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Original Article

# Antioxidant potential of underutilized parts of salam (*Syzygium polyanthum*) using DPPH and CUPRAC methods

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## ABSTRACT

Antioxidants can combat free radicals that can cause degenerative diseases. Natural antioxidant agents can be found in salam (*Syzygium polyanthum*). Thus, the purpose of this study is to assess the salam's underutilized roots and twigs as a natural antioxidant source. The study also aims to identify and determine the flavonoid compound levels in the selected extract. Salam's antioxidant properties were demonstrated by the detection of flavonoids and phenolic substances by phytochemical screening. Using UV-visible spectrophotometry, the amounts of antioxidant activity, total flavonoid, and total phenolic were determined. The cupric ion-reducing antioxidant capacity (CUPRAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) techniques were used to measure antioxidant activity. Using high-performance liquid chromatography, the flavonoid components of the chosen extract were identified and quantified. The most antioxidant activity using DPPH was found in the ethanol of salam root extract, which included rutin, apigenin, and apigenin-7-O-glucoside molecules. It is possible to develop salam roots as a novel potential antioxidant agent source.

**Keywords:** Antioxidant, Flavonoid, Phenolics, *S. polyanthum*, Unused parts

## Introduction

Unpaired electrons are found in compounds known as free radicals. These molecules are mostly chemically reactive and unstable because they always try to obtain electrons from other molecules [1]. Oxidative stress, which is a major factor in degenerative processes including aging, cancer, arteriosclerosis, ischemic/reperfusion phenomena, and inflammatory illnesses, can be brought on by free radicals [2-4]. By giving free radicals electrons, antioxidants are chemicals that may neutralize them and bring them down to a stable state [5]. With the presence of antioxidants, oxidative stress that can cause degenerative disease can be inhibited [6, 7].

The biggest chemicals with antioxidant properties found in plants are flavonoids and phenolic compounds [8]. Flavonoid and phenolic compounds are abundantly found in salam (*S. polyanthum*). There is another scientific name for Salam. It has been demonstrated that the plant Eugenia polyantha possesses anti-inflammatory, anti-hypertensive, anti-ulcer, anti-diabetic, and anti-diarrheal properties [9]. Salam is an evergreen plant or a plant that will continue to grow throughout the year or season, and widely distributed in Southeast Asia, including Malaysia, Thailand, Singapore, and Indonesia [10]. Salam leaves also have the potential to be used as a natural preservative because salam leaves contains phenols, tannins, flavonoids, and hydroquinone, which are antioxidant and antimicrobial agents [11]. The present research proposes to investigate the unused parts (roots and twigs) of salam as source of natural antioxidant. The study also

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aims to identify and determine the flavonoid compound levels in the selected extract.

## Materials and Methods

### Chemicals

Sodium carbonate, n-hexane, ethyl acetate, ethanol, distilled water, gallic acid, aluminum chloride, cupric chloride, neocuproine, ammonium acetate, ascorbic acid, DPPH, methanol, rutin, kaempferol, apigenin, and apigenin-7-O-glucoside. The instruments included a UV-visible spectrophotometer (Thermo Fisher Scientific, Inc.; Trace 1300), UV light (Camag), high-performance liquid chromatography (HPLC) equipment (Shimadzu Corporation), an oven, a water bath, a rotary evaporator, and a grinder.

### Sample collection

The collection and preparation of materials consisted of several stages, including material collection, plant determination, drying, and grinding materials into crude drug powder [12-15]. Five kilograms of each organ part—leaves, roots, and twigs—were gathered from Ciwidey in Bandung City, West Java. Materials were cleaned by washing them with water and letting them dry after plants were identified at the Bandungense Herbarium, Biology Study Program, School of Life Sciences and Technology, Bandung Institute of Technology. Once dried, the materials were chopped and dried using an oven. The dried leaves, roots, and twigs of salam were then ground into coarse powder by a grinder. The powder was stored in a sealed container under dry conditions.

### Extract preparation

Ethanol, n-hexane, and ethyl acetate were used as solvents in the reflux approach to extract around 300 grams of S. polyanthum leaves, roots, and twigs. Three extractions were performed on each solvent; each cycle lasted two hours. Following that, a rotary evaporator was used to evaporate the solvent.

### Total phenolic content (TPC)

As a standard, gallic acid was used to determine the total phenolic content. To create solutions with concentrations ranging from 60 to 130 µg/mL, gallic acid was first produced as a 1000 µg/mL stock solution and then diluted. 500 µL of 10% Folin-Ciocalteu reagent, 400 µL of 1 M sodium carbonate, and 50 µL of each diluted gallic acid solution were placed in an Eppendorf tube. The combination was incubated for 30 minutes, and then its absorbance at a wavelength of 765 nm was measured using a UV-visible spectrophotometer. A calibration curve for gallic acid was created using the absorbance values that were obtained. A blank solution was created by mixing 50 µL of methanol with 500 µL of 10% Folin-Ciocalteu reagent and 1 M sodium carbonate [16]. Following the same protocol as the gallic acid standard, the total phenol content of the n-hexane, ethyl acetate, and ethanol extracts was ascertained. To create a stock solution, each extract was treated with pro-analysis methanol. Each extract was measured six times. The total phenol content of each extract was calculated using the linear regression equation of the gallic acid calibration curve and displayed as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g).

### Total flavonoid content (TFC)

The Chang technique was used to calculate the total flavonoid content [17]. In order to create concentrations ranging from 40 to 110 µg/mL, 500 µg/mL of quercetin was produced in a stock solution and then diluted. Five hundred microliters of pure water, 300 microliters of methanol, 20 microliters of 10% aluminum chloride, 20 microliters of 1 M sodium acetate, and 100 microliters of each diluted quercetin solution were combined. The combination was incubated for 30 minutes, and then its absorbance at a wavelength of 415 nm was measured using a UV-visible spectrophotometer. A blank solution was created by combining 400 µL of methanol, 20 µL of 1 M sodium acetate, 560 µL of distilled water, and 20 µL of 10% aluminum chloride. Next, using the acquired absorbance values, the quercetin calibration curve was produced.

Each extract was expressed as milligrams of quercetin equivalent (QE) per gram of extract (mg QE/g) after the total flavonoid content was determined using the linear regression equation of the quercetin calibration curve. Measurements of each extract's total flavonoid content were conducted in six duplicates using the same approach as the standard.

### Antioxidant activities

#### DPPH method

The DPPH method was used to measure antioxidant activity using ascorbic acid as a reference. A stock solution was made with 50 µg/mL of DPPH. The ascorbic acid stock solution was made by dissolving 20 mg of ascorbic acid in 100 mL of pro-analysis methanol. Ascorbic acid solutions at a range of concentrations (10 µL, 12.5 µL, 15 µL, 20 µL, 25 µL, 30 µL, 35 µL, and 40 µL) were made using the stock solution. Pro-analysis methanol was then added to each solution until the final volume was 125 µL. Following the addition of 750 µL of the DPPH solution, the mixture was sealed and left in a dark location for half an hour. The UV-visible spectrophotometer was adjusted at 517 nm to measure the absorbance. To obtain a regression equation, the percentage of inhibition was computed at every concentration, and a calibration curve of the ascorbic acid inhibition % was made.

The extract solution was made by dissolving it in pro-analysis methanol, filtering it, and using the filtrate as the sample. The ascorbic acid standard's treatment process was applied to the extract as well [18-23]. Six duplicates of each extract were used for the measurements. After the DPPH inhibition percentage of the sample was established, the antioxidant activity of the sample was calculated by utilizing the ascorbic acid regression equation

to input the inhibition % value. According to Celep *et al*. [24], the antioxidant activity was then translated into milligrams of ascorbic acid equivalent antioxidant capacity (AEAC) per gram of sample (mg AEAC/g for short).

### *CUPRAC method*

The CUPRAC approach employed ascorbic acid as a standard to measure antioxidant activity. Five milliliters of cupric chloride (1705 µg/mL) and five milliliters of neocuproine (1562 µg/mL) were combined to create a CUPRAC stock solution. CUPRAC solution 100 µg/mL was made from the stock solution. Five concentration series of the standard were established by extracting 15 µL, 17.5 µL, 20 µL, 22.5 µL, 25 µL, 27.5 µL, 30 µL, and 35 µL from a 200 µg/mL ascorbic acid stock mixture and placing them in separate Eppendorf tubes. Each tube received 750 µL of the CUPRAC solution after ammonium acetate buffer was added until the final volume was 250 µL. The mixture was incubated for 30 minutes, and the absorbance at 450 nm was measured using a UV-visible spectrophotometer. An ascorbic acid concentration calibration curve was produced by processing the three measurements of each standard concentration.

The extract solution was prepared by dissolving the extract in pro-analysis methanol, filtered, and used as a sample. Six duplicates of the extract solution were tested for absorbance, and the regression equation for the standard solution was used to calculate the antioxidant activity. Milligrams of AEAC per gram was used to quantify the antioxidant activity [25].

### *Determination of flavonoids by HPLC*

Using the HPLC LC-20AD equipment, the flavonoid compounds in the selected S. polyanthum extract were identified and determined using a mobile phase made of methanol and water with a linear gradient of 40–60% methanol for 5 minutes, 70% methanol for 10 minutes, and 40% methanol for 15 minutes. As the stationary stage, LiChrospher® 100 RP-C18 5 µm (100 mm length, 4 mm diameter, 20 mm pre-column (Merck)) was used. At 30°C and a flow quantity of 1 mL/min, a 20 µL injection volume was administered. 360 nm was the wavelength of the UV-visible detector that was employed for detection. The one-point approach was utilized for the measurement of flavonoids. Apigenin, kaempferol, rutin, quercetin, and apigenin-7-O-glucoside were the requirements. A syringe was used to combine the criteria and the chosen extract in methanol for liquid chromatography. A 0.45 µm membrane filter was used to filter the mixture prior to HPLC analysis.

### *Statistical analysis*

To determine the significance of the data and the correlations between the variables, statistical analysis was performed using Minitab software. Every result was presented using the mean ± standard deviation. Pearson's correlation analysis was used to determine the link between antioxidant activity, total phenolic content (TPC), and total flavonoid content (TFC), as well as the correspondence between different antioxidant activity test techniques [26].

## Results and Discussion

### *Determination of plants*

The plant was recognized by the Bandungense Herbarium Laboratory, Biology Study Program, School of Life Sciences and Technology, Bandung Institute of Technology, based on an analysis of its morphology. Based on the findings of the determination, the plant was identified as S. polyanthum (Wight) Walp., a member of the Myrtaceae family.

### *Extraction*

Salam leaves have potential to be used as a natural preservative because salam leaves contains phenols, tannins, flavonoids which are antioxidant and antimicrobial agents [11]. The unused parts of salam namely roots and twigs may be contained similar compounds and give similar activity. Therefore, sample in this study were leaves, roots and twigs of salam (*S. polyanthum*). The reflux technique was used to extract the powdered crude drug from the salam leaves, roots, and twigs using n-hexane, ethyl acetate, and ethanol, which are increasing polarity solvents. The "like dissolves like" concept, which states that a substance will dissolve in a solvent with a comparable polarity, is applied during the extraction process. As a result, substances were separated according to their polarity using these three solvents with varying polarities. largely nonpolar molecules were recovered from n-hexane, largely semi-polar compounds from ethyl acetate, and principally polar compounds from ethanol.

### *Total phenolic content (TPC) and total flavonoid content (TFC)*

The TPC values, which were determined using gallic acid as a standard, are displayed in **Table 1**. For gallic acid, the linear regression formula was $y = 0.0054x + 0.0385$, with $R^2 = 0.9963$. The TPC values of each salam extract sample ranged from 14.963 to 292.284 mg GAE/g. At 292.284 ± 12.309 mg GAE/g, the salam root ethanol extract exhibited the highest total phenol content (TPC). At 14.963 ± 2.271 mg GAE/g, the n-hexane extract of salam roots exhibited the lowest total phenol content (TPC). According to Luliana *et al*. [27], the TPC values for methanol extracts of salam leaves vary depending on the extraction method used. The extract obtained using the Soxhlet method had a TPC value of 227.72 ± 21.6 mg GAE/g, the maceration method yielded 338.62 ± 21.3 mg GAE/g, and the infusion method resulted 144.48 ± 8.20 mg GAE/g. The TPC value of methanol-water extract from salam bark was reported as 856 ± 28.2 mg GAE/g [28]. The TPC value of ethanol extract from salam leaves obtained by maceration was 2.21 ± 0.24 mg GAE/g [29]. It was not comparable to the current study, which showed the TPC of ethanol leaves extract of salam around 258.436 mg GAE/g. Another investigation found that the

methanol extracts of salam root bark and stem bark had TPC values of 189.80 ± 2.0 mg GAE/g and 187.50 ± 1.3 mg GAE/g, respectively [30]. Compared to the current investigation, which revealed that the TPC of salam ethanol roots extract was about 292.284 mg GAE/g, it was lower.

TFC values were calculated using quercetin as a standard and are shown in **Table 1**. The $R^2$ value for quercetin was 0.9938, and the linear regression equation was y = 0.0074x - 0.0417. Each salam extract sample had a different TFC, which ranged from 5.593 to 84.467 mg QE/g. At 84.467 ± 7.87 mg QE/g, the salam leaves ethyl acetate extract exhibited the greatest total flavonoid content (TFC). The salam root ethanol extract had the lowest total flavonoid content (TFC), measuring 5.593 ± 1.625 mg QE/g. Dewijanti *et al*. [31] reported that the TFC values for salam leaf aqueous extracts differed by area, with extracts from East Java having 20.80 ± 2.36 µg QE/mg, Central Java having 52.05 ± 6.25 µg QE/mg, and West Java having 33.90 ± 3.61 µg QE/mg. The methanol extracts of salam root bark and stem bark had TFC values of 134.85 ± 0.5 mg QE/g and 114.82 ± 2.3 mg QE/g, respectively, according to Sabandar *et al*. [30]. It was not comparable to the current study, which showed the TFC of ethanol extract of salam roots around 5.593 mg QE/g. Similar to the current study, which revealed the TFC of ethanol extract of salam leaves at about 28.402 mg GAE/g, the other study's TFC value for the extract of salam leaves made with 70% ethanol and extracted by maceration was 30.57 mg QE/g [32].

The differences in TPC and TFC values between the conducted experiment and the literature can occur for several reasons. These differences may arise due to variations in extraction methods, including the solvents used, extraction temperature, and extraction techniques [33]. Additionally, differences in the extracted compounds can also be influenced by the plant's location of origin, as atmospheric and environmental factors such as rainfall and sunlight intensity may vary [34].

Total phenol content (TPC) is measured in alkaline circumstances using the Folin-Ciocalteu reagent. The hydroxyl groups in gallic acid phenolate ions react with the Folin-Ciocalteu reagent to form a blue molybdenum-tungsten complex, which may be identified by reducing heteropoly acid (phosphomolybdate-phosphotungstate) using a spectrophotometer calibrated to 765 nm. The measurement techniques for total flavonoid content using aluminum chloride are based on the colorimetric methodology. Aluminum chloride reacts with the hydroxyl group at the C3'-C4', the keto group at the C-4 atom, and the hydroxyl group at the adjacent C-3 or C-5 atoms of flavones and flavonols to cause the solution to become yellow. This complex shifts the wavelength toward the visible spectrum [35]. Sodium carbonate is added to maintain alkaline condition in this reaction because complex aluminum chloride with C3'-C4' will be broken in acid condition [36].

Natural phenolic and flavonoid compounds are secondary metabolites in which at least one hydroxyl group and an aromatic ring are present [37]. Because they donate electrons, phenolic substances are the source of antioxidant activity, and their hydroxyl groups directly contribute to their antioxidant properties [38]. These substances can help prevent oxidative illnesses brought on by free radicals by inhibiting them [39]. Secondary metabolites called flavonoids have antioxidant properties that are dependent on the quantity and location of free OH groups [40]. The flavonoid group exhibits the best antioxidant activity when there is a higher presence of hydroxyl groups in the structure mainly in C3' and C4', and or a double bond between C2 and C3, and or hydroxyl group in C3, and or a 4-carbonyl group [41].

## *Antioxidant activities (DPPH and CUPRAC method)*

**Table 1** showed the antioxidant activity that was measured using DPPH methods. Ascorbic acid was utilized a standard in DPPH method. Using the DPPH technique, the ascorbic acid linear regression equation was y = 12.19x + 10.034, and $R^2$ = 0.9913. The antioxidant activity by DPPH in all salam extract samples showed varying results, ranging from 4.559 to 502.989 mg AEAC/g. The ethanol extract of salam roots had the top antioxidant activity, measured 502.989 ± 23.422 mg AEAC/g. Meanwhile the n-hexane extract of salam roots had the lowest antioxidant activity, measured 4.559 ± 0.151 mg AEAC/g.

Using the DPPH test, Lelono *et al*. [28] found that the methanol-water extract of *S. polyanthum* bark had an antioxidant activity of 449 ± 23.5 mg AEAC/g. The 96% ethanol extract of *S. polyanthum* leaves, extracted using maceration, had an $IC_{50}$ value of 88.21 ± 1.38 µg/mL, whereas the extract obtained using the ultrasound-assisted extraction method had an $IC_{50}$ value of 41.23 ± 6.11 µg/mL [42]. The $IC_{50}$ value of the ethanol extract of *S. polyanthum* leaves extracted through maceration varies depending on the leaves condition. The $IC_{50}$ value of the young leaves extract was 37.441 µg/mL, while the half-mature leaves extract was 14.889 µg/ mL, and the mature leaves extract was 11.001 µg/mL [43]. The $IC_{50}$ value of *S. polyanthum* leaves methanol extract was 90.85 µg/mL [44]. The $EC_{50}$ value for the DPPH of the methanol extract from *S. polyanthum* leaves obtained via maceration was 20.90 ± 0.26 µg/mL [45]. The $IC_{50}$ values for different extracts of *S. polyanthum* leaves were as follows: n-hexane extract 136.7 µg/mL, dichloromethane extract 126.1 µg/mL, ethyl acetate extract 56.7 µg/mL, and methanol extract 44.35 µg/mL [28].

Antioxidant activity was determined by CUPRAC methods and presented in **Table 1**. The CUPRAC method was determined using ascorbic acid as a standard. The linear regression equation for ascorbic acid with the CUPRAC method was y = 7.9501x + 16.888, with $R^2$ = 0.9905. The CUPRAC method in all salam extract samples, ranging from 27.417 to 108.069 mg AEAC/g. Based on the CUPRAC method, the highest antioxidant activity was found in the salam leaves ethyl acetate extract, measuring 108.069 ± 3.797 mg AEAC/g. The n-hexane extract of salam roots had the lowest antioxidant activity, measured 27.417 ± 3.354 mg AEAC/g.

Researchers [46] reported that the antioxidant activity of all fractions of *S. polyanthum* leaves was as follows: n-hexane extract 0.50 ± 0.01 TEAC (mmol/g)$^c$, dichloromethane extract 0.46 ± 0.01 TEAC (mmol/g)$^c$, ethyl acetate extract 0.40 ± 0.01 TEAC (mmol/g)$^c$, methanol residue extract 2.26 ± 0.01 TEAC (mmol/g)$^c$, and water extract 0.19 ± 0.01 TEAC (mmol/g)$^c$. TEAC (mmol/g)$^c$ refers to the trolox equivalent antioxidant capacity in mmol/g at 200 mg/mL. The ethanol extract of *Syzygium aqueum* leaves and twigs had the best antioxidant activity using the CUPRAC method, measured 221.47 ± 9.18 mg AEAC/g and 222.51 ± 10.44 mg AEAC/g [43].

Determining antioxidant activity using the DPPH and CUPRAC methods yields different results due to the distinct mechanisms involved. The DPPH method consists of a hydrogen transfer mechanism, whereas the CUPRAC method involves an electron transfer mechanism [47]. The stable free radical DPPH that interacts with antioxidants through an electron transfer process. This approach measures the decrease of DPPH radicals to estimate antioxidant activity. When reacting with antioxidants, the DPPH free radicals are reduced, changing the color from purple to yellow [48]. The DPPH assay relies on scavenging DPPH by antioxidants, which can donate hydrogen [49].

The determination of antioxidant activity using the CUPRAC method employs the copper (II)-neocuproine reagent as the chromogenic oxidant, which facilitates the reduction of $Cu^{2+}$ - neocuproine to $Cu^{+}$ - neocuproine through the action of antioxidants present in the plant extracts. This reduction results in a chromophore with a maximum absorbance at a wavelength of 450 nm [45]. Neocuproine is 2,9-dimethyl-1,10-phenanthroline. In this method, a color change occurs from light blue (due to the $Cu^{2+}$-neocuprine to orange-yellow to the reduced $Cu^{+}$-neocuproine). A compound that can exhibit antioxidant activity if it can reduce or possesses an $E^0$ value lower than the $Cu^{2+}$- neocuproine /$Cu^{+}$-neocuproine system (0.6 V) [43].

Based on the present study, the TFC values of the n-hexane extract samples from the roots and twigs were similar, measuring 30.623 and 30.734 mg QE/g, respectively. However, the two samples showed different results in the antioxidant activity tests using the DPPH and CUPRAC methods. In the DPPH method, both samples exhibited antioxidant capacity values of 4.559 and 19.759 mg AEAC/g. In contrast, the CUPRAC method yielded antioxidant capacity values of 27.417 and 33.333 mg AEAC/g for the two samples.

According to Pourmorad *et al*. [16], a sample's high TFC value does not always mean that it has strong antioxidant activity, which might lead to this outcome. The chemical composition of a sample's flavonoid components, rather than just the quantity of flavonoids present, plays a significant role in its antioxidant activity. Flavonoids with hydroxyl groups at positions C3' and C4' on ring B, a hydroxyl group at C3, a ketone group at C4, and a double bond between C2 and C3 tend to exhibit strong antioxidant activity [42]. This structure enables flavonoids to effectively neutralize free radicals. The DPPH method works by measuring the ability of antioxidants to donate hydrogen atoms or to contain hydroxyl groups. Therefore, if a sample has a high TFC value but shows low antioxidant activity in the DPPH assay, it may be due to the absence of key hydroxyl groups in its flavonoid compounds. In other words, even if two samples have similar TFC values, their DPPH antioxidant activities may differ because the flavonoid components in each sample may have different structural characteristics.

In the CUPRAC method, metal ions are reduced by antioxidants for example, $Cu^{2+}$ is reduced to $Cu^{+}$. The ability of a compound to reduce metal ions depends on its standard reduction potential ($E^0$). A compound that can exhibit antioxidant activity if it can reduce or possesses an $E^0$ value lower than the $Cu^{2+}$- neocuproine /$Cu^{+}$-neocuproine system (0.6 V) [43]. Therefore, if a sample contains a high amount of flavonoids but does not have an $E^0$ value lower than 0.6 V (lacks strong reducing power), it will not expose high antioxidant activity. This also explains why two samples with similar TFC values may show different antioxidant activities in the CUPRAC assay; the flavonoid structures in each sample may vary, resulting in different reducing capacities. Furthermore, the existence of additional active chemicals such tannins, alkaloids, or other bioactive substances may be the cause of a sample's low TFC value but significant antioxidant activity.

**Table 1. TPC, TFC, and Antioxidant Activity of various salam extracts**

| | Extract | TPC (mg GAE/g) | TFC (mg QE/g) | DPPH (mg AEAC/g) | CUPRAC (mg AEAC/g) |
|---|---|---|---|---|---|
| Leaves | n-Hexane | 36.728 | 39.619 | 43.440 | 51.168 |
| | Ethyl acetate | 87.5 | 84.467 | 132.028 | 108.069 |
| | Ethanol | 258.436 | 28.402 | 436.712 | 59.819 |
| Roots | n-Hexane | 14.963 | 30.623 | 4.559 | 27.417 |
| | Ethyl acetate | 90.401 | 5.519 | 133.502 | 55.441 |
| | Ethanol | 292.284 | 5.593 | 502.989 | 54.472 |
| Twigs | n-Hexane | 31.559 | 30.734 | 19.759 | 33.333 |
| | Ethyl acetate | 55.103 | 44.374 | 45.112 | 55.125 |
| | Ethanol | 127.222 | 7.668 | 123.076 | 54.907 |

## *Determination of flavonoids by HPLC*

Generally, many literatures stated that flavonoid is the natural compounds that related with antioxidant activity. Then, identification and quantification of flavonoid constituent levels in the chosen extract using HPLC. The ethanolic salam roots extract had the top antioxidant with DPPH method were choosing as the selected extract. In *S. polyanthum*, particularly in the leaves, the commonly found flavonoid compounds are kaempferol, quercetin, and rutin [49]. The two most prevalent flavonoids in the Myrtaceae family are quercetin and kaempferol [32]. Since the primary flavonoids in the Syzygium genus are apigenin-7-O-glucoside, quercetin, apigenin, and kaempferol, the reference compounds—which included rutin, quercetin,

kaempferol, apigenin, and apigenin-7-O-glucoside—were used as standards [46, 50].

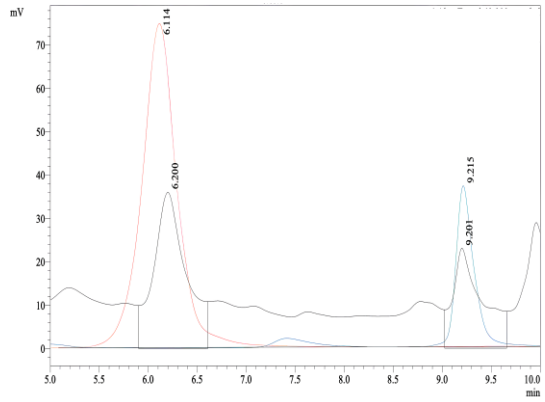


**Figure 1.** Black line indicates sample (40000 µg/mL), red line indicates apigenin-7-O-glucoside (50 µg/mL), and green line indicates apigenin (100 µg/mL) in this high performance liquid chromatogram of ethanolic salam root extract and references.

**Table 2. Level of apigenin and apigenin 7-O- glucoside in salam roots ethanol extract**

| Compound | AUC | | |
|---|---|---|---|
| | Reference | Sample | Level (mg/g) |
| Apigenin-7-O-glucoside | 1888779 | 828585<br>809638<br>761794 | 0.529 ± 0.023 |
| Apigenin | 487856 | 484448<br>475189<br>426481 | 2.368 ± 0.160 |

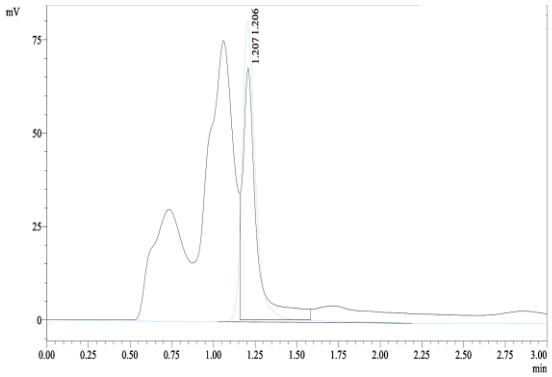


**Figure 2.** ethanolic salam root extract and references in a high performance liquid chromatogram; Green line: rutin (25 µg/mL); black line: sample (5000 µg/mL).

**Table 3. Level of rutin in salam roots ethanol extract**

| Compound | AUC | | |
|---|---|---|---|
| | Reference | Sample | Level (mg/g) |
| Rutin | 463962 | 583763<br>376469<br>393085 | 4.861 ± 1.241 |

**Figures 1 and 2** displayed the HPLC chromatograms of the ethanol extract of S. polyanthum roots in contrast to the standard, while **Tables 2 and 3** displayed the amounts of flavonoid components in the selected extract. The extract's flavonoid components exhibited retention periods that were in line with the benchmarks. The chromatogram showed that just one peak in the extract coincided with the peaks of the flavonoid standard chromatogram. At 6.2 minutes, the extract's chromatogram matched that of the apigenin-7-O-glucoside standard, which had a 6.114-minute retention period. A concentration of 0.529 ± 0.023 mg/g of apigenin-7-O-glucoside was found in the extract when it was quantified using the one-point technique. The chromatogram, which had a retention duration of 9.215 minutes, was comparable to the apigenin standard at 9.201 minutes. It was determined that the extract's apigenin concentration was 2.368 ± 0.160 mg/g using the one-point technique.. Additionally, the chromatogram at 1.207 minutes resembled the rutin standard, with a retention time of 1.206 minutes. The rutin content in the extract, as determined by the one-point method, was 4.861 ± 1.241 mg/g.

According to Hartati *et al*. [46], the ethanol extract of green honey deli apple (Syzygium aqueum) contains 0.16% and 0.39% of two flavonoid components, quercetin and kaempferol, respectively. Discrepancies in the identification and quantification of flavonoid compounds in the extract might be due to differences in species, growing regions, and harvest seasons [51-54].

## *Correlation analysis between TPC, TFC, and antioxidant activities*

The two antioxidant activities, TPC and TFC, were tested for linearity using Pearson's correlation, which yields linear findings if both demonstrate significant and positive results.

**Table 4. Correlation of TPC and TFC with antioxidant parameters**

| | | Pearson's Correlation Coefficient (r) | | | |
|---|---|---|---|---|---|
| Extract | | Antioxidant DPPH | | Antioxidant CUPRAC | |
| | | TPC | TFC | TPC | TFC |
| n-Hexane | Leaves | 0.987**** | 0.952**** | 0.968**** | 0.878*** |
| | Roots | 0.933**** | 0.902**** | 0.916**** | 0.887*** |
| | Twigs | 0.763*** | 0.874*** | 0.808*** | 0.796*** |
| Ethyl | Leaves | 0.863*** | 0.704*** | 0.911**** | 0.819*** |
| | Roots | 0.935**** | 0.878*** | 0.987**** | 0.915**** |
| | Twigs | 0.694** | 0.848*** | 0.886*** | 0.838*** |
| Ethanol | Leaves | 0.951**** | 0.879*** | 0.870*** | 0.907**** |
| | Roots | 0.976**** | 0.768*** | 0.736*** | 0.915**** |
| | Twigs | 0.989**** | 0.522** | 0.965**** | 0.693** |

**Table 5. Correlation of DPPH and CUPRAC methods**

| Antioxidant DPPH | | Pearson's Correlation Coefficient (r) Antioxidant CUPRAC |
|---|---|---|
| n-Hexane | Leaves | 0.944**** |
| | Roots | 0.991**** |
| | Twigs | 0.963**** |
| Ethyl Acetate | Leaves | 0.892*** |
| | Roots | 0.936**** |
| | Twigs | 0.850*** |
| Ethanol | Leaves | 0.975**** |
| | Roots | 0.766*** |
| | Twigs | 0.969**** |

The results of TPC and TFC's antioxidant activity and Pearson's correlation were shown in **Table 4**. DPPH and CUPRAC's Pearson's correlation was shown in **Table 5**. The correlation analysis was conducted using Minitab software, and results showed moderate to extremely strong correlations, with values ranging from 0.522 to 0.991. According to Schober *et al*. Pearson's correlation coefficient values of 0.00-0.10 signal a very weak relationship, 0.10-0.39 a weak correlation, 0.40-0.69 a moderate correlation, 0.70-0.89 a strong correlation, and 0.90-1.00 a very high correlation [55].

According to the DPPH and CUPRAC techniques, phenolic and flavonoid molecules are important contributors to antioxidant activity. TPC and TFC showed moderate to very substantial associations with these antioxidant values. Additionally, TPC and TFC demonstrated a strong and favorable association with antioxidant activity (DPPH and CUPRAC). The methanol extract from salam bark's TPC showed a very strong correlation with DPPH antioxidant activity, with a value of 0.9031, while the TFC showed a moderate correlation with DPPH antioxidant activity, with a value of 0.5639, as reported by Sabandar *et al*. [30]. Additionally, a linear connection in the antioxidant activity as determined by the DPPH and CUPRAC tests was suggested by the significant to very strong correlation that was found between them.

## Conclusion

Using the CUPRAC technique, the salam leaf ethyl acetate extract exhibited the best antioxidant activity, measuring 108.069 ± 3.797 mg AEAC/g, whereas the salam root ethanol extract showed the highest DPPH antioxidant activity, with a value of 502.989 ± 23.422 mg AEAC/g. The extracts of salam leaves, roots, and twigs included phenolic and flavonoid substances that moderately to strongly correlated with antioxidant activity. The DPPH and CUPRAC techniques' measurements of antioxidant activity revealed a linear relationship with strong to extremely significant correlations. The unused parts of salam, namely roots contained rutin, apigenin and apigenin-7-O-glucoside. The roots of salam have potential for development as a novel source of antioxidant agent.

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**Ethics statement:** None

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