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Identification of Flavonoid Compounds in Ethanol Extract of *Chromolaena odorata* L. Leaves Using Infrared Spectrophotometry

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Abstrak

Flavonoid merupakan kelompok metabolit sekunder penting yang banyak ditemukan pada tanaman obat dan memiliki berbagai aktivitas biologis seperti antioksidan, antiinflamasi, dan antibakteri. Penelitian ini bertujuan untuk mengidentifikasi dan mengonfirmasi keberadaan senyawa flavonoid pada ekstrak etanolik daun *Chromolaena odorata* L. menggunakan metode spektrofotometri inframerah (FTIR) sebagai pendekatan *fingerprinting* struktural. Daun kering diekstraksi secara maserasi menggunakan etanol 90%, kemudian ekstrak yang diperoleh dianalisis pada rentang bilangan gelombang 4000–400 cm⁻¹. Hasil FTIR menunjukkan adanya serapan khas gugus hidroksil (–OH), karbonil (C=O), ikatan rangkap aromatik (C=C), dan eter (C–O–C), yang merupakan ciri utama struktur flavonoid. Pola spektrum ini konsisten dengan data literatur serta hasil uji fitokimia pendukung, sehingga menegaskan peran FTIR sebagai alat konfirmasi kualitatif dan *fingerprint* kimia senyawa flavonoid dalam ekstrak etanolik daun *C. odorata*. Temuan ini memperkuat potensi tanaman tersebut sebagai sumber alami senyawa bioaktif untuk pengembangan obat herbal terstandar dan penelitian fitofarmaka lebih lanjut.

Kata kunci: *Chromolaena odorata L., metabolit sekunder, flavonoid, ekstrak etanol, spektrofotometri inframerah (FTIR).*

Abstract

Flavonoids are an important group of secondary metabolites found in medicinal plants and possess various biological activities such as antioxidant, anti-inflammatory, and antibacterial properties. This study aims to identify and confirm the presence of flavonoid compounds in the ethanolic extract of Chromolaena odorata L. leaves using infrared spectrophotometry (FTIR) as a structural fingerprinting approach. Dried leaves were extracted by maceration using 90% ethanol, then the obtained extract was analyzed at a wavenumber range of 4000–400 cm⁻¹. FTIR results showed the presence of typical absorption of hydroxyl (–OH), carbonyl (C=O), aromatic double bonds (C=C), and ether (C–O–C) groups, which are the main characteristics of flavonoid structure. This spectral pattern is consistent with literature data and supporting phytochemical test results, thus confirming the role of FTIR as a qualitative confirmation tool and chemical fingerprint of flavonoid compounds in the ethanolic extract of C. odorata leaves. These findings strengthen the potential of this plant as a natural source of bioactive compounds for the development of standardized herbal medicines and further phytopharmaceutical research.

Key words: *Chromolaena odorata L., secondary metabolites, flavonoids, ethanol extract, infrared spectrophotometry (FTIR).*

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INTRODUCTION

The use of medicinal plants is a crucial pillar in the development of natural ingredients to support pharmaceutical and health innovation. Plants produce various secondary metabolites that function in defense against environmental and microbial stressors, while also possessing biological potential for humans. One of the most extensively studied metabolite groups is flavonoids, polyphenolic compounds with broad pharmacological activities such as antioxidant, anti-inflammatory, antibacterial, and anticancer properties. This activity is related to their ability to stabilize free radicals through electron donation and binding to transition metal ions (Krysa et al., 2022). In the context of modern pharmaceuticals, flavonoids are widely developed as natural active ingredients for preventive therapy of degenerative diseases and as the basis for standardized herbal medicine formulations (Utami et al., 2023).

One of the plants rich in flavonoids and has the potential as a source of natural active ingredients is balakacida (*Chromolaena odorata* L.), a member of the Asteraceae family. This plant is empirically used as a wound medicine, anti-inflammatory, and natural antiseptic, and is known to contain flavonoids, terpenoids, saponins, and tannins (Aminnulloh et al., 2025) Flavonoid compounds in *C. odorata* play an important role in antioxidant and antibacterial activity through cell membrane protection mechanisms and oxidation inhibition (Indrianingsih et al., 2025) However, most previous studies are still limited to qualitative phytochemical tests and quantification using UV-Vis spectrophotometry, so that specific identification of functional groups of flavonoid compounds has not been widely explored.

In this context, Fourier Transform Infrared Spectrophotometry (FTIR) becomes a relevant and strategic method. FTIR works based on the principle of infrared radiation absorption by molecules at specific wavelengths, producing a spectrum that describes the vibrations of the bonds and functional groups of the compound (Krysa et al., 2022).

Its advantage over other methods such as UV-Vis or HPLC lies in its ability to detect the main functional groups (–OH, C=O, C–O–C) directly without the need for derivatization or special solvents. FTIR is also non-destructive, fast, and solvent-saving, making it efficient for analyzing complex mixtures such as the ethanol extract of *C. odorata leaves* . Unlike UV-Vis which only provides quantitative information on total flavonoids and HPLC which requires specific standard comparators, FTIR is able to provide structural profiles and qualitative identification of functional groups that contribute to biological activity.

Based on this background, this study aims to identify the functional groups of flavonoid compounds in the ethanol extract of balakacida leaves using the FTIR method. This study is expected to strengthen the scientific basis for the use of spectroscopic techniques for the identification of bioactive compounds, while also supporting efforts to standardize natural ingredients in the development of plant-based pharmaceutical preparations.

RESEARCH METHODS

Research Design

This research is a laboratory experiment with a descriptive-analytical approach that aims to identify flavonoid compounds in the ethanol extract of *Balakacida leaves* (*Chromolaena odorata* L.) using Fourier Transform Infrared Spectrophotometry (FTIR). Identification is carried out based on the analysis of typical flavonoid functional groups that appear in the infrared spectrum of the measurement results.

Time and Place

The research was conducted at the Pharmaceutical Biology Laboratory of Kader Bangsa University, Palembang, during the period 25 September – 24 December 2025.

Materials and tools

The materials used in this study included Balakacida (Chromolaena odorata L.) leaves randomly collected from the Palembang region, 90% ethanol, distilled water, chloroform, ether, $FeCl_3$ reagent, 2N H_2SO_4, HCl, Mayer's reagent, Dragendorff's reagent, Liebermann–Burchard's reagent, Mg metal, ice cubes, and petroleum jelly.

The equipment used included a blender, maceration container, glass stirring rod, gauze or filter paper, beaker glass, Erlenmeyer flask, volumetric flask, analytical balance, rotary evaporator, water bath, oven, test tube, test tube rack, dropper pipette, dropper plate, petri dish, wooden clamp, Fourier Transform Infrared (FTIR) spectrophotometer, parchment paper, and silicate crucible.

Making Simple Drugs

Balakacida leaves were collected from the Asri Residence housing complex, Banyuasin Regency, South Sumatra. Fresh, healthy, pest-free leaves were harvested in the morning (8:00–10:00 a.m. WIB), then wet sorted to separate damaged or contaminated leaves. Selected leaves were washed using running water until clean, drained, and chopped into uniform sizes to speed up the drying process. Drying was carried out by airing in a shaded place until the water content was reduced, followed by dry sorting. The dried simplicia were then weighed, stored in a tightly closed, light-proof container, and ground using a laboratory blender to a fine powder. The powder was sieved using an 80 mesh sieve to obtain a uniform particle size, then weighed according to research requirements.

Extraction Process

Extraction was carried out using the maceration method using 90% ethanol as a solvent. A total of 145 grams of simplicia powder was put into a clean and dry maceration container, then 1,500 mL of 90% ethanol was added with a ratio of material to solvent of 1:10. The mixture was stirred homogeneously, tightly closed, and left for 3 × 24 hours at room temperature, while stirring periodically. The filtrate was filtered to separate the dregs, then re-maceration was carried out with the same volume of solvent. The two filtrates were combined and evaporated using a rotary evaporator at a temperature of 40–45°C until a thick ethanol extract of *Balakacida leaves was obtained* . The extract was stored in a closed container to prevent degradation due to light and air.

Phytochemical Screening

Identification of Alkaloid Compounds

A 500 gram sample of fresh sample was weighed and placed into a beaker glass. 1 mL of 2 N HCl solution and 9 mL of distilled water were added to the sample, then heated in a water bath for 2 minutes. After cooling, the solution was filtered using filter paper into a test tube or dropper plate.

Three drops of the filtrate were taken and dripped into each test tube or dropper plate well. Next, two drops of alkaloid reagents, namely Mayer's reagent, Dragendorff's reagent, and Liebermann–Burchard's reagent, were added. The formation of a precipitate or a certain color change was observed as a positive indication of the presence of alkaloid compounds.

Identification of Phenolic Compounds and Their Derivatives

Fresh samples were placed in Erlenmeyer flasks, then 25 mL of ethanol was added and heated in a water bath for 15 minutes. The extract was filtered through cotton wool into a test tube, then evaporated over a water bath to dryness to obtain a concentrated extract. Next, the extract was dissolved in 10 mL of a 1:1 chloroform:water mixture, shaken gently, and left to form two layers.

a. The top layer (polar) is used for identification of phenolic and flavonoid compounds.
b. The bottom layer (non-polar) is used for the identification of terpenoid and steroid compounds.

Specific color reactions to chemical reagents (e.g. $FeCl_3$ for phenols or $AlCl_3$ for flavonoids) are observed as qualitative indicators.

Sample Preparation for FTIR Analysis

The thick extract from the isolation was dried at 40–50°C until solvent-free. The dried sample was then ground using a mortar and pestle until homogeneous. A total of ±10 mg of the sample was mixed with ethanol to obtain a homogeneous semi-solid consistency. This mixture was then applied to the infrared spectrophotometer measurement cell using the Attenuated Total Reflectance (ATR) mode. The ATR crystal surface (usually diamond or ZnSe) was first cleaned with absolute ethanol, then the sample was dropped and pressed using the ATR pressing arm until optimal contact was achieved. The sample was then ready for infrared spectroscopy measurements.

Infrared Spectrophotometry (FTIR) Analysis

FTIR analysis was performed using a Fourier Transform (FTIR) infrared spectrophotometer in the wavenumber range of 4000–400 cm^{-1}, with a resolution of 4 cm^{-1}, and 32 scans to increase the signal-to-noise ratio (S/N). The obtained infrared spectra were analyzed to identify the typical functional groups of flavonoid compounds, such as:

a. Hydroxyl group (–OH): strong and broad absorption at 3200–3600 cm^{-1}
b. Carbonyl group (C=O): strong absorption at 1650–1700 cm^{-1}
c. Aromatic double bond (C=C): moderate absorption at 1500–1600 cm^{-1}
d. C–O bond (phenolic or ether): absorption at 1000–1300 cm^{-1}

The measured spectrum was compared with standard flavonoid spectrum reference data from scientific literature for qualitative confirmation of compound identity.

Data analysis

The infrared spectra were analyzed qualitatively by interpreting the positions and intensities of the absorption peaks. Compound identification was based on the matching of characteristic wavenumbers to flavonoid spectra in the literature. The analysis results are presented in the form of FTIR spectra and descriptive descriptions of the detected functional groups.

RESULTS AND DISCUSSION

Table 1. Determination Results Shrinkage Drying Balakacida Leaf Simplicia

Plant Name	The Part Taken	Initial Weight	Weight of Dry Simplicia	Drying Loss
Balakacida (*Chromolaena odorata* L.)	Leaf	700 grams	210 grams	70%

Based on Table 1, balakacida leaf simplicia was obtained from 700 grams of fresh leaves which after the drying process produced 210 grams of dried simplicia, resulting in a weight loss of 490 grams or a drying loss of 70%. This high drying loss value reflects the high natural water content in fresh leaves, generally ranging from 60–80%, as reported by (Khaled et al., 2024) in other tropical leaf species.

These results align with research by Utami et al., 2022, which reported that the leaves of herbal plants such as *Andrographis paniculata* exhibit drying losses of 65–75%, depending on leaf tissue thickness and drying conditions. The drying process causes the evaporation of free water and some bound water without damaging key chemical components such as flavonoids and tannins, because the water bath temperature is relatively low (40–50°C). The thin, spongy structure of balakacida leaves accelerates the water evaporation process compared to other plant parts such as stems or roots, resulting in a relatively high percentage of drying losses.

Thus, the drying loss value of 70% in this study is still within a reasonable range for leaf simplicia and indicates that the drying method used has succeeded in reducing the water content to a limit suitable for storage and further extraction processes.

Table 2. Results of Balakacida Leaf Extraction Using the Maceration Method

Weight of Simple Ingredients	Solvents Used	Amount of Solvent	Extract Weight	Rendamen
145 grams	Ethanol 90%	3 L	5.6 grams	3.862%

Extraction of balakacida leaf simplicia using the maceration method with 90% ethanol solvent produced a thick extract of 5.6 grams, with a yield of 3.862%. This yield shows the efficiency of the ethanol solvent in extracting polar to semipolar active compounds such as flavonoids, tannins, and saponins.

These results are consistent with research by Do et al., 2014, which showed that 80–90% ethanol is the optimal solvent for extracting flavonoids from *Chromolaena odorata leaves* with a yield of 3–5%. Research by Okeke et al., 2020 also reported a yield of 3.5–4.2% in the extraction of tropical herbal leaves using 90% ethanol, which is consistent with the results of this study.

The relatively low yield value can be caused by several factors, including the ratio of material to solvent, soaking duration, and temperature during extraction. Furthermore, the chemical characteristics of the active compounds in the herbal medicine greatly determine extraction efficiency. Ethanol 90% is semipolar, making it effective in dissolving secondary metabolites such as flavonoids and tannins, but less optimal for highly nonpolar compounds such as terpenoids. Decreased efficiency can also occur if the solvent used is too polar (for example, water), because some active compounds in balakacida leaves are semipolar and do not dissolve completely in such solvents.

Table 3. Phytochemical Screening Test of Simplex and Ethanol Extract of Balakacida Leaves

No	Compound	Reagent	Color According to Literature	Screening Results			
				Simple ingredients		Extract	
				Color	Note	Color	Note
1.	Alkaloid	Liebermann - Burchard	Brown, reddish brown to blackish brown	Reddish brown	+	Yellow	-
		Mayer	White mist, sediment or clumps form	White sediment	+	White sediment	+
		Dragendorff	Orange-yellow sediment	Orange-yellow sediment	+	Orange-yellow sediment	+
2.	Phenolic	Water + FeCl3	Purplish blue to dark blue	Purplish blue to dark blue	+	Purplish blue to dark blue	+
3.	Flavonoid	Water + HCl + Mg Metal	Orange red	Orange red	+	Orange red	+
4.	Saponin	Water (Water layer is shaken)	The foam that forms does not disappear immediately	No foam formed	-	No foam formed	-
5.	Steroids and Terpenoids	Chloroform + Activated carbon + Liebermann	Green (+terpenoid)	Green	+ Terpenoid	Green	+ Terpenoid

Based on the results of the phytochemical screening test in Table 3, it was found that the simplex and ethanol extract of balakacida leaves contained alkaloid, phenolic, flavonoid, and terpenoid compounds, but did not show the presence of saponins.

The observed color changes are the result of chemical reactions between functional groups of secondary metabolite compounds and specific reagents.

In the alkaloid test, the formation of a white precipitate in the Mayer reagent and an orange-yellow precipitate in the Dragendorff reagent is caused by the formation of a complex between metal ions (Hg^{2+} or Bi^{3+}) with the nitrogen group in the alkaloid (Wijianto et al., 2022). Meanwhile, the reddish-brown color in the Liebermann–Burchard reagent comes from the oxidation reaction of unsaturated compounds or sterol structures in the sample with the strong acid contained in the reagent.

However, the results of the alkaloid test in this study showed inconsistency between the Liebermann–Burchard reagent and two other reagents (Mayer and Dragendorff).

This discrepancy can occur due to differences in the sensitivity and selectivity of each reagent toward certain types of alkaloids. Some reagents, such as Liebermann–Burchard, are generally more sensitive to sterols and triterpenoids, and therefore can produce false positives if these compounds are present in the sample. Conversely, false negatives can occur if the alkaloid concentration in the extract is low or if the compound undergoes thermal or oxidative degradation during the drying and extraction processes.

In the phenolic test, a color change from purplish blue to dark blue due to the addition of $FeCl_3$ indicates the presence of a phenolic –OH group that forms a complex with the Fe^{3+} ion. This test is relatively sensitive, but can produce false positives if other compounds with strong hydroxyl groups (e.g., ascorbic acid or non-phenolic tannins) react with the same reagent.

In the flavonoid test, the formation of an orange-red color indicates a reaction between Mg metal and HCl, which reduces the carbonyl group of the flavonoid to a characteristically colored flavyl compound. Consistent positive results between the drug and the extract indicate the relative stability of the flavonoid during ethanol extraction. as also reported by (Khaled et al., 2024) in leaf extracts of other tropical plants.

Phytochemical screening tests are qualitative and indicative, not conclusive. Positive results only indicate the possible presence of a particular compound, but do not provide quantitative information or definitive chemical structure. Therefore, screening results should be further confirmed with instrumental analysis, such as FTIR, LC-MS, or HPLC, to confirm the compound's identity.

Furthermore, the potential for false-positive or false-negative results must be recognized as a major limitation of phytochemical testing. Factors such as reaction pH, thermal degradation, solvents used, and sample matrix complexity can influence results. Therefore, phytochemical screening serves as an initial stage in secondary metabolite exploration, providing a foundation for more specific and quantitative follow-up analyses.

Table 4 TLC Results of Balakacida Leaf Fraction

No	Fractionation	The Noda Ke-	Rf value	Stain Color
1	Extract	1	0.69	Green
		2	0.61	Blue
		3	0.52	Purple
		4	0.47	Purple
		5	0.4	Purple
		6	0.25	Purple
2	Ethyl Acetate	1	0.54	Purple
		2	0.56	Purple
3	n-Hexane	1	0.30	Purple
4	Water	1	0.45	Purple

Separation of secondary metabolite compounds was carried out using thin layer chromatography (TLC). The plate used was silica gel GF254 (Merck, Germany) on an aluminum plate with a thickness of 0.25 mm as the stationary phase. The mobile phase used was a mixture of *n-hexane: ethyl acetate: methanol: water (4:3:2:1)* . The sample was spotted using a micropipette at a distance of 0.5 cm from the bottom edge of the plate, then eluted ascending until the solvent reached a distance of 5.5 cm. After development, the plate was dried at room temperature and observed under UV light at wavelengths of 254 nm and 366 nm. Furthermore, to reveal the color of the spot. *The retardation factor* (Rf) value was calculated using the formula:

$$Rf = \frac{\text{Stain Travel Distance}}{\text{Solvent Travel Distance}}$$

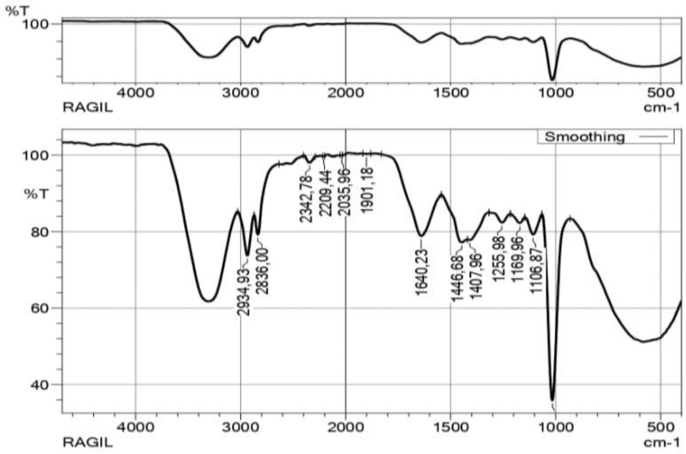
Ethanol extract of balakacida leaves produced six spots with Rf values ranging from 0.25–0.69 and variations in green, blue, and purple. Spots with high Rf values (0.61–0.69) indicate the presence of compounds with low to moderate polarity, possibly lipophilic pigments such as chlorophyll and terpenoid derivatives. This is in line with the findings of (Abdullahi et al., 2023) who reported that the green spot with Rf 0.65–0.70 in *Chromolaena odorata extract* is related to nonpolar chlorophyll.

The 2nd spot (Rf = 0.61, blue color) has a high affinity to the mobile phase, indicating compounds with low-moderate polarity. The blue color indicates the presence of certain phenolic or flavonoid compounds, which commonly show blue fluorescence after spraying the reagent. This supports that the spot is one of the dominant active components in balakacida leaf extract (Usman Yusnita & Muin Rahmatullah, 2023) Spots with medium Rf values (0.40–0.52) indicate the presence of semipolar compounds such as flavonoids and phenolics, which produce a purple color under UV light. According to (Sharma et al., 2025), flavonoid compounds in *C. odorata* flower extract appear with a purplish purple color in the Rf range of 0.45–0.60. The 5th spot (Rf = 0.40, purple) indicates compounds with medium polarity, possibly flavonoid glycosides or oxygenated terpenoids.

Meanwhile, spots with low Rf values (0.25–0.30) indicate polar compounds or compounds that interact strongly with the stationary phase, such as alkaloids or polar glycosides. These results are consistent with those of Suliani et al. (2021) who reported a single purple spot (Rf ≈ 0.32) in the *n-hexane fraction of C. odorata* leaves , identified as a nonpolar flavonoid aglycone.

The ethyl acetate fraction displayed two purple spots (Rf = 0.54 and 0.56), indicating semipolar compounds, in accordance with the characteristics of flavonoids that are easily soluble in ethyl acetate (Odion et al., 2023). The n-hexane fraction produced one purple spot at Rf = 0.30, indicating flavonoid aglycones or simple terpenoid derivatives, while the water fraction displayed one purple spot (Rf = 0.45), indicating polar compounds such as phenolic glycosides.

Overall, the TLC profile indicates that balakacida leaves contain a variety of secondary metabolites with a wide range of polarities. Purple and blue colors indicate aromatic compounds (flavonoids, phenolics, alkaloids), while green indicates chlorophyll pigments. This finding is consistent with (Popoola et al., 2024) who reported that *C. odorata extracts* exhibited a fluorescent purple-blue stain at Rf = 0.45–0.68 representing biologically active aromatic compounds.



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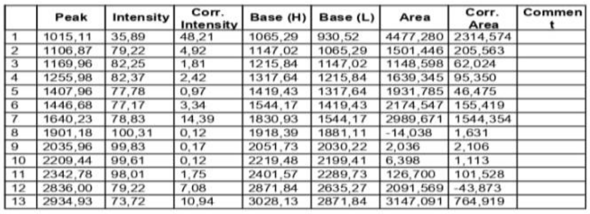
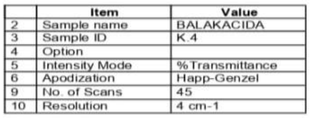


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	1015,11	35,89	48,21	1065,29	930,52	4477,280	2314,574	
2	1106,87	79,22	4,92	1147,02	1065,29	1501,446	205,563	
3	1169,96	82,25	1,81	1215,84	1147,02	1148,598	62,024	
4	1255,98	82,37	2,42	1317,64	1215,84	1639,345	95,350	
5	1407,96	77,78	0,97	1419,43	1317,64	1931,785	46,475	
6	1446,68	77,17	3,34	1544,17	1419,43	2174,547	155,419	
7	1640,23	78,83	14,39	1830,93	1544,17	2989,671	1544,354	
8	1901,18	100,31	0,12	1918,39	1881,11	-14,038	1,631	
9	2035,96	99,83	0,17	2051,73	2030,22	2,036	2,106	
10	2209,44	99,61	0,12	2219,48	2199,41	6,398	1,113	
11	2342,78	98,01	1,75	2401,57	2289,73	126,700	101,528	
12	2836,00	79,22	7,08	2871,84	2635,27	2091,569	-43,873	
13	2934,93	73,72	10,94	3028,13	2871,84	3147,091	764,919	



	Item	Value
2	Sample name	BALAKACIDA
3	Sample ID	K.4
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	45
10	Resolution	4 cm-1

FIGURE 1. Results of Infrared Spectrophotometry Observations

Table 6. Analysis of Functional Groups of Extracted Compounds Using Infrared Spectophotometry

Peak	Number Waves (cm⁻¹)	Intensity (%)	Interpretation of Functional Groups
2934.9	73.72	Aliphatic C–H stretching absorption (–CH_2, –CH_3)	Alkane groups of flavonoid side chains
2836	79.22	C–H stretch aldehyde	Possibility existence aromatic CHO group
2342.8	98.01	CO_2 or C≡C (if from dry sample)	Air interference or oxidized compounds
2096	99.83	C≡C or C≡N stretch	Compound oxidized flavonoid derivatives
1901.2	100.31	Overtone or combination vibration aromatic	Characteristics system aromatic ring

1640.2	78.83	C=O stretch (carbonyl) and C=C aromatic	Characteristics of flavonoids (benzopyrone structure)
1468.7	77.17	Aromatic C–C stretching and –CH_2 bending	Ring phenolic aromatic
1417.6	77.78	C–O–H bending phenolic	Hydroxyl group aromatic (flavonol / flavone)
1256	82.37	C–O stretch of ether or phenolic groups	Characteristic O–CH_3 or C–O–C flavonoid groups

Analysis spectrum infrared (FTIR) against extract ethanol leaf Balakacida show the presence of absorption bands typical flavonoid compounds , with a number of peak main identified in the spectrum region infrared medium (4000–400 cm^{-1}).

a. strong band at 1640 cm^{-1} indicates existence group aromatic carbonyl (C=O) , which is characteristics typical flavonoid structures such as flavonols and flavanones . According to Noh et al. (2017), the peak at 1660–1640 cm^{-1} indicates C=O stretch on the 3-hydroxyflavone skeleton and becomes indicator main the presence of flavonoid core.
b. Absorption at 1255–1015 cm^{-1} indicates C–O–C and C–O–H stretching which are common in phenolic compounds and glycosides. (Agatonovic-Kustrin et al., 2021) reported that a strong band around 1036 cm^{-1} in olive leaf extract indicates the presence of flavonoid glycosides, related to the C–O–C stretching of the pyranoid ring of the glucose unit.
c. The weak peak around 2934 cm^{-1} is related to the asymmetric C–H stretching of the –CH_2 group, which is commonly found in alkyl chains of aromatic substituents. This finding is in accordance with the results (Krysa et al., 2022) on *Radix Scutellariae* extract , which attributed the peak at 2930–2940 cm^{-1} to aliphatic-aromatic interactions in the flavonoid structure.
d. The absence of a sharp band at around 1700 cm^{-1} indicates that the compounds in this extract are not dominated by free ketone groups, but rather by a carbonyl-aromatic conjugation system that shifts to a lower frequency due to the formation of intramolecular hydrogen bonds. This is in accordance with the explanation (Singh & Mendhulkar, 2015) that 4-keto-3-hydroxy flavonoids (e.g., quercetin, kaempferol) show a weakened C=O band that shifts to 1650 cm^{-1} due to hydrogen bonds between the –OH (C3) and C=O (C4) groups.
e. The 3400–3200 cm^{-1} range, which typically represents phenolic O–H vibrations, is not clearly visible in this spectrum. The absence of the O–H band may be due to overlap with the residual water band, low intensity due to low –OH group concentration, or background noise in the instrument. In some cases, the broad O–H band may be masked by the water spectrum from atmospheric humidity or residual solvent (ethanol) (Singh & Mendhulkar, 2015). stated that the O–H band of polyphenols tends to be broad and sensitive to humidity conditions, so its detection is not always consistent in natural extract samples.

Some minor peaks such as those at 2096 cm^{-1} and 2342 cm^{-1} are most likely not derived from bioactive compounds, but rather spectral artifacts due to atmospheric CO_2 absorption (2342 cm^{-1}) and instrumental background compensation (baseline distortion) around 2100 cm^{-1}. Therefore, these bands are not interpreted as active functional groups of the extract. This phenomenon is common in FTIR analysis of solid samples containing moisture or trapped air .

As a qualitative method, FTIR only indicates the presence of functional groups based on the position and intensity of absorption bands, not the identity of specific molecular structures or quantitative composition. Therefore, FTIR results should be interpreted in a complementary manner with other spectroscopic data such as UV-Vis, LC-MS, or NMR to confirm the presence of flavonoids structurally.

CONCLUSION

This study shows that balakacida (*Chromolaena odorata* L.) leaves contain various secondary metabolites, especially flavonoids, which have the potential to be a source of natural bioactive compounds. The drying process resulted in a drying loss of 70%, while extraction using the maceration method with 90% ethanol gave a yield of 3.862%, confirming the effectiveness of semipolar solvents in extracting active compounds from leaf tissue.

Phytochemical screening and TLC results showed the presence of flavonoids, phenolics, alkaloids, and terpenoids with an Rf value range of 0.25–0.69, reflecting the distribution of compounds based on differences in polarity. FTIR analysis confirmed this finding through the identification of typical flavonoid absorption bands, namely aromatic C=O (~1640 cm^{-1}), C–O–C and C–O–H (1250–1015 cm^{-1}), as well as indications of phenolic O–H groups (3400–3200 cm^{-1}).

Methodologically, these results demonstrate that the combination of TLC and FTIR is an effective and economical qualitative approach for identifying flavonoid compounds in plant extracts. However, this method has limitations in structural resolution and specificity, as it cannot distinguish isomers or determine molecular structure with certainty.

To deepen our understanding of the chemical profile of balakacida leaves, further research is recommended using advanced quantitative and structural analysis methods such as HPLC or UPLC to determine the main flavonoid content, as well as LC-MS/MS and NMR for molecular characterization and identification of compounds. Toxicological studies and biological activity tests (e.g., antioxidant, antibacterial, or anti-inflammatory) are also needed to support the development of the pharmacological and phytopharmaceutical potential of this plant.

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