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Acute Toxicity Determination and Compound Changing with Chemometric Procedures from Komba-Komba Leaf (*Chromolaena odorata* L.)

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ABSTRACT

The employing of komba-komba leaves (*Chromolaena odorata* L.), a medicinal plant that can prevent and treat many ailments, is one example of the expanding use of traditional medicine in the health field. To increase their use as traditional medicine, Komba-komba leaves must have complete information about their safety due to the abundance of secondary metabolite substances they contain. The goal of this work was to classify the extract components and fractions of komba-komba leaves using FTIR spectra and a PCA chemometric technique, as well as to estimate the hazardous potential of komba-komba leaves, total flavonoid and phenolic levels from komba-komba leaves. The BSLT (Brine Shrimp Lethality Test) method was used for acute toxicity testing. The toxicity test results revealed that the LC_{50} values of the komba-komba leaves (*Chromolaena odorata* L.) *n*-hexane fraction, ethyl acetate fraction, and insoluble ethyl acetate fraction had LC_{50} values 1000 ppm, indicating that the extract and *n*-hexane fractions were poisonous. The total flavonoid concentration was determined to be 243.4 mgEQ/g in the methanol extract, 380.1 in the n-hexane fraction, 512.8 in the ethyl acetate fraction, and 189 mgEQ/g in the ethyl acetate insoluble fraction. The methanol extract's total phenolic content was 146.3 mgEAG/g, whereas that of the *n*-hexane, ethyl acetate, and insoluble ethyl acetate fractions was 269.6, 360.9, and 109.3 mgEAG/g, respectively. From the results of the score plot, it can be seen that the PCA analysis was successful in demonstrating group differences between extracts and fractions of komba-komba leaves. Extracts and fractions of *C.odorata* L. leaves have moderate toxicity due to the presence of flavonoid and phenolic content, making them potential candidates for development as natural antioxidants.

Keywords: Toxicity, *Chromolaena odorata* L., BSLT, FTIR, Chemometrics

ABSTRAK

Penggunaan obat tradisional dalam dunia kesehatan semakin berkembang, salah satunya penggunaan daun Komba-komba (*Chromolaena odorata* L.) yang merupakan salah satu tanaman obat yang berpotensi untuk mencegah dan mengobati berbagai penyakit. Banyaknya kandungan senyawa metabolit sekunder dalam daun Komba-komba menyebabkan perlunya informasi lengkap mengenai keamanan pemakaian daun Komba-komba untuk meningkatkan pemanfaatannya sebagai obat tradisional. Tujuan penelitian ini untuk mengetahui potensi toksik dari daun komba-komba, kadar total flavanoid dan fenolik dari daun komba-komba serta pengelompokan senyawa ekstrak dan fraksi daun komba-komba menggunakan spektra FTIR dan juga dengan pendekatan kemometrik PCA. Pengujian toksisitas akut dilakukan dengan menggunakan metode BSLT (*Brine Shrimp Lethality Test).* Hasil uji toksisitas menunjukan nilai LC_{50} dari ekstrak metanol, fraksi *n*-heksan, fraksi etil asetat dan fraksi tidak larut etil asetat daun komba-komba (*Chromolaena odorata* L.) memiliki nilai $LC_{50} \leq$ 1000 ppm yang menunjukan bahwa ekstrak dan fraksi *n*-heksan bersifat toksik. Hasil penetapan kadar flavanoid total menunjukan ekstrak metanol sebesar 243,4 mgEQ/g, fraksi *n*-heksan sebesar 380,1, fraksi etil asetat sebesar 512,8 ppm dan fraksi tidak larut etil asetat sebesar 189 mgEQ/g. Untuk kandungan fenolik total dari ekstrak metanol sebesar 146,3, fraksi *n*-heksan sebesar 269,6, fraksi etil asetat sebesar 360,9 dan fraksi tidak larut etil asetat sebesar 109,3 mgEAG/g. Hasil analisis PCA berhasil menunjukan perbedaan kelompok antara ekstrak dan fraksi daun komba-komba yang dapat dilihat dari hasil *score plot.* Ekstrak dan fraksi-fraksi daun *C. odorata* L. memiliki toksisitas sedang dengan adanya kandungan flavonoid dan fenoliknya sehingga dapat dikembangkan sebagai antioksidan alami.

Kata Kunci: Toksisitas, *Chromolaena odorata* L., BSLT, FTIR, Kemometrik

INTRODUCTION

The implementation of traditional medicine in medical treatment is expanding, especially in light of suggestions that people should get back to nature (Abriyani *et al.*, 2022). Over eighty percent of people throughout the world still rely on conventional medicine to keep them healthy, according to the WHO. Traditional medicine is a substance or mixture of substances made from plants, animals, minerals, extract preparations (galenic), or combinations of these substances that have been used for treatment for generations and can be used in accordance with social norms. The use of traditional medicine experienced an increase in 2014 (20.99%), with the most commonly used dosage form being in the form of cut or sliced preparations (55.2%) (Pane et al., 2021).

Komba-komba (*Chromolaena odorata* L.), a plant that grows wild and is frequently regarded as a weed, is one of the plants that can be used as medicine. *Chromolaena odorata* L. is known by the names "kirinyuh" in the West Java region, while in Southeast Sulawesi, the Muna ethnic group refers to it as "komba-komba". Finding out in-depth information on the compound content and the safety of utilizing komba-komba leaves (*Chromolaena odorata* L.) as a traditional medicine is one of the initiatives being made to boost their use. The leaves contain a number of important substances, including tannins, phenols, flavonoids, saponins, and steroids (Eriadi *et al.*, 2016). Komba-komba leaves have historically been used as a remedy for rheumatism, amenorrhea, leech bites, nose inflammation, decongestants, and diarrhea in diabetics (Ance *et al.*, 2018).

Toxicology studies, such as acute toxicity testing, sub-acute toxicity tests, chronic toxicity tests, and particular toxicity tests, must be performed in order to evaluate safety (Nirwanto *et al.*, 2014). Furthermore, the chemical makeup of plant extracts is extremely complicated, necessitating the development of analytical methods that can fully explain their chemical properties. The Fourier Transform Infrared (FTIR) approach is one of these analysis methods. The resulting FTIR fingerprint spectrum contains a wealth of complicated data that can fully characterize a material's chemical properties (Purwakusumah *et al.*, 2014). Earlier research on the toxicity test of the ethanol extract of komba-komba leaves revealed that the LC_{50} value of 225.81 ppm for komba-komba leaf extract (*Chromoleana odorata* L.) might result in up to 50% mortality of shrimp larvae (Armadany *et al.*, 2022). The ethanol extract of komba-komba leaves (*Chromolaena odorata* L.) was tested for acute toxicity by Eriadi *et al*. in 2016 on male white mice. The results showed that the extract falls into the practically non-toxic category because it has an LD_{50} > 15 kg /BB.

Acute toxicity tests of extracts and fractions of komba-komba leaves (*Chromolaena odorata* L.) using the Brine Shrimp Lethality Test (BSLT) method in conjunction with Fourier Transform Infra Red (FTIR) spectrophotometry and chemometrics were of interest to the researchers as a result of this background information.

RESEARCH METHODS

Tools

The tools used in this study were rotary vacuum evaporator (Rotavapor, Buchi), analytical balance (Precisa®), hot plate (Stuart®), beaker (Pyrex®), measuring flask (Pyrex®), oven (Gallenkamp Civilab-Australia®), porcelain cup, droppings, stirring stick, jar, spectrophotometer, object glass, cover glass.

Materials

The materials to be used in the research are extracts of komba-komba leaves, the required solution is ether water, methanol, *n*-hexane, and ethyl acetate.

Preparation of Extract and Fractionation of Komba-komba leaves

With a sample of 800 grams of komba-komba leaf simplicia, the maceration method of extraction was used. The simplicia was placed in a jar and exposed to methanol for

three consecutive days. To create a thick extract, the resulting extract was placed into an evaporator flask to evaporate the solvent present in it (Munte *et al.*, 2016). The extract was fractionated using a trituration procedure with *n*-hexane and ethyl acetate as the solvents (1:10). 350 mL of *n*-hexane solvent was then added to the 35 g of komba-komba leaf extract in the mortar, which was then repeatedly crushed and filtered until the solvent's color changed to clear. A rotary evaporator is used to collect and evaporate the filtrate.

Phytochemical screening of extracts and fractions

The following phytochemicals were screened in the komba-komba leaves (*Chromolaena odorata* L.) methanol extract, *n*-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction:

1. Alkaloid Test

The color change and precipitate that happened after adding two drops of Mayer's reagent to the methanol extract and individual fractions of 1 mL each were noted. The production of a yellowish-white precipitate is a sign of success (Ekayani *et al.*, 2021).

2. Tannin Test

The color changes were then noticed after adding 1-2 drops of $FeCl_3$ to each 1 ml of methanol extract and fractions. If the color changes to blue, purple, or a dark green, this is a good sign (Ekayani *et al.*, 2021).

3. Flavanoid Test

When sodium hydroxide was applied to each 1 mL of methanol extract and fraction, the resulting color shift was seen. If the color switches to red, orange, or yellow, results are seen as being favorable (Anwar et al., 2023; Ekayani et al., 2021).

4. Terpenoid Test

The color change was noticed after adding 1 drop of sulfuric acid and 3 drops of strong HCl to each 1 mL of methanol extract and fractions. If a red, brownish red, or purple color forms, the outcome will be favorable (Ekayani *et al*., 2021).

5. Saponin Test

10 mL of hot water were mixed with 1 mL of the methanol extract and its fractions, and the mixture was then violently agitated for a short while. One or two drops of 2 N hydrochloric acid (HCl) added to persistent foam signifies a positive saponin test result (Munte *et al.*, 2016).

Brine Shrimp Lethality Test Method Toxicity Test

The Brine Shrimp Lethality Test (BSLT) is a technique used to assess the toxicity of an extract (Vitalia *et al*., 2016). Each concentration involved three replications with three groups of ten *Artemia salina* larvae each. Three prepared containers must be used for assessing each sample extract concentration, with one serving as a control for each replication. Different concentrations were used during the test, including 10, 50, 100, 250, 500, 750, 1000, and 2000 ppm. Then, 10 *Artemia salina* larvae were added to the solution at each concentration. Artemia salina larvae deaths were monitored for 24 hours, with each concentration reproduced three times in comparison to the control. If *Artemia salina* larvae do not move for a few seconds after being observed, that is the conventional criterion for determining whether they have died (Sondakh *et al*., 2017).

Determination of Levels of Flavonoids

Each concentration series of the quercetin standard solution (10, 20, 30, 40, 50, 60, and 70 ppm) was taken in 1 mL, added to 3 mL of methanol, 0.2 mL of $AlCl_3$ 10%, and 0.2 mL of 1 M potassium acetate, and filled with distilled water to 10 mL before being incubated for the operating time. The full range of standard solution concentrations was measured at the highest attainable wavelength. There was developed a calibration curve for the relationship between quercetin and absorbance (Ahmad, *et al*., 2015).

The extract was weighed at 50 mg, and the fraction was dissolved in 50 mL of methanol each hour. Next, 1 mL was divided into 10 mL by adding 3 mL of methanol, 0.2

mL of 10% $AlCl_3$, 0.2 mL of 1 M potassium acetate, and 0.2 mL of distilled water. After 30 minutes of incubation, the absorbance was measured using UV-Vis spectrophotometry performed three times. Each sample's flavonoid concentrations were measured before being added to the equation for the quercetin standard curve.

$$\text{Levels of total flavonoids per sample weight} = \frac{(C \times V \times Fp)}{m}$$

Information:
C = Total flavonoid concentration
Fp = Dilution factor
V = Sample volume (L)
m = Extract mass (g)

Determination of Total Phenolic Content

The 50 mg of methanol extract was measured and 50 mL of methanol was used to dissolve it to create each methanol extract solution and fraction. Pipetting 1 mL of the extract and fraction solution into each container was followed by adding 0.4 mL of Folin-Ciocalteau reagent, shaking the mixture for 4–8 minutes, adding 4 mL of 7% Na_2CO_3 solution, shaking the mixture again for homogeneity, increasing the volume of the solution with distilled water to 10 mL, and letting the mixture stand for the operating time at room temperature. Three measurements of absorbance were taken, and the resultant phenolic content was reported as gallic acid equivalent (Ahmad *et al*, 2015). Using the formula, the value is multiplied by the sample's whole volume before being compared to the weight (Wardhani *et al*., 2018):

$$\text{Total phenolic content per sample weight} = \frac{C \times V \times Fp}{m}$$

Information:
C = Total flavonoid concentration
Fp = Dilution factor
V = Sample volume (L)
m = Extract mass (g)

Measurement of FTIR Spectra and Clustering of Chemometric Data

An FTIR spectrophotometer was used to measure the FTIR spectra of komba-komba leaves extracts and fractions in the mid-IR region (4000-650 cm^{-1}). Software will automatically eliminate the background spectra from the sample measurement results to produce the sample spectra that will be evaluated. The results of the FTIR study were then used to do compound grouping using the Mini tap program.

RESULTS AND DISCUSSION

Extraction and Fractionation

Results of the maceration method for extracting komba-komba leaf simplicia produced an methanol extract with a 15% yield; the yield was obtained using 800 grams of komba-komba leaf simplicia. Differences in polarity levels and variations in specific gravity between two samples serve as the basis for the separation principle in the fractionation procedure. Using two solvents *n*-hexane and ethyl acetate, the methanol extract of komba-komba leaves was fractionated using the trituration method. *n*-hexane and ethyl acetate were used as solvents to fractionate the 35 grams of methanol extract from komba-komba leaves. According to table 1, the *n*-hexane fraction was obtained in a weight yield of 25.71%, the ethyl acetate fraction was obtained in a weight yield of 36%, and the remainder fraction was obtained in a weight yield of 38.17%.

Table 1. Results of the methanol extract fraction of komba-komba leaves

No.	Fraction	Extract weight (g)	Fraction Weight (g)	Yield (%)
1	*n*-hexane	35	9	25.7
2	Ethyl acetate	35	12.6	36
3	Ethyl acetate insoluble	35	13.36	38.17

The findings of the fractionation process revealed that the greatest insoluble fraction of ethyl acetate had different values. Due to variations in polarity values for each

class of chemical substances, fractionation outcomes can vary. The polarity of a solution can significantly affects chemical substances because it influences their solubility and interactions with other molecules. Polarity is a measure of the separation of electric charge in a molecule. The polar compounds are drawn to the methanol extract whereas the nonpolar compounds are drawn to the *n*-hexane fraction, the semi-polar compounds are drawn to the ethyl acetate fraction, and the polar compounds are drawn to the insoluble ethyl acetate fraction. Due to the weight of the fraction and the higher yield in the ethyl acetate insoluble fraction, it was determined that the komba-komba leaf sample included more polar chemicals (Purwanto, 2015).

Botanical Chemical Screening

Through a color reaction with a specific reagent that can reveal distinctive traits of each group of secondary metabolites, a qualitative phytochemical screening method can be carried out (Vifta & Advistasari, 2018). Table 2 displays the findings of the phytochemical analysis of the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction.

The results presented in of Table 2 demonstrate that secondary metabolite chemicals such as alkaloids, flavonoids, tannins, terpenoids, and saponins were present in significant amounts in the extracts and fractions of the komba-komba leaves under study. It is predicted that in the alkaloid test using Meyer's reagent, the nitrogen in the alkaloid will combine with the K^+ metal ion from potassium tetraiodomercurate (II) to generate a precipitating potassium alkaloid complex.

Table 2. Summarizes the results of a phytochemical analysis of komba-komba leaves in methanol extract, n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction

No.	Compound Classes	Sample	Results	Information
1	Alkaloids	Methanol extract	+	There develops a whitish precipitate.
		n-hexane fraction	+	
		Ethyl acetate Fraction	+	
		ethyl acetate insoluble fraction	+	
2	Flavonoids	Methanol extract	+	Orange color is formed
		n-hexane fraction	+	Yellow color is formed
		Ethyl acetate Fraction	+	Orange color is formed
		ethyl acetate insoluble fraction	+	Orange color is formed
3	Terpenoids	Methanol extract	+	A brown color form
		n-hexane fraction	+	
		Ethyl acetate Fraction	+	
		ethyl acetate insoluble fraction	+	
4	Saponins	Methanol extract	+	Foam is produced
		n-hexane fraction	+	
		Ethyl acetate Fraction	+	
		ethyl acetate insoluble fraction	+	
5	Tannin	Methanol extract	+	A dark green color develops
		n-hexane fraction	+	
		Ethyl acetate Fraction	+	
		Ethyl acetate insoluble fraction	+	

The outcomes of the testing revealed positive flavonoids with the samples' colors changing to yellow and orange. When the sample reacts with NaOH, acetophenone molecules are formed, which is what causes the alterations. It can be claimed that all positive samples included terpenoid compounds since the results showed a shift in color from green to brown as a result of oxidation in the terpenoid group through the production of conjugated double bonds. By adding hot water, it was possible to identify the saponin compounds present in the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction of komba-komba leaves. Foam is created when the non-polar groups in the micelle structure face inward and the polar groups face outward. The results of the komba-komba leaves' methanol extract, *n*-hexane fraction, and ethyl acetate insoluble fraction created foam, indicating that saponin was present.

Test for Acute Toxicity

Testing for acute toxicity using extracts and fractions of komba-komba leaves in solutions at concentrations of 10, 50, 100, 250, 500, 750, 1,000, and 2,000 ppm. To ensure accuracy, this toxicity test treatment was performed three times in repetition and replication. Table 3 displays the findings of the toxicity tests conducted on the corresponding komba-komba leaf fractions and extracts.

Table 3. The LC_{50} values of extracts and fractions of komba-komba leaves (*Chromolaena odorata* L.) that were calculated

No.	Sample	Concentration (ppm)	Mortality (%)	LC_{50} (ppm)
1	Methanol extract	10	0	175.91
		50	16.66	
		100	36.66	
		250	56.66	
		500	76.66	
		750	100	
		1000	100	
		2000	100	
2	*n*-hexane fraction	10	0	148.98
		50	26.66	
		100	36.66	
		250	66.66	
		500	86.66	
		750	100	
		1000	100	
		2000	100	
3	Ethyl acetate Fraction	10	0	661.09
		50	6.66	
		100	13.33	
		250	16.66	
		500	50.00	
		750	66.66	
		1000	83.33	
		2000	100	
4	Ethyl acetate insoluble fraction	10	0	465.87
		50	13.33	
		100	16.66	
		250	23.33	
		500	53.33	
		750	63.33	
		1000	100	
		2000	100	

It is evident from the table above that the mortality of *A. salina* Leach larvae increase with sample concentration. The amount of toxicity decreases with increasing LC_{50} value and increases with decreasing LC_{50} value, respectively. If an extract or fraction has an LC_{50} value of 1000 ppm and kills up to 50% of test animals, it is considered active and hazardous. The n-hexane fraction sample had the lowest LC_{50} value, which was 148.98 ppm. The proportion of ethyl acetate in the sample with the highest LC_{50} value has a value of 661.09 ppm. The ethyl acetate insoluble fraction has an LC_{50} value of 465.87 ppm, while the methanol extract has an LC_{50} value of 175.91 ppm. A sample is considered to be extremely hazardous if its LC_{50} value is below 30 ppm and it has the ability to fight cancer, toxic if it is between 30 and 1000 ppm and it has the capacity to fight bacteria and oxidative stress, and non-toxic if it is greater than 1000 ppm.

The secondary metabolite chemicals, including flavonoids, alkaloids, tannins, terpenoids, and saponins, discovered in samples of komba-komba leaves (C. *odorata* L.) are linked to the poisonous effects of extracts and fractions. The mechanism of death of *Artemia salina* larvae is related to the function of phenolic compounds, specifically inhibiting *Artemia Salina* larvae's ability to eat and acting as a stomach poison. Phenolic compounds also inhibit *Artemia salina* larvae's taste receptors in the mouth, preventing the larvae from receiving a taste stimulus and preventing the larvae from recognizing their food. As a result, the larvae lack nutritional intake and die (Abriyani *et al*., 2022; Armadany *et al*., 2022; Cahya *et al*., 2022).

Counting the amount of Total flavonoids Levels

Using the aluminum chloride colorimetric method, total flavonoid components in komba-komba leaves extracts and fractions were quantitatively analyzed. Quercetin, a flavonoid from the flavonol group containing a keto group and a hydroxyl group, is the substance used as a standard because it can create a color complex with $AlCl_3$. Table 4 displays the results of the total flavonoid levels.

Quarcetin equivalents (EQ), which represent the total amount of flavonoid compounds, are measured and represented in units of mgEQ/g. This indicates that 1 g of the test sample contains around 1 mg of EQ compounds.

Table 4. Results of total flavonoid levels of komba-komba leaf extracts and fractions

No.	Sample	Flavonoid Levels (ppm)			X±SD (ppm)	Total Flavonoid Levels (mgEQ/g)
		I	II	III		
1	Methanol extract	24.81	23.64	24.58	24.32±0.620	243.4
2	*n*-hexane fraction	38.45	37.76	37.82	25.53±1.928	380.1
3	Ethyl acetate Fraction	52.39	50.11	51.35	51.28±1.139	512.8
4	Ethyl acetate insoluble fraction	19.66	18.05	19	18.90±0.808	189

The amount of flavonoid compounds in komba-komba leaves ranges from 189 mgEQ/g in the insoluble ethyl acetate fraction to 512.8 mgEQ/g in the ethyl acetate fraction. This demonstrates that the flavonoid compounds in komba-komba leaves can be extracted successfully using the solvent ethyl acetate, demonstrating that the flavonoid chemicals present in komba-komba leaves are semi-polar flavonoids. One of the functions of flavonoid compounds is as antioxidants, which work by donating hydrogen ions to neutralize the toxic effects of free radicals, transforming them into more stable forms (Hasan et al., 2022).

Finding the Total Phenolic Content

The Follin-Ciocalteau reagent was used to calculate the total phenolic content.

An inorganic reagent called Follin-Ciocalteau can combine with phenol compounds, namely the blue molybdenum tungstant, to generate a complex solution. The fraction's phenolic content increases with a darker color intensity. The concentration of phenolic ions generated is exactly proportionate to the blue color that results. The more phenolic chemicals present, the more phenolic ions will develop, increasing the intensity of the resulting blue color. Because this reaction proceeds slowly in an acidic environment, sodium carbonate was introduced in the experiment to create an alkaline environment, which allowed the reaction to proceed more quickly (Huda *et al*., 2022).

Table 5. Results of komba-komba leaf extract and fraction total phenolic content

No.	Sample	Phenolic Levels (ppm)			X±SD (ppm)	Total Flavonoid Levels (mgEAG/g)
		I	II	III		
1	Methanol extract	14.76	13.52	15.59	14.63±1.042	146.3
2	*n*-hexane fraction	27.82	26.13	26.95	26.96±0.640	269.6
3	Ethyl acetate Fraction	36.52	35.26	36.5	36.09±0.631	360.9
4	Ethyl acetate insoluble fraction	11.30	10.47	11.04	10.93±0.422	109.3

The total phenolic content results show that the ethyl acetate fraction has the highest concentration of phenolic compounds, at 36.09 ppm with a total phenolic content of 360.9 mgEAG/g sample, followed by the insoluble ethyl acetate fraction, which has 10 phenolic compounds with a total phenolic content of 109.3 mgEAG/g fraction sample, 26.96 ppm with a total phenolic content of 269.6 mgEAG/g fraction sample in the three n-hexane fractions, and 14.63 ppm with a total phenolic content of 146.3 mgEAG/g fraction sample in the four methanol extracts.

The whole phenol content produced will vary depending on the type of solvent used. This demonstrates that the ethyl acetate fraction contains the largest concentration of phenolics, indicating that the phenolic chemicals present in komba-komba leaves are semi-polar phenolic compounds. Given the high concentration of total polyphenols in the ethyl acetate solvent, tannins and flavanols are two examples of polyphenol groups that may share the same molecular weight as the solvent. Compared to ethyl acetate, methanol extract contains fewer phenolic chemicals. This is due to the phenolic substances still having a connection to biomolecules like proteins, polysaccharides, lipids, and other organic components.

Based on Dewatisari's research (2020), solvents with relatively high polarity, such as ethanol, can attract polar compounds, including phenolic compounds like flavonoids (Dewatisari, 2020). The results obtained from the fractionation process in this study were influenced by various factors, such as the use of *n*-hexane and ethyl acetate, where these solvents have different polarities. Ethyl acetate can attract larger phenolic compounds compared to *n*-hexane due to its higher polarity. The high flavonoid content in the ethyl acetate fraction suggests its potential use as a medicinal ingredient. Flavonoid compounds in plants are considered essential components in various applications, including nutraceuticals, pharmaceuticals, and cosmetics. This is because flavonoids possess antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties.

Based on FTIR spectra, komba-komba leaves are grouped

Each komba-komba leaves simplicia's wave numbers and absorbance are the data collected during the FTIR test. Figure 1 displays the results of the FTIR spectra of

komba-komba leaf samples. The FTIR spectroscopy test findings demonstrate that komba-komba leaves contain a variety of functional groups.

Based on the interpretation of FTIR data, it is indicated that methanol extract, *n*-hexane fraction, and ethyl acetate fraction contain functional groups such as O-H (Hydroxyl), N-H (Amine), C-H (Alkane), C=O (Carbonyl), C=C (Alkene, Aromatic), C-O (Alcohol, Ester, Carboxylic Acid), C-H (Aromatic), and C-H (Alkene). These functional groups suggest the presence of flavonoid compounds in both the extract and fractions. A study by Indarto (2015) revealed that the IR spectrum of flavonoid compounds includes O-H, aliphatic C-H, C=O, C=C, and C-O groups (Indarto, 2015).

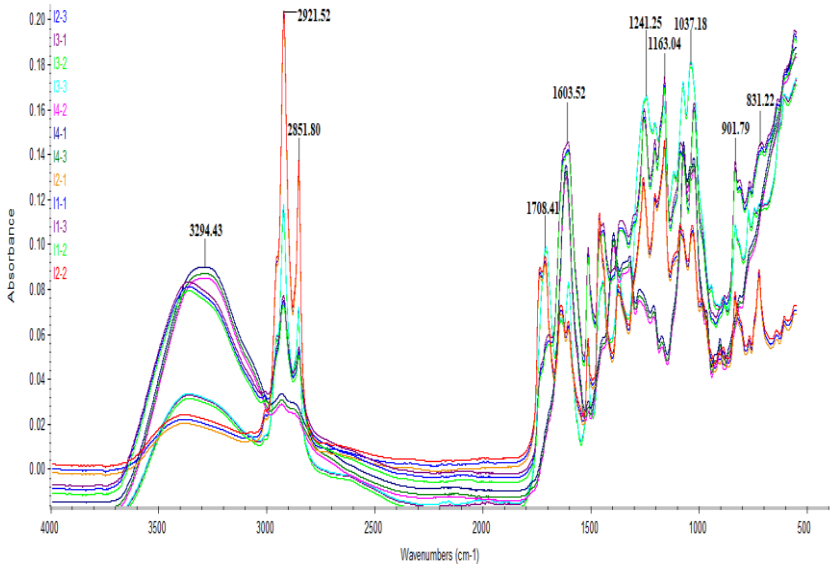

Figure 1. Attenuated total reflectance measurement was used to scan the spectra of methanol extract (I1), *n*-hexane fraction (I2), ethyl acetate fraction (I3), and ethyl acetate insoluble fraction (I4)

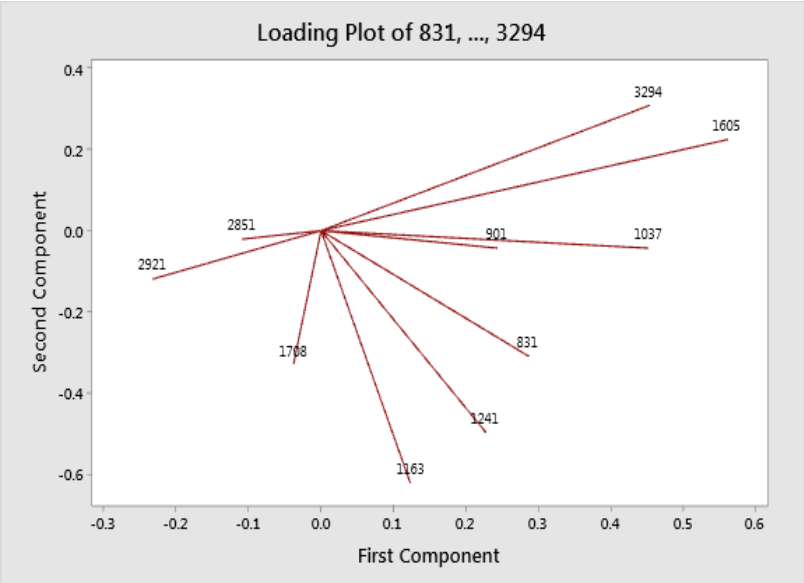

Figure 2. Grafik *loading plot* daun komba-komba

The FTIR diversity fingerprint profiles were then created by utilizing the PCA approach to examine the FTIR spectra. The spectra of komba-komba leaves can be categorized using PCA based on the various solvents that were employed. Methanol extract, *n*-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction were the variables employed in this investigation. The groups of each sample were successfully displayed by PCA based on the results of the score plot. The similarity

between the samples increases with closer proximity on the score plot. The methanol extract and ethyl acetate fraction of komba-komba leaves contain neighboring points that have a similar profile, as illustrated in Figure 2, according to the findings of the score plot graph.

CONCLUSION

Extracts and fractions of *C.odorata* L. leaves have moderate toxicity due to the presence of flavonoid and phenolic content, making them potential candidates for development as natural antioxidants.

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