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Antioxidant Properties of Lemuni Leaves (*Vitex trifolia var. purpurea)* in Different Concentrations of Ethanol-Water Solvent Extraction

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Abstract. Vitex trifolia var. purpurea leaves (Lemuni leaves) is considered as a traditional medicine due to their antioxidant property. The antioxidant properties of Lemuni leaves depends on the concentration of ethanol-water solvent. Therefore, this current study intended to identify the antioxidant activity of Lemuni leaves (Vitex trifolia var. purpurea) in different concentrations of ethanol-water solvent extraction. Four ethanol-water concentrations were prepared (water extract (0% ethanol), 25% ethanol extract, 50% ethanol extract, and 75% ethanol extract). The antioxidant properties of Lemuni leaves were evaluated by Folin-Ciocalteu assay, aluminium chloride calorimetric method while 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay were used to measure the antioxidant activities of the extracts. The experimental results showed that 75% ethanol extract had the highest ($p<0.05$) total phenolic content (94.69 ± 4.75mg GAE/g DW) and total flavonoid content (30.76 ± 2.85mg QE/g DW) significantly. The 75% ethanol extract also had the significantly lowest ($p<0.05$) IC_{50} value for DPPH (3.56 ± 0.11 mg/mL) and ABTS (2.01 ± 0.05 mg/mL) assays. Strong correlations were discovered between the total flavonoid content, total phenolic content with antioxidant properties of Lemuni leaves at different ethanol-water ratio by DPPH ($r = -0.816$; $r = -0.824$) and ABTS assays ($r = -0.929$; $r = -0.920$). The significantly elevated antioxidant properties of the 75% ethanol extract suggest promising applications in health supplements, pharmaceuticals, skincare products, biomedical research, and commercial ventures.

Keywords: antioxidant activity, ethanol-water solvent, okra fruits, total phenolic content, total flavonoid content

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1. Introduction

Traditionally, plants have been utilised as drugs and medicines to treat specific illnesses. Plants used as traditional medicine can be traced back to 5000 years ago [1]. Even currently, plants have often been treated as a traditional medicine that could treat disease. Lalhminghlui and Jagetia [2] stated that traditional medicine is still in high demand by human beings today because of the

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safetyness, dependability and lesser side effects compared to modern medicine. In Malaysia, it has been reported that approximately 2000 medicinal plant species contain health-beneficial properties for the human body [3]. Most plants consist of bioactive phytochemicals, including flavonoids, alkaloids, and others that may benefit health. Therefore, medicinal property research, such as antioxidant property research, is the gate of exploring ways to improve the overall health status of human beings. *Vitex trifolia var. purpurea*, also commonly known as Lemuni, is a type of plant and is one of the species from the family Lamiaceae in the genus Vitex [4]. Traditionally, *Vitex trifolia var. purpurea* is classified as a medicine and is widely used against fever, pain, allergy, and inflammation [5]. In Traditional Chinese Medicine, *Vitex trifolia var. purpurea* is applied to cure headaches, migraine, colds, and eye pain [6]. Besides, it is also used as an antioxidant, anti-asthmatic, anti-microbial, anticancer, antidiabetic, analgesic, antiemetic, antituberculosis, hepatoprotective, anti-premenstrual syndrome, and antihistaminic [4]-[6]. Numerous studies have reported that antioxidants act as free radical scavengers that could mitigate the harm created by oxidative stress [7]-[9]. Because of unpaired electrons, free radicals and reactive oxygen species (ROS), are erratic and extremely reactive, henceforth, antioxidants work to stabilise these substances by giving an electron to neutralise the free radicals [10]. As a result, degenerative illnesses such as cancer, diabetes, and cardiovascular disease can be avoided. The extraction method using different solvents will affect the antioxidant properties of *Vitex trifolia var. purpurea* [11]-[12]. According to Xu *et al.* [9], one of the most significant aspects that could affect extraction efficiency is the type and concentration of solvents. The solvent type depends on the polarity and chemical nature of antioxidant contents that need to be extracted. For instance, polar and medium hydrophilic solvents such as ethanol and water are typically used for the extraction of hydro-soluble antioxidants like anthocyanin. Meanwhile, lipid-soluble antioxidants use common organic solvents such as hexane and ethyl acetate for extraction [9]. Ethanol is the most frequently used solvent extract for antioxidants. This is because, compared to other organic solvents like methanol, hexane, and ethyl acetate, ethanol is comparatively inexpensive, easily accessible, and primarily acknowledged as a green solvent. However, the best ethanol concentration in extracting the antioxidant content in *Vitex trifolia var. purpurea* leaves is yet to be identified. Thus, this study aims to investigate the effects of different concentrations of ethanol on the total polyphenol content, total content of flavonoid and antioxidant activity in Lemuni leaves *(Vitex trifolia var. purpurea)*.

2. Materials and Methods

2.1. Materials

In Taiping, Perak, the leaves of *Vitex trifolia var. purpurea* were harvested by the seller.The leaves were purchased when the plant was three months old.

2.2. Experimental Design

The fresh leaves of *Vitex trifolia var. purpurea* were purchased and transported to the laboratory in UPM for sample preparation. The leaves were freeze-dried (-80ºC) for three days, blended into powder form and sieved to obtain homogenous particles. Four ethanol-water concentrations were prepared, including water extract (0% ethanol), 25% ethanol extract, 50% ethanol extract, and 75% ethanol extract. Water extract [13]: Exactly 15g of *Vitex trifolia var. purpurea* leaves powder (sample) was homogenized with 100 ml of water at room temperature. Then, the mixture was stirred at 120 rpm in an orbital shaker for 2 hours at room temperature. The mixture was centrifuged at 4000 rpm for 15 minutes. The mixture was filtered using a Whatman No. 1 filter paper. After that, the filtrate was freeze-dried. The filtrate was kept at -20℃ until further use. Similar treatments were used for the ethanol extract [13]: 75% aqueous ethanol, 50% aqueous ethanol and 25% aqueous ethanol. All the filtrates were kept at -20℃ until further use.

2.3. Determination of Total Phenolic Content (TPC)

With a few adjustments, the modified Folin-Ciocalteu technique [8] was used to identify the extracts' total phenolic content (TPC). 0.5 mL of the Folin-Ciocalteu reagent, 7.5 mL of distilled water, and 0.5 mL of plant extract were blended together. The resultant combination was then allowed to sit at ambient for ten minutes. After adding 1.5 mL of a 20% sodium carbonate solution, the mixture was left to incubate for an additional 20 minutes at 40°C in a water bath. To produce colour, the cuvettes containing the mixtures were vortexed for 15 seconds and then left to stand at 40°C for 30 minutes. The absorbance was then measured using the UV-VIS spectrophotometer at 765 nm. The concentration vs. absorbance of gallic acid was plotted as the standard to create a calibration curve. A standard curve was developed using various gallic acid concentrations (0.001, 0.005, 0.01,0.02, 0.04, 0.06, 0.08, & 0.1 mg/mL). Every test was conducted in triplicate. Results were reported using the equation based on the calibration curve as mg gallic acid equivalent (GAE) per g dry weight. The samples' TPC was determined using the following formula:

$$\text{Total Phenolic Content, TPC} = \frac{R \times V}{W} \quad (1)$$

where R = x value acquired from the curve of standard; V = samples' volume used; W = samples' weight used for extraction

2.4. Determination of Total Flavonoid Content

With slight adjustments, colorimetry was used to evaluate the flavonoid content (TFC) using the aluminium chloride calorimetric assay [12]. Prior to adding 0.15 mL of sodium nitrite (5% $NaNO_2$, w/v), 10 mg of plant extracts were first dissolved in 1 mL of distilled water and allowed to stand for five minutes. Following a 6-minute incubation period, 0.15 mL of aluminium trichloride (10% $AlCl_3$) was added. Next, 2 mL of sodium hydroxide (NaOH, 4% w/v) was added, and volume was increased to 5 mL with distilled water. Following a 15-minute incubation period,

the mixture took on a pink colour, and a spectrophotometer was used to detect its absorbance at 510 nm. As a blank, distilled water was utilised. The quercetin equivalents (QE) per gram of extract (mg) used to express the TFC. A standard curve was created using various quercetin concentrations (0.025, 0.05, 0.01, 0.02, 0.04 & 0.08 mg/mL). Every test was conducted in triplicate. The following formula was used to determine the samples' TFC:

$$\text{Total Flavonoid Content, TFC} = \frac{R \times V}{W} \tag{2}$$

where R = x value acquired from the curve of standard; V = total volume of sample used; W = weight of sample used for extraction

2.5. Determination of Antioxidant Activity

2.5.1. 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay

With certain adjustments, the DPPH free radical scavenging technique was used to find the antioxidant activity of the plant extracts [7]. At the same amounts as the extract, vitamin C served as the standard for antioxidants. After adding 1 mL of the extract and 3 mL of the corresponding solvent, 0.5 mL of 1 mM DPPH in methanol was added to the test tube. After giving the mixture a vigorous shake, it was darkened for half an hour. The equal quantity of DPPH and methanol were added to a blank solution. The sample was prepared at the following concentrations: 0.05, 0.1, 0.5, and 1.0 mg/mL in a cuvette that was placed in the spectrophotometer and the solvents used were water, 25% ethanol, 75% ethanol, and 50% ethanol. With a UV-vis spectrophotometer, the absorbance of the final solution was determined at 517 nm. Following the triplicate execution of each test, the following equation was used to determine the radical scavenging activity:

$$\% \text{ DPPH radical scavenging} = \{[A_b - A_a]/A_b\} \times 100 \tag{3}$$

where A_b = absorption of the blank sample; A_a = absorption of the extract

Next, the extractions' concentration was plotted against the percentage of DPPH radical scavenging activity on a graph, which allowed for the determination of the graph's IC_{50} value, the concentration at which 50% inhibition occurs. The amount of antioxidants required overall to reduce the concentration of DPPH radicals by 50% is known as the IC_{50} value.

2.5.2. 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

With minor adjustments, the ABTS radical cation decolorization method was applied to determine the activity of antioxidant of the extracts of the plants [8]. Before being used, ABTS (7 mM) and potassium persulfate (2.45 mM) were allowed to react at room temperature in the dark for 12 to 16 hours, producing the ABTS cation. Methanol was added to the ABTS solution to achieve a final absorbance of 734 nm. The absorbance of the blue-green colour of the produced cation was measured by mixing 990 µL of ABTS solution with 10 µL of test solution. After six minutes, the absorbance decreased and was compared to the control. The standard was trolox, while the blank control was distilled water. Every test was conducted in triplicate. The following equation was

used to compute the ABTS radical cation scavenging activity, which was represented in milligram Trolox equivalent per gram of dry weight (mgTE/g dry Weight):

$$\% \text{ ABTS radical scavenging} = \{[A_b - A_a]/A_b\} \times 100 \quad (4)$$

where A_b = absorption of the blank sample; A_a = absorption of the extract

To find the concentration for 50% inhibition (IC_{50} values), a graph was created by plotting the proportion of ABTS radical cation decolorization activity against the extractions' concentration. The amount of antioxidants required overall to reduce the starting ABTS concentration by 50% is known as the IC_{50} value.

2.6. Statistical Analysis

The difference between various amounts of ethanol-water solvent extraction for *Vitex trifolia var. purpurea* leaves with the TPC, TFC and antioxidant activity was assessed using a one-way ANOVA with posthoc Tukey HSD Test. Also, employing varying concentrations of ethanol-water solvent extraction for *Vitex trifolia var. purpurea* leaves, the Pearson correlation test was applied to determine correlations between antioxidant content and antioxidant capability. All statistical data should be reviewed and analysed using the statistical programme SPSS version 25 (IBM et al., USA). A corrected p-value of less than 0.05 indicated that a difference was significant.

3. Results and Discussion

3.1. Total Phenolic Content (TPC)

The TPC in different concentrations of ethanol-water solvent extraction of *Vitex trifolia var. purpurea* leaves was arranged in descending order; 75% ethanol (94.69 ± 4.75mg GAE/g DW) > 50% ethanol (45.85 ± 9.88mg GAE/g DW) > 25% ethanol (22.58 ± 5.03mg GAE/g DW) > Water (8.58 ± 1.86mg GAE/g DW). There were significant differences ($p < 0.05$) in TPC between the 75% ethanol with 50% ethanol, 25% ethanol and water, and 50% ethanol with 25% ethanol and water. In contrast, one-way ANOVA showed that TPC between 25% ethanol and water was not significantly different ($p > 0.05$).

Table 1. Total Phenolic Content (TPC) of *Vitex trifolia var. purpurea* Leaves in Different Concentration of Ethanol-Water Solvent Extraction

Extract of the Sample	Total Phenolic Content (mg GAE/ g DW)
Water	8.58 ± 1.86[a]
25% ethanol	22.58 ± 5.03[a]
50% ethanol	45.85 ± 9.88[b]
75% ethanol	94.69 ± 4.75[c]

Note: Mean ± standard deviation were used to express value (n = 4). Means with different letters are significantly different at $p < 0.05$

In this study, it was found that 75% ethanol extraction had the greatest amount of total phenolic content in *Vitex trifolia var. purpurea* leaves. This may be because Junka *et al.* [14] reported that the optimum ethanol-water solvent extraction could have the highest amount of phenolics due to the polarity index. Nguyen *et al.* [15] explained that the solubility of the solvent would be strongly affected by the ethanol concentration due to the polarity of the solvent. Besides, aqueous solvents such as ethanol-water are more appropriate for extracting bioactive compounds with a broad polarity range [16]. Variations in ethanol content changed the solvent's degree of polarisation and hydrogen bond strength, impacting the extraction yield process [15]. Furthermore, ethanol can penetrate the cell wall easily as it is permeable to the cell wall, and water further enhances the solubility of the polar substances, resulting in a high amount of total phenolic content in ethanol-water extraction [17]. According to Yusof *et al.* [18], the extraction efficiency will be elevated when the solvent polarity is low, as low solvent polarity increases the solubility of the phenolic compounds. Thus, 75% ethanol extract has the greatest total phenolic content contrasted with other concentration and water extracts as it could facilitate efficient polar substances.

3.2. Total Flavonoid Content (TFC)

The TFC in different concentrations of ethanol-water solvent extraction of *Vitex trifolia var. purpurea* leaves was arranged in descending order; 75% ethanol (30.76 ± 2.85mg QE/g DW) > 50% ethanol (18.05 ± 4.17mg QE/g DW) > 25% ethanol (7.96 ± 2.57mg QE/g DW) > Water (3.58 ± 1.77mg QE/g DW). There were significant differences ($p < 0.05$) in TFC between the 75% ethanol with 50% ethanol, 25% ethanol and water, and 50% ethanol with 25% ethanol and water extract. TFC between 25% ethanol and water was not significantly different ($p > 0.05$) according to one-way ANOVA. Most flavonoids are semi- or medium-polar, and ethanol might be a better option than aqueous; hence, solvents with lower polarity are recommended to increase extraction efficiency [18].

Table 2. Total Flavonoid Content of *Vitex trifolia var. purpurea* Leaves in Different Concentration of Ethanol-Water Solvent Extraction

Extract of the Sample	Total Flavonoid Content (mg QE/g DW)
Water	3.58 ± 1.77^{a}
25% ethanol	7.96 ± 2.57^{a}
50% ethanol	18.05 ± 4.17^{b}
75% ethanol	30.76 ± 2.85^{c}

Note: Mean ± standard deviation were used to express value (n = 4). Means with different letters are significantly different at $p < 0.05$

A higher affinity solvent for the samples allowed the solvent to penetrate the solid matrix more effectively, increasing the solutes' efficiency and, consequently, the extraction efficiency [18]. It is exceedingly improbable that flavonoids will dissolve in the highly polar extraction solvent. Flavonoids became more soluble as the polarity of the solution drew closer to them, according to the "like dissolves like" principle [19]. Moreover, purple leaves contain a high amount of anthocyanin, one of the subgroups of flavonoid compounds. Anthocyanins contain a hydrocarbon that is resistant to water, but they also consist of polyphenol compounds that are very soluble in both polarised solvents and water [20]. Thus, the appropriate organic solvent for extracting anthocyanins should be chosen, followed by a water mixture. A rise in ethanol content reduced the polarity difference between the anthocyanins and solvent, which in turn caused the reduction of other water-soluble components, such as pectin and polysaccharide that prevent the anthocyanins from dissolving [21]. The yield of anthocyanin extraction tended to rise gradually as ethanol concentration rose. A higher ethanol concentration may cause some liposoluble elements to dissolve, whereas a lower ethanol concentration may result in an incomplete extraction [22]. Since the extraction yields highly rely on the solubility of flavonoids in an ethanol solution, the proportion of ethanol is a crucial variable.

3.3. 2,2'-diphenyl-1-picrylhydrazyl (DPPH) Assay

The quantity of antioxidants needed to reduce the concentration of DPPH by 50% is expressed by the IC_{50} of a chemical, which is calculated by extrapolating the results of a linear regression study. A lower IC50 indicates a chemical's more potent antioxidant activity [23]. The IC_{50} value in different concentrations of ethanol-water solvent extraction of *Vitex trifolia var. purpurea* leaves was arranged in ascending order, 75% ethanol (3.56 ± 0.11 mg/mL) < 50% ethanol (3.97 ± 0.08 mg/mL) < 25% ethanol (4.66 ± 0.21 mg/mL) < water (4.97 ± 0.02 mg/mL). There were significant differences ($p < 0.05$) in IC_{50} between 75% ethanol extract with 50% ethanol extract, 25% ethanol extract and water extract, 50% ethanol extract with 25% ethanol and water extract, and 25% ethanol extract with water extract, according to one-way ANOVA. This is because the phenolic compounds belong to a class of phytochemicals whose molecules are simple to oxidize and can give reactive compounds hydrogen atoms while protecting against free radicals [24].

Table 3. IC_{50} in Different Concentration of Ethanol-Water Solvent Extraction of *Vitex trifolia var. purpurea* Leaves

Extract of the Sample	IC_{50} (mg/ mL)
Water	4.97 ± 0.02^{a}
25% ethanol	4.66 ± 0.21^{b}
50% ethanol	3.97 ± 0.08^{c}
75% ethanol	3.56 ± 0.11^{d}

Note: Mean ± standard deviation were used to express value (n = 4). Means with different letters are significantly different at $p < 0.05$

Boo *et al.* [25] stated that the higher the content of phenolic compounds, the higher the electron donor capacity. Numerous distinct chemicals with various polarities were present in the plant extracts, giving them their antioxidant activity. As the solubility of polyphenols varies with ethanol concentration, the ethanol concentration has a considerable impact on antioxidant activity [26]. It was clear that the antioxidant chemicals in extracts varied in polarity. Antioxidant activity can be altered by altering the solvent's polarity [27]. It proposed that a certain amount of water added to organic solvents could make it easier to extract antioxidants from plants. There is also evidence that polyphenols can be extracted more effectively from aqueous organic solvents than pure organic solvents [27]. Thus, it was reasonable to assume that the *Vitex trifolia var. purpurea* leaves extract treated with 75% ethanol would contain significant phenolic compounds that can serve as hydrogen-donating radical scavengers, which is utilised as antioxidant. This is because IC_{50} in DPPH assay represents the concentration of an antioxidant required to scavenge 50% of the DPPH radicals present in the solution. It is a measure of the antioxidant potency of the compound being tested, with lower IC_{50} values indicating higher antioxidant activity.

3.4. 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Assay

The quantity of antioxidants needed to reduce the concentration of ABTS by 50% is expressed by the IC_{50} value of a chemical, which is calculated by extrapolating the results of a linear regression study. According to Faisal and Handayani [28], the IC_{50} value negatively correlates with antioxidant activity; the lower the IC_{50} value, the higher the antioxidant activity. The IC_{50} in different concentrations of ethanol-water solvent extraction of *Vitex trifolia var. purpurea* leaves were arranged in ascending order, 75% ethanol (2.01 ± 0.05 mg/mL) < 50% ethanol (2.05 ± 0.03 mg/mL) < 25% ethanol (2.16 ± 0.03 mg/mL) < water (2.34 ± 0.06 mg/mL). According to one-way ANOVA, there were significant differences ($p < 0.05$) in IC_{50} between 75% ethanol extract with 25% ethanol extract and water and 50% ethanol extract with 25% ethanol and water. In comparison, IC_{50} between 75% ethanol and 50% ethanol were not significantly different ($p > 0.05$). Most plants primarily consist of phenolic chemicals, which have been found to have antioxidant and free radical scavenging properties [29].

Table 4. IC_{50} in Different Concentration of Ethanol-Water Solvent Extraction of *Vitex trifolia var. purpurea* Leaves

Extract of the Sample	IC_{50} (mg/ mL)
Water	2.34 ± 0.06^{a}
25% ethanol	2.16 ± 0.03^{b}
50% ethanol	2.05 ± 0.03^{c}
75% ethanol	2.01 ± 0.05^{c}

Note: Mean ± standard deviation were used to express value (n = 4). Means with different letters are significantly different at $p < 0.05$

The solvent's polarity may have been caused by the solubility of phenolic compounds having antioxidant properties [30]. Their solubility was extremely sensitive to the polarity of the solvent,

and antioxidant capabilities were immediately polar [31]. In addition, Ra *et al.* [32] reported that greater ethanol concentration showed higher radical scavenging activity. In contrast, lower ethanol concentrations of the extract tended to be related to lower ABTS radical scavenging activity. Moreover, distilled water has the highest dielectric constant compared with other ethanol extracts with different concentrations [15]. This affected the solvent's level of polarization and hydrogen bond strength, thus influencing the extract's antioxidant activity. The capacity to scavenge free radicals was higher in all the extracts produced using an organic solvent that was pure and aqueous than in the water extract [23]. The varying polarity levels of the mixture of ethanol and water increase the antioxidant activity obtained when using different solvents [14]. However, too much water also accelerated the extraction of other substances, thus lowering the quantity of phenols in the extracts [33]. Therefore, *Vitex trifolia var. purpurea* leaves extract treated with 75% ethanol contains significant phenolic compounds that can serve as hydrogen-donating radical scavengers. The value for both assays is different because although antioxidant activity is measured by both assays, there may be subtle differences in the specific radicals targeted and the underlying mechanisms [23]. For the same molecule, it is therefore feasible that the IC_{50} values obtained from the DPPH test and the ABTS assay will differ [23].

3.5. Correlation Between Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity

As indicated in Table 5, the association between TPC, TFC, and antioxidant activity was investigated using the Pearson correlation test. TPC and DPPH radical scavenging activity showed a significant negative correlation ($r = -0.816$, $p < 0.05$). Furthermore, DPPH radical scavenging activity and total flavonoid concentration showed a statistically significant negative correlation ($r = -0.824$, $p < 0.05$). Furthermore, there was a high and negative association between the phenolic content ($r = -0.929$, $p < 0.001$) and the flavonoid content ($r = -0.920$, $p < 0.001$) and the ABTS radical scavenging activity. Consequently, TPC and TFC have a significant impact on and influence the antioxidant activity of *Vitex trifolia var. purpurea*. Lemuni leaves have a significant level of antioxidant activity, as evidenced by the decreasing IC_{50} value with increasing TPC and TFC.

Table 5. Pearson Correlation Between Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of *Vitex trifolia var. purpurea* Leaves

Antioxidant Activity	Total Phenolic Content (TPC)	Total Flavonoid Content (TFC)
DPPH Radical Scavenging Activity (IC_{50})	-0.816*	-0.824*
ABTS Radical Scavenging Activity (IC_{50})	-0.929**	-0.920**

Note: **Correlation is significant at p<0.001, *Correlation is significant at p<0.05

The result from this study can be supported by Boo *et al.* [25], who reported there was a strong and negative association between DPPH radical scavenging activity with the TFC as well as the TPC of *Brassica oleracea L. var. Capitata f. Rubra*, due to the DPPH radical scavenging activity,

was depended on the concentration of antioxidant. Moreover, Boo *et al.* [25] demonstrated a robust correlation between the total phenolic content and the total flavonoid content of *Brassica oleracea L. var. Capitata f. Rubra* with ABTS radical scavenging activity. Other than that, the study by Ri *et al.* [34] discovered that the TPC and TFC of *Aurea Helianthus* were directly proportional to their antioxidant activity. Furthermore, there was a potent correlation between TFC and TFC with DPPH radical scavenging activity and ABTS radical scavenging activity for *Ocimum sanctum Linn* [35]. In short, the antioxidant activity of *Vitex trifolia var. purpurea* leaves will be affected by TPC and TFC. The amount of polyphenols and flavonoids in a plant extract determines how effective an antioxidant it is [28].

4. Conclusion and Recommendation

The study aimed to assess the antioxidant activity and content of *Vitex trifolia var. purpurea* leaves using various ethanol-water solvent concentrations for extraction. According to the results, when compared to other ethanol concentrations, the 75% ethanol extract had the highest total phenolic and flavonoid content and the lowest IC_{50} values for DPPH and ABTS scavenging activities. Strong negative correlations were found between IC_{50} values and total phenolic/flavonoid contents, indicating that higher phenolic and flavonoid contents led to stronger radical scavenging capacity. Additionally, the significantly elevated antioxidant properties of the 75% ethanol extract suggest promising applications in health supplements, pharmaceuticals, skincare products, biomedical research, and commercial ventures. However, further research considering factors like plant maturity, origin, and location is necessary for a comprehensive understanding. In conclusion, the study suggests that adding less water to ethanol extracts enhances the antioxidant activity of *Vitex trifolia var. purpurea* leaves, making the 75% ethanol extract a promising source of natural antioxidants for utilization and processing.

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