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Research Paper

Assessment of Antioxidant Activity, Total Phenolic and Flavonoid Contents of *Albizia saponaria* L. Bark Extract

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Abstract
Langir (*Albizia saponaria* L.), belonging to the family of the Fabaceae, is a medicinal endemic plant of South Sulawesi, Indonesia. The Albizia genus shows antioxidant, antibacterial, anthelmintic, antidandruff, and anti-inflammatory properties. In this study, total phenolic (TP) and total flavonoid (TF) extracts from the bark of *A. saponaria* were screened for robust antioxidant activity in order to identify potential sources of new compounds for use in pharmaceutical formulations in the future. TP and TF of the 96% ethanol extract and fractions (hexane, ethyl acetate, butanol, and water) were calculated by the Folin–Ciocalteu and aluminum chloride procedures, respectively. Furthermore, the antioxidant activity was determined by DPPH free radical scavenging method and ABTS assay. Generally, both extract and fractions showed significant radical scavenging activities. Ethyl acetate fraction exhibited more potent radical scavenging activity in the DPPH method (IC_{50} $35.27 \pm 2.85\,\mu$g/mL) and ABTS assay (IC_{50} $60.04 \pm 0.98\,\mu$g/mL), followed by 96% ethanol extract, and hexane, butanol, and water fractions. Furthermore, the highest TP (4.50 ± 0.01 mg/g GAE) and TF (3.55 ± 0.04 mg/g QE) were obtained from ethyl acetate fraction. There was a strong correlation between antioxidant activity with TP (DPPH, R^2 = 0.6436; ABTS, R^2 = 0.7676) and TF content (DPPH, R^2 = 0.5533; ABTS, R^2 = 0.5961). The extract's antioxidant properties may be attributable to its higher phenolic and flavonoid content. In summary, the phenolic and flavonoid content of the ethyl acetate fraction indicates its potential utility as a source of antioxidants.

Keywords
Albizia saponaria, Antioxidant, Total Flavonoid, Total Phenolic

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1. INTRODUCTION

Free radicals are an atom or molecule that consist of odd number of electron(s) or uncoupled electron makes them unstable, short lived and very reactive. In order to attain molecular stability, free radicals react with nearby molecules to produce electron pairs. Diabetes, cancer, cardiovascular disease, and other degenerative disorders will follow from the reaction, which the body undergoes continuously if it is not stopped (Cenini et al., 2019; Sharifi-Rad et al., 2020). Antioxidants are necessary to counteract and stop the harm that free radicals do. Antioxidants can supplement the lack of electrons needed by free radicals and inhibit the chain reaction of free radical formation (Chaudhary et al., 2023; Jena et al., 2023).

One of the primary sources of antioxidants comes from plants. One plant whose antioxidant activity has not been widely exported is *Albizia saponaria* L. The pharmacological potential of *A. saponaria* has not yet been explored. A few papers reported that the bark of *A. saponaria* has antifungal activity to treat dandruff. Furthermore, the active fraction of *A. saponaria* can stimulate hair growth *in vivo*. The bark of *A. saponaria* was also reported to contain alkaloids, flavonoids, saponins, tannins, and triterpenoids (Arba et al., 2022). Plant phenolics have excellent antioxidant activity and other health advantages, making them essential to the human diet. Epidemiological studies have shown that consume food and beverage that rich in antioxidant significantly turn down the incidence of several oxidative stress-related illnesses, such as Alzheimer, atherosclerosis, cardiovascular, and Parkinson's (Kumar and Goel, 2019). Flavonoids exhibit several different health-promoting bioactivities. The most notable of these has continued to be their ability to function as antioxidants (Speisky Cosoy et al., 2022).

The taxonomic approach to the Albizia genus shows that the methanol extract of the root of *A. richardiana* has antioxidant

activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) with an IC_{50} value of 155.32 μg/mL (Islam et al., 2020). The anti-inflammatory properties of the wood from *A. myriophylla* can be attributed to its capacity to scavenge free radicals and impede the production of nitric oxide (Bakasatae et al., 2018). Based on this emphasis, research has been conducted to measure the total phenolic (TP), total flavonoid (TF), and antioxidant activity of the bark of *A. saponaria* Further, the correlation analysis determines the components causative to the antioxidant properties.

2. EXPERIMENTAL SECTION

2.1 Collection of Sample

The stem bark of *A. saponaria* was collected from the district of Bone's tropical forest in South Sulawesi, Indonesia (-4.67697, 119.94605) in December 2022 (rainy season). The authenticity of the plant was confirmed, and the specimen was stored in the Department of Biological Pharmacy, Almarisah Madani University.

2.2 Preparation of Extracts

The bark was dried in the oven at 40°C for 3 days and milled into powder using a milling machine. A total of 1.2 kg of the dried powder was weighed and extracted by maceration with 3 × 5.0 L of ethanol (96%). Maceration was carried out for 3 days and stirred occasionally, avoiding direct sunlight. Following filtration, the extract was evaporated at 30°C under vacuum pressure (Buchi) to obtain a dried extract. The liquid-liquid extraction method was used to process the ethanol extract fractionally using hexane, ethyl acetate, butanol, and water (residue). About 100 g of ethanol dried extract was dissolved in 1.0 L of water and successively partitioned with hexane (500 mL × 3), ethyl acetate (500 mL × 3), and butanol (500 mL × 3) to provide the corresponding fractions. All the fractions were evaporated under vacuum pressure (Buchi) at 30°C to get a dry extract. The final weight was recorded to calculate the percentage of yield of extract, following the formula:

$$\text{Yield}\,\% = \frac{\text{Weight of Extract}}{\text{Weight of Dried Bark}} \times 100\% \quad (1)$$

2.3 Determination of Phenolic Contents

The total phenolic (TP) content was ascertained utilizing the modified Folin-Ciocalteu test, as described by Pakki et al. (2020). In summary, 0.5 mL of Na_2CO_3 (10% w/v) and 0.5 mL of Folin-Ciocalteu (7.5% v/v) were added to 1.0 mL of each sample (10 mg/mL) and thoroughly mixed. The mixture was filtered after 30 minutes, and water was added to bring the volume up to 5.0 mL. The mixture was left in a dark condition for 30 minutes. The absorbance was measured with a Shimadzu spectrophotometer at λ 730 nm. Gallic acid was used in 5 gradient differential concentrations (0.5, 1.0, 2.0, 3.0, and 4.0 μg/mL) to generate a calibration curve. The TP was expressed as the equivalent of mg/g of gallic acid (mg/g GAE).

2.4 Determination of Flavonoid Contents

The total flavonoid (TF) was determined using the modified aluminum chloride colorimetric ($AlCl_3$) method described by Shraim et al. (2021). Firstly, 0.5 mL of each sample (10 mg/mL) was incubated with 1.0 mL of $AlCl_3$ (5%w/v) and 1.0 mL of sodium acetate solution (1.8 g/mL) for 30 minutes. The absorbance was measured with a Shimadzu spectrophotometer at λ 415 nm. The TF was calculated as mg of quercetin equivalent (QE) per gram (mg/g QE). Quercetin was used in 5 gradient differential concentrations (3.0, 6.0, 9.0, 12.0, and 15.0 μg/mL) to generate a calibration curve.

2.5 Determination of Antioxidant Activity

2.5.1 DPPH Analysis

The ability of the sample to reduce the free radical was evaluated using the method proposed by Pakki et al. (2020). Approximately 10 mg of each sample was dissolved in 10 mL of ethanol (1.000 μg/mL), consequently pipetted and diluted until found to be 7.87, 15.75, 31.25, 62.50, 125.00, 250.00, and 500.00 μg/mL. One hundred milliliters was transferred to a 96-well microplate, and 100 μL of 240 μM DPPH was added. The mixture was let to stand at room temperature for 30 minutes in a relatively dark place. The absorbance was recorded at λ 515 nm using a microplate reader (Biotek EL×808, USA). Vitamin C was the positive control in 7 gradient differential concentrations (2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 μg/mL).

2.5.2 ABTS Analysis

The method outlined by Chaves et al. (2020) assessed the antioxidant activity of the extracts against ABTS radicals. Through the oxidation of ABTS by potassium persulfate, radical $ABTS^{\bullet+}$ was created. A 1:1 v/v combination of potassium persulfate (4.95 mM) and ABTS (7 mM) was made and allowed to stand at room temperature for 16 hours in the dark. After that, methanol was added to dilute the mixture. Aliquots of 100 μL of extract of each sample (at 7 different concentrations: 7.87, 15.75, 31.25, 62.50, 125.00, 250.00, and 500.00 μg/mL in 96-wells microplate and added 100 μL of ABTS. The absorbance decrease was measured at λ 734 nm in a microplate reader (Biotek EL×808, USA). Vitamin E analog, trolox, was used as the positive control in 7 gradient differential concentrations (5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0 μg/mL).

2.5.3 Statistical Analysis

Each experiment was conducted in triplicate, and the mean values along with the standard deviation (SD) were presented for the results. In SPSS version 18, the researchers employed analysis of variance (ANOVA) to determine the difference between the groups. To further assess the significance level of $p < 0.05$, Tukey's post hoc test was utilized. The correlation analysis of TP with TF, DPPH, and ABTS; TF with DPPH and ABTS; and DPPH with ABTS were expressed as R^2 coefficients using MS Excel 365 from Microsoft (Redmond, USA).

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

Table 1. Effect of Different Solvents on Yield of Bark Extracts and Fractions of *A. saponaria*

Solvent	Extraction Yield (%)
96% Ethanol extract	3.38
Hexane fraction	0.60
Ethyl acetate fraction	17.39
Butanol fraction	47.45
Water fraction	33.87

Table 2. The IC_{50} Values of the Antioxidant Activity of Bark Extracts and Fractions of *A. saponaria* Using DPPH and ABTS Method (n= 3)

Sample	IC_{50} (μg/mL)	
	DPPH	ABTS
96% Ethanol extract	96.25 ± 5.34	168.87 ± 6.92
Hexane fraction	237.83 ± 12.71	433.89 ± 4.75
Ethyl acetate fraction	35.27 ± 2.85	60.04 ± 0.98
Butanol fraction	110.22 ± 2.01	128.17 ± 14.48
Water fraction	254.06 ± 16.97	323.66 ± 7.75
Vitamin C	7.61 ± 0.04	-
Trolox	-	17.93 ± 0.29

The biological activity of the extract is closely related to the content of active compounds in the sample. Extraction is the process of withdrawing active compounds from the plant material matrix into the solvent (Chemat et al., 2019; Akinmoladun et al., 2022). After initial extraction with 96% ethanol, the resulting extract yield was 3.38%. After fractionation, it was found that the highest yield was shown by the butanol fraction (47.45%), followed by the water fraction (33.87%) and ethyl acetate fraction (17.39%), as shown in Table 1. The lowest yield was shown by the hexane fraction (0.60%). The result showed that the active compounds contained in the bark of *A. saponaria* L are polar.

3.2 Total Phenolic and Flavonoid

Plants are rich in secondary metabolites, including phenolics and flavonoids. Plant-derived phenolic compounds possess antioxidant properties that support the antioxidant capacity of blood plasma and the body's immune system. As a result, chronic and degenerative diseases are less common when these substances are consumed in high amounts. Phenolic substances possess antioxidant properties as their hydroxyl groups can donate electrons (Mutha et al., 2021; Rudrapal et al., 2022). By colorimetric assay, this study determined the phenolic and flavonoid contents in 96% ethanol extract and hexane, ethyl acetate, butanol, and water fractions. The TP was expressed in gallic acid equivalent using the regression equation y = 0.1519 + 0.2677x (R^2 = 0.9995), as in Figure 1a. Phytochemical analyses performed on bark extract of *A. saponaria* have shown the presence of phenolic. Ethyl acetate fraction showed the highest TP (4.50 ± 0.01 mg/g GAE), while the lowest TP was shown by hexane fraction (0.44 ± 0.07 mg/g GAE) as in Figure 1b. According to the current study, the phenolic compounds in the bark of *A. saponaria* accumulate in polar solvents. In line, the ethyl acetate fraction of *A. odoratissima* produced the highest amount of TP compared to hexane, chloroform, and methanol extract (Banothu et al., 2017).

The well-known antioxidant properties of phenolic compounds and flavonoids, among many other significant bioactive substances, have long piqued attention because of their potential to improve human health and both prevent and treat variety of illnesses. Studies reported that plants naturally contain about 8000 different types of phenolic compounds (Tungmunnithum et al., 2018). Furthermore, in this research, flavonoid levels are expressed in quercetin equivalents. The TF was determined using the regression equation y = 0.1485 + 0.0313x, R^2 = 0.9967, Figure 1c. According to the present data, the ethyl acetate fraction had the highest flavonoid content, followed by butanol and water fractions, while the lowest level was observed in hexane fraction, 3.55 ± 0.04, 1.41 ± 0.10, 1.26 ± 0.07, and 0.45 ± 0.07 mg/g QE, respectively (Figure 1d). Similar to phenolics, flavonoids accumulate in the ethyl acetate fraction, indicating that the active compounds accumulate in polar solvents. Ibrahim and Abdul-Hafeez reported that chrysoeriol, hesperidin, and quercetin were predominant flavonoids in *A. lebbeck* stem bark, while naringin, catechin, rutin, luteolin, and kaempferol were also identified but in low concentrations (Ibrahim and Abdul Hafeez, 2023).

3.3 Antioxidant Activity

According to several studies, the antioxidant activity of plants has been attributed to phenolic and flavonoid compounds. The redox characteristics, which also serve as a metal chelator, hydrogen donor, and singlet oxygen quencher, have been linked to antioxidant action (Kadum et al., 2019; Okello et al., 2021). DPPH is a straightforward technique for determining a compound's antioxidant activity. The DPPH method is based on fading DPPH color after contact with antioxidants, which is determined using a spectrophotometer. The ethanolic bark extract and fractions from *A. saponaria* in this investigation could decolorize the DPPH free radicals. Generally, the data illustrated that the extract and all fractions showed inhibition against DPPH free radicals (Figure 2a). The higher concentration used showed a higher percentage of inhibition. At the same concentration, the highest antioxidant activity was shown by ethyl acetate fraction. At the lowest concentration of 7.87 μg/mL, ethyl acetate fraction had a percent inhibition of 20.36 ± 17.15%, followed by butanol (6.26 ± 0.70%), hexane (5.42 ± 1.43%), and water fractions (4.04 ± 0.73%). Meanwhile, at a medium concentration of 62.5 μg/mL, the highest percent inhibition was obtained at 64.82μ9.41% for the ethyl acetate fraction, followed by butanol 35.37 ± 0.93%, hexane 27.05 ± 0.56%,

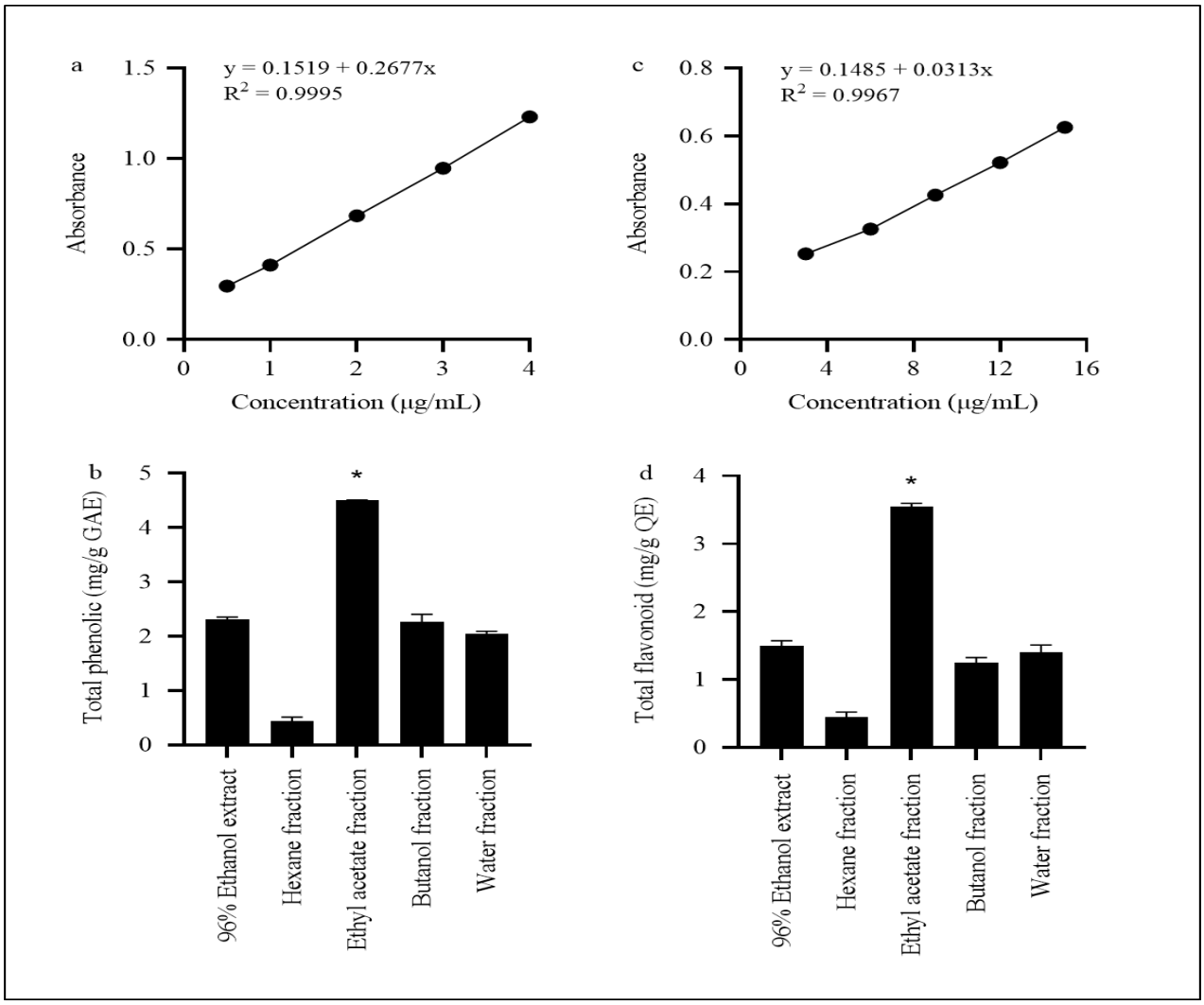

Figure 1. The Phytochemical Content in Different Bark Extracts and Fractions of *A. saponaria* (a) Calibration Curve of TP; (b) Total Phenolic; (c) Calibration Curve of TF; And (d) Total Flavonoid. Moreover, the Data Were Presented As Mean ± SD (n = 3). Furthermore, the Difference between the Groups Was Evaluated Using Anova Followed by Tukey's Post Hoc Test, with a Significance Level of $p < 0.05$. Here, Superscript (*) Signifies to All Groups.

and water fractions 26.17 ± 2.34%. However, vitamin C, a positive control, still showed better activity than the extract and all fractions. Antioxidant activity in beverages, foods, vegetables, and extracts has been quantified using vitamin C as reference. Moreover, hydrogen atom transfer mechanism can neutralize the DPPH free radical and vitamin C can donate hydrogen (De Menezes et al., 2021).

Moreover, the ATBS test was also conducted to evaluate the capability of extracts to eliminate the free radicals. The reduction of the ABTS color from deep bluish-green to colorless indicates the presence of antioxidants. Commonly, all the samples have antioxidant capability against ABTS radical (Figure 2b). At the lower concentration, 7.87 μg/mL, ethyl acetate fraction showed the highest activity of 19.18 ± 3.62%, followed by butanol (9.94 ± 1.71%), hexane (3.60 ± 3.60%), and water fractions (1.27 ± 1.20%). Furthermore, at the middle concentration, 62.5 μg/mL, ethyl acetate fraction showed the highest activity, followed by butanol > hexane > water. In contrast to trolox at 20 μg/mL, can inhibit the ABTS free radical to 54.94 ± 0.37%. Trolox is an analogue of vitamin E that is soluble in water. It is used as the standard to measure the ABTS antioxidant capacity. Through direct reduction and electron donation, TROX can neutralize the radical cation ABTS (Shahidi and Zhong, 2015).

Table 2 indicates the IC_{50} values of DPPH and ABTS radical scavenging assays. The IC_{50} means a concentration can inhibit fifty percent of free radical activity. The lower IC_{50} value means the higher antioxidant ability. Antioxidant activity can be divided into 5 categories: very strong ($IC_{50} < 50 \mu$g/mL), strong (IC_{50} 50 to 100 μg/mL), moderate (IC_{50} 100 to 150

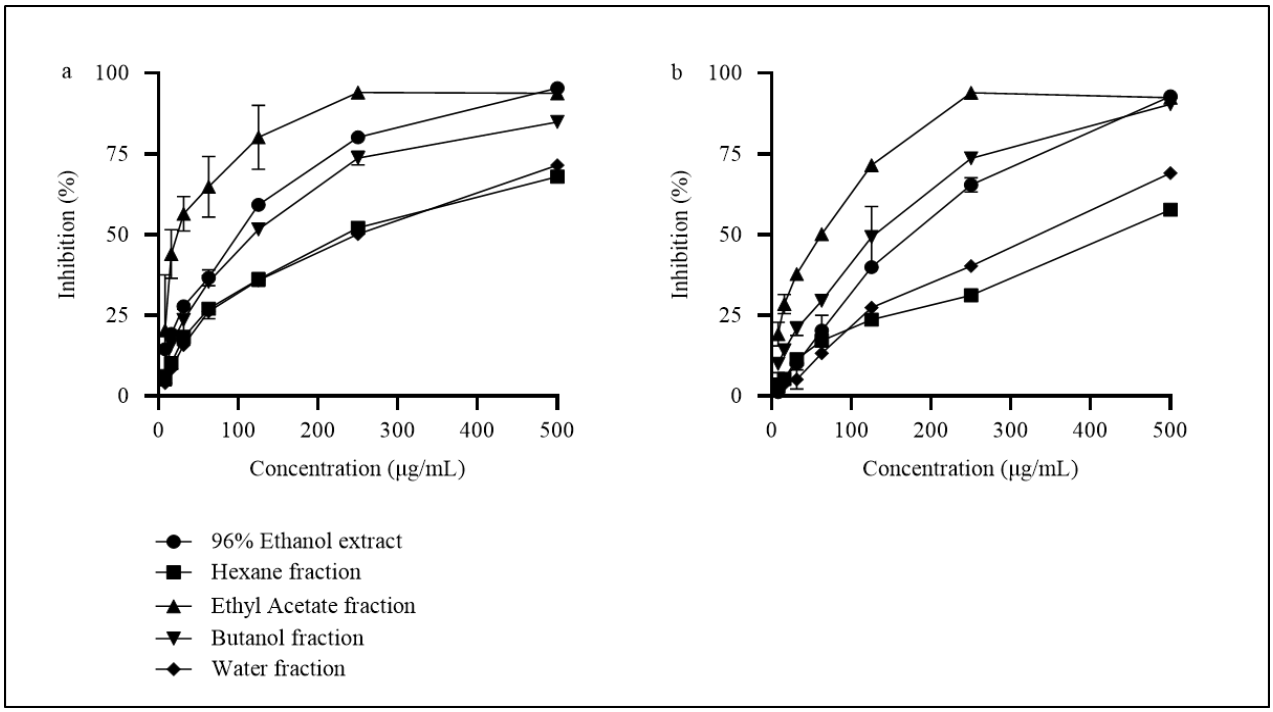

Figure 2. Antioxidant Activity of *A. saponaria* Bark Extract and Fractions against (a) DPPH Free Radicals and (b) ABTS Free Radicals

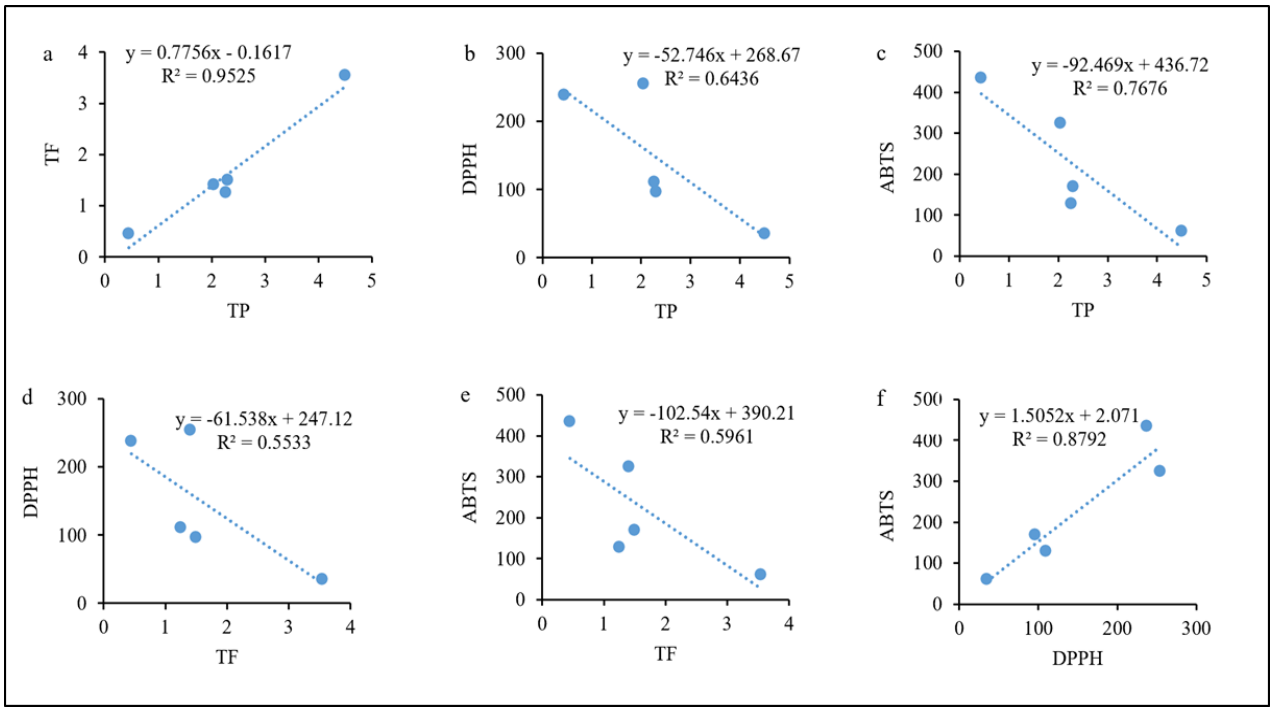

Figure 3. The Simple Scatter Plot Regression to Measure the Correlation between (a) TP and TF, (b) TP and DPPH, (c) TP and ABTS, (d) TF and DPPH, (e) TF and ABTS, and (f) DPPH and ABTS

μg/mL), weak (IC_{50} 150 to 200 μg/mL) and very weak when the IC_{50} more than 200 μg/mL (Maryam et al., 2023). On the DPPH assay, ethyl acetate fraction obtained a higher DPPH radical scavenging activity than all the other samples with an

IC_{50} value of 35.27 ± 2.85 μg/mL (very strong category). Butanol fraction (110.22 ± 2.01 μg/mL) had lower IC_{50} values compared to hexane fraction (237.83 ± 12.71 μg/mL) and water fraction (254.06 ± 16.97 μg/mL). However, the most potential antioxidant was vitamin C, with an IC_{50} value of 7.61 ± 0.04 μg/mL (very strong category) or 4.63 times more potent than the ethyl acetate fraction.

Additionally, in the ABTS radical method, the average IC_{50} value of the ethyl acetate fraction was 60.04 ± 0.98 μg/mL (strong category) or more potent than extract and fractions, as in Table 2. Butanol fraction and 96% ethanol extract showed a similar activity of 128.17 ± 14.48 μg/mL (moderate category) and 168.87 ± 6.92 μg/mL (weak category). The lowest activity was the hexane fraction of 433.89 ± 4.75 μg/mL (very weak category). However, the IC_{50} value of the sample was reasonably high compared to trolox, with an IC_{50} value of 17.93 ± 0.29 μg/mL (very strong category).

The extract's antioxidant properties might result from elevated TP and TF concentrations. The findings showed that the ethyl acetate fraction of *A. saponaria* exhibited more active than 96% ethanol extract and hexane, butanol, and water fractions. According to the ABTS assay results, the ethyl acetate fraction also showed the most significant inhibition. The 96% ethanol extract wood of *A. myriophylla* demonstrated antioxidant activities on the ABTS assay, with an IC_{50} value of 57.14 ± 0.13 μg/mL (Bakasatae et al., 2018).

Since phenolic hydroxyls form the core of phenolic acids' antioxidant capacity, the quantity and orientation of phenolic hydroxyls affect the compounds' antioxidant activity. Furthermore, the methoxy and carboxylic acid groups significantly impact the antioxidant capacity of phenolic acids (Chen et al., 2020). Concurrently, the fundamental structure of flavonoids is the flavan nucleus, including three rings containing fifteen carbon atoms (C6−C3−C6). By chelating metal ions and/or scavenging free radicals, flavonoid functional hydroxyl groups mediate their antioxidant actions. In order to stop radicals from damaging target biomolecules, metal chelation may be essential (Alara et al., 2021; Łodyga Chruscińska et al., 2018).

3.4 Antioxidant Activity in Relation to the Total Flavonoid and Phenolic Profiles

Phenolics and flavonoids are widely recognized as the most sizable phytochemical compounds derived from plants that possess antioxidant properties. Flavonoids are a group of phenolic compounds, but not all phenolics are flavonoid (Chen et al., 2020). Correlation between phenolics and flavonoids are needed to confirm whether phenolic, or flavonoid contribute to their antioxidant activity. Since the polarity of the compounds in each solvent used to measure antioxidant activity varies, the results are frequently consistent. This study investigated the correlation between TP, TF, and antioxidant activities of *A. saponaria* (Figure 3). The relationships were calculated using a simple regression using MS Excel 365. The TP positively correlated with TF (R^2 = 0.9525) but negatively correlated with DPPH (R^2 = 0.6436) and ABTS (R^2 = 0.7676). Like TP, TF negatively correlated with DPPH and ABTS, R^2 = 0.5533 and 0.5961, respectively. DPPH and ABTS, despite being antioxidant methodologies, exhibit a positive correlation (R^2) of 0.8792. A positive correlation indicates that as TP increases, so does TF.

On the other hand, a negative correlation means that the higher the TP, the lower the IC_{50} value of DPPH and ABTS. The extracts' antioxidant properties may be attributable to their higher phenolic and flavonoid content. The findings showed that the bark ethyl acetate fraction of *A. saponaria* had greater efficacy when compared to 96% ethanol extract and hexane, butanol, and water fractions. Additionally, Sobeh et al. (2017), reported that *A. harveyi* leaves are dominated by flavonoid compound (kaempferol glycosides, myricetin, and quercetin) and are probably responsible for the antioxidant properties. As has been reported for other species, the strong positive correlation between phenolic and flavonoid compounds and antioxidant activity indicated that phenolic and flavonoid compounds might contribute to antioxidant activity (Khiya et al., 2021; Muflihah et al., 2021).

4. CONCLUSION

The data in this paper supports the genus claims made for *A. saponaria* and demonstrates the bark's strong antioxidant properties. Our study showed that using the bark ethyl acetate fraction of *A. saponaria* resulted in higher antioxidant activity. These findings support that a diet high in plants and herbs may lower oxidation and protect against related illnesses. Even though our research suggests that *A. saponaria* has antioxidant qualities, more study needs to be done to separate and formulate the bark's active components for potential medicinal uses.

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