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Assessment of Total Phenolic and Flavonoid Content from Nine Different Families of Herbal Medicines Originated from West Java, Indonesia

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Abstract
Indonesia is known as a country with abundant natural resources, one of which is herbal plants. These herbal plants contain secondary metabolites such as polyphenol and flavonoid that have some health advantages. In this study, 18 herbal plants from 9 different families were used to calculate the total phenolic content calculated as gallic acid equivalent and the total flavonoid content calculated as quercetin equivalent. . The quantitative result shown that five plant extracts from *Lauraceae, Fabaceae, Myrtaceae,* and *Melastomataceae* family were obtained which had the highest total phenolic content, namely cinnamon cortex (*Cinnamomum burmannii*), angsana leaves (*Pterocarpus indicus* Willd.), bay leaves (*Syzygium polyanthum* (Wight.) Walp.), harendong bulu leaves (*Clidemia hirta* (L.) D. Don), and clove leaves (*Syzygium aromaticum* L.) Meanwhile, the five plant extracts from *Zingiberaceae, Fabaceae, and Lamiaceae* family that had the highest total flavonoid levels were red ginger (*Zingiber officinale* cv rubra), temu ireng (*Curcuma aeruginosa*), temu giring (*Curcuma heyneana*), gayam leaves (*Inocarpus fagiferus* Fosb.), and nilam leaves (*Pogostemon cablin* Benth.) The results were supported by qualitative results, indicated by spots aligning with the quercetin standard for flavonoids and the gallic acid standard for polyphenols in all mentioned plants.

Keywords: Extract, Thin layer chromatography, Total flavonoid, Total phenolic, UV-Vis spectrophotometer.

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Total Kandungan Fenolik dan Flavonoid dari Sembilan Keluarga Obat Herbal Asal Jawa Barat, Indonesia

Abstrak
Indonesia dikenal sebagai negara dengan sumber daya alam (SDA) yang melimpah, salah satunya tanaman herbal. Tanaman herbal mengandung metabolit sekunder, seperti polifenol dan flavonoid yang memiliki beberapa manfaat bagi kesehatan. Dalam penelitian ini digunakan delapan belas tanaman herbal dari sembilan famili berbeda untuk menghitung kandungan total fenolik yang dihitung sebagai asam galat dan total kandungan flavonoid yang dihitung sebagai kuersetin. Hasil penelitian menunjukkan diperoleh lima ekstrak tumbuhan dari famili *Lauraceae, Fabaceae, Myrtaceae,* dan *Melastomataceae* yang memiliki kandungan total fenolik tertinggi, yaitu kulit kayu manis (*Cinnamomum burmannii*), daun angsana (*Pterocarpus indicus* Willd.), daun salam (*Syzygium polyanthum* (Wight.) Walp.), daun harendong bulu (*Clidemia hirta* (L.) D. Don), dan daun cengkeh (*Syzygium aromaticum* L.) . Sedangkan lima ekstrak tumbuhan dari famili *Zingiberaceae, Fabaceae, dan Lamiaceae* yang memiliki kadar flavonoid total tertinggi adalah jahe merah (*Zingiber officinale* cv rubra), temu ireng (*Curcuma aeruginosa*), temu giring (*Curcuma heyneana*), daun gayam (*Inocarpus fagiferus*). Fosb.), dan daun nilam (*Pogostemon cablin* Benth.) . Hasil tersebut didukung dengan hasil analisis kualitatif bahwa seluruh tanaman memiliki spot yang sejajar dengan standar kuersetin untuk pengujian flavonoid dan standar asam galat untuk pengujian polifenol.

Kata Kunci: Ekstrak, Flavonoid total, Fenolik total, Kromatografi lapis tipis, Spektrofotometer UV-Vis

1. Introduction

Secondary metabolites are produced from biosynthetic processes such as hydroxylation, methylation, and glycosylation which are used as a means of self-defense against the plant's environment, such as pathogens, UV radiation, etc. One example of secondary metabolites in plants is polyphenolic compounds.[1] Polyphenol compounds have a structure of ≥ 2 phenol groups which are formed from two metabolic pathways, namely shikimic acid and acetic acid. Examples of polyphenolic compounds are flavonoids, tannins, and phenolic acids.[2] Phenolic compounds are the largest phytochemical group and have great antioxidant activity through electron transfer, hydrogen atoms, and transition metal chelation.[3] This compound can also maintain immunity, and has anti-ageing, antifungal, and anti-inflammatory effects.[4,5] In addition, flavonoid compounds have antioxidant, anticancer, antiallergic, antiviral, and antibacterial activity.[6,7] Based on the biological activity of the phenol group, the content of phenolic compounds in plants is determined as gallic acid equivalent (GAE) and flavonoid compounds as quercetin equivalent (QE), namely the equivalent amount of mg of gallic acid or quercetin in 1 gram of sample and the equivalent amount of mg of quercetin in 1 gram of sample.[8]

A literature review has been carried out to screen medicinal plants in Indonesia that have high phenolic or flavonoid content. From the plants obtained, they are then grouped by family. Families that have plants with high phenolic or flavonoid content were selected as samples for this research. *Curcuma zedoaria* (temu putih), *Curcuma aeruginosa* (temu ireng), *Curcuma xanthorrhiza* Roxb. (temulawak), *Curcuma heyneana* (temu giring), *Zingiber officinale* cv rubra (red ginger), and *Zingiber aromaticum* (lempuyang wangi) which belong to the *Zingiberaceae* family, are known to exhibit activities as antibacterial, antiviral, anti-aging, antioxidant, and anti-inflammatory agents in diseases such as osteoarthritis, rheumatoid arthritis, and others.[9] *Pterocarpus indicus* Willd (angsana), *Parkia speciosa* Hassk. (petai), and *Inocarpus fagiferus* Fosb (gayam) are plants that belong to the *Fabaceae* family known to possess antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, and immunomodulatory activities, and can be efficacious as treatments for fever, cough, liver issues, and as anthelmintic drugs.[10,11] *Urena lobata* (pulutan) and *Guazuma ulmifolia* (jati belanda) are plants that belong to the *Malvaceae* family, known to have immunostimulant activity and are often used for the treatment of diarrhoea, cough, and immune system maintenance.[12] *Piper bettle* (betel leaf or daun sirih) is a plant belonging to the *Piperaceae* family, known to have anti-inflammatory and bacteriostatic activities.[13] *Pogostemon cablin* Benth. (nilam) is a plant that belongs to the *Lamiaceae* family, known to have activities as analgesic, anti-inflammatory, antimicrobial, and antiemetic agents.[14] *Sonneratia caseolaris* L. (rambai laut) is a plant that belongs to the *Lythraceae* family, known to have activities as an antibacterial, wound healer, and chickenpox medicine.[15] *Syzygium aromaticum* L. (clove) and *Syzygium polyanthum* (Wight.) Walp., and *Melastomataceae* (bay leaves or daun salam) are plants that belong to the *Myrtaceae* family, known to have antioxidant, antibacterial, antidiabetic, and anti-inflammatory properties.[16] *Clidemia hirta* (L.) D. Don. (harendong bulu) is a plant that belongs to the *Melastomataceae* family, known to have properties as antioxidants, antibacterials, and antidiarrheal.[17] *Cinnamomum burmannii* (cinnamon cortex) is a plant that belongs to the *Lauraceae* family, known to have activities as antioxidants, antifungals, and in preventing Alzheimer's disease.[18]

Until now, there has been no research that combined the determination of phenolic and flavonoid compounds on the Indonesian plants selected for our study. Considering the benefits provided by these phenolic and flavonoid compounds, it is necessary to carry out screening processes and determine their levels in these plants through qualitative and quantitative tests, thus enabling their more effective utilization as an alternative treatment for various diseases. Qualitative testing involves thin-layer chromatography (TLC) methods, and quantitative testing employs UV-Vis spectrophotometry.

2. Materials and Methods

2.1. Tools

UV-Vis spectrophotometer (Specord 200 double beam), portable UV 254 and 366 nm (CAMAG), rotary evaporator (IKA RV 8).

2.2. Materials

A number of fresh rhizomes harvested from April until September 2023 were collected from various regions in West Java, Indonesia (Sumedang, Bandung, Tasikmalaya, and Garut) from the *Zingiberaceae* family (temu putih, *Curcuma zedoaria* (No.31/HB/04/2023); temu ireng, *Curcuma aeruginosa* (No.29/HB/04/2023); temulawak, *Curcuma xanthorrhiza* Roxb (No.30/HB/04/2023); temu giring, *Curcuma heyneana* (No.28/HB/04/2023); red ginger, *Zingiber officinale* cv rubra (No.27/HB/04/2023); lempuyang wangi, *Zingiber aromaticum* (No.33/HB/04/2023); leaves from *Fabaceae* family (angsana, *Pterocarpus indicus* Willd (No.35/HB/04/2023); petai, *Parkia speciosa* Hassk. (No.34/HB/04/2023); gayam, *Inocarpus fagiferus* Fosb (No.36/HB/04/2023), *Malvaceae* (pulutan, Urena

lobata (No.32/HB/04/2023); jati belanda, *Guazuma ulmifolia* (No.55/HB/09/2023), *Piperaceae* (betel leaf, *Piper betle* (No.30/HB/07/2023), *Lamiaceae* (nilam, *Pogostemon cablin* Benth. (No.27/HB/07/2023), *Lythraceae* (rambai laut, *Sonneratia caseolaris* L. (No.37/HB/04/2023), *Myrtaceae* (clove, *Syzygium aromaticum* L. (No.48/HB/08/2023); bay leaves, *Syzygium polyanthum* (Wight.) Walp. (No.46/HB/08/2023), *Melastomataceae* (harendong bulu, *Clidemia hirta* (L.) D. Don (No.47/HB/08/2023)); and a cortex from *Lauraceae* family (cinnamon, *Cinnamomum burmannii* (No.45/HB/08/2023)). *Folin-Ciocalteu* (FC) reagent, sodium carbonate (Na_2CO_3), gallic acid, quercetin, aluminium chloride ($AlCl_3$), ferric chloride (FeCl3), sodium acetate (CH_3COONa), and citroborate reagent were analytical grade and obtained from Merck (Germany).

2.3. Methods

2.3.1. Sample Preparation

Each fresh sample was sorted, washed, and then dried in a drying cabinet to obtain dried plant material, which were subsequently ground into a powder. The powdered samples were then extracted using the maceration method with 70% ethanol for 3×24 hours, while being occasionally stirred for the first 6 hours. The macerate was then collected, and liquid extract was evaporated using a rotary evaporator at a temperature of 60°C and a rotation speed of 75 rpm. The extract was further concentrated using a waterbath at the same temperature to obtain a thick extract, and its yield was calculated.

2.3.2. Qualitative Analysis of Phenolic and Flavonoid Content Using TLC

The presence of flavonoid and phenolic compounds in each sample was analysed qualitatively using thin-layer chromatography (TLC) with the stationary phase of silica gel GF254. The method used was determined using the method described by Yasmin et al. (2019)[19], with several adjustments. About 50 mg of extract were dissolved in 5 mL of 70% ethanol, and then spotted on a silica gel TLC plate and eluted using appropriate eluent as the mobile phase. For the identification of phenolic compounds using gallic acid as a reference, the eluent used was ethyl acetate:methanol (5:5). Meanwhile, for the identification of flavonoid compounds using quercetin as a reference, two types of eluents were employed: chloroform:methanol (9:1) and ethyl acetate:n-hexane (5:5). The specific reagent was then applied to enhance the appearance of their spots. $FeCl_3$ 10% (w/v) was used for the detection of phenolic compounds, with positive results indicated by a change in the colour of the spot, turning from blue to dark blue to almost black.[20] Meanwhile, to detect flavonoids, the specific reagents employed were ammonia vapour and citroborate 10% (w/v), with positive outcomes being successive color changes in the spot, turning to reddish yellow and yellow.[21]

2.3.3. Determination of Total Phenolic and Flavonoid Content

Total Phenolic Content (TPC)

Total phenol content (TPC) in each extract was determined using the FC method described by Do et al. (2014)[22], with minor modifications. The extract was dissolved in aqua pro injection (API) to a concentration of 50 µg/mL. The calibration curve was established using gallic acid in API (50 – 600 ppm). The diluted extract or gallic acid (1.6 mL) was added to 0.2 mL FC reagent (5-fold diluted with distilled water) and mixed thoroughly for 3 minutes. Sodium carbonate (0.2 mL, 10% w/v) was added to the mixture and the mixture was allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was measured at 760 nm using a UV-Vis spectrophotometer. TPC was expressed as milligram gallic acid equivalent per gram sample (mg GAE/g sample). All measurements were done in triplicate.

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of each extract was investigated using the aluminum chloride colorimetric method described by Do et al. (2014)[22], with slight modifications. In brief, the extract sample was dissolved in methanol to a concentration of 100 µg/mL. The calibration curve was prepared by diluting quercetin in methanol (1 – 18 ppm). The diluted extract or quercetin (2.0 mL) was mixed with 0.1 mL of 10% (w/v) aluminium chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 minutes. Then the maximum absorbance of the mixture was measured at 415 nm using a UV-Vis spectrophotometer. TFC was expressed as milligram quercetin equivalent per gram sample (mg QE/g sample). All measurements were done in triplicate.

3. Results

3.1. Extraction Yields

All the dried plant materials was extracted through a cold process, maceration, as it is easier to perform by immersing the dried plant materials in a solvent without heating, while also preventing damage to the chemical compounds contained within (Kurniawan et al., 2021). The solvent used was 70% ethanol due to its suitable

polarity for extracting compounds from samples that are both polar and nonpolar. It is safer, efficient, capable of extracting compounds faster, inhibits the growth of other microbes, and does not cause swelling in cell membranes.[23,24] The process was conducted for 3×24 hours to obtain the maximum yield.[25] The yield values of the obtained extracts are shown in Table 1.

3.2. Qualitative Analysis of Phenolic and Flavonoid Content Using TLC

Qualitative Analysis of Phenolic and Flavonoid Content Using TLC were demonstrated in the Table 2 and Table 3, respectively.

3.3. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Table 4 shows the quantitative analysis and TPC and TFC of the extracts measured using the FC method and the aluminium chloride colorimetric method. TPC values were obtained from the calibration curve y = 0.0007x + 0.0037 with R^2 = 0.9981, where x is the absorbance and y is the concentration of gallic acid solution (50 – 600 µg/mL expressed as mg GAE/g extract. The LOD (Limit of Detection) and LOQ (Limit of Quantitation) values of the calibration curve for the phenolic standard are 1572.14 and 5240.47 mg/g, respectively obtained from statistic calculation. Whereas TFC values were obtained from the calibration curve y = 0.0522x + 0.0183 with R^2 = 0.9976, where x is the absorbance and y is the concentration of quercetin solution (1 – 18 ppm) expressed as mg GAE/g extract. The LOD and LOQ values of the calibration curve for the flavonoid standard are 61.47 and 204.91 mg/g, respectively obtained from statistic calculation.

4. Discussion

4.1. Extraction Yields

The percentage of yield collected from all samples is compared with the data from literature. There are several extracts that obtain less % yield than those reported in the literature, such as *P. indicus* Willd. with 10.64%[26], *P. speciosa* Hassk. with 13.85%[27], and *S. caseolaris* L. Leaf with 27.25% yield.[28] This can be caused by differences in the extraction method and solvent used. Other than that, % yield from the other extracts are around the value of those written in Farmakope Herbal Indonesia (FHI) literature.

4.2. Qualitative Analysis of Phenolic and Flavonoid Content Using TLC

Detection of a compound from an extract sample can be done using the TLC method and in TLC, if the sample contains the same content as the standard used, it will exhibit parallel spots or stains.[29] The detection of phenolic compounds relied on the appearance of $FeCl_3$ spots, where hydroxyl groups from plants react with Fe^{3+} ions. A positive result for phenolic compounds is indicated by a colour change to dark blue, as well as strong red, green, blue, or black fluorescence under a UV lamp at 366 nm.[20] In the identification of phenolic compounds, *Z. officinale* cv rubra rhizome, *C. zedoaria* rhizome, *C. aeruginosa* rhizome, *C. heyneana* rhizome, and *C. zanthorriza* rhizome exhibit a light

Table 1. Extract yield value

Plant Species	Colour of Extract	Dried Plant Weight (g)	Total Extract (g)	Yield (%)
C. zedoaria Rhizome	Light brown	295.47	144.90	49.04
C. aeruginosa Rhizome	Blackish brown	298.40	43.27	14.50
C. heyneana Rhizome	Dark yellow	228.08	192.45	84.38
C. xanthorrhiza Roxb. Rhizome	Yellowish red	386.65	202.73	52.43
Z. officinale cv rubra Rhizome	Dark red	266.87	142.70	53.47
P. indicus Willd. Leaf	Blackish brown	285.00	22.47	7.88
U. lobata Leaf	Blackish green	245.00	30.36	12.39
P. speciosa Hassk. Leaf	Blackish brown	200.00	22.82	11.41
I. fagiferus Fosb. Leaf	Blackish green	800.00	119.26	14.91
Z. aromaticum Rhizome	Light brown	245.00	43.42	17.72
P. betle Leaf	Dark brown	230.00	17.13	7.45
P. cablin Benth. Leaf	Dark brown	375.00	45.79	12.21
S. caseolaris L. Leaf	Dark brown	390.00	81.33	20.85
S. aromaticum L. Leaf	Dark brown	225.00	86.59	38.48
C. hirta (L.) D. Don. Leaf	Blackish green	270.00	112.24	41.57
G. ulmifolia Leaf	Dark brown	295.00	36.05	12.22
C. burmannii Cortex	Blackish red	495.00	137.63	27.80
S. polyanthum (Wight.) Walp. Leaf	Dark brown	245.00	56.05	22.88

Table 2. Qualitative analysis of phenolic content using TLC

Sample	Retardation Factor (Rf)	Eluent Ethyl acetate : Methanol (5:5) Colour of Spot(s)					
		Before spraying by $FeCl_3$			After spraying by $FeCl_3$		
		Visible	UV 254 nm	UV 366 nm	Visible	UV 254 nm	UV 366 nm
C. zedoaria Rhizome	0.88	-	Blue (faded)	Purple (faded)	-	Dark Blue	Light Blue
C. aeruginosa Rhizome	0.90	Dark Blue (faded)	Blue (faded)	Purple (faded)	-	Dark Blue	Light Blue
C. heyneana Rhizome	0.85	-	Green	Yellow	Dark Brown	Dark Green	Dark Yellow
	0.80	Brown (faded)	Green (faded)	Yellow (faded)	-	Dark Blue	Light Blue
C. xanthorrhiza Roxb. Rhizome	0.82	-	Greenish Brown (faded)	Yellow (faded)	Dark Brown	Dark Green	Dark Yellow
	0.80	Brown (faded)	Blue (faded)	Purple (faded)	-	Dark Blue	Light Blue
Z. officinale cv rubra Rhizome	0.90	-	Blue (faded)	Purple (faded)	Orange	Dark Blue	Light Blue
	0.80	-	-	-	-	-	Light Blue
P. indicus Willd. Leaf	0.88	Brown	Blackish Brown	Reddish Purple	Blackish Blue	Blackish Blue	Purple
	0.62	Light Brown	Blackish Brown	Light Purple	Blackish Blue	Blackish Blue	Purple
U. lobata Leaf	0.88	Greenish Brown (faded)	Greenish Brown (faded)	Purple	Blackish Blue	Blackish Blue	Purple
	0.62	Greenish Brown (faded)	Greenish Brown (faded)	Purple	Blackish Blue	Blackish Blue	-
P. speciosa Hassk. Leaf	0.89	-	-	-	-	Blackish Blue	-
	0.78	Greenish Brown (faded)	-	Dark Purple	-	Blackish Blue	-
	0.71	Greenish Brown (faded)	-	Dark Purple	Blackish Blue	Blackish Blue	Dark Purple
I. fagiferus Fosb. Leaf	0.93	Greenish Brown (faded)	-	-	Blackish Blue	Blackish Blue	-
	0.81	Brown (faded)	Blackish Blue (faded)	Reddish Purple	Blackish Blue	Blackish Blue	-
	0.66	Brown (faded)	Blackish Blue (faded)	Reddish Purple	Blackish Blue	Blackish Blue	Dark Purple
Z. aromaticum Rhizome	0.86	Yellow (faded)	Blackish Blue	Yellow	Grey (faded)	Grey (faded)	-
P. betle Leaf	0.75	Brown (faded)	Blackish Blue	Yellow	Blackish Blue	Deep Blackish Blue	Dark Purple
	0.71	Brown (faded)	Blackish Blue	Purple	Blackish Blue	Deep Blackish Blue	Dark Purple
P. cablin Benth. Leaf	0.85	Brown	Greenish Brown	Purple	Blackish Brown	Greenish Blue	Dark Purple
S. caseolaris L. Leaf	0.80	Brown (faded)	Greenish Brown	Purple	Blackish Blue	Blackish Blue	Dark Purple
S. aromaticum L. Leaf	0.91	Greenish Brown	Greenish Brown	Orange	Deep Blackish Blue	Deep Blackish Blue	Deep Orange
	0.79	Greenish Brown	Greenish Brown	Orange (faded)	Deep Blackish Blue	Deep Blackish Blue	Orange
	0.54	Greenish Brown (faded)	Greenish Brown (faded)	-	Deep Blackish Blue	Deep Blackish Blue	-
C. hirta (L.) D. Don. Leaf	0.86	Brown (faded)	Blackish Blue	Orange (faded)	Dark Blue (concentrated)	Blackish Blue	Orange
G. ulmifolia Leaf	0.90	Greenish Brown	Blackish Blue	Orange	Bluish Grey (faded)	Bluish Grey (faded)	Orange

C. burmannii Cortex	0.75	Brown	Blackish Blue	Orange (faded)	Black	Black	Dark Purple
S. polyanthum (Wight.) Walp. Leaf	0.91	Greenish Brown (faded)	Blackish Blue	Orange (faded)	Blackish Blue	Blackish Blue	Orange (faded)
	0.75	Greenish Brown (faded)	Blackish Blue	Orange (faded)	Blackish Blue	Blackish Blue	Dark Purple
Gallic Acid (Standard)	0.75	-	Blackish Blue	Purple	-	Blackish Blue	Purple

blue colour under the UV lamp at 366 nm. Therefore, those samples contain a small number of phenolic compounds because it exhibits a light blue colour. Sample with a larger number of phenolic compounds will exhibit a strong blue or black under the UV lamp at 366 nm. The value of the phenolic compounds in each sample will be determined later from UV-Vis spectrophotometry results.

In Table 2, the calculation results of the Rf values for gallic acid show that the Rf values of samples that closely match the gallic acid reference Rf value, with a difference of Rf ≤ 0.05, are only found in the *P. betle* leaf, *S. caseolaris* L. leaf, and *C. burmannii* cortex samples. Hence, it can be stated that these three samples positively contain gallic acid compounds. Other samples which are positive for phenolic compounds are assumed to not have gallic acid in them. This research result is consistent with the research done by Nguyen (2020)[30] for *P. betle* leaf, Jubaidah (2019)[28] for *S. caseolaris* L. leaf, and Pagliari (2023)[31] for *C. burmannii* cortex which showed that all of these samples have gallic acid content.

The flavonoid contained in each sample detected by TLC are compared by using the spots appearing from quercetin as a standard. A positive flavonoid result is indicated by the appearance of fluorescence spots under a UV lamp at 366 nm, which typically exhibit a yellow, blue, and green colour that indicate different flavonoid typesWhereas the spots will not appear or the fluorescence from the spots will be reduced under a UV lamp at 254 nm.[32] Based on the test results on Table 3, it can be seen that the positive results for flavonoids with the appearance of ammonia vapour spots are a change in colour to reddish yellow because the flavonoid compounds in the plant will react to become quinoid compounds in the β ring which are red. Meanwhile, the positive result of the appearance of citroborate spots is a change in colour to yellow.[21]

Based on the flavonoid identification results in Table 3, all test samples from the *Zingiberaceae* family produced fluorescence under 366 nm light with different colour and intensities. *C. heyneana* rhizome, *C. xanthorrhiza* Roxb rhizome, and *C. aeruginosa* rhizome emit yellow fluorescence, while *Z. officinale* cv rubra rhizome and *C. zedoria* rhizome emit blue fluorescence. However, for *C. zedoria* rhizome and *C. aeruginosa* rhizome the colour released has a lower intensity than the others; this shows that the amount of flavonoids contained in them is less than the other samples produced fluorescence under a 366 nm UV lamp with different colour intensities. All test samples emit light yellow fluorescence.

The difference in TLC spot colors for each sample indicates that, even though both flavonoid and polyphenol compounds are present (as shown by spots aligning with standards like quercetin and gallic acid),

Table 3. Qualitative Analysis of Flavonoid Content Using TLC

Eluent Chloroform : Methanol (9:1)

Sample	Rf	Colour of Spot(s)								
		Before Spraying with Reagent(s)			After Spraying by Ammonia Vapour			After Spraying by Citroborate		
		Visible	UV 254 nm	UV 366 nm	Visible	UV 254 nm	UV 366 nm	Visible	UV 254 nm	UV 366 nm
C. zedoaria Rhizome	1.00	-	Yellow (faded)	-	-	Yellow (faded)	-	-	Yellow (faded)	-
	0.60	-	Yellow (faded)	Yellow (faded)	-	Yellow (faded)	Yellow (faded)	-	Yellow (faded)	Yellow (faded)
C. aeruginosa Rhizome	1.00	-	-	-	Yellow (faded)	Yellow (faded)	-	-	Yellow (faded)	-
	0.90	-	-	Light Blue	Brown (faded)	Yellow (faded)	Light Blue	Orange (faded)	Yellow (faded)	Light Blue
	0.62	-	-	Yellow	-	-	Yellow	-	-	Yellow (faded)

C. heyneana Rhizome	1.00	Yellow	Yellow	-	Brown (faded)	Dark Yellow	-	Orange	Dark Yellow	Yellow (faded)
	0.90	Yellow	Yellow	-	Dark Brown	Dark Yellow	-	Orange	Dark Yellow	Dark Yellow
	0.72	Brown (faded)	-	Yellow (faded)	Brown	Yellow	Yellow	Orange	Yellow	Yellow
C. xanthorrhiza Roxb. Rhizome	1.00	Yellow (faded)	-	-	Yellow (faded)	-	-	Orange	Dark Yellow	Yellow (faded)
	0.90	Orange	-	-	Dark Orange	-	-	Orange	Dark Yellow	Dark Yellow
	0.75	Orange (faded)	-	-	Orange	Yellow	Light Blue	-	Brown	Dark Yellow
	0.72	Orange (faded)	-	-	Orange	Yellow	Yellow	-	Brown	Dark Yellow
	0.53	-	-	-	Blackish Blue	Blackish Blue	Greenish Blue	-	-	-
Z. officinale cv rubra Rhizome	1.00	Yellow (faded)	-	-	Orange (faded)	-	-	Yellow (faded)	Yellow	Yellow
	0.98	Yellow (faded)	Brown (faded)	Light Blue	Brown (faded)	Brown (faded)	Light Blue	Yellow (faded)	Dark Blue (faded)	Light Blue
	0.62	Yellow (faded)	Dark Blue (faded)	Yellow	Yellow	Dark Blue (faded)	Yellow	Yellow (faded)	Yellow	Yellow
P. indicus Willd. Leaf	1.00	Green	Brown	Purple	Light Brown	Brown (faded)	Purple	Brown (faded)	-	Purple
	0.94	-	-	Yellow	Light Brown	Brown (faded)	Yellow	Brown (faded)	-	Yellow
	0.93	-	-	Green	-	-	Yellow	-	-	Yellow
	0.76	-	-	Purple	-	-	Yellow	-	-	Purple
	0.54	-	-	Purple (faded)	-	-	Yellow	-	-	-
	0.52	-	-	Yellow (faded)	-	-	Yellow	-	-	Purple
	0.46	-	Brown (faded)	Reddish Purple	-	-	Yellow	-	-	-
U. lobata Leaf	1.00	Green	Brown	Reddish Purple	Brown	Brown (faded)	Purple	Brown	Yellow	Light Purple
	0.76	-	-	Purple	-	-	Purple	-	-	Purple
	0.54	-	-	Purple	-	-	Purple	-	-	Purple
	0.44	Yellow (faded)	Yellow (faded)	Purple	-	-	Purple	-	-	Light Blue
P. speciosa Hassk. Leaf	1.00	Green	Brown	Purple	Light Brown	Yellowish Green	Yellow	Brown (faded)	Yellow	Purple
	0.97	-	-	White	-	-	Purple	-	-	-
	0.65	-	-	Purple (faded)	-	-	Yellow	Brown	Yellow	Dark Blue
	0.42	-	-	Yellow (faded)	-	Brown (faded)	Yellow	-	-	Yellow
I. fagiferus Fosb. Leaf	1.00	Green	Brown	Purple	Brown	Brown (faded)	Purple	Brown (faded)	Yellow	Purple
	0.65	-	-	Purple (faded)	-	-	Purple	-	-	Purple

	0.43	-	-	Purple	-	-	-	-	-	Yellow (faded)
Z. aromaticum Rhizome	0.76	Light Yellow	Blue	Yellow	Light Yellow	Brown (faded)	Yellow	Yellow (faded)	-	Yellow
	0.62	-	-	Yellow	-	-	Light Yellow	-	-	Light Yellow
	0.40	-	Blue	-	Light Yellow	Brown (faded)	Yellow	Yellow (faded)	-	-
	0.20	-	Blue (faded)	-	-	-	Light Yellow	-	-	Light Yellow
P. betle Leaf	1.00	Light Brown	Blue	Purple	Brown (faded)	Greenish Yellow	Purple	Brown (faded)	Yellow	Purple
	0.81	-	Blue	Purple	Dark Brown	Greenish Yellow	Blackish Purple	Brown	Yellow	Black-ish Purple
	0.62	-	-	Purple	-	-	Light Purple	-	-	Light Purple
	0.56	-	-	Yellow	-	-	Light Purple	-	-	Light Purple
	0.50	Light Brown	Blue	Purple	Brown (faded)	Greenish Yellow	Purple	-	-	-
	0.20	-	Blue	-	-	-	-	-	-	-
P. cablin Benth. Leaf	1.00	Light Brown	Blue	Purple	Orange Brown	Yellowish Green	Purple	Brown (faded)	Yellow	Black-ish Purple
	0.83	Light Brown	Blue	Purple	-	Greenish Brown	Purple	-	-	Purple (faded)
	0.67	-	Blue	-	-	-	Purple	-	-	Purple (faded)
	0.56	-	Blue	Purple	-	Brown (faded)	Yellow	-	-	Yellow
	0.50	Light Brown	Blue	Purple	-	Brown (faded)	Purple	-	-	Purple (faded)
	0.48	-	-	Yellow	-	Brown (faded)	Purple	-	-	-
	0.45	-	-	Purple	-	Brown (faded)	Purple	-	-	
	0.39	-	-	Yellow	-	Brown (faded)	Yellow	-	-	Yellow
	0.28	-	-	Yellow	Brown (faded)	-	Yellow	-	-	Yellow
S. caseolaris L. Leaf	0.32	Light Brown	Brown (faded)		Light Brown	Yellow (faded)	Purple	Yellow (faded)	Yellow	Yellow (faded)
S. aromaticum L. Leaf	1.00	Brown	Dark Blue	Purple	Brown	Yellow	Purple	Brown (faded)	Yellow	Purple
	0.75	Brown (faded)	-	Purple	-	-	-	-	-	Purple

C. hirta (L.) D. Don. Leaf	1.00	Brown	Brown	Purple	Brown	Yellow	Yellow	Brown (faded)	Yellow	Purple (faded)
G. ulmifolia Leaf	1.00	Brown	Brownish Blue	Purple	Brown	Yellow	Purple	Brown (faded)	Yellow	Purple
	0.70	-	-	Purple	-	-	-	-	-	Light Purple
C. burmannii Leaf	1.00	Brown	Dark Blue (faded)	-	Reddish Brown	-	Yellow	Brownish Red	Yellow	Yellow
S. polyanthum (Wight.) Walp. Leaf	1.00	Brown	Dark Blue	-	Brown	Yellow	Yellow	Brown (faded)	Yellow	Yellow

Eluent Ethyl Acetate : n-Hexane (5:5)

Sample	Rf	Colour of Spot(s)								
		Before Spraying with Reagent(s)			After Spraying by Ammonic Vapour			After Spraying by Citroborate		
		Visible	UV 254 nm	UV 366 nm	Visible	UV 254 nm	UV 366 nm	Visible	UV 254 nm	UV 366 nm
C. zedoaria Rhizome	1.00	-	Dark Blue	Light Blue	-	Dark Blue	Light Blue	-	Blue	Yellow (faded)
	0.94	-	Dark Blue	Light Blue	-	Dark Blue	Light Blue	-	Yellow (faded)	Yellow (faded)
	0.80	-	Dark Blue	Light Blue	-	Dark Blue	Light Blue	-	-	Yellow (faded)
C. aeruginosa Rhizome	1.00	-	Dark Blue	Light Blue (faded)	-	Dark Blue	Light Blue (faded)	-	Blue	Yellow (faded)
	0.94	-	Dark Blue	-	-	Dark Blue	-	-	Yellow (faded)	Yellow (faded)
	0.79	-	Dark Blue	Dark Blue	-	Dark Blue	Dark Blue	-	Yellow (faded)	Yellow (faded)
C. heyneana Rhizome	1.00	-	Blue	Yellow	-	Blue	Yellow	-	Blue	Yellow (faded)
	0.94	-	Brown (faded)	Orange (faded)	-	Brown (faded)	Orange (faded)	-	Brown	Yellow (faded)
	0.82	Orange	Brown	Brownish Yellow	Reddish Orange	Brown	Brownish Yellow	Orange	Yellow	Yellow
	0.78	-	Yellow (faded)	Light Blue	-	Yellow (faded)	Light Blue	Orange	Yellow (faded)	Yellow
	0.74	Orange (faded)	Brown	Brownish Yellow	Orange	Brown	Brownish Yellow	Orange	-	Blue
	0.58	-	-	-	Orange (faded)	-	Yellow	Orange (faded)	-	Yellow
C. xanthorrhiza Roxb. Rhizome	1.00	-	Blue	Yellow (faded)	-	Blue	Yellow (faded)	-	Blue	Yellow (faded)
	0.82	Orange	Orange	Brownish Yellow	Reddish Orange	Brown	Brownish Yellow	-	Brown	Yellow
	0.78	Brown	Yellow (faded)	Blue	Brown	Yellow (faded)	Blue	Orange	Yellow	Yellow
	0.74	-	Brown	Brownish Yellow	-	Brown	Brownish Yellow	Orange	Brown (faded)	Blue
	0.62	-	Blackish Brown	Yellow	-	Blackish Brown	Yellow	Orange (faded)	-	Yellow
	0.59	-	-	Purple (faded)	-	-	Purple (faded)	Orange (faded)	-	Brown

Z. officinale cv rubra Rhizome	1.00	Yellow (faded)	Blue	-	Yellow	Blue	-	-	Blue	Blue
	0.89	Orange (faded)	Yellow (faded)	Light Blue	Orange (faded)	Yellow (faded)	Light Blue	Yellow	Yellow	Yellow
	0.74	Orange (faded)	-	Yellow	Orange (faded)	-	Yellow	-	Yellow (faded)	Yellow
	0.60	-	Yellow (faded)	Yellow	-	Yellow (faded)	Yellow	Yellow	-	Yellow (faded)
	0.33	-	-	-	-	-	Yellow (faded)	-	Brown (faded)	Brown (faded)
P. indicus Willd. Leaf	0.87	-	-	Yellow	-	-	Yellow	-	-	-
	0.82	-	Light Brown	Yellow	Light Brown	Light Brown	Yellow	-	Brown	Yellow
U. lobata Leaf	0.86	-	-	Yellow	-	-	Yellow	-	-	Yellow
	0.81	-	Light Brown	Purple	Brown	Brown	-	-	Brown	-
	0.35	Green (faded)	-	Purple	Brown	-	Purple	-	-	Yellow
P. speciosa Hassk. Leaf	0.78	-	Brown	Purple	Greenish Brown	Brown	Purple	Brown	Brown	Purple (faded)
	0.22	Brown (faded)	-	Dark Blue (faded)	Blackish Brown	-	-	-	-	Yellow
I. fagiferus Fosb. Leaf	0.81	-	-	Purple (faded)	-	Yellow (faded)	Purple	-	Yellow	Purple
Z. aromaticum Rhizome	0.93	-	Dark Blue (faded)	-	-	-	Yellow (faded)	-	-	Yellow (faded)
P. betle Leaf	0.80	Brown (faded)	Brown	Purple	Dark Brown	Dark Brown	Dark Purple	Dark Brown	Dark Brown	Blackish Purple
P. cablin Benth. Leaf	0.92	-	-	Yellow	-	-	Yellow	-	-	Yellow
	0.83	Brown (faded)	Dark Blue	Purple (faded)	Yellow	Brown	Purple (faded)	Greenish Yellow	Yellow	Purple
S. caseolaris L. Leaf	0.23	-	-	-	Brown	-	Purple (faded)	-		Greenish Yellow
S. aromaticum L. Leaf	1.00	Brown	Blue	Purple	Brown	-	Purple	Brown	Brown	Purple
	0.88	-	-	Purple	-	-	-	-	Brown	Purple
C. hirta (L.) D. Don. Leaf	1.00	Brown	Blue	-	Brown	-	-	Brown	-	Light Purple
G. ulmifolia Leaf	1.00	Brown	Blue	-	Brown	-	Blue	Brown	Brown	Purple
	0.85	-	-	-	-	-	-	-	-	Light Purple
C. burmannii Cortex	1.00	Reddish Brown	Blue	-	Dark Brown	-	-	Reddish Brown	Brown	-
S. polyanthum (Wight.) Walp. Leaf	0.06	Brown	Blue	-	Brown	-	-	Brown	-	-

Quercetin (Standard)	0.40	Yellow	Yellow	Yellow	Reddish Yellow	Reddish Yellow	Yellow	Reddish Yellow	Reddish Yellow	Yellow

the compounds in each plant may exhibit different colors. This variation is due to differences in chemical structures and their interactions with the reagents used. For example, an orange spot may indicate the presence of isoflavones (a flavonoid derivative), while a light yellow to brown spot could indicate flavonols (another flavonoid derivative), and so on. Further identification was not conducted as the aim of this study was solely to determine which plants had the highest TFC and TPC levels among the candidates used in the research.[33]

Based on the calculated Rf value for quercetin in Table 3, it can be seen that no samples showed positive results for quercetin, since with no Rf value was close to the standard Rf value of quercetin used. However, when irradiated with a 366 nm lamp, and by spraying the spots with ammonia vapour or citroborate, the sample produced spots with a colour that was more intense than before spraying, namely a yellowish color. This can occur due to a reaction between flavonoids and the reagents used to form the kinoid structure.[34] Therefore, it can be seen that each sample contains flavonoids, but not quercetin. As an example, *C. burmannii* cortex is known to contain flavonoids such as kaempferol[35] and *S. polyanthum* (Wight.) Walp. leaf contains flavonoids such as kaempferol and myricetin.[36]

4.3. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

All samples were tested using TLC (qualitative) and UV-Vis spectrophotometry (quantitative) to analyze their gallic acid and quercetin content. The data obtained from standard measurements were plotted into concentration-response curves to derive the equation and linear regression, with concentration on the x-axis and absorbance on the y-axis. Gallic acid was used as the standard solution for determining total phenolic content, while quercetin was used for total flavonoid content. Gallic acid is a derivative of hydroxybenzoic acid found in almost all plants and can react with Folin-Ciocalteu reagent.[37] Meanwhile, quercetin is a flavonoid compound with a keto group at C-4 and hydroxyl groups at C-3 or C-5 of the flavone and flavonol, which can absorb light at wavelengths between 400 – 500 nm.[38]

The determination of TFC, calculated as quercetin, was performed using UV-Vis spectrophotometry with $AlCl_3$ reagent. Flavonoids contain conjugated aromatic compounds that exhibit strong absorption in the UV and visible light spectra.[39] Samples containing flavonoids will react with $AlCl_3$ to form a stable complex between ketone or hydroxyl groups, which is indicated by a yellow color. The addition of CH_3COOK is used to stabilize the formed complex reaction.[40,41]

Meanwhile, for the determination of TPC, the standard

Table 4. Quantitative analysis of TPC and TFC using UV-Vis spectrophotometry

Extract	Total Phenolic Content (mgGAE/g extract)	Total Flavonoid Content (mgQE/g extract)
C. zedoaria Rhizome	1232.86	41.39
C. aeruginosa Rhizome	904.29	107.91
C. heyneana Rhizome	842.38	125.74
C. xanthorrhiza Roxb. Rhizome	1666.19	17.31
Z. officinale cv rubra Rhizome	828.10	130.47
P. indicus Willd. Leaf	2059.37	39.34
U. lobata Leaf	1592.70	57.92
P. speciosa Hassk. Leaf	1618.10	40.02
I. fagiferus Fosb. Leaf	1580.00	82.94
Z. aromaticum Rhizome	1132.86	34.91
P. betle Leaf	1665.71	25.68
P. cablin Benth. Leaf	1580.00	79.25
S. caseolaris L. Leaf	1551.43	41.19
S. aromaticum L. Leaf	1837.14	51.75
C. hirta (L.) D. Don. Leaf	1989.52	12.97
G. ulmifolia Leaf	1770.48	35.19
C. burmannii Cortex	2119.68	10.16
S. polyanthum (Wight.) Walp. Leaf	2049.84	30.57

solution, gallic acid, is reacted with Folin-Ciocalteu reagent based on the strength of the reduction of phenolic hydroxyl groups, indicated by a color change to blue or deep black. Following that, Na_2CO_3 is added to create a more basic condition to induce the proton dissociation in phenolic compounds into phenol ions. Both the samples and standards are measured at a maximum wavelength of 760 nm.[22,42]

From the results of quantitative tests for determining the total phenolic and total flavonoid content using UV-Vis spectrophotometry, it is known that *C. burmannii* cortex, *P. indicus* Willd., *S. polyanthum* (Wight.) Walp., *C. hirta* (L.) D. Don., and *S. aromaticum* L. leaves are the five plants with the highest total phenolic content. In qualitative tests using TLC, among these five plants, when observing the colour of the spots that appear after the application of a 10% $FeCl_3$ reagent, all five plants show a positive result for phenolic compounds as they exhibit a blue or dark colour. However, based on the intensity of the colour of the spots, the C. burmannii cortex sample, which produces greyish spots, indicates a relatively lower presence of detected phenolic compounds. This contrasts with the quantitative results obtained from both UV-Vis spectrophotometer testing and Rf value calculations using TLC for the same samples. When examining the Rf values generated by these five samples, only the Rf value of *C. burmannii* cortex similar to the reference Rf value of gallic acid, with a difference of 0.0375. These results confirm that only the *C. burmannii* cortex shows a positive result for containing phenolic compounds in the form of gallic acid. On the other hand, the other four samples also test positive for phenolic compounds; however, the phenolic compounds they contain may not be gallic acid. From the results of this testing, it can be inferred that the five plants with high phenolic content likely have the best antioxidant activity. This is because phenolic compounds are secondary metabolites that serve as natural antioxidants and can function as anti-aging, antifungal, and anti-inflammatory agents. Based on literature, *C. burmanni* cortex, *P. indicus* Willd., *S. polyanthum* (Wight.) Walp., *C. hirta* (L.) D. Don., and *S. aromaticum* L. leaf have IC50 (Inhibitory Concentration) values of 35.9[43] ; 27.4[44] ; 25.06[45] ; 5[46] ; and 21.5[47] µg/mL, respectively. The values show that *C. burmannii* cortex has the highest antioxidant activity, which is in line with the phenolic content contained in it. However, this IC50 value is not comparable in other samples. Nevertheless, the low IC50 value in each sample indicates the strength of high antioxidant activity, so it can be fair to say that the high content of phenolic compounds in a plant will make the plant have high antioxidant activity.

Meanwhile, the five plants with the highest TFC, as seen from the measurements using UV-Vis spectrophotometry, are found in *Z. officinale* cv rubra, *C. heyneana*, and *C. aeruginosa* rhizomes and in *I. fagiferus* Fosb. and *P. cablin* Benth. leaves. Based on the TLC results using both chloroform:methanol (9:1) and ethyl acetate:n-hexane (5:5) as solvents, none of the samples produce an Rf value close to the reference quercetin used. However, based on the colour of the spots generated by these five plants with the highest flavonoid concentrations, it is known that all five samples test positive for flavonoids, exhibiting colours ranging from yellow to reddish yellow or brownish. The highest intensity of colour is produced by the *Z. aromaticum* rhizome sample, with a deep red colour after being sprayed with citroborate and a reddish orange colour after exposure to ammonia vapour. From these results, it can be deduced that the *Z. aromaticum* rhizome sample contains a high amount of quercetin when compared to *Z. officinale* cv rubra rhizome. However, quantitatively, the *Z. officinale* cv rubra rhizome sample contains a higher amount of flavonoids, indicating that the high flavonoid content in *Z. officinale* cv rubra rhizome is likely a different type of flavonoid and not quercetin. Based on research by Manuhara48, *Z. officinale* cv rubra has a higher flavonoid content (104.14 mgQE/g) compared to *Z. aromaticum* rhizome that only has 97.67 mgQE/g flavonoid content. However, in the same literature, it was reported that the *Zingiberaceae* family contains the highest flavonoid counted as quercetin is *C. xanthorrhiza*, with TFC of 141.70 mgQE/g. Differences in the results obtained by this research and the literature can occur due to differences in sampling locations since this has quite an influence on the size of the content of a compound in the plant sample.

5. Conclusions

Total phenolic and flavonoid content analysis has been carried out on a total of 18 medicinal plants from nine families. The five plant extracts that had the highest total phenolic content were namely *C. burmannii* cortex, *P. indicus* Willd. leaf, *S. polyanthum* (Wight.) Walp. leaf, *C. hirta* (L.) D. Don. leaf, and *S. aromaticum* L. leaf with respective levels of 2120.00, 2056.19, 2049.52, 1989.52, and 1837.14 mgGAE/g. Meanwhile, the five plant extracts that had the highest total flavonoid levels were *Z. officinale* cv rubra rhizome, *C. aeruginosa* rhizome, *C. heyneana* rhizome, *I. fagiferus* Fosb. leaf, and *P. cablin* Benth. leaf with respective levels of 130.47, 125.74, 107.91, 82.89, and 79.25 mgQE/g.

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Conflict of Interest

The authors declare no conflicts of interest.

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