FTIR-ATR based fingerprinting and chemometrics analysis of metabolites profile of Phyllanthus niruri L. affected by fertilization with NPK-chitosan nanopolymer and harvesting age

by Shaum Shiyan

EXPERIMENTAL PAPER

FTIR-ATR based fingerprinting and chemometrics analysis of metabolites profile of *Phyllanthus niruri* L. affected by fertilization with NPK-chitosan nanopolymer and harvesting age (*ahead of print*)

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Summary

Introduction: *Phyllanthus niruri* L. (PnL) is a herbaceous plant containing flavonoid quercetin and can be used as an immunomodulator to prevent Covid-19. However, the flavonoid content and yield of herbs extract were not maximized. Therefore, PnL herbs were planted in various harvest periods and application of NPK-chitosan nanopolymer fertilizer to estimate these parameters.

Objectives: Determine the effect of NPK-chitosan nanopolymer fertilizer and harvesting age on herb extracts also determine the grouping pattern and correlation between responses based on FTIR-ATR spectral pattern using a chemometric approach.

Methods: Each group consisted of 50 plants. The formulation of NPK-chitosan nanopolymer fertilizer based on the dose of NPK consisting of the first dose is 15.5 grams/group, the second dose is 31 grams/ group, and the third dose is 7.5 grams/group. Grouping of differences in harvesting age for plants consisted

of 4, 6, and 8 weeks after the plant (WAP). Extraction used ultrasound-assisted extraction, and data were analyzed using a chemometric approach.

Results: Extract with the highest yield was found in second harvest time and third doses of fertilizer (W2D3) which is 9.73 %, and the highest TFC obtained in an extract with second harvest time and first doses of fertilizer (W2D1) is 17.34 mg QE/g. Total flavonoid content and extract yield were influenced by functional groups at wavenumbers 3486.77–3157.12 cm^{-1} (1); 1740.96–1670.34 cm^{-1} (3); 1425.02–1272.62 cm^{-1} (5); 1257,753–1138,81 cm^{-1} (6); 1131.38–945.53 cm^{-1} (7); 711.36–529.23 cm^{-1} (8).

Conclusions: The results showed that harvest time and fertilizer dose affected the growth parameters of PnL, total flavonoid content, and yield of extract. Functional groups in IR spectra also have positive and negative correlations with total flavonoid and yield extract responses.

Key words: *chemometrics, chitosan nanopolymer, flavonoids, FTIR, Phyllanthus niruri*

Słowa kluczowe: *chemometria, monopolimer chitosanowy, flawonoidy, FTIR, Phyllanthus niruri*

INTRODUCTION

Herbal products with claims of immunomodulators experienced a very high increase in demand during the Covid-19 pandemic. *Phyllanthus niruri* L. (PnL) is one of the herbal plants that has been clinically tested as an immunomodulator and flavonoid, especially quercetin as a dominant phytochemical compound responsible for this mechanism [1]. Flavonoids in PnL leaves also have antiviral and anti-inflammatory activity [2]. *Phyllanthus niruri* herbs have extraordinary potential. Based on research, FTIR analysis on *Phyllanthus* genes such as *Phyllanthus amarus* and *Phyllanthus maderaspatensis* was carried out to determine differences in phytochemical compound content on differences in growth locations [3]. However, the use of *P. niruri* (PnL) herbs before they become extracts and final products still stays problematic, especially regarding cultivated sources' metabolite content, and there is no study about this research in available literature.

Agricultural engineering for traditional medicinal plants has been widely developed. Applying NPK fertilizer to plants can minimize production cost, maximize yield, reduce fertilizer wastage, reduce soil toxicity, maximize growth and dry matter production [4]. However, there is no scientific report on the fertilization pattern using NPK-nano polymer technology based on chitosan on PnL to increase extract yields and total flavonoid content. The application of chitosan nanopolymer NPK has been carried out on coffee plant seeds. Research shows that the application of chitosan nanopolymer can increase the number of leaves, plant height, and leaf area of coffee seedlings. Each

plant leaf with different nitrogen, phosphorus, and potassium fertilizers contains different compounds [5]. Therefore, the preparation of PnL as traditional medicine ingredients since cultivation is exciting to obtain high flavonoid content.

FTIR-ATR analysis enables identifying the main functional groups and chemical bonds, thereby providing information on the biochemical compounds present in the sample [6]. Fertilization variation is expected to give functional groups results the chemical content of different PnL herbs. The *Fourier transform infrared-attenuated total reflection* (FTIR-ATR) instrumentation will be chemometric analysis based on the functional group with the fingerprinting spectra pattern obtained.

The objective obtained from the FTIR characterization is to know the characteristics of the metabolite compounds contained in the PnL extract, which have been given in various doses and in different harvest times. Therefore, the purpose of this evaluation was to determine the relationship between harvest age and the effect of fertilization dose on the metabolite profile, total flavonoid content, and extract yield.

MATERIAL AND METHODS

Chemicals and reagents

Phyllanthus niruri L. seedlings were obtained from the Agrotech Training Center, Faculty of Agriculture, Universitas Sriwijaya (Indonesia). Supporting chemicals such as sodium tripolyphosphate, chitosan, glacial acetic acid, and quercetin were purchased from Sigma-Aldrich (Singapore). Other

ingredients such as distilled water, NPK 16-16-16® fertilizer, ethanol were purchased from a local distributor in Palembang (Indonesia).

Seeding process

The prepared soil is being watered. Plant seeds were placed on the seedling media and being sown also treated until the seeds sprouted and ready to be transferred to polybags. The seeding media consisted of trays and polybags. The formulation of NPK-chitosan nanopolymer fertilizer was divided into three doses consisting of different NPK fertilizer compositions. Doses are calculated and divided in table 1.

Table 1.
Dosage of NPK-chitosan nanopolymer fertilizer in the planting process

Doses	NPK for 1 plant [g]	Amount for 50 plants [g]
D1: dose 1	0.31	15.50
D2: dose 2	0.62	31.00
D3: dose 3	0.15	7.50

Preparation of NPK-chitosan nanopolymer

NPK-chitosan nanopolymer fertilizer was made by the modified ionic gelation method (tab. 2). Chitosan solution with 0.1% concentration was prepared with 0.35% acetic acid as a solvent and stored overnight at room temperature. NPK fertilizer is dissolved with mineral water in a ratio of 1:1 until dissolved. The chitosan solution was stirred with a magnetic stirrer for 1 hour, then mixed with NPK which had been dissolved according to the dose. Then, 0.25% Na TPP solution was dripped into chitosan solution with a ratio of chitosan: Na TPP 6:1 [5] and mixed in magnetic stirrer to form liquid fertilizer NPK-chitosan nanopolymer.

Table 2.
NPK-nanopolymer chitosan fertilizer formulation design

Substance name	Amount
Chitosan	0.1 g
Acetic acid 0.35%	100 ml
Sodium TPP	0.25%
NPK	according to 1st, 2nd, and 3rd dose

Particle size analysis

The particle diameter of the NPK-nanopolymer fertilizer was evaluated using the DLS-PSA Zetasizer Nano ZSP instrumentation (Malvern Panalytical, UK).

Grouping of several harvest ages on plants

Plants were divided into harvest age groups consisting of 150 plants. Each group of plants is given a different dose of fertilizer. Every 50 plants in 1 group were given doses of 1, 2, 3, and similarly for plants in groups 2 and 3. Plant group 2 were harvested six weeks after the plant (WAP), and plant group 3 were harvested at 8.

Plant cultivation process

The prepared soil is placed on a tray, watered with water, and cleaned of litter and dirt. Plant seeds are sown on seedling media and treated until the seeds grow and are ready to be transferred to polybags. As determined, each group of plants was given a different dose of NPK-chitosan nanopolymer liquid fertilizer.

Extraction and yield calculation

Extraction was performed using the ultrasound-assisted extraction method [7], comparing PnL powder and 70% ethanol solvent, 1:10 *w/v*. The result was concentrated to obtain an extract. The yield obtained was calculated, weighed, and recorded [8].

Determination of total flavonoid content (TFC)

The wavelength used is 400–450 nm with a standard solution of 10 ppm quercetin. The standard solution of quercetin was 1000 ppm, then several concentrations of the standard solution were made, namely 10, 20, 30, 40, and 50 ppm. The absorbance was determined using the UV-Vis spectrophotometric method at the maximum wavelength to obtain a linear regression equation [9].

The extracted sample for which the total flavonoid was determined was made with 2000 ppm concentration. Incubation was carried out at an

average temperature of 25-30°C for 30 minutes. The absorbance was determined using the UV-Vis spectrophotometric method at the maximum wavelength. Samples were made in three replications for each analysis, and the flavonoid content was calculated [9].

FTIR-ATR analysis

The analysis was carried out by measuring the infrared absorption of the extract with three replications at a wavelength of 4000-500 cm^{-1}, and the resulting IR spectra were analyzed. The IR spectrophotometer instrumentation used was Nicolet iS10 (Thermo Scientific, USA).

Chemometrics analysis

Data were analyzed using a chemometrics approach with principal component analysis (PCA) and cluster analysis (CA) methods with the help of Minitab series 17 software (Minitab, State College, PA, USA).

Ethical approval: The conducted research is not related to either human or animal use.

RESULT AND DISCUSSION

Fertilizers that have been formulated with chitosan nano polymer technology have good effectiveness. The data obtained from plant height, extract yield, and total flavonoid content proves an effect of giving nanopolymer fertilizer formula. The results showed that the application of fertilizers showed maximum results. The fertilizer formula at dose 1 had a droplet size of 98.45 ± 2.56 nm with a PDI value of 0.434. Dose 2 had a particle size of 92.84 ± 3.56 nm with a PDI value of 0.389 and dose 3 96.27 ± 3.58 nm with a PDI value of 0.415.

Cultivation of *Phyllanthus niruri*

Cultivation of plants according to harvest time and dose of fertilizer provides data on plant height. Plant height data was measured on ten plant samples in each group, and one plant sample was taken from each treatment at first harvest time group and first doses fertilizer to third harvest time and third

doses of fertilizer, then compared the plant heights for each week of planting as shown in figure 1 and table 3.
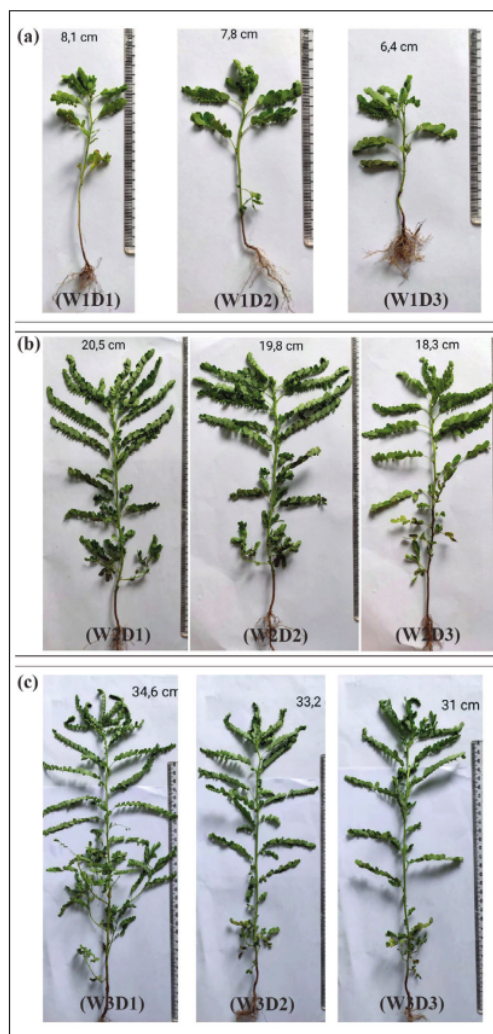

Figure 1.

Phyllanthus niruri L. obtained after harvesting

The results showed that the maximum plant height growth was shown in plants given a dose of 1 fertilizer (15.5 grams). It shows that N, P, and K are essential factors due to their function in metabolic and biochemical processes in plant cells. Nitrogen (N), phosphorus (P), and potassium (K) are heavily needed for plant growth [10]. The contribution of nitrogen to plant growth is 40% – 50%. Phosphate (P) is an indispensable element in synthesizing

nuclear proteins, lecithin, promoting cell division, and accelerating aerial and underground growth. K is considered a quality factor in plant production because it is closely related to plant development and metabolism [11, 12].

Table 3.

Comparison of plant height resulting from the fertilization process

Harvest time	Fertilizer dose	Sample code	Plant height [cm]
W1 (4 weeks)	Normal	–	3.40±0.10
	D1: dose 1	W1D1	8.10±0.10
	Dose 2	W1D2	7.80±0.20
	Dose 3	W1D3	6.40±0.10
W2 (6 weeks)	Normal	–	20.50±0.10
	Dose 1	W2D1	16.80±0.20
	Dose 2	W2D2	19.80±0.20
	Dose 3	W2D3	18.30±0.20
W3 (8 weeks)	Normal	–	34.60±0.10
	Dose 1	W3D1	29.30±0.20
	Dose 2	W3D2	33.20±0.20
	Dose 3	W3D3	31.70±0.10

Yield of *Phyllanthus niruri* L. extract

PnL from each group was extracted so that nine samples of extracts with different yields were produced in table 4. The extracts with the highest yield were found in the extracts of the PnL group at second harvest time and third fertilizer dose with a yield of 9.73% and having higher yields than the research conducted [13], which resulted in a yield of 8.04%. The results obtained indicate that the harvest time and the dose of fertilization given during plant cultivation affect the yield of extracts with the second and third fertilization doses that produce maximum results. Plant mineral nutrition provided by NPK fertilizer promotes growth and influences secondary metabolite content [14].

Dry weight of plant is also affected by the application of fertilizer during the plant cultivation process. NPK fertilizer was proven to increase seedling height, stem diameter, number of leaves, dry weight, and leaf area of seedlings [15]. The dry weight of the plant was obtained mainly by plants in the second harvest time group and third doses fertilizer with weight result 9.37 grams.

Table 4.

Extraction results and total flavonoid content analysis

Harvest time	Fertilizer dose	Sample code	Yield [%]	Percentage [%] TFC
W1 (4 weeks)	Normal	–	1.66±0.03	0.66±0.03
	Dose 1	W1D1	6.40±0.16	1.46±0.01
	Dose 2	W1D2	1.00±0.17	0.83±0.01
	Dose 3	W1D3	5.86±0.35	1.36±0.02
W2 (6 weeks)	Normal	–	3.60±0.29	0.71±0.03
	Dose 1	W2D1	6.27±0.15	1.73±0.01
	Dose 2	W2D2	5.60±0.15	0.76±0.00
	Dose 3	W2D3	9.73±0.26	1.25±0.01
W3 (8 weeks)	Normal	–	2.19±0.11	0.65±0.07
	Dose 1	W3D1	8.95±0.06	1.27±0.36
	Dose 2	W3D2	1.90±0.11	1.60±0.12
	Dose 3	W3D3	3.35±0.04	0.88±0.02

Total flavonoid content (TFC)

The content of flavonoids is one of the most important elements in PnL plants. Flavonoids become stronger antioxidant compounds compared with vitamin E. Flavonoid compounds can stimulate immunity [19]. The analysis process is performed by setting the maximum wavelength, making a calibration curve, and testing the extracted sample. The analysis results showed that the maximum wavelength of the quercetin standard was at a wavelength of 435 nm. Obtained the maximum wavelength and then made a standard quercetin solution calibration curve with 10, 20, 30, 40, 50 g/ml concentrations. The results obtained absorbance data, respectively as follows 0.282; 0.453; 0.605; 0.754; 0.8630.

The quercetin calibration curve is useful to determine the levels of flavonoid compounds in the sample through a linear regression equation from the quercetin calibration curve. The linear regression equation obtained is $y=0.0146x-0.1523$, with a correlation coefficient (r) of 0.9943. The extract in the W1D2 group had the highest flavonoid content, 17.34 mgQE/g or 1.73%. Total flavonoid levels were calculated, and the following data were obtained in Table 4.

Spectra FTIR-ATR

Measurement of FTIR-ATR on PnL extract obtained a typical spectrum pattern of each sample (fig. 2, tab. 5). Analysis of the IR spectra of 9 extract

Table 5.

Absorbance data at selected peak

Sample code	Wave numbers [cm^{-1}]							
	1: 3486.77-3157.12	2: 2974.85-2800.29	3: 1740.96-1670.34	4: 1688.92-1562.54	5: 1425.02-1272.62	6: 1257.75-1138.81	7: 1131.38-945.53	8: 711.36-529.23
W1D1	0.198	0.099	0.077	0.139	0.178	0.158	0.159	0.165
W1D2	0.177	0.116	0.049	0.119	0.155	0.223	0.166	0.181
W1D3	0.109	0.128	0.150	0.115	0.152	0.192	0.153	0.165
W2D1	0.107	0.124	0.229	0.117	0.178	0.165	0.154	0.166
W2D2	0.151	0.104	0.266	0.149	0.241	0.205	0.198	0.215
W2D3	0.122	0.103	0.157	0.138	0.233	0.170	0.180	0.195
W3D1	0.098	0.113	0.016	0.155	0.169	0.195	0.173	0.179
W3D2	0.153	0.145	0.030	0.121	0.213	0.182	0.172	0.189
W3D3	0.184	0.124	0.019	0.133	0.233	0.167	0.178	0.193
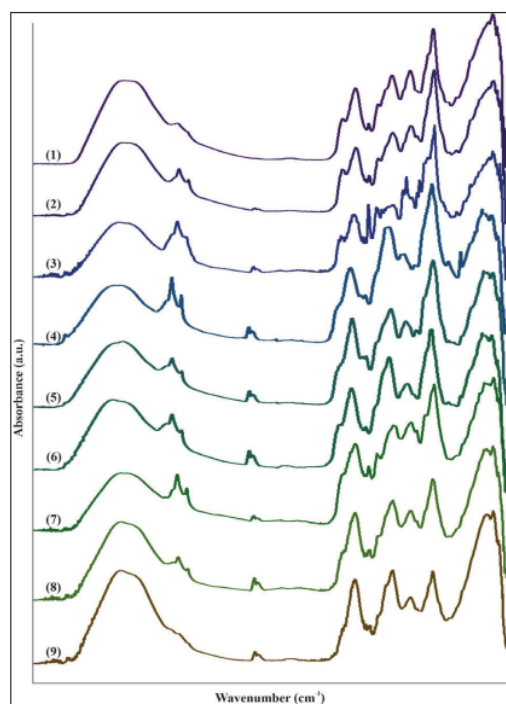
Figure 2.

FTIR-ATR spectra of extracts by grouping harvest time and dose. (1) W1D1; (2) W1D2; (3) W1D3; (4) W2D1; (5) W2D2; (6) W2D3; (7) W3D1; (8) W3D2; (9) W3D3

samples using FTIR was carried out three times. Replication is used to minimize spectral analysis errors and see the similarities or differences of each replication. The FTIR spectra results are very complex information and describe all the characteristics.

IR spectra produces data in the form of peaks at certain wavenumbers based on the vibration of the functional groups of the extract and the absorbance value. This data is used to identify one data with another data. Absorbance data that produces numbers are then used for chemometric analysis (PCA-CA) to evaluate the nature and character of the FTIR spectral pattern of each formula and see the similarity of all treatments [16].

FTIR spectra are very rich in molecular structure information with a series of absorption bands specific for each molecule, so they can be used to distinguish a similar raw material. The FTIR spectra of the harvested PnL herb extract samples showed absorption at a wavenumber of 1704.99 cm^{-1}, which was at peak number 3, indicating the presence of a carboxylic acid group in the absorption range of 1725-1705 cm^{-1} and a frequency area of 1540.47 cm^{-1} with a weak intensity indicating the presence of C=C. 1527.34 cm^{-1} indicates the presence of C=C- group, the peak at a frequency of 2992.98 cm^{-1} indicates the presence of an alkane in the observed compound [17, 18].

The results of further analysis showed the presence of free O-H groups in the range of 3650–3600 cm^{-1}, namely at a wavelength of 3648.57 cm^{-1}, hydrogen-bond O-H at peak 1, which was in the range of 3486.77–3157.12 cm^{-1} with medium intensity. The absorption band in the area of 2931.26 cm^{-1} where falls into the absorption band range in the IR spectra of 2974.85–2800.29 cm^{-1}, which indicates that isolate C contains alkanes, and the absorption band at 1643 cm^{-1} indicates the presence of compounds -C=C-. Data at peak 8 in the range of 711.36–529.23 cm^{-1} contains alkene compounds [18, 19].
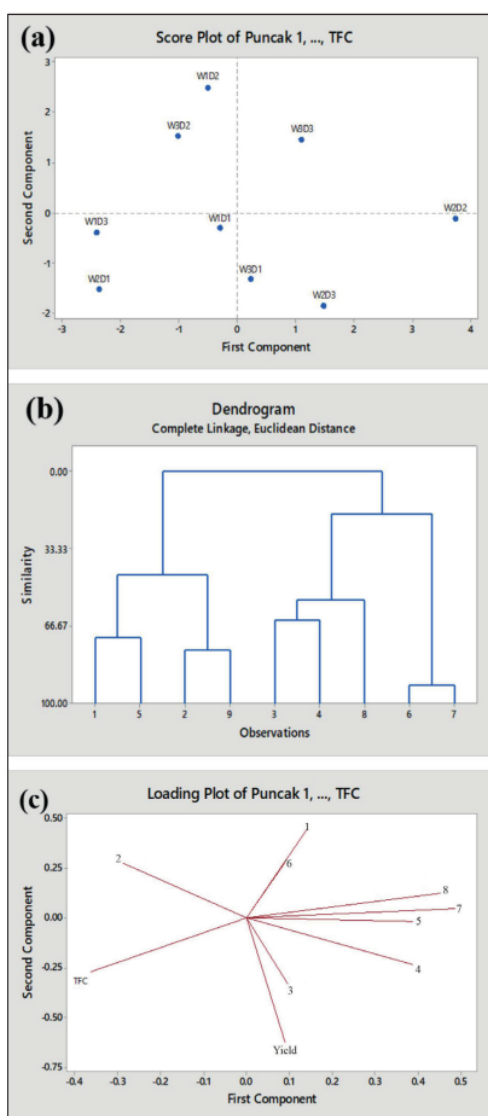
Chemometric analysis

Chemometric approaches using principal component analysis-cluster analysis (PCA-CA) methods to classify and evaluate correlations between responses [20, 21]. The analysis results using a chemometric approach resulted in a score plot and a dendrogram. The score plot obtained indicates the formation of 3 categories. Figure 2 shows the PCA score plot analysis results showing that each extracted sample is grouped into different distances between points. The farther apart the sample distances between the points, the less similarity each sample has.

The analysis results showed that the first harvest time group and second-dose fertilizer, third harvest time and two fertilizer doses, and third harvest time and third-dose fertilizer showed similarities in components due to the distance in the adjacent score plots. The extracts of the W1D3 and W2D1 groups showed high similarity. Further data showed that the extract at one harvest time first dose, third harvest time and first fertilizer dose, also third harvest time doses of fertilizer also showed similarities because of their proximity. The extracted sample at second harvest time with second fertilizer doses was not similar because it was very far from other samples.

The degree of similarity between samples is shown in the dendrogram (fig. 3b). Based on the FTIR-ATR spectra profile, treatment results using modified NPK fertilization using chitosan nanopolymer can be grouped. Samples 6 and 7 have a closeness value (similarity level) of 92.06%, samples 2 and 9 have a closeness of 77.09%, samples 1 and 5 have a closeness of 71.41%. Samples 3 and 4 have a closeness value of 63.79%, samples 3 and 8 have a proximity value of 55.76%, samples 1 and 2 have a closeness of 44.48%.

The loading plot diagram shows the correlation between the variables or responses being evaluated. The correlation between responses was evaluated based on the distance between the vectors on the loading plot (fig. 3c). The farther the distance from the starting point, the more significant the contribution of the variables to the PCA process. Vector lines close together in figure 3c indicate a directly proportional relationship, while opposite variables or vectors show an inverse relationship. Correlation can be known from the angle formed between the variable vectors. Supposed angle formed from the two variable vectors is close to 0° or getting narrower and has the same direction. In that case, the variable has a positive correlation and vice versa. Meanwhile, the uncorrelated variables are depicted with two lines forming an angle close to 90° [21-23].

Figure 3.

Results of chemometric analysis, (A) score plot, (B) dendrogram, (C) loading plot

The results of loading plot analysis showed the correlation between variables, namely TFC, eight absorption peaks, and extract yield. The result shows that the response of total flavonoid levels from the PnL extract samples positively correlated with peak 5, peak 7, and peak 8 in the IR absorption region. The phenomenon corresponds to the functional group at each peak. The alkene group is at peak

8 with an absorption range of 711.36–529.29 cm^{-1}, the amine group, is at peak 7 with an absorption range of 1131.37–945.53 cm^{-1}, and the presence of –CH$_2$ at peak 5 with a value of the wavelength range is 1465.02–1271.62 cm^{-1}. Based on these data, these groups influenced the flavonoid content as an evaluated response. The more groups and compounds, the total flavonoid content will increase.

The response to the extract yield on the loading plot graph data shows a positive correlation with peak 3 with an absorption area range of 1740.96–1562.54 cm^{-1}; there is a C=O or aldehyde group in the quercetin compound contained in the extract [18]. This condition indicated that the yield response would increase along with the number of C=O groups in the quercetin contained in the extract. The TFC response shows negative correlation with absorption peaks 1 and 6 because the farthest distance from the starting point to the Y-axis contributes to the formation of PC2.

CONCLUSION

Harvest time and fertilizer dose affect the quality of the extract from the *Phyllanthus niruri*. The high flavonoid content of the plant is highly dependent on the accuracy of the harvest age. Modification of NPK fertilizer in the chitosan nanopolymer formulation provided better results than conventional NPK. Overall, the FTIR-ATR spectra pattern combined with chemometric analysis gave a good evaluation of the metabolite profile of the extract and could be applied in developing the assessment of other biopharmaceutical plants.

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FINAL GRADE

/100

GENERAL COMMENTS

Instructor

CLAIM

Take an arguable position on the scientific topic and develop the essay around that stance.

ADVANCED	The essay introduces a precise, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay develops the claim and counterclaim fairly, distinguishing the claim from alternate or opposing claims.
PROFICIENT	The essay introduces a clear, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay effectively acknowledges and distinguishes the claim from alternate or opposing claims.
DEVELOPING	The essay attempts to introduce a qualitative and/or quantitative claim, based on the scientific topic or text(s), but it may be somewhat unclear or not maintained throughout the essay. The essay may not clearly acknowledge or distinguish the claim from alternate or opposing claims.
EMERGING	The essay does not clearly make a claim based on the scientific topic or text(s), or the claim is overly simplistic or vague. The essay does not acknowledge or distinguish counterclaims.

EVIDENCE

Include relevant facts, definitions, and examples to back up the claim.

ADVANCED	The essay supplies sufficient relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
PROFICIENT	The essay supplies relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
DEVELOPING	The essay supplies some qualitative and/or quantitative data and evidence, but it may not be closely related to the scientific topic or text(s), or the support that is offered relies mostly on summary of the source(s), thereby not effectively supporting the essay's claim and counterclaim.
EMERGING	The essay supplies very little or no data and evidence to support its claim and counterclaim, or the evidence that is provided is not clear or relevant.

REASONING

Explain how or why each piece of evidence supports the claim.

ADVANCED	The essay effectively applies scientific ideas and principles in order to explain how or why the cited evidence supports the claim. The essay demonstrates consistently logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations anticipate the audience's knowledge level and concerns about this scientific topic.

PROFICIENT	The essay applies scientific reasoning in order to explain how or why the cited evidence supports the claim. The essay demonstrates logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations attempt to anticipate the audience's knowledge level and concerns about this scientific topic.
DEVELOPING	The essay includes some reasoning and understanding of the scientific topic and/or text(s), but it does not effectively apply scientific ideas or principles to explain how or why the evidence supports the claim.
EMERGING	The essay does not demonstrate clear or relevant reasoning to support the claim or to demonstrate an understanding of the scientific topic and/or text(s).

FOCUS

Focus your writing on the prompt and task.

ADVANCED	The essay maintains strong focus on the purpose and task, using the whole essay to support and develop the claim and counterclaims evenly while thoroughly addressing the demands of the prompt.
PROFICIENT	The essay addresses the demands of the prompt and is mostly focused on the purpose and task. The essay may not acknowledge the claim and counterclaims evenly throughout.
DEVELOPING	The essay may not fully address the demands of the prompt or stay focused on the purpose and task. The writing may stray significantly off topic at times, and introduce the writer's bias occasionally, making it difficult to follow the central claim at times.
EMERGING	The essay does not maintain focus on purpose or task.

ORGANIZATION

Organize your writing in a logical sequence.

ADVANCED	The essay incorporates an organizational structure throughout that establishes clear relationships among the claim(s), counterclaims, reasons, and evidence. Effective transitional words and phrases are included to clarify the relationships between and among ideas (i.e. claim and reasons, reasons and evidence, claim and counterclaim) in a way that strengthens the argument. The essay includes an introduction and conclusion that effectively follows from and supports the argument presented.
PROFICIENT	The essay incorporates an organizational structure with clear transitional words and phrases that show the relationship between and among ideas. The essay includes a progression of ideas from beginning to end, including an introduction and concluding statement or section that follows from and supports the argument presented.
DEVELOPING	The essay uses a basic organizational structure and minimal transitional words and phrases, though relationships between and among ideas are not consistently

clear. The essay moves from beginning to end; however, an introduction and/or conclusion may not be clearly evident.

EMERGING   The essay does not have an organizational structure and may simply offer a series of ideas without any clear transitions or connections. An introduction and conclusion are not evident.

LANGUAGE

Pay close attention to your tone, style, word choice, and sentence structure when writing.

ADVANCED   The essay effectively establishes and maintains a formal style and objective tone and incorporates language that anticipates the reader's knowledge level and concerns. The essay consistently demonstrates a clear command of conventions, while also employing discipline-specific word choices and varied sentence structure.

PROFICIENT   The essay generally establishes and maintains a formal style with few possible exceptions and incorporates language that anticipates the reader's knowledge level and concerns. The essay demonstrates a general command of conventions, while also employing discipline-specific word choices and some variety in sentence structure.

DEVELOPING   The essay does not maintain a formal style consistently and incorporates language that may not show an awareness of the reader's knowledge or concerns. The essay may contain errors in conventions that interfere with meaning. Some attempts at discipline-specific word choices are made, and sentence structure may not vary often.

EMERGING   The essay employs language that is inappropriate for the audience and is not formal in style. The essay may contain pervasive errors in conventions that interfere with meaning, word choice is not discipline-specific, and sentence structures are simplistic and unvaried.