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Chemometric Evaluation of Antioxidant Activity in Libo Fruit Extracts: Correlation with Phenolic and Flavonoid Content

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ABSTRACT

Libo fruit (*Ficus septica* Burm. F.) is traditionally used for its medicinal properties, including antioxidant activity. This study assessed the antioxidant potential of methanol (EM) and purified (EP) extracts of Libo fruit collected from four regions in Southeast Sulawesi, Indonesia. Antioxidant activities were measured using ABTS and CUPRAC assays, while total phenolic and flavonoid contents were quantified by Folin–Ciocalteu and aluminum chloride methods. EM from Sampara and EP from Katobu showed the strongest antioxidant capacities, which were inversely correlated with IC_{50} values. Principal Component Analysis (PCA) grouped the samples into three clusters based on physicochemical similarities, supported by FT-IR spectral data. These findings suggest that *F. septica* fruit extracts are potent natural antioxidants and that chemometric approaches effectively differentiate extracts by chemical composition.

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ABSTRAK

Buah Libo (*Ficus septica* Burm. F.) telah lama dimanfaatkan dalam pengobatan tradisional karena memiliki berbagai aktivitas farmakologis, termasuk sebagai antioksidan. Penelitian ini mengevaluasi potensi antioksidan dari ekstrak metanol (EM) dan ekstrak terpurifikasi (EP) buah Libo yang dikumpulkan dari empat wilayah di Sulawesi Tenggara. Aktivitas antioksidan diuji menggunakan metode ABTS dan CUPRAC, sedangkan kandungan total fenolik dan flavonoid ditentukan dengan metode Folin–Ciocalteu dan kolorimetri aluminium klorida. EM dari Sampara dan EP dari Katobu menunjukkan aktivitas antioksidan tertinggi yang berkorelasi negatif dengan nilai IC_{50}. Analisis Principal Component Analysis (PCA) berhasil mengelompokkan sampel ke dalam tiga klaster berdasarkan kesamaan sifat fisiko-kimia, didukung oleh data spektrum FT-IR. Temuan ini menunjukkan bahwa ekstrak buah *F. septica* memiliki potensi kuat sebagai antioksidan alami dan pendekatan kemometrik efektif dalam membedakan profil kimia ekstrak berdasarkan komposisinya.

Keywords: Aktivitas Antioksidan; Analisis Kemometrik; ABTS; CUPRAC; *Ficus septica*

1. Introduction

Antioxidants are compounds that function to stabilize the increase of free radicals, protect cells from oxidative damage, and help prevent degenerative diseases caused by oxidative stress [1]. Free radicals with unpaired electrons in their outermost orbitals tend to form unstable and reactive molecules, which in turn trigger oxidative stress. These radicals, particularly reactive oxygen species (ROS), can damage DNA, lipids, and proteins by forming bonds with vital cellular components. Under normal physiological conditions, ROS generation and antioxidant defense mechanisms remain balanced. However, when this balance is disrupted, oxidative stress ensues, leading to cell damage [2].

Oxidative damage is a major contributing factor to various degenerative diseases such as arthritis, cancer, asthma, atherosclerosis, diabetes, Parkinson's disease, and Alzheimer's disease [3], [4]. Antioxidants neutralize free radicals by donating electrons or hydrogen atoms, converting them into more stable and less reactive metabolites that are easily eliminated from the body [4],[5]. Common synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylated hydroquinone (TBHQ) have been shown to inhibit oxidative reactions associated with these diseases [5],[6]. However, concerns over their long-term safety have increased interest in natural antioxidants.

Natural products, especially those derived from medicinal plants, are rich sources of bioactive compounds with antioxidant potential. One such plant is Libo (*Ficus septica* Burm. f.), which is traditionally used for its hepatoprotective, antibacterial, antiviral, antimalarial, and antioxidant properties [7]–[13]. Extracts from *F. septica* leaves have been reported to effectively scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, with an IC_{50} value of 4.45 µg/mL [14]. Furthermore, Taeri et al. [13] demonstrated that the ethyl acetate fraction of Libo fruit exhibited strong antioxidant activity using the ABTS and CUPRAC assays, with IC_{50} values of 6.33 ± 0.01 µg/mL and 11.64 ± 0.28 µg/mL, respectively.

The antioxidant activity of plants is often attributed to their total phenolic and flavonoid content, which act as hydrogen donors to neutralize free radicals and prevent oxidative cell damage [15]–[17]. The 70% ethanol extract of *F. septica* has been shown to contain 8.11% total phenolic content expressed as EAG (equivalent to gallic acid) [18]. Additionally, Wahyuni and Hertiani [19] reported that the leaves and fruits of *Ficus carica* L. and *Ficus parietalis* Bl. contain significant amounts of phenolic and flavonoid

compounds: 1.46 ± 0.006 GAE and 0.61 ± 0.010 RE (leaves), and 0.51 ± 0.001 GAE and 0.20 ± 0.001 RE (fruits), respectively.

Variability in the secondary metabolite composition of plants is influenced by environmental and geographical factors such as altitude, climate, and soil conditions [20], [21]. To assess the complex relationship between antioxidant activity and bioactive compound content, chemometric tools such as Principal Component Analysis (PCA) have been employed. PCA allows the simplification of multivariate data sets and enables the grouping of samples based on chemical similarity [22], [23]. Additionally, Fourier Transform Infrared (FT-IR) spectroscopy can identify functional groups involved in antioxidant activity, and when combined with PCA, provides a comprehensive chemometric approach.

Therefore, this study aims to evaluate the antioxidant activity of Libo fruit (*F. septica* Burm. f.) using ABTS and CUPRAC methods, determine the total phenolic and flavonoid contents, and classify the samples from various geographical locations in Southeast Sulawesi using PCA and FT-IR spectroscopy.

2. Methods

Sample Preparation

Libo fruit (*Ficus septica* Burm. f.) was collected from four regions in Southeast Sulawesi: Parigi District (Muna Regency), Benu-benua District (Kendari City), Sampara District (Konawe Regency), and Katobu District (Muna Regency), Indonesia. The plant material was taxonomically identified as Ficus septica Burm. F. by Mrs. Murni Sabilu at the Biology Laboratory, Faculty of Teacher Training and Education (FKIP), Halu Oleo University and deposited in the herbarium of the same laboratory with a voucher specimen number BIO 268 (**Figure 1**). The samples were thoroughly washed and air-dried at room temperature until the moisture content was below 10%. The dried samples were then ground using a food processor to obtain powdered *simplicia*, commonly used in traditional medicine as crude herbal material.

(a) (b)

Figure 1. (a) *Ficus septica* (Libo) tree; (b) Libo fruit samples collected from four different growing locations in Southeast Sulawesi, Indonesia

Extraction and Purification

Extraction of Libo fruit powder (*Ficus septica* Burm. f.) was performed using a maceration method with 70% methanol as the solvent. Each sample (500 g) from the four different collection sites was soaked in 70% methanol for 72 hours with occasional stirring. The resulting macerate was filtered, and the filtrate was concentrated under reduced pressure using a rotary vacuum evaporator to yield a thick methanolic extract

(EM).

The obtained EM was further purified via a liquid-solid extraction method using *n*-hexane. Specifically, 10 g of each methanolic extract was placed in a mortar, combined with 100 mL of *n*-hexane, and thoroughly ground. The *n*-hexane layer was then separated and collected. This purification step was repeated until the *n*-hexane layer became transparent, indicating the removal of non-polar impurities such as lipids and pigments. The final *n*-hexane fraction was evaporated to dryness under vacuum, producing a purified condensed extract (EP) (**Figure 2**).

Figure 2. Purified extracts (EP) of Libo fruit (*Ficus septica* Burm. f.) derived from four different growing sites in Southeast Sulawesi, Indonesia

Antioxidant Activity Assay Using ABTS Method

The antioxidant activity of the samples was evaluated using the ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation assay, as described by previous studies [13], [24], [25] , with slight modifications. A 7 mM ABTS stock solution was prepared by dissolving 18 mg of ABTS in 5 mL of methanol and mixing it with 5 mL of 2.45 mM potassium persulfate. The mixture was incubated in the dark at 23°C for 16 hours to form a stable dark blue ABTS radical solution.

For the antioxidant test, 1 mL of each sample was mixed with 3 mL of methanol and then 1 mL of the ABTS radical solution. The mixture was shaken and incubated at room temperature for 10 minutes. The absorbance was measured at 745 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). The antioxidant activity was expressed as percent inhibition, calculated using the formula:

$$\%\ Inhibition = \frac{Abs\ Blank - Abs\ Sample}{Abs\ Blank} x\ 100\%$$

The IC_{50} value (the concentration required to inhibit 50% of radicals) was determined by substituting y = 50 in the linear regression equation (y = bx + a), and solving for x [24].

Antioxidant Activity Assay Using CUPRAC Method

The antioxidant capacity of the samples was measured using the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) method, with modifications based on previous studies [13],[26],[27]. A 7.5 mM neocuproine reagent served as the chromogenic oxidizing agent, while vitamin C was used as the reference standard.

For the assay, 1 mL of each sample was mixed with 1 mL of 7.5 mM neocuproine reagent and 1 mL of 10 mM $CuCl_2$ solution. The mixture was incubated at room temperature for 30 minutes. After incubation, absorbance was measured at a wavelength of 453 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). The antioxidant activity was expressed as percent inhibition based on absorbance changes compared to the control.

Determination of Total Phenolic Content

The total phenolic content of methanolic (EM) and purified (EP) extracts was determined using the Folin–Ciocalteu method, following previously published procedures [28]–[30] with gallic acid as the calibration standard. One milliliter of the sample was placed in a test tube and mixed with 0.4 mL of 7% Na_2CO_3 solution. The mixture was vortexed until homogeneous, then incubated at room temperature for 30 minutes. The absorbance was measured at 750 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE/g).

Determination of Total Flavonoid Content

The total flavonoid content of *Ficus septica* Burm. f. extracts was determined using the aluminum chloride ($AlCl_3$) colorimetric method, following standard procedures [31]–[33] with slight modifications. One milliliter of sample was mixed with 3 mL of methanol, followed by the addition of 1 mL of 10% $AlCl_3$ solution and 200 µL of 1 M potassium acetate. The mixture was transferred to a 10 mL volumetric flask and diluted with distilled water. After homogenization, the solution was incubated at room temperature for 30 minutes, and the absorbance was measured at 435 nm using a UV-Vis spectrophotometer. The flavonoid content was expressed as milligrams of quercetin equivalent per gram of sample (mg QE/g).

Chemometric Analysis (Principal Component Analysis)

Principal Component Analysis (PCA) was employed to explore the correlation between antioxidant activity (IC_{50} values from ABTS and CUPRAC assays), total phenolic and flavonoid contents, and spectral data from Fourier Transform Infrared (FT-IR) analysis. The FT-IR data, obtained in the 4000–600 cm^{-1} range, were used to identify functional groups contributing to sample variation.

All data were input into Minitab version 22 (Minitab LLC, USA), where PCA was performed using default eigenvalue-based dimensionality reduction. The score plot was used to classify samples based on similarities in chemical composition and purification type, while the loading plot identified the most influential variables, including specific wavenumbers. Variables forming acute angles were interpreted as positively correlated, obtuse angles as negatively correlated, and right angles as uncorrelated [22], [23]. This chemometric approach was used to validate grouping patterns seen in FT-IR and antioxidant profiles and is in line with previous studies on herbal extract classification.

3. Results and Discussion

Antioxidant Activity Test Using ABTS Method

The antioxidant activity of the samples was assessed using the ABTS method, which relies on the ability of antioxidant compounds to neutralize ABTS radicals by donating protons to the $ABTS^{\bullet+}$ radical cation. This reaction involves the oxidation of potassium persulfate and is visually indicated by a color change from greenish-blue to colorless [34],[35].The rate of decolorization reflects the antioxidant capacity in scavenging free radicals [37],[38].

The ABTS assay is suitable for both organic and inorganic antioxidants with rapid reaction times and is applicable across a wide pH range. It is effective for evaluating both hydrophilic and lipophilic compounds. Based on the results of this assay, both methanol extract (EM) and purified extract (EP) of Libo fruit (*Ficus septica* Burm. f.) exhibited notable antioxidant activity, as presented in **Table 1**.

Testing with the ABTS method is suitable for organic and inorganic compounds with fast reaction times and a wide pH range. This method is also tested on compounds

that are hydrophilic and lipophilic. The ABTS assay results (**Table 1**) indicated that both methanolic (EM) and purified (EP) extracts of *Ficus septica* Burm. f. possessed notable free radical scavenging activity. Among the samples, EP from Sampara District showed the highest potency, with an IC_{50} value of 30.337 ± 0.644 mg/mL, compared to 42.694 ± 0.655 mg/mL for EM from the same location. Vitamin C was used as a positive control in this study. These findings align with previous reports [38] indicating that the growing location of mahogany (*Swietenia mahagoni* L.) influences flavonoid levels and, consequently, antioxidant potential. Similarly, research by [39] confirmed that members of the *Ficus* genus (Moraceae) exhibit pharmacological activity, particularly those growing in specific ecological conditions such as on the island of Java.

Antioxidant Activity Test Using CUPRAC Method

The CUPRAC (Cupric Ion Reducing Antioxidant Capacity) assay was also employed to evaluate the antioxidant activity of methanolic (EM) and purified (EP) extracts of Ficus septica Burm. f. This method is based on the reduction of copper(II)-neocuproine complex (Cu^{2+}–Nc) to copper(I)-neocuproine complex (Cu^{+}–Nc) by antioxidant compounds [27],[41],[42]. The chromophoric ligand neocuproine (Nc) reacts with $CuCl_2$ under physiological pH to form a stable yellow-orange Cu^{+}–Nc complex, which can be quantitatively measured by a UV-Vis spectrophotometer at 453 nm.

Based on the results presented in **Table 1**, the purified extract (EP) from Katobu District exhibited the strongest antioxidant activity among all samples tested, with an IC_{50} value of 29.199 ± 0.150 mg/mL. This was lower than the IC_{50} value of the corresponding methanolic extract (44.251 ± 0.528 mg/mL), indicating that purification enhanced the antioxidant potential. These values were also comparable to the IC_{50} of vitamin C (9.660 ± 0.150 mg/mL), which served as the positive control.

The relatively low IC_{50} values of the EP, particularly from Katobu, suggest a high capacity to reduce Cu^{2+} to Cu^{+}, reflecting strong antioxidant power. This finding aligns with the results of ABTS assay and is consistent with previous studies [42], which reported that antioxidant activity is closely correlated with total phenolic and flavonoid content. Geographic variation, including factors such as altitude and microclimate, may influence secondary metabolite levels, as also reported in *Celosia argentea* Linn.

Table 1. IC_{50} Values (mg/mL) of Methanol and Purified Extracts of Libo Fruit (*Ficus septica* Burm. f.) Based on ABTS and CUPRAC Antioxidant Assays

Sample		IC_{50} value (mg/mL)	
		ABTS	**CUPRAC**
Methanol Extract	Parigi	45.259 ± 0.618	46.166 ± 0.541
	Benu-benua	45.775 ± 0.512	49.705 ± 0.722
	Sampara	42.694 ± 0.655	44.606 ± 0.152
	Katobu	44.215 ± 0.601	44.251 ± 0.528
Purified extract	Parigi	35.912 ± 0.595	29.801 ± 0.826
	Benu-benua	35.244 ± 0,355	29.455 ± 0.440
	Sampara	30.337 ± 0.644	30.276 ± 0.020
	Katobu	31.269 ± 0.500	29.199 ± 0.150
Vitamin C		7.714 ± 0.108	9.660 ± 0.150

Determination of Total Phenolic and Flavonoid Content

The total flavonoid content in the methanolic (EM) and purified (EP) extracts of *Ficus septica* Burm. f. was determined using the aluminum chloride ($AlCl_3$) colorimetric method [43].]. The color change from red to orange after the addition of $AlCl_3$ indicates complex formation between hydroxyl and ketone groups at the ortho position of the flavonoid structure. Quercetin was used as a standard, and the flavonoid content was expressed as milligrams of quercetin equivalent (mg QE/g sample). Based on the data presented in **Table 2**, the highest flavonoid content was observed in the samples from Katobu District, with values of 233 ± 0.49 mg QE/g for EM and 283 ± 0.31 mg QE/g for EP.

The total phenolic content was determined using the Folin–Ciocalteu method [13]. This assay involves the reduction of the phosphomolybdate-phosphotungstate reagent by phenolic compounds in an alkaline medium, forming a blue molybdenum-tungsten complex. The intensity of the blue color, measured at 750 nm, correlates with the phenolic concentration. According to **Table 2**, the Katobu District samples also exhibited the highest phenolic content, with values of 94.33 ± 0.85 mg GAE/g (EM) and 111.00 ± 0.70 mg GAE/g (EP).

Table 2. Total phenolic and flavonoid contents of *Ficus septica* Burm. f. methanolic and purified extracts

Sample		Total Level ± SD	
		Flavonoids (mg QE/g)	**Phenolic (mg GAE/g)**
Methanol Extract	Parigi	230 ± 0.56	87,67 ± 0.57
	Benu-benua	228 ± 0.31	81,00± 0.62
	Sampara	232 ± 0.38	91,00± 0.62
	Katobu	233 ± 0.49	94,33± 0.85
Purified extract	Parigi	280 ± 0.56	104,30± 0.15
	Benu-benua	278 ± 0.31	97,67± 0.57
	Sampara	282 ± 0.38	107,67± 0.47
	Katobu	283 ± 0.31	111,00± 0.70

Note: *GAE = gallic acid equivalent; QE = quercetin equivalent. Phenolic and flavonoid contents were measured using Folin–Ciocalteu and $AlCl_3$ colorimetric methods, respectively.*

Variations in total phenolic content can be attributed to several environmental factors, including geographic location, rainfall, temperature, and sunlight exposure, all of which influence the biosynthesis of secondary metabolites through photosynthesis [44]. This result is supported by Istiqomah et al. [45], who reported that the purified extract of *Luffa acutangula* (L.) Roxb. had higher phenolic content than its crude extract due to the removal of interfering substances such as lipids and pigments during purification.

The antioxidant capacity of natural plant extracts is often associated with the concentration of phenolic and flavonoid compounds. Fokunang et al. (2020) [46] emphasized that phenolic and flavonoid constituents play a central role in neutralizing free radicals in plant-based antioxidants. Qi et al. (2021) [47] further confirmed that higher levels of these secondary metabolites are positively correlated with antioxidant

potential in various species of the *Ficus* genus. In addition, Rohman (2021) [48] reported that the total phenolic and flavonoid content significantly contributes to the radical scavenging activity of herbal extracts, including *Ficus septica* Burm. f.

Data Analysis with Chemometric Technique

The correlation between antioxidant activity (measured using ABTS and CUPRAC) and the levels of total phenolics and flavonoids was analyzed using the Principal Component Analysis (PCA) technique. PCA reduces multivariate, intercorrelated data into a few principal components, grouping samples with similar physicochemical characteristics [49]. In this study, PCA was used to evaluate how total phenolic and flavonoid contents relate to IC_{50} values from antioxidant assays.

The results are visualized in the loading plot curve **(Figure 2)**, which illustrates the strength of each variable's influence on the principal components. Each variable is represented by a vector, and the angle between vectors indicates their correlation: angles <90° represent positive correlations, angles close to 90° indicate no correlation, and angles >90° indicate negative correlations [49]. In this context, a negative correlation implies that higher phenolic and flavonoid levels are associated with lower IC_{50} values (i.e., stronger antioxidant activity), while a positive correlation suggests the opposite.

As shown in **Figure 3**, the vectors for total phenolics and flavonoids form angles greater than 90° with the IC_{50} values of both ABTS and CUPRAC methods, confirming a negative correlation. This suggests that the antioxidant activity of *Ficus septica* Burm. f. is strongly influenced by its phenolic and flavonoid contents. This finding is in line with the report by Hartono et al. (2020) [50], who found that the antioxidant activity of *Curcuma mangga* Val & Zijp is also affected by the concentration of these secondary metabolites.

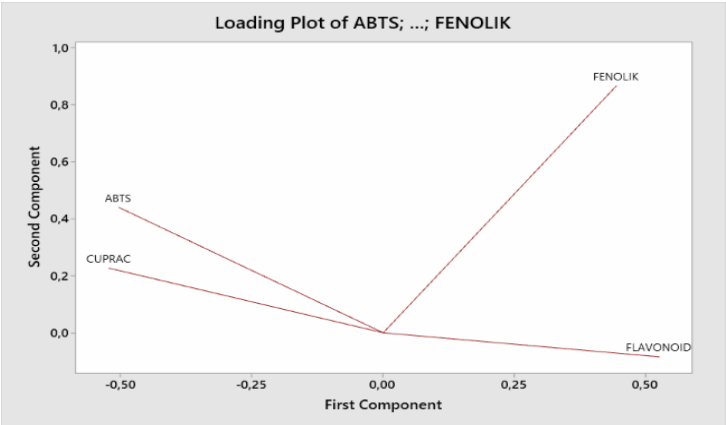


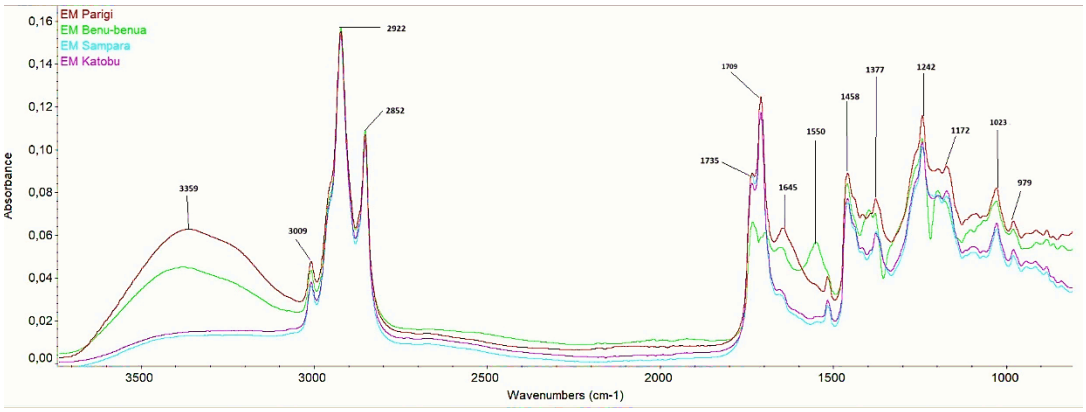
Figure 3. Loading plot of ABTS showing the correlation between IC_{50} values and total phenolic and flavonoid contents.

In this study, methanolic (EM) and purified (EP) extracts of *Ficus septica* Burm. f. collected from four different locations in Southeast Sulawesi were analyzed using Fourier Transform Infrared (FT-IR) spectroscopy with the Attenuated Total Reflectance (ATR) technique in the mid-infrared region (4000–600 cm^{-1}). The FT-IR spectra, shown in **Figure 4**, illustrate the functional groups present in the EM and EP samples based on their absorbance patterns.

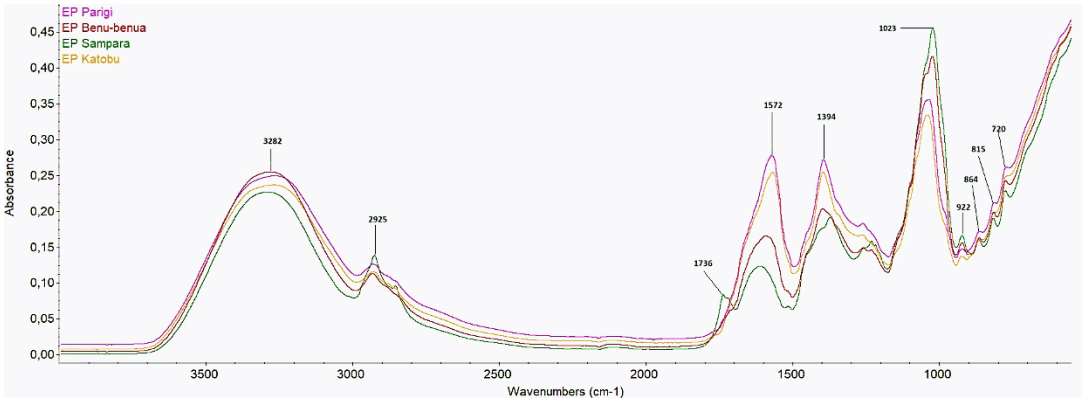
As observed in **Figure 4a**, the EM spectra from different growing sites showed similar patterns. A notable absorption peak was observed at 1458 cm^{-1}, corresponding to C≡C bending vibrations, and at 1242 cm^{-1} and 1172 cm^{-1}, associated with C–O

stretching vibrations. Meanwhile, the EP spectra (**Figure 4b**) exhibited peaks at 864 cm^{-1}, 815 cm^{-1}, and 720 cm^{-1}, which are attributed to C–H bending vibrations. These spectral differences reflect variations in chemical composition due to the purification process.

The interpretation of these functional group differences is further used in chemometric analysis. A summary of the characteristic wave numbers for both EM and EP samples is presented in **Table 3**.



(a)



(b)

Figure 4. FT-IR spectra of Libo fruit (*Ficus septica* Burm. f.) extracts from Southeast Sulawesi. (a) Methanolic extract (EM); (b) Purified extract (EP). Spectra obtained using ATR-FTIR in the region of 4000–600 cm^{-1}.

Table 3. Interpretation of functional groups of Libo fruit (*Ficus septica* Burm F..) samples

Wave Number (cm^{-1})	Vibration	Functional groups
3282	O-H stretch	Amina
2952	C-H stretch	Alkanes
1735	C=O groove	Ester
1572	N-H bending	Amina
1377	O-H bending	Alcohol
1023	C-N groove	Amina
979	C=H bending	Alkenes

FT-IR spectroscopy combined with the Attenuated Total Reflectance (ATR) technique was used to identify the functional groups in methanolic (EM) and purified (EP) extracts of *Ficus septica* Burm. f. This technique provides reliable spectral data by capturing the fingerprint region, which is highly specific and allows for accurate differentiation between samples [51]. Additionally, ATR reduces interference from moisture and other impurities that commonly affect the reading of infrared spectra [52]. The resulting spectra in the mid-infrared region (4000–600 cm^{-1}) revealed characteristic absorption bands. As presented in **Table 3**, the main functional groups identified include amines (3282, 1572, and 1023 cm^{-1}), alkanes (2952 and 979 cm^{-1}), esters (1735 cm^{-1}), and alcohols (1377 cm^{-1}), all of which are commonly associated with phenolics and flavonoids.

Table 4. Interpretation of functional groups of Libo fruit (*Ficus septica* Burm F.) samples

Eigenvalue	0,071951	0,005606	0,000752	0,000346	0,000018	0,000008	0,000003	0,000000
Proportion	0,914	0,071	0,010	0,004	0,000	0,000	0,000	0,000
Cumulative	0,914	0,986	0,995	1,000	1,000	1,000	1,000	1,00
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**

Eigenvalue	-0,000000	-0,000000	-0,000000	-0,000000	-0,000000	-0,000000	-0,000000
Proportion	-0,000	-0,000	-0,000	-0,000	-0,000	-0,000	-0,000
Cumulative	1,000	1,000	1,000	1,000	1,000	1,000	1,000
	PC9	**PC10**	**PC11**	**PC12**	**PC13**	**PC14**	**PC14**

Eigenvalue	-0,000000	-0,000000
Proportion	-0,000	-0,000
Cumulative	1,000	1,000
	PC15	**PC16**

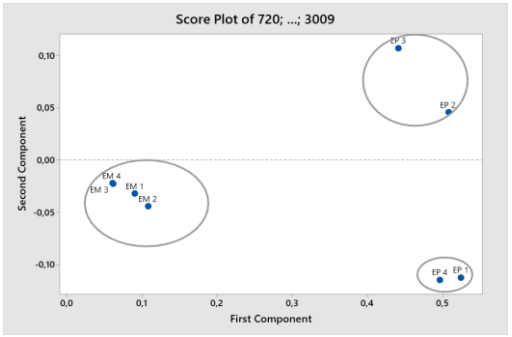


Figure 5. Score plot using FT-IR ATR absorbance data (3600–400 cm^{-1}), showing principal component separation of methanolic (EM) and purified (EP) extracts of *Ficus septica* Burm. f. EM1–EM4 represent methanol extracts from Parigi, Benu-benua, Sampara, and Katobu, respectively. EP1–EP4 refer to purified extracts from the same locations in the same order

To evaluate the variability among FT-IR spectra of EM and EP samples, PCA was applied to the fingerprint region data. PCA reduces the complexity of multivariate spectral data into principal components (PCs), which capture meaningful variation among samples and allow visualization of chemical differences. As presented in **Table 4**, PC1 and PC2 account for 91.4% and 7.1% of the total variance, respectively, with a cumulative contribution of 98.6%. This high level of explained variance indicates that most of the spectral variation is concentrated in the first two components, making the

dataset suitable for effective sample discrimination [53]. In the PCA score plot, the closer the samples appear, the greater their similarity in chemical composition.

The score plot in **Figure 5** displays the grouping of samples based on their principal component scores. Methanolic extracts (EM1–EM4) from Parigi, Benu-benua, Sampara, and Katobu cluster closely, suggesting similar spectral characteristics across geographic locations. In contrast, purified extracts (EP1–EP4) separate into two distinct clusters: EP1 (Parigi), EP4 (Katobu), and EP2 (Benu-benua) group together, while EP3 (Sampara) forms a separate cluster. Although EP2 and EP3 appear in the same quadrant, their spatial distance indicates lower chemical similarity. This result demonstrates that purification and origin both influence the chemical profile of the extracts.

These chemometric findings are consistent with previous research by Salim et al. (2023) [54], which used FT-IR and PCA to group samples of *Ceiba aesculifolia* collected from different sites in South Sulawesi based on their phytochemical fingerprint profiles.

Further interpretation was conducted using the loading plot in Figure 6, which illustrates the contribution of each wave number to the formation of PC1 and PC2 based on FT-IR absorbance data (3600–400 cm^{-1}). In this plot, variables located farther from the origin exert greater influence on sample discrimination. Among all variables, the wave number 1023 cm^{-1} contributed most significantly, indicating its strong role in differentiating phenolic-associated chemical structures within the extracts. EM1–EM4 refer to methanolic extracts from Parigi, Benu-benua, Sampara, and Katobu, respectively, while EP1–EP4 refer to the corresponding purified extracts.

Figure 6. Loading plot curve using FT-IR ATR absorbance value (3600-400 cm^{-1}). EM1 = Parigi methanol extract; EM2 = Benu-benua methanol extract; EM3 = Sampara methanol extract; EM4 = Katobu methanol extract; EP1 = Parigi purified extract; EP1 = Benu-benua purified extract; EP3 = Sampara purified extract; EP3 = Katobu purified extract.

This study has several limitations that should be considered when interpreting the findings. First, the samples of *Ficus septica* Burm. f. were collected from only four locations in Southeast Sulawesi. This limited geographic range may not fully represent the phytochemical variability of the species across different environments or growing conditions, thereby restricting the generalizability of the results. Second, antioxidant activity was assessed exclusively using in vitro chemical assays (ABTS and CUPRAC), without in vivo or cellular-level validation. Therefore, the biological relevance of the antioxidant potential observed remains to be confirmed in more physiologically complex systems. Third, while the study successfully identified correlations between antioxidant activity and total phenolic and flavonoid content using chemometric

analysis, it did not isolate or structurally characterize specific active compounds. Such characterization would be important for understanding mechanisms of action and for future pharmacological applications.

4. Conclusion

The methanolic (EM) and purified (EP) extracts of Libo fruit (*Ficus septica* Burm. f.) demonstrated strong antioxidant activity, with variations influenced by the levels of total phenolics and flavonoids across different growing locations. The highest antioxidant capacity was found in samples from Sampara and Katobu districts in Southeast Sulawesi, which also exhibited the highest concentrations of phenolic and flavonoid compounds, confirming their contribution to antioxidant potential. Chemometric analysis using PCA successfully grouped the extracts into three distinct clusters based on their physicochemical characteristics, indicating consistency in antioxidant-related spectral markers. These findings highlight the potential of *Ficus septica* as a natural antioxidant source. To strengthen its application in pharmaceutical or nutraceutical development, further studies are recommended to expand geographic sampling, assess seasonal variation, and evaluate in vivo efficacy and safety.

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Conflicts of Interest:

The authors declare no conflicts of interest.

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