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Phytochemical Profile and Antioxidant Activity of Ethanolic Extracts from Selected Wild Medicinal Plants: *Azadirachta indica, Acalypha indica*, and *Muntingia malabura*

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Abstract. *Azadirachta indica, Acalypha indica, and Muntingia calabura are wild plants with antioxidant content capable of treating various diseases. This study aims to evaluate phytochemical compound screening using qualitative tests and to characterize them using FT-IR (Fourier Transform Infrared Spectroscopy) in Azadirachta indica, Acalypha indica, and Muntingia Calabura, as well as to assess total phenolic content (TPC), flavonoid content (TFC), and antioxidant potential. The results showed that Azadirachta indica and Acalypha indica contained alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids, and carbohydrates but lacked proteins and triterpenoids. In contrast, Muntingia Calabura leaf extract lacked glycosides, triterpenoids, and carbohydrates. The highest TPC content in Muntingia Calabura extract was 269.88±0.00 mg GAE/gr extract, and the highest TFC was found in Azadirachta indica extract at 676.34±21.85 mg CE/gr extract. The ethanol extract of Azadirachta indica and Muntingia Calabura leaves has very strong antioxidant activity, with IC_{50} values of 32.76 ± 3.02 ppm and 31.16 ± 1.96 ppm, respectively, while Acalypha indica had an IC_{50} value of 107.71 ± 3.55 ppm, indicating a moderate level of antioxidant activity. These results support the potential of the three medicinal plants as sources of natural antioxidants. The findings can stimulate further research to isolate key bioactive compounds and perform toxicity tests to support potential applications in the pharmaceutical and food industries.*

Keywords: *Acalypha indica; antioxidant; Azadirachta indica; FTIR; Muntingia calabura.*

Type of the Paper: Regular Article.

1. Introduction

Phytochemicals are non-nutritive bioactive compounds produced by plants as a defense mechanism against pathogen attack or oxidative stress [1]. They are classified as secondary metabolites, generally possessing complex chemical structures such as aromatic rings (phenolics), isoprene groups (terpenoids), heterocyclic nitrogen (alkaloids), and steroids [2,3]. Although not directly involved in primary metabolites, these compounds have bio-pharmacological activities, including antioxidant, anti-inflammatory, anticancer, antibacterial, and cardioprotective effects [4,5]. Their antioxidant activity is closely related to their ability to neutralize free radicals or Reactive Oxygen Species (ROS) that can damage cells.

Reactive oxygen species (ROS), also referred to as free radicals, are generated through normal metabolic activities or by external influences such as exposure to UV light, medications,

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cigarette smoke, air pollution, and industrial chemicals. The effects of radicals on the body are a significant concern in human health. To a certain extent, free radicals are essential for normal physiological functions, including metabolism and the immune system. However excessive amounts can cause severe cellular and tissue damage, leading to oxidative stress. These disorders lead to several diseases such as infections, cardiovascular (CV), diabetes, stroke, cancer, heart disease, Alzheimer's, liver cirrhosis, symptoms of aging, and so on [6]. Therefore, antioxidants are crucial for neutralizing, preventing, and treating diseases caused by free radicals.

As oxidation reaction inhibitors, antioxidant substances including flavonoids, phenolics, and polyphenols can protect against free radicals and hold considerable potential for treating diseases caused by ROS [7,8]. Antioxidant compounds occur naturally in plants, including fruits, vegetables, grains, and other plant sources [9]. Currently, there is increasing interest in exploring phytochemical compounds, particularly polyphenols, flavonoids, and phenolics in medicinal plants as natural antioxidants. In the pharmaceutical, food, and cosmetic industries, these natural antioxidants are viewed as safer alternatives to synthetic ones [6–11].

Azadirachta indica (known as neem or mimba), *Acalypha indica* (Indian Acalypha or anting-anting), and *Muntingia Calabura* (Jamaican cherry kersen) are wild plants with antioxidant content that can help treat various diseases. Several studies have shown that *Azadirachta indica* leaf extract possesses antioxidant, blood sugar-regulating, inflammation-reducing, anxiety-relieving, stress-reducing, and antibacterial properties [12–14]. *Acalypha indica* has high phenolic and flavonoid contents, which have an important role in human therapeutics, including antibacterial, antioxidant, antifungal, and anti-ulcer [15,16]. *Muntingia Calabura* has antidiabetic, antioxidant, and anti-inflammatory properties due to its high phenolic content in the leaves and stems. In Peru and Malaysia, its bark and flowers of *Muntingia Calabura* are traditionally used to treat headaches, colds, and infections [17,18].

Although traditional medicine makes extensive use of these herbs, comprehensive information on their chemical composition has not been fully reported. Research is limited, and no phytochemical screening using FTIR spectroscopy of standardized leaf samples or leaf extract of *Azadirachta indica, Acalypha indica*, and *Muntingia Calabura* has been conducted. Previous studies have primarily employed qualitative phytochemical tests without characterizing compound structures, and none have examined wild plants from Pasuruan, East Java, where these species are abundant. This indicates a research gap, as the phytochemical compounds responsible for their biological activities remain unidentified. Therefore, this study is urgently needed to validate their therapeutic potential and support their development as local medicinal resources by combining qualitative phytochemical screening, compound characterization using FTIR (Fourier

Transform Infrared Spectroscopy), antioxidant activity testing, and quantification of phenolic and flavonoid contents.

2. Materials and Methods

2.1. Botanical materials

Azadirachta indica (neem or mimba), Acalypha indica (Indian Acalypha or anting-anting), and Muntingia Calabura (Jamaican cherry or kersen) were purchased from a farmer in Pasuruan, Indonesia (-7.6472, 112.9075). The sample was dried at 40ºC for approximately one day, or until completely dry. Moisture content was not quantitatively measured. The dried samples were pulverized using a blender and sieved to obtain particles larger than 100 µm. Fig. 1 shows the wild plant powder.

(a) (b) (c)

Fig. 1. Raw materials (a). *Azadirachta indica,* (b). *Acalypha indica* and (c). *Muntingia calabura*

2.2. Chemicals

Wako Pure Chemical Industries Inc. provided the following reagents: ethanol (C_2H_5OH 96%), sodium hydroxide (NaOH, 97%), hydrochloric acid (HCl), ferric chloride ($FeCl_3$), acetic acid (CH_3COOH), sulfuric acid (H_2SO_4), sodium carbonate (Na_2CO_3, 99,8%), aluminum trichloride hexahydrate ($AlCl_3.6H_2O$, 98%), sodium nitrite ($NaNO_2$, 98,5%), dragendorff, wagner and molish reagents. Catechin ($C_{15}H_{14}O_6$, C217500) was purchased from Toronto Research Chemicals, Inc. Sigma-Aldrich provided the gallic acid ($C_7H_6O_5$, 97,90%) and folin ciocalteu's phenol reagent.

2.3. Preparation of extracts

The powdered samples of three medicinal wild plants were extracted individually using a Soxhlet technique with slight modification [14], at a powder-to-solvent ratio of 1:5 (gr/ml). For each extraction, filter paper containing 5 grams of dried plant powder was placed in the extractor tube. A total of 250 mL 96% Ethanol was added to a two-necked flask, and the extraction apparatus was assembled. The heating mantle was activated to elevate the temperature to 70ºC, which took approximately 7-10 minutes. Each extraction lasted 240 minutes for each sample. The extracts (Fig. 2) were then evaporated for approximately 3 hours using a rotary evaporator. The concentrated residue obtained after evaporation, referred to as crude ethanol extract, was a thick

liquid used for further analysis. The solid residues remaining from the Soxhlet extraction were also collected and dried. These residues, which differed slightly in color among the three species, are presented in Fig. 3 as supporting documentation of the extraction process.

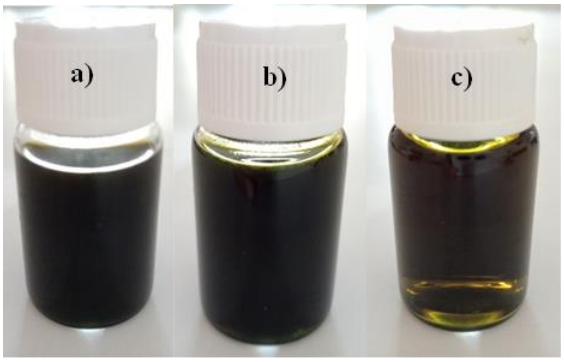

Fig. 2. Ethanol extracts a). *Azadirachta indica*, b). *Acalypha indica* and c). *Muntingia Calabura*

2.4. Screening for Phytochemical Compounds

Qualitative analysis of phytochemical content in crude ethanol extracts of *Azadirachta indica, Acalypha indica,* and *Muntingia calabura* was conducted separately. The analysis included tests for alkaloids, flavonoids, tannins, saponins, phenols, proteins, glycosides, steroids/terpenoids and carbohydrates using established methods with slight modifications [19–25]. Each plant sample was analyzed twice.

(a) (b) (c)

Fig. 3. Solid residues (a). *Azadirachta indica*, (b). *Acalypha indica* and (c). *Muntingia calabura*

2.4.1. Alkaloid test (Wagner's and Dragendroff's Kraut's Test)

Two milliliters of crude ethanol extract were dried in a porcelain dish, mixed with 8 ml of 1% HCl solution, warmed, and filtered. The solution was then divided into two portions. In the first tube, 3 drops of Dragendorff's solution were added; a positive result for alkaloids was indicated by a brownish-orange precipitate. In the second tube, 3 drops of Wagner's solution were added; a brownish precipitate indicated the presence of alkaloids [21,22].

2.4.2. Flavonoid test

Two milliliters of crude ethanol extract were mixed with five drops of concentrated HCl and 0.2 gr of Mg Powder. The appearance of yellow, blue, orange, or red indicated a positive result

[20,24].

2.4.3. Tannin test

Two milliliters of crude ethanol extract were added with three to four drops of 1% FeCl3, a blackish green or black indicated the presence of tannin [23,25].

2.4.4. Saponin test

Two milliliters of crude ethanol extract was combined with six milliliters of deionized water and agitated until blended. The appearance of bubbles or foam indicated saponin [19].

2.4.5. Phenol test

Two milliliters of crude ethanol extract was mixed with three to four drops of 5% FeCl3, a significant green or blue color appeared, indicating the presence of phenol [23].

2.4.6. Protein test

Two milliliters of crude ethanol extract was mixed with two milliliters of 4% NaOH. A purple-pink color indicated the presence of protein [22].

2.4.7. Glycosides test

One milliliter of crude ethanol extract was mixed with 0.5 ml of glacial CH3COOH and three drops of 1% FeCl3. A brown color indicated the presence of glycosides [19].

2.4.8. Steroid and triterpenoid test

A porcelain dish was used to evaporate 1 ml of crude ethanol extract. The residue was then treated with ten drops of glacial CH3COOH and two drops of H2SO4 and left for a few minutes. A blue or green color indicated the presence of steroids, while a red or purple color indicated the presence of triterpenoids [23].

2.4.9. Carbohydrate test (Molisch's test)

1 ml of crude ethanol extract was mixed with a few drops of molish reagent, followed by the addition of 1 ml of concentrated H2SO4. The mixture was left to stand for 2 - 3 minutes. A red or purple color indicated the presence of carbohydrates [21].

2.4.10. Characterization using FTIR (Fourier Transform Infrared)

Analysis was conducted on three types of samples from each medicinal plant: powdered raw material, crude ethanol extract, and solid residues from the soxhlet extraction process. To ensure consistency in the FTIR analysis, the crude ethanol extracts, initially in a viscous form, were dried in an oven at 40°C for several hours to reduce the ethanol content. The resulting semi-solid extracts were then subjected to FTIR spectroscopy using a SHIMADZU instrument.

The FTIR analysis aims to identify functional groups, compare compound profiles, and evaluate compound extraction efficiency. Spectral comparison of the three samples provided an overview of the phytochemical compounds stability during the extraction process. However, qualitative phytochemical, TPC, TFC, and antioxidant analyses were conducted on the crude

ethanol extract, as this fraction contains the highest concentration in bioactive compounds compared with the unprocessed raw material powder and the solid residue, which has lost most of its compounds.

2.5. Total Phenolic Content Quantification (TPC analysis)

This analysis followed the method reported in previous research Rizkita et al. [8] with slight modifications. The total phenolics extract was measured calorimetrically using the folin ciocalteu reagent. A 0.5 ml portion of the extract solution was combined with 2.5 ml of folin ciocalteu reagent (diluted at a 1:10 ratio with deionized water), shaken, and left for four minutes. Subsequently, 2.5 mL of sodium carbonate (7.50% w/v) was added, and the mixture was diluted with deionized water to a final volume of 10 mL. The solution was then incubated in the dark for 90 minutes at room temperature. Absorbance was measured at 765 nm using a B-One UV-Vis spectrophotometer. The total phenolic content was calculated from a standard calibration curve prepared using five known concentrations of gallic acid (0–25 ppm).

2.6. Quantifying the Total Flavonoid Content (TFC analysis)

Flavonoids were analyzed using a colorimetric technique. A 0.5 mL extract solution was mixed with 0.3 mL of $NaNO_2$ solution (7% w/v), vortexed, and incubated in the dark chamber at room temperature for 6 minutes. Subsequently, 0.3 mL AlCl3.6H2O solution (10% w/v) was added and incubated in the dark at room temperature for 5 minutes. Then, 2 ml of 1 M NaOH solution was added, followed by dilution with demineralized water to a final volume of 10 mL. The treated solution was analyzed at 507 nm using a UV-Vis spectrophotometer to determine the total flavonoid content in the extracts. Catechin is used as the standard.

2.7. Antioxidant Activity

The DPPH method (2,2-diphenyl-1-picrylhydrazyl hydrate) was used to assess antioxidant activity. Extract concentrations of 100 ppm were pipetted at volumes of 0.5 mL; 1 mL; 1.5 mL; 2 mL: 2.5 mL into separate flasks. Methanol was then added to each flask to a final volume of 5 mL, producing solutions with concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 100 ppm. Then, 2.5 mL of DPPH solution (50 ppm) was added to each flask. The mixtures were kept in the dark for 30 minutes, after which absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm [26,27]. A positive control containing 2 mL methanol and 1 mL DPPH was utilized for comparison.

The inhibition activity percentage was computed using the equation below:

$$\% \text{ Inhibition} = \left(\frac{A_b - A_s}{A_b}\right) \times 100\% \quad (1)$$

Where A_b represents the absorbance without the sample and A_s represents the absorbance of the tested sample.

The IC50 value was determined by calculating the inhibition percentage of DPPH radicals. The IC50 figure represents the extract concentration required to reduce 50% of the total DPPH. Thus, the value 50 was substituted for y, and x was calculated to obtain the IC50 value.

All analyses (TPC, TFC, and antioxidant activity) were conducted in triplicate for each plant sample, and the results were expressed as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Phytochemical Analysis

Screening for phytochemical compounds is an important step in evaluating wild plants for their potential as antioxidants, anticancer, and various pharmacological activities. The results of phytochemical screening of *Azadirachta indica*, *Acalypha indica* and *Muntingia Calabura* leaf extracts are presented in Table 1.

Table 1. Screening of wild plants phytochemical compounds

Compounds	*Azadirachta indica* (mimba)	*Acalypha indica* (Anting-anting)	*Muntingia Calabura* (Kersen)
Alkaloid	++	++	++
Flavonoid	+++	+++	+++
Tannin	+++	+++	+++
Saponin	++	+++	++
Phenol	+++	+++	+++
Protein	++	-	+++
Glycosides	+++	++	-
Steroid/Triterpenoid	Steroid = + Triterpenoid = -	Steroid = + Triterpenoid = -	Steroid = + Triterpenoid = -
Carbohydrate	+++	+++	-

Note: The intensity of phytochemical reactions is expressed in symbols: (+++) indicates strong presence, (++) moderate, (+) weak, and (–) not detected.

The intensity of phytochemical reaction was determined through visual observation of color changes, precipitate formation, or other visible changes following the addition of specific reagents. A semi-quantitative intensity scale (+++, ++, +, -) was applied based on the relative comparisons among samples within the same test group.

Data in Table 1 shows that ethanol extracts of *Azadirachta indica*, *Acalypha indica,* and *Muntingia Calabura* leaves contain alkaloids, flavonoids, tannins, saponins, and phenols. Protein compounds were detected only in *Muntingia Calabura* leaves, while glycoside and carbohydrate compounds were found in *Azadirachta indica* and *Acalypha indica* leaves. Steroids were present in *Azadirachta indica* and *Muntingia Calabura*, whereas triterpenoids were absent in all ethanol extract samples. The occurrence of phenolic and flavonoid compounds in the ethanol extract of wild plants shows their potential as antioxidants [9]. Furthermore, the detection of tannins, saponins, flavonoids, alkaloids, phenolic, and steroids suggest that these plants have potential as therapeutic plants.

Meanwhile, previous studies (Table 2) have reported phytochemical screening results for

Azadirachta indica, Acalypha indica, and *Muntingia calabura* plants using different methods and solvents.

Table 2. Comparison with previous research for Screening of wild plants phytochemical compounds

Plant Species	Extraction Method	Process	Solvent	Detected Metabolites	Reference
Azadirachta indica	Soxhlet	1:7.5 (w/v), 3x48h	70% Ethanol	Alkaloids, saponins, flavonoids, tannins, steroids/triterpenoids	[28]
	MAE	1:20 (w/v), 5 min, 70 °C, 2450 Hz	Deionized water	Alkaloids, phenols, flavonoids, saponins, tannins, terpenoids	[29]
	Maceration	1:10 (w/v), 24 h	Distilled water	Alkaloid, flavonoid, saponins, terpenoids, polyphenols and tannins, steroids	[30]
	Soxhlet	1:5 (w/v), 4h, 70°C	96% ethanol	Alkaloids, flavonoids, tannins, saponins, phenols, protein, glycosides, steroids, carbohydrate.	Present research
Acalypha indica	Maceration	24 h	96% ethanol	Saponins, tannins, quinones, alkaloids, flavonoids, phenolics	[31]
	Maceration	1:20 (w/v), 5 days,	ethanol and methanol 50%	Phenolics, flavonoids, alkaloids, terpenoids, steroids,	[32]
	Soxhlet	1:5 (w/v), 4h, 70 °C	96% ethanol	Alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids, carbohydrate	Present research
Muntingia calabura	Maceration	-	Hexane, ethyl acetate and methanol	Methanol extract (alkaloids, flavonoids, phenols, protein, carbohydrate, saponins), Ethyl acetate extract (flavonoids, diterpines, phenol), n-hexane extract (flavonoids, diterpines)	[33]
	Maceration	3x24 h	ethanol, ethyl acetate and n-hexane	Ethanol extract (alkaloids, flavonoids, tannin, phenols, steroids, saponins, terpenoids), Ethyl acetate extract (alkaloids, flavonoids, tannin, phenols, steroids, saponins, terpenoids), n-hexane extract (alkaloids, tannin, phenols, steroids, saponins, terpenoids)	[34]
	Soxhlet	1:5 (w/v), 4h, 70 °C	96% ethanol	Alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids, carbohydrate	Present research

The results of phytochemical screening from various previous studies (Table 2) indicated similar secondary metabolite content, although most studies reported compounds presence qualitatively without specifying abundance levels. Extraction outcomes are influenced by method, solvent type, sample-to-solvent ratio, extraction conditions, and storage time [35]. Microwave-

Assisted Extraction (MAE) has been reported to be more efficient in obtaining bioactive compounds than maceration. However, Soxhlet extraction remains the standard in phytochemical studies due to its ability to continuously extract polar compounds over extended periods while maintaining a stable temperature below the solvent's boiling point. Higher temperatures can increase the solubility of bioactive compounds and accelerate the diffusion from plant matrices into the solvent [8], enabling optimal extraction of flavonoids, phenols, and alkaloids compared with maceration.

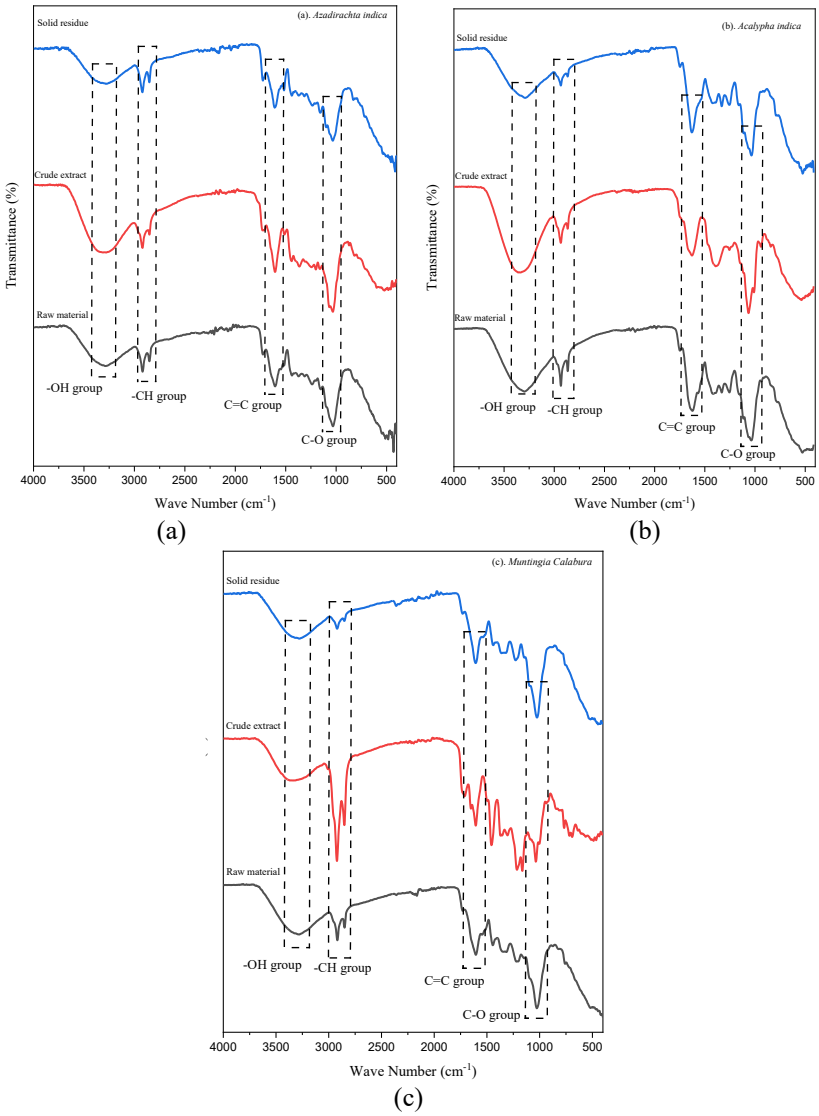

Fig. 1. FT–IR spectrum of wild plant leaves (a) raw material, (b) crude extract, (c) solid residue

The existence of secondary metabolites in the sample extract indicates that the leaves of *Azadirachta indica*, *Acalypha indica,* and *Muntingia Calabura* possess therapeutic properties. This potential is supported by ethnobotanical data showing their traditional use in treating various health conditions, including diabetes, inflammation, stress, infections, skin diseases, digestive disorders, and fever [12–15,17,18]. All three wild plants contain phytochemical compounds with varying levels, such as tannins, phenols, and flavonoids, which include polyphenolic compounds

that can donate hydrogen and act as antioxidants [36]. All ethanol extract of the wild plants contained phenolic and flavonoid compounds; therefore, further analyses were conducted to quantify their total phenolic and flavonoid contents.

3.2. FTIR characterization

Fig. 3 shows the FT-IR plant spectra before and after soxhlet extraction, as well as the resulting crude extract. The functional groups were detected in the range of 4000 to 400 cm^{-1}. Tables 3-5 display the observed peaks and the corresponding functional groups identified through infrared spectroscopy.

Several prominent spectral peaks indicate the existence of certain functional groups common to all samples. Fig. 4 shows high absorption in the wavelength regions 3400–3000, 2900–2600, 1800–1200 and 1200–700 cm^{-1}. FTIR spectroscopy provides information through spectral fingerprints to identify phytochemical compounds or functional groups in samples, including plant extracts, making it an accurate, fast, and simple technique [37,38]. The 3400–3000 cm^{-1} region shows symmetric (sim) and asymmetric (asim) stretching of the hydroxyl group (-OH) and the -H bond characteristic of polyphenolic compounds [39]. The O-H group significantly contributes to antidiabetic, antioxidant, and antibacterial activities [40]. The C=O carbonyl group, a functional group of flavonoids, is located between 1750 – 1700 cm^{-1} [37] and is associated with the compounds pheophytin and chlorophyll [41].

Table 3. FT-IR spectrum wavenumbers and functional groupings collected from *Azadirachta indica*

Functional Groups	Peak Values (cm^{-1})			**Compounds Identified**
	Powdered Raw Material	Crude Ethanol Extract	Solid Residue	
-OH stretching	3290.27	3278.86	3281.71	Alcohol and hydroxy compound
C-H asymmetric stretching	2919.46	2920.88	2920.88	Alkyl/alkene, aromatic compounds, lipids
C-H symmetric stretching	2851.00	2851.00	2852.42	Lipids, proteins
C=O carbonyl group	1725.72	1715.73	1725.72	Ketone, ester, ether, carboxylic acids, aldehyde
C=C stretching	1604.49	1605.91	1608.77	Aromatic compounds, alkene, amino acids
Aliphatic nitro compounds	1547.44	-	-	Nitrogen-oxy compounds
C=C-C stretching	1516.06	1513.21	1517.49	Aromatic ring
C-H bending	1437.62	1441.90	1439.05	Alkyl/alkene
	1373,44	1364.88	1372.02	
-OH bending	1236.53	1243.66 1200.87	1233.67	Alcohol and hydroxy compound
	1152.38	1160.94	1158.08	
C-O group	1032.58	1066.81 1032.58	1099.61 1034.00	Alcohol and hydroxy compound, ether
C-O-O stretching	-	871.42	-	Ether and oxy compound
Aromatic C-H out-of-plane bending	808.66	818.65	830.06	aromatic ring, benzenoid

Table 4. FT-IR spectrum wavenumbers and functional groupings collected from *Acalypha indica*

Functional Groups	Peak Values (cm^{-1})			**Compounds Identified**
	Powdered Raw Material	Crude Ethanol Extract	Solid Residue	
-OH stretching	3281.71	3320.22	3271.73	Alcohol and hydroxy compound
C-H asymmetric stretching	2918.03	2919.46	2919.46	Alkyl/alkene, aromatic compounds, lipids
C-H symmetric stretching	2851.00	2851.00	2851.00	Lipids, proteins
C=O carbonyl group	1732.85	1702.90	1731.42	Ketone, ester, ether, carboxylic acids, aldehyde
C=C stretching	1608.77	1611.62	1614.47	Aromatic compounds, alkene, amino acids
Aliphatic nitro compounds	1551.72	-	-	Nitrogen-oxy compounds
C-H bending	1399.11 1316.39	1377.72 -	1399.11 1316.39	Alkyl/alkene
-OH bending	1239.38 1139.54	1239.38	1239.38 1145.25	Alcohol and hydroxy compound
C-O group	1099.61 1021.17	1098.18 1048.27	1098.18 1019.74	Alcohol and hydroxy compound, ether
Vinyl C-H out-of-plane bending	-	994.07	-	Olefinic (alkene)
C-C skeletal vibration	-	924.19	-	Alkyl/alkene
Aromatic C-H out-of-plane bending	-	827.20	-	Aromatic ring, benzenoid
C-H Monosubstitution (phenyl)	761.60	-	760.17	Aromatic ring, benzenoid
C-I stretching	512.01	-	509.16	Aliphatic iodo compounds

The presence of functional groups (Tables 3-5) confirms the secondary metabolites identified in the screening test. The high intensity of the -OH, C-H, C=C, and C-O functional groups in fresh leaves, crude extracts, and residues indicates the existence of aromatic compounds such as flavonoids and phenols. Numerous low-intensity single bands in the 1500 – 500 cm^{-1} range, known as the fingerprint regions [42], are associated with specific functional groups, including C–H, O–H, C–O bonds, and aliphatic organohalogen compounds (C–Br and C–I), as presented in Tables 3-5. A decrease in peak intensity in wild plants before and after the extraction process, with fresh medicinal plants showing higher peak intensity than solid residues. The decrease in FTIR peak intensity spectrum shows that the extraction process successfully removed phytochemical compounds from the plants.

3.3. Total Phenolic and Flavonoid Compounds

The phenolic content of the extract was determined using the Folin-Ciocalteu reagent, while the total flavonoid content was measured via a reaction between flavonoids and $AlCl_3$, producing a yellow solution. The total phenolic content as expressed as mg Gallic Acid Equivalent per gram of extract (mg GAE/g extract), and the total flavonoid content (TFC) as mg catechin equivalent per gram of extract (mg CE/g extract). Calibration curves were established for gallic acid ($y=19.366x - 0.027$, $R^2 = 0.9876$) and catechin ($y=89.568x-7.995$, $R^2=0.9716$). The results for

phenolic and flavonoid content are presented in Table 6.

Table 5. FT-IR spectrum wavenumbers and functional groupings collected from *Muntingia calabura*

Functional Groups	Peak Values (cm^{-1})			**Compounds Identified**
	Powdered Raw Material	Crude Ethanol Extract	Solid Residue	
-OH stretching	3280.29	3338.76	3317.37	Alcohol and hydroxy compound
C-H asymmetric stretching	2918.03	2922.31	2920.88	Alkyl/alkene, aromatic compounds, lipids
C-H symmetric stretching	2851.00	2852.42	2852.42	Lipids, proteins
C=O carbonyl group	1731.42	1712.88	1731.42	Ketone, ester, ether, carboxylic acids, aldehyde
C=C stretching	- 1605.91	1651.55 1605.91	- 1605.91	Aromatic compounds, alkene, amino acids
Aliphatic nitro compounds	1543.16	-	1536.03	Nitrogen-oxy compounds
C=C-C stretching	-	1497.52	-	Aromatic ring
C-H bending	1444.75 1340.64	1457.59 1370.59	1439.05 1362.03	Alkyl/alkene
Vinylidene C-H in-plane bend	-	1304.98	1320.67	Olefinic (alkene)
-OH bending	1220.84 1146.67	1215.13 1163.79	1227.97 1145.25	Alcohol and hydroxy compound
C-O group	1026.87	1035.43	1025.45	Alcohol and hydroxy compound, ether,
C-F stretching	-	1002.63	-	Aliphatic fluoro compounds
C-C skeletal vibration	-	937.02	-	Alkyl/alkene
Aromatic C-H out-of-plane bending	-	841.46	-	Aromatic ring, benzenoid
C-H Monosubstitution (phenyl)	758.74	767.30	753.04	Aromatic ring, benzenoid
OH out-of-plane bend	-	717.38	-	Alcohol and hydroxy compound
C-Br stretching	-	691.71	-	Aliphatic bromo compounds
C-I stretching	-	-	520	Aliphatic iodo compounds

The highest total phenolic content was identified in *Muntingia calabura* leaf sample (269.88±0.00 mg GAE/gr sample), exceeding that of *Azadirachta indica* (258.26±2.05 mg GAE/gr extract) and *Acalypha indica* (220.16±5.00 mg GAE/gr extract). These findings align with previous research reporting that *Muntingia calabura* is rich in phenolic compounds, particularly phenolic acids such as gallic acid, ferulic acid, and vanillic acid, which are associated with biological activities including vasodilation, antibacterial, anti-inflammatory, antiviral, and anticarcinogenic effects [45,46].

Meanwhile, *Azadirachta indica* has the highest flavonoid content (676.34±21.85 mg CE/gr extract), followed by the ethanol extract of *Muntingia Calabura* and *Acalypha indica* (577.82±0.32 and 259.40±15.52 mg CE/gr extract). This finding aligns with previous studies Kumar et al. [43] reporting high flavonoid content in *Azadirachta indica* leaves. The high flavonoid content is attributed to the presence of rutin, kaempferol, naringenin, and taxifolin in the *Azadirachta indica* leaf extract [47]. These flavonoids possess an aromatic structure with at least one hydroxyl group,

which plays a key role in antioxidant activity [8]. In addition, the high flavonoid content may be associated with enhanced biosynthesis as a physiological adaptation of plants to tropical environments characterized by intense biotic and abiotic stresses, prompting the production of bioactive compounds such as flavonoids as part of the plant's natural defense mechanism against environmental stresses [48].

Table 6. Total phenolic and flavonoid content in extract and its comparison with previous studies

Plant Species	Extraction Method	Process	Solvent	Total phenolic content (mg GAE/gr ekstrak)	Total flavonoid content	Reference
Azadirachta indica	Soxhlet	60 °C, until colorless	80% methanol	70	119 mg QE/g extract	[43]
	Soxhlet	1:5 (w/v), 4h, 70 °C	96% ethanol	258.26±2.05	676.34±21.85 mg CE/g extract	Present research
Acalypha indica	maceration	24 h	Ethanol	203.065	-	[31]
	Multilevel maceration	1:15 (w/v), 3 days	Chloroform methanol	9.89 ± 0.77 45.11 ± 4.86	5.87 ± 1.40 mg QE/g 19.87 ± 0.61 mg QE/g	[15]
	Soxhlet	1:5 (w/v), 4h, 70 °C	96% ethanol	220.16±5.00	259.40±15.52 mg CE/g extract	Present research
Muntingia calabura	maceration	1:8 (w/v), 2 days	96% ethanol	361.22±0.26	42.46±0.15 mg QE/g	[44]
	Soxhlet	1:5 (w/v), 4h, 70 °C	96% ethanol	269.88±0.00	577.82±0.32 mg CE/g extract	Present research

The results of the total phenolic and flavonoid content analysis confirmed the qualitative screening test for phytochemical compounds previously conducted. The high phenolic and flavonoids content in plants is associated with antioxidant activity, which plays a role in preventing diseases caused by oxidative stress [49]. The comparison of phenolic and flavonoid content is supported by previous research (Table 6), which shows variations in bioactive compound levels. The concentrations of phenolic and flavonoid compounds in the extracts depend on various factors, including the extraction method, solvent type, and operating conditions during extraction [35]. In addition, biological variations, genetic factors, and environmental conditions also influence the production of plant-derived secondary compounds [50].

3.4. Antioxidant Activity

Scavenging of DPPH free radicals is a common method used for evaluating antioxidant

activity. DPPH, a stable free radical, changes color from purple to yellow upon accepting a hydrogen atom during free radical scavenging, leading to reduced absorbance in spectrophotometer measurements [51]. The extract's free radical inhibitory activity, expressed as %inhibition or %scavenging, is presented in Table 7.

Table 7. %inhibition in DPPH Radical Scavenging Assay of Ethanolic Leaf Extracts

Concentration (ppm)	%inhibition		
	Azadirachta indica	*Acalypha indica*	*Muntingia Calabura*
10	21.50	5.86	0.78
20	31.82	13.32	4
30	46.50	17.05	78.11
40	64.16	21.14	84.44
50	81.29	24.33	85.44

Table 8. IC50 of extracts

Species	Straight line equation	R^2	IC50 (ppm)
Azadirachta indica	y = 1.5192x + 3.479	0.9905	32.76±3.02
Acalypha indica	y = 0.4476x + 2.913	0.9710	107.71±3.55
Muntingia Calabura	y = 2.4978x – 24.378	0.8029	31.16±1.96

Antioxidant activity is determined by the IC50 value derived from the linear equation. The IC50 represents the extract concentration required to decrease 50% of the total DPPH. The antioxidant activity of the extract is presented in Table 8.

Based on the result, the ethanol extract of *Azadirachta indica* and *Muntingia Calabura* leaves exhibit very strong antioxidant activity, while *Acalypha indica* shows a moderate level of antioxidant activity. Substances with high antioxidant activity have low IC50 values [52]. An IC50 value below 50 ppm indicates extremely potent antioxidant activity; values between 50 and 100 ppm are considered strong, 101–150 ppm moderate, and 151–200 ppm weak [53]. The abundance of secondary metabolite molecules in plants influences their antioxidant potential. The phytochemical screening of the ethanol extracts of *Azadirachta indica*, *Acalypha indica*, and *Muntingia calabura* leaves revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols and steroids, indicating potential free radical scavenging properties. Phenolic and flavonoid substances contribute proportionally to antioxidant potential [35], as the hydroxyl groups in phenolic compounds make them effective electron donors, thereby enhancing their antioxidant activity [54].

4. Conclusions

This study analyzed the phenolic content, flavonoid levels, and antioxidant properties of ethanol extracts from three wild plants: *Azadirachta indica, Acalypha indica*, and *Muntingia calabura*. All extracts contained various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids, and carbohydrates. *Muntingia calabura* showed

the highest phenolic content (269.88±0.00 mg GAE/g extract), while *Azadirachta indica* had the highest flavonoid concentration (676.34±21.85 mg CE/g extract). Antioxidant activity analysis showed that *Azadirachta indica* and *Muntingia calabura* exhibited very strong antioxidant activity, with IC_{50} values of 32.76±3.02 µg/mL and 31.16±1.96 µg/mL, respectively, while *Acalypha indica* exhibited moderate activity (IC_{50}: 107.71±3.55 mg/mL). FTIR analysis confirmed the presence of functional groups related to bioactive compounds in raw materials, crude ethanol extracts, and solid residues, indicating the potential of these plants as natural antioxidant sources.

Abbreviations

TPC	Total Phenolic Content
TFC	Total Flavonoid Content
DPPH	2,2-diphenyl-1-picrylhydrazyl hydrate
GAE	Gallic Acid Equivalent
CE	Catechin Equivalent
FTIR	Fourier Transform Infrared
IC50	Inhibitory Concentration 50%

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Nadya Rizkita: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Diana Novita Sari**: Investigation, Writing – review and editing. **Daning Kinanti Sutama**: Validation, Writing – review and editing.

Declaration of Competing Interest

The authors declare no competing interests or personal relationships that could influence this research.

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