obtained $y = 0.993x + 0.384$ with R^2 of 0.993 and the RMSEC of 2.563 and RMSECV of 5.057.

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Conflicts of interest: The authors declare no conflict of interest

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