Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 13, Issue 2, 2023, 153
https://doi.org/10.33263/BRIAC132.153

FTIR Spectroscopy Combined with Chemometrics for Evaluation of Gambir Extract – Self Nano Emulsifying Formulation from *Uncaria gambir* Roxb

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Scopus Author ID 57204864081
Received: 11.01.2022; Accepted: 11.02.2022; Published: 28.03.2022

Abstract: Gambir leaf (*Uncaria gambir* Roxb) has potential as an antidiabetic through the inhibition of α-glucosidase and amylase enzymes. However, natural extracts have disadvantages such as solubility, stability, and bioavailability. Therefore, gambir leaf extract was formulated to be self-nano emulsifying (GE-SNE) with several different concentrations of constituent components (12 formulas). Several GE-SNE formulas were evaluated using a Fourier Transform Infrared (FTIR) Spectrophotometer to classify spectral patterns. The absorbance value of the wavenumber that appears in all GE-SNE was analyzed using a chemometric approach. In chemometric analysis, fingerprints have a broad influence on the results of the spectrum of gambir leaf extract and self-emulsifying formulations. The classification results provide helpful information in the development and optimization of GE-SNE. Different concentrations of GE-SNE affect the viscosity and specific gravity of each formula. In addition, there are differences in the group of variables in the chemometric analysis based on the similarity of properties. FTIR-ATR combined with chemometric analysis can classify GE-SNE based on differences in their composition.

Keywords: Chemometrics; FTIR-ATR; nanoemulsion; PCA-CA; self-nano emulsifying; *Uncaria gambir* Roxb.

1. Introduction

High blood sugar levels cause hyperglycemia and lead to diabetes. This disease is caused by several conditions such as impaired insulin secretion and action or both disorders of fat, protein, and carbohydrate metabolism. The effects felt by diabetics in the long term are nephropathy, neuropathy, retinopathy, and other complications. Diabetic patients also tend to be at increased risk of other diseases such as heart disease, obesity, cataracts, kidney dysfunction, erection, liver damage, and some liver diseases [1,2].

Drug interactions increase with the increasing variety and number of drugs in therapy, and this condition affects poor blood glucose level (BGLs) control and therapeutic outcome. The most common drugs involved in drug interactions were insulin with angiotensin receptor blocker, aspirin, quinolone, and ace inhibitor. It was dissimilar to the study that reported aspirin, clopidogrel, simvastatin, amlodipine, beta-blockers, NSAIDs agents, and ACE inhibitors were most implicated in drug interactions [3]. Gambir plant (*Uncaria gambir* Roxb.) can be the alternative to control the decrease in blood sugar levels in diabetic patients. The results showed that the average blood sugar level in the intervention group given gambir was 199.88 mg/dL, the control group was 326.25 mg/dL. Based on existing publications, giving *Uncaria gambir* has reduced BGLs [4,5].

Phytochemical analysis showed that the significant antioxidant compound in *Uncaria gambir* is catechin, as the predominant secondary metabolite. Catechins are capable to obstructed and scavenging free radicals. In general, gambir extracts have high antioxidant activities [5]. Gambir has antioxidants caused by the polyphenol group, such as catechins. Gambir can inhibit free radical activity and act as an inhibitor of the α-glucosidase enzyme. The IC_{50} value of catechins extracted with ethyl acetate for the inhibition of DPPH is 4.60-18.20 g/mL, and the IC_{50} value for the inhibitory effect of α-glucosidase is 40.45-52.43 g/mL so that gambir can be classified as antidiabetic [6]. Based on the research above, in this study, the polyphenols of gambir leaves, namely catechins, are an alternative herbal treatment for diabetes mellitus, taken by extraction using the UAE method using 10% ethyl acetate that diluted in aqua dest as a solvent with a ratio of 1:20 (w/v). Ethyl acetate is used as a solvent because the content of total phenols of the extract is higher than other solvents and can obtain the higher catechins in gambir leaves extract [7]. However, these active polyphenol compounds show less than 5% oral bioavailability, low membrane permeability, and poor absorption in the digestive tract (GIT) [8]. Therefore, catechins are formulated in self-emulsifying.

The results of previous studies have proven that catechins and other polyphenolic compounds gambir leaves extract have pharmacological potential. However, the solubility of extract has been a problem in the formulation. Gambir leaves extract has low solubility and bioavailability. There are no previous studies that can solve the problems of gambir leaves extract in the formulation. Therefore through this study, gambir leaves extract is formulated using a medicinal technology delivery system in the form of self-emulsion (SE) that can be improved solubility and bioavailability of gambir leaves extract.

Lipid-based formulations, especially drug delivery systems self-emulsifying (SE), especially nano and micro size, have been reported can increase oral bioavailability. Gentle agitation spontaneously forms an oil-in-water nanoemulsion in the aqueous phase with 200 nm of globule size. Because its size is in the nano range, it can avoid various physicochemical barriers (solubility, sedimentation, and creaming) and barriers to biologics first pathway metabolism, *P*-glycoprotein, and efflux) [9,10]. The development of pharmaceutical preparations in self-emulsifying is potential for hydrophobic materials. Self-emulsifying is an isotropic mixture of oil, surfactant, and cosurfactant, which spontaneously forms a nanoemulsion when mixed with water through mild agitation in the digestive tract [11,12]. The formed nanoemulsion has less than 200 nm [13,14]. Currently, many studies approach using the design of experiment (DoE). However, there are no reports regarding the application of chemometric combinations of infrared spectra in the evaluation of SE.

Fourier transforms infrared (FTIR) spectroscopy in this study was carried out to determine the presence of catechin compounds, interactions, and stability in the preparation of

self-emulsifying formulation with three different essential components in the form of oil surfactants and co-surfactants. FTIR spectrophotometer is used because of chemical analysis acquired fast spectra with large quantities. This analysis is mostly used for qualitative analysis. FTIR is usually used to identify a compound where the parameter is the wavenumber that arises due to the absorption by a unique functional group in the compound. Infrared spectrum data processing is carried out using a chemometric approach as the multivariate statistical method [15,16]. PCA analysis helps reduce the dimensions on the graph generated by FTIR to be easier to analyze. CA analysis was used to classify the types of oil based on the similarity between samples [17–19].

2. Materials and Methods

2.1. Chemical materials.

Gambir leaves obtained from Babat Toman-Banyuasin, Sumatera Selatan (Indonesia). Materials needed in this study include catechins obtained from Sigma-Aldrich (Singapore), oleic acid from Bratachem (Jakarta, Indonesia), tween 80 purchased from Croda (United Kingdom), propylene glycol from Dow Chemical (Indonesia). Other materials (such as distilled water, aqua pro injection, ethyl acetate, and ethanol) are purchased from local distributors in Palembang, Indonesia.

2.2. Extraction from gambir leaves.

The powder was sieved with a 60 mesh sieve to form a fine, uniform powder. The extraction process is carried out with the Ultrasonic Processor Qsonica–Q500. A total of 5 g samples of gambir leaf powder were extracted by the ultrasound-assisted extraction (UAE) method using water and 10% ethyl acetate as a solvent with a ratio of 1:20 (w/v) material and solvent.

2.3. Preparation of self nano emulsifying.

The extract was dissolved in oleic acid using a vortex and ultrasonication for 5 minutes. The mixture was added to propylene glycol until homogeneous, and then tween was added until homogeneous [11,20,21]. The mixing process can be assisted using ultrasonication for 5 minutes. The developed formula only varies the concentration of the constituent components. The complete design for the self-emulsifying formulas is presented in Table 1.

Table 1. Design formula of gambir extract – self-nano emulsifying (GE-SNE).

Formula	Oleic acid (%)	Tween 80 (%)	Propylene glycol (%)
F1	47.18	42.81	10.00
F2	10.00	67.77	22.22
F3	60.00	30.00	10.00
F4	47.09	42.90	10.00
F5	47.18	30.00	22.81
F6	25.48	44.51	30.00
F7	47.18	30.00	22.81
F8	33.61	52.52	13.86
F9	33.61	52.52	13.86
F10	10.00	67.66	22.33
F11	10.00	80.00	10.00
F12	23.01	66.95	10.03

2.4. Transmittance determination.

Samples in GE-SNE and the obtained emulsion form were put into a cuvette; the volume of each sample is 3 mL. Clarity was measured as percent transmission (%T) at a maximum wavelength of 650 nm using a Genesys 10s series Spectrophotometer (Thermo Scientific, USA). The blank used to measure the percent transmission was distilled water [22].

2.5. Evaluation of self-nano emulsifying.

Evaluation of pH test in the self-emulsifying formulation of gambir leaves extracts is used pH paper by dipping the pH paper into the formula. The color that obtained is matched with the standard color in the container. A self-emulsifying viscosity test was carried out with an Ostwald viscometer. The Ostwald viscometer requires fewer samples than other viscometers. The principle used is to measure the time it takes for the liquid to pass through two predetermined points on a vertical capillary tube [11,23].

2.6. Centrifugation assay.

Centrifugation evaluated stability using the BKC-TL4IV Biobase series instrumentation (Shandong, China). Samples were tested at a speed of 3500 rpm for 30 minutes. In addition, observation of instability parameters such as separation, precipitation, creaming, and cracking was carried out.

2.7. Determination droplet using particle size analyzer.

The nanoemulsion formed was evaluated from droplet diameter and zeta potential using the Zetasizer Nano ZSP particle size analysis tool (Malvern Panalytical, UK). GE-SNE samples were emulsified in aqueous media. The emulsion formed was measured according to predetermined specifications, and Zetasizer 7.12 software was used to process the data obtained [24,25].

2.8. FTIR-ATR spectra.

The infrared spectral pattern of the GE-SNE samples was measured using a Nicolet iS10 series infrared spectrophotometer (Thermo Scientific, USA). The type of detector used in the measurement is deuterated triglycine sulfate. The measurement is 4000-500 cm^{-1}, the resolution is 4 cm^{-1}, and the reading is 16 scans/minute. Omnic 4.2 software (Thermo Scientific, USA) was used to translate and evaluate the obtained spectral patterns. The absorbance value of the sample spectra was used as quantitative data and statistically analyzed [26].

2.9. Chemometrics analysis.

The absorbance at the specified wavenumber of the FTIR-ATR spectrum was analyzed using principal component and cluster analysis (PCA-CA) techniques. Classification and response evaluation data were analyzed using a chemometric approach using the Minitab series 17 software (Minitab, State College, PA, USA) [27,28].

3. Results and Discussion

3.1. Self-emulsifying loaded gambir leaves extract.

Self-emulsifying preparations were carried out by extracting dried powder which had become powder by the maceration method. Catechins are soluble in cold alcohol, ethyl acetate, hot water, glacial acetic acid, and acetone [29]. Based on this, the selected solvent was distilled water and 10% ethyl acetate with a ratio of 1:20. The results of the extraction of 175 grams of a sample of gambir leaf powder in 1750 mL of solvent obtained gambir leaves extract as much as 3.17 grams with a yield of 3.96%. Based on the results of obtaining extracts that meet the yield requirements from the Indonesian Herbal Pharmacopoeia, the yield is not less than 2.9%.

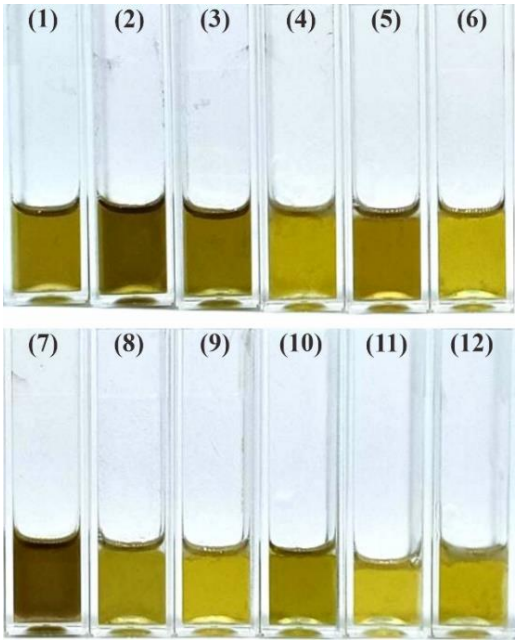

Figure 1. Visualization of GE-SNE from 12 formulas.

The results of the self-emulsifying preparation showed different colors. This phenomenon was formed due to differences in oil concentration, surfactant, and co-surfactant in oleic acid, tween 80, and propylene glycol. The GE-SNE preparation has been carried out several tests, and the results of these tests are carried out by chemometric analysis. The visual test results of the self-emulsifying preparation are in Table 2.

Table 2. Characteristics of 12 formulas (GE-SNE).

Formula	Phase	Color	Viscosity (mPa·s)	pH	Density	Droplet (nm)	Zeta Potential (mV)
F1	no separation	brownish-yellow	46.94	7	0.836	164.7	26.1
F2	no separation	blackish yellow	79.15	7	1.406	147.9	23.2
F3	no separation	greenish-yellow	48.36	7	1.018	371.6	24.1
F4	no separation	yellow	61.30	7	1.302	587.7	31.6
F5	no separation	brownish-yellow	58.18	7	0.902	266.6	21.7
F6	no separation	yellow	70.16	7	0.666	262.4	22.7
F7	no separation	blackish yellow	84.17	7	0.902	125.3	26.5
F8	no separation	greenish-yellow	52.76	7	1.310	408.5	24.5
F9	no separation	yellow	78.41	7	1.382	112.6	26.6
F10	no separation	greenish-yellow	73.06	7	1.162	273.0	25.9
F11	no separation	yellow	102.20	7	0.966	81.7	29.6
F12	no separation	yellow	82.92	7	1.238	103.1	26.3

The results show no phase separation in each formula, meaning that each formula had a stable formulation. The viscosity test results showed that all formulas had low viscosity and qualified the requirements. The pH test showed results in all formulas are 7, and the requirements are 4,5-6, and these results still can be accepted. Droplet tested qualified the requirements for almost all formulas below 200 nm. The zeta potential measurement in each formula showed results below 30 mV which means that all formulas had system stability and had no flocculation.

3.2. FTIR-ATR-based fingerprinting of GE-SNE.

GE-SNE stability is thermodynamically tested using heating-cooling, freeze-thaw, and centrifugation methods. FTIR spectrum of ethyl acetate extract catechins that appeared in the wavenumber region of 1000-1300 cm^{-1} at 1288.05 indicated the presence of stretching vibrations from the C-O group. Furthermore, the absorption that appears is 1628.28 in 1620-1680 cm^{-1}, indicating the presence of aromatic C=C bonds. Another absorption at 2935.24 cm^{-1} is in the range of 2850-3000 cm^{-1} (stretching vibration bonds from the C-H alkane group) and absorption at 3301.08 cm-¹ in the wavenumber region 3200-3350 cm^{-1}, indicating the presence of O-H bonds. The results obtained in this study also produce wave numbers in this range, indicating that GE-SNE contains catechin compounds. Figure 2a shows the absorption peaks and waves in the GE-SNE preparation, with ten selected peaks.

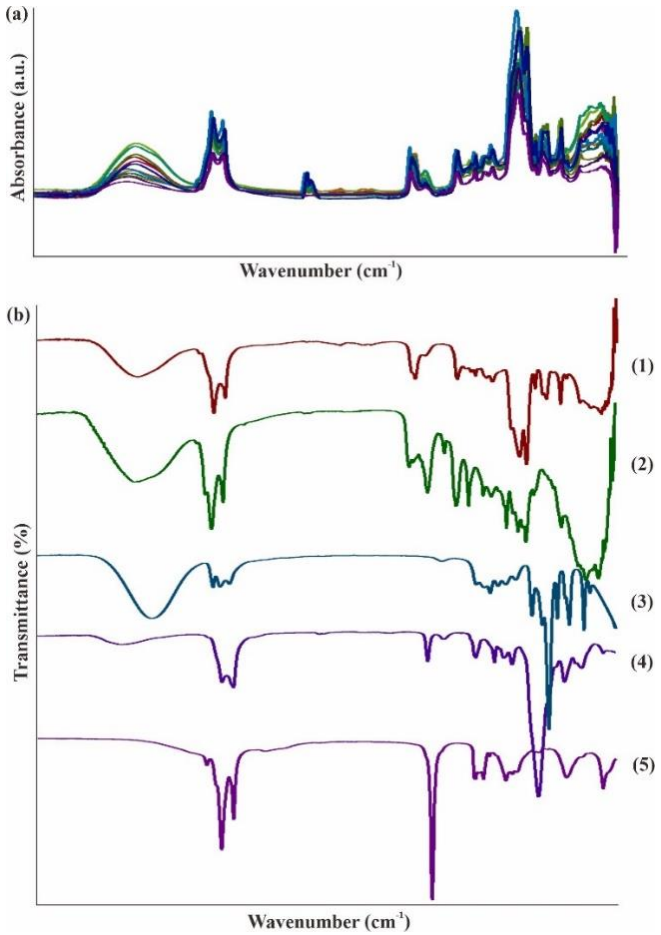

Figure 2. (**a**) FTIR-ATR spectra of GE-SNE and constituent components; (**b**) Gambir leaf extract and self-emulsifying; (**1**) self-emulsifying; (**2**) gambir leaf extract; (**3**) propylene glycol; (**4**) tween 80; (**5**) oleic acid.

The data from the formulation and characterization of the 12 GE-SNE formulas that have been obtained were processed using the chemometric analysis method. One of the

chemometric analysis techniques applied to evaluate the FTIR-ATR spectra in this study is PCA-CA. The multivariate approach used is a system or process of chemical analysis by applying mathematical and statistical methods. Chemometrics provides a method to automatically reduce the amount of data generated by the instrument [18,29].

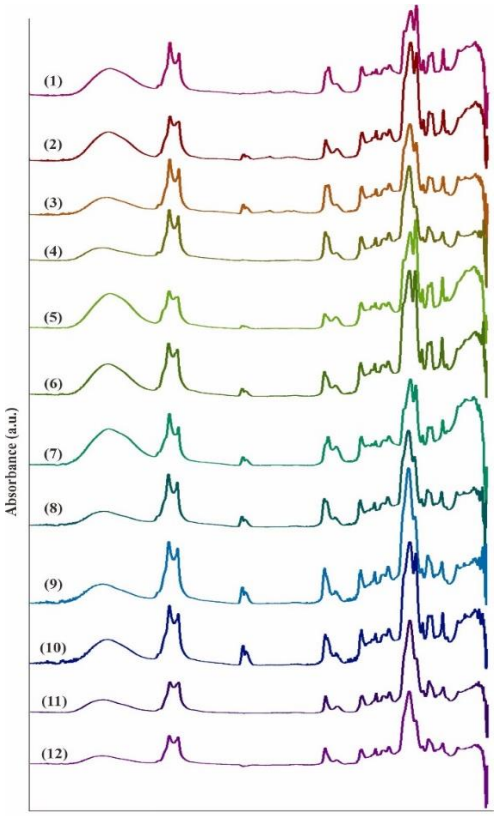

Figure 3. The spectra of GE-SNE formulas.

PCA analysis aims to simplify the observed variables by reducing data dimensions consisting of many interrelated variables without changing the information [27,28,31]. CA technique is a method based only on the information found in the data that describes the relationship and the object or based on the similarity of the characteristics of the object. The purpose of this analysis is so that the objects that join the cluster describe objects that are similar or related and not related to objects in other clusters.

Table 3. The absorbance values in the FTIR-ATR spectra of GE-SNE are 12 formulas.

Formula	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
F1	0.031	0.085	0.067	0.049	0.023	0.158	0.170	0.081	0.089	0.102
F2	0.045	0.071	0.063	0.036	0.024	0.213	0.195	0.093	0.107	0.111
F3	0.026	0.089	0.075	0.051	0.023	0.168	0.138	0.068	0.082	0.088
F4	0.025	0.087	0.078	0.057	0.027	0.186	0.120	0.069	0.088	0.079
F5	0.050	0.068	0.058	0.037	0.035	0.192	0.214	0.108	0.119	0.137
F6	0.038	0.075	0.068	0.042	0.033	0.198	0.199	0.097	0.107	0.115
F7	0.051	0.088	0.067	0.057	0.045	0.160	0.151	0.076	0.090	0.102
F8	0.031	0.092	0.079	0.062	0.039	0.187	0.146	0.080	0.097	0.094
F9	0.031	0.087	0.046	0.058	0.031	0.202	0.140	0.074	0.093	0.086
F10	0.042	0.086	0.076	0.057	0.039	0.191	0.176	0.090	0.101	0.104
F11	0.036	0.079	0.080	0.047	0.025	0.253	0.162	0.084	0.110	0.087
F12	0.021	0.083	0.083	0.054	0.022	0.230	0.149	0.077	0.098	0.086

The average data in Table 3 obtained from the absorbance of each wavenumber is the data that will be used for later processing in the Minitab software. Based on the pattern and the absorbance value of the FTIR-ATR spectra, the GE-SNE formula has many characteristics in common. It can be seen that the two samples have almost the same characteristics. The

chemometric analysis approach was used for classification based on the absorbance value of FTIR-ATR.

3.3. Principal component analysis and cluster analysis to assess response correlation and its classification.

PCA analysis on the Score plot figure 4a shows four groups, group 1 consists of runs 1, 7, and 10, group 2 consists of runs 3, 4, 8, and 9, group 3 consists of runs 11 and 12, and group 4 consists of runs 2, 6, and 5. Each group is grouped based on the proximity between points. The closeness between these points is due to the similarity of the characteristics of the FTIR-ATR spectra pattern. The farther the distance indicates, the less similarity [17,18,31]. The PCA analysis on the loading plot shows the angle correlation between the run formulas. Angles that are close together or coincide indicate that the correlation is positive.

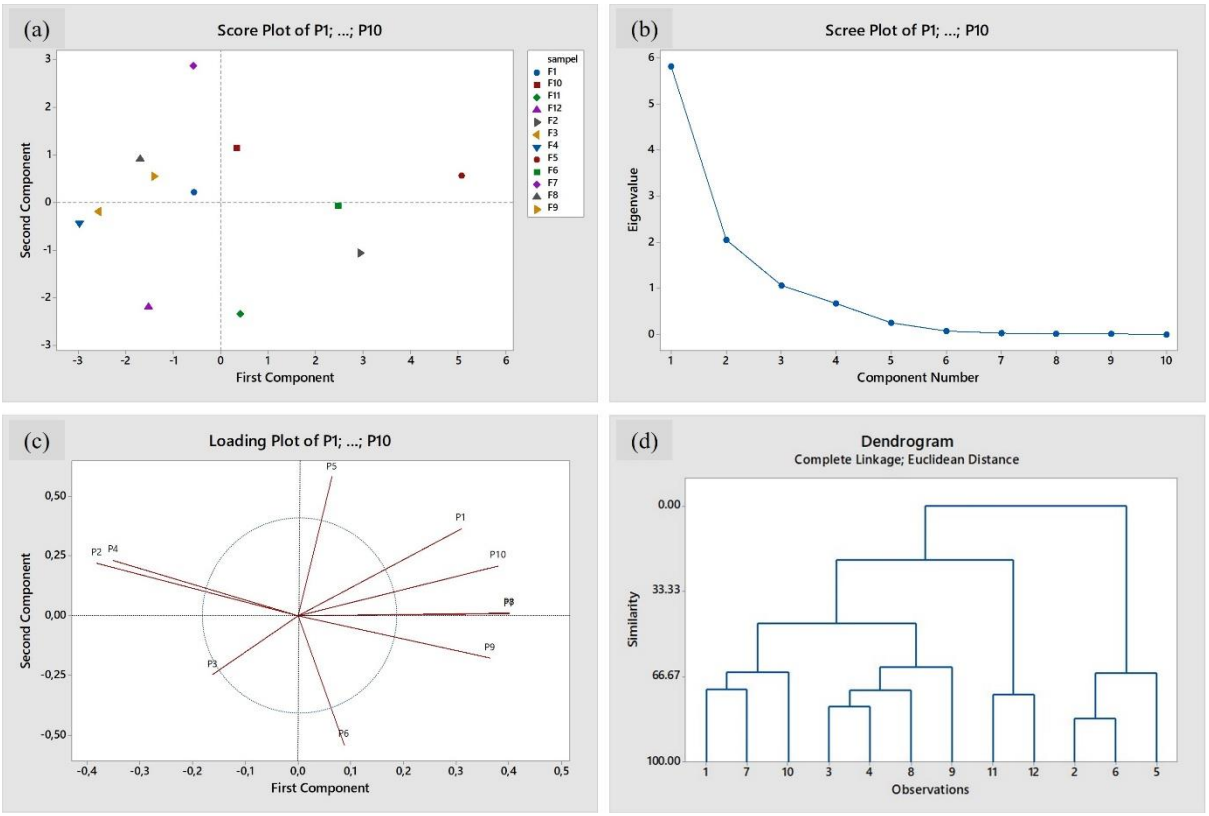

Figure 4. Chemometric analysis: (**a**) score plot; (**b**) scree plot; (**c**) loading plot; (**d**) dendrogram.

The scree plot in Figure 4b shows the eigenvalues that can show the main components. The requirement to get the main component of the eigenvalues must be that the data from Figure 4b shows only samples 1 to sample 4, which show the most influential sample in the classification, by showing the eigenvalues in sample 1 of 0.0018, sample 2 of 0.0008, sample 3 of 0.0001, and sample 4 of 0.0001. Subsequent samples 5 to 10 were excluded.

Figure 4c loading plots are used to evaluate the correlation between variables based on the angle formed between the variables used. If two vectors form a slight angle, the second variable is positively correlated. On the other hand, if two vectors form an angle of about 90°, then the two variables are not correlated. If the two vectors form an angle of about 180°, the two variables are negatively correlated [27,30,32].

PCA analysis on the dendrogram Figure 4d shows the same group of variables, and there is a bond in one group based on the value of proximity (similarity) [17,32]. The group of

variables on formulas 1, 7, and 10 has a similarity value of 64.96%. The variables in formulas 3 and 4 (78.13%), 8, and 9 have a similarity value of 62.88%. Meanwhile, run numbers 2, 5, and 6 have a similarity level of 82.94%, presented in the CA analysis results (Figure 4d).

4. Conclusions

There is an influence in the fingerprint area between spectrum result extract gambir leaves and self-emulsifying formulation. This difference indicates the success of gambir leaf extract into the system, self-emulsifying to load. There was no significant difference in oil, surfactant, and co-surfactant concentration in the FTIR spectrum. However, the difference in concentration affects the viscosity and density of each run of the self-emulsifying formula. In addition, there are differences in the group of variables in the chemometric analysis based on the similarity of properties.

Funding

This study was supported by PKM-RE Belmawa DIKTI No 99/E2/KM/2021.

Acknowledgments

The author is grateful, and this research is facilitated by the Laboratory of Pharmaceutical Biology. Thanks to the Phytopharmaceutic Research Center (PRC), LP2M Universitas Sriwijaya, and PT DKSH Indonesia.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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