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Research Article

Mechanisms of action of total phenolics and flavonoids in kalangkala (*Litsea angulata* Blume) leaf extract and fractions, and their correlation with in vitro antioxidant potential

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Abstract

Phenolic and flavonoid compounds are widely found in a variety of plant species, including kalangkala (*Litsea angulata* Blume), a species endemic to South Kalimantan, Indonesia. Phenolic and flavonoid compounds are the compounds responsible for antioxidant activity. The objective of this study was to examine the mechanisms of action of total phenolic content (TPC) and total flavonoid content (TFC) in kalangkala leaves, as well as their correlation with in vitro antioxidant activity against ABTS free radicals. The study entailed the extraction of kalangkala leaves through maceration with 70% ethanol, followed by multilevel fractionation. Determination of the total phenolic content and total flavonoid content was also conducted, along with the assessment of antioxidant activity and the determination of their respective correlations. The results showed that the total phenolic content of the ethanol extract was 51.65 ± 4.04 mg GAE/g, the n-hexane fraction was 64.39 ± 7.63 mg GAE/g, the ethyl acetate fraction was 97.05 ± 1.46 mg GAE/g, and the methanol fraction 28.96 ± 2.22 mg GAE/g. The total flavonoid content of the ethanol extract was 22.19 ± 0.91 mg QE /g, the n-hexane fraction was 20.27 ± 2.38 mg QE /g, the ethyl acetate fraction was 88.07 ± 1.34 mg QE /g, and the methanol fraction was 99.62 ± 1.97 mg QE /g. The antioxidant activity of the ethanol extract was 51.23±3.86 ppm/ml, the n-hexane fraction was 159.44±2.93 ppm/ml, the ethyl acetate fraction was 136.01±6.47 ppm/ml, and the methanol fraction was 93.28±0.66 ppm/ml. The total phenolic content of the extract and fractions of kalangkala leaves exhibited a significant correlation ($p<0.05$) with the antioxidant activity of the leaves, as indicated by a correlation coefficient of 0.580. In contrast, the total flavonoid content of the extract and fractions of kalangkala leaves did not demonstrate a significant correlation with the antioxidant activity the plant material, as evidenced by a correlation coefficient of -0.224. The study concluded that a strong antioxidant is present in the 70% ethanol extract of kalangkala leaves, with a value of 51.65±4.04, as well as in the methanol fraction, with a value of 93.28±0.66 ppm/ml. Additionally, the study establishes a correlation between the total phenolic and flavonoid content present in the ethanol extract, n-hexane fraction, ethyl acetate fraction and methanol fraction of the kalangkala leaves and the antioxidant activity of the plant material.

Keywords: total phenolic, total flavonoid, antioxidant, ABTS, kalangkala leaves (*Litsea angulata* Blume)

Introduction

Polyphenol compounds are abundant in plants and have been identified as as potential antioxidants capable of combating oxidative stress and various diseases, such as metabolic diseases [1]. These compounds represent a class of active compounds found in natural ingredients [2]. Phenolic compounds produced by plants have activity in response to environmental stress. These compounds have the potential to act as protectors against UV-B rays and to preserve DNA integrity, preventing cell damage and death [3]. Flavonoid compounds are a group of phenolic compounds that are found in abundance in plants and have been reported to have antioxidant activity [4, 5]. These compounds have been identified in virtually all parts of plants, including fruits, leaves, flowers, seeds, and roots [6].

Flavonoid compounds have been reported as antioxidants with the capacity to impede adipose tissue accumulation, thereby reducing the risk of obesity, diabetes mellitus, cancer, heart disease, and degenerative diseases [7, 8]. The aim of

administering antioxidants is to prevent the formation of intracellular free radicals by increasing the levels of endogenous enzymes or defense mechanisms against free radicals, thereby inhibiting the occurrence of oxidative stress related to diabetes [9]. Antioxidant activity is based on the capacity of a compound to scavenge free radicals and inhibit damage caused by oxidative stress. The flavonoid and total phenolic compounds present in kalangkala leaf extract are hypothesized to have antioxidant activity mechanisms in vitro, potentially addressing the antioxidant needs of individuals with diabetes mellitus and reducing oxidative stress levels in these individuals. The use of antioxidants in diabetes precipitated by oxidative stress has been reported to demonstrated efficacy. Nevertheless, the effectiveness and safety of antioxidant-containing supplements for treating diabetes require further investigation.

One Indonesian endemic plant that has been reported for its medicinal properties is kalangkala. Kalangkala (*Litsea angulata* Blume) is classified within the genus Litsea, which is part of the Lauraceae family. Kalangkala has been observed to grow in certain regions of Kalimantan, notably South Kalimantan and East Kalimantan, as well as Sumatra Island, and Java Island [10]. It has been reported to contain secondary metabolites such as flavonoids, phenolic, alkaloids, saponins, tannins, terpenoids, carotenoids, and coumarins [11, 12]. Several studies have documented the pharmacological activities of kalangala, including its properties as an antioxidant, antibacterial agent, spermicide, and antidiabetic agent [13]. A number parts of kalangkala have been the subject of scientific study. For instance, the bark has been found to possess antioxidant properties, with an IC_{50} value of 85.33 ppm [14]. However, the mechanisms of action of total phenolic and flavonoid content of *Litsea angulata* Blume leaf extract and fraction have never been reported. Therefore, this study aims to examine the mechanisms of action of total phenolic and flavonoid content in *Litsea angulata* BIume leaf extract and fractions, as well as the potential correlation between the mechanisms and in vitro antioxidant activity.

Materials and Methods

Preparation of 70% ethanol extract of *Litsea angulata* BIume leaves

This study used leaf samples of kalangkala (*Litsea angulata* BIume) obtained in September 2024 from South Kalimantan, Indonesia. The samples were identified in the Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, South Kalimantan, with the number 208/ LAB.LABDASAR/IX/2024. Three hundred grams of kalangkala leaf powder were macerated using 70% ethanol at a ratio of 1:7 (w/v) and stirred for 1 hour using a top stirrer each day for a total of three consecutive days at room temperature. The collected filtrate was filtered using a Buchner funnel. The residual material was then subjected to a second round of maceration for two consecutive days. Subsequently, all extracted filtrates were concentrated using a rotary evaporator (Heidolph, Thermo Fisher Scientific, USA) at a temperature ranging from 50°C to 60°C with a speed of 40-70 rpm. The obtained extract was re-concentrated using a water bath at a temperature of 50°C until a thick ethanol extract of kalangala leaves with a content of 70% was obtained [11].

Preparation of fractionation of *Litsea angulata* BIume leaves

The obtained 10 grams of 70% thick ethanol extract of *Litsea angulata* BIume leaves were subjected to fractionation with n-hexane at a ratio of 1:4 (ethanol extract:n-hexane) using a magnetic stirrer to facilitate the dissolution of the extract during the fractionation process [11]. The insoluble n-hexane portion was fractionated with ethyl acetate solvent, utilizing a ratio of 1:4 (extract: ethyl acetate). The insoluble ethyl acetate fraction was fractionated with a methanol solvent at a ratio of 1:4 (ethanol extract: methanol). Each fraction was then evaporated over a water bath at 50°C in order to yield the n-hexane fraction, the ethyl acetate fraction, and the methanol fraction [15].

Determination of total phenolic content

The ethanol extract and leaf fractions of *Litsea angulata* BIume were each weighed at a maximum of 80 mg and then dissolved with 10 ml of pro-analysis methanol. A volume of 1 ml of the test solution was dispensed by means of a pipette. Thereafter, 5.0 ml of Folin reagent, 4.0 ml of 1% NaOH, and 2.8 ml of distilled water were added. The soultion was then left to incubate according to the prescribed operating time. Absorbance measurement was conducted by UV-Vis spectrophotometry (Shimadzu UV-1800, USA) at a wavelength of 764.80 nm. The replication process was conducted three times

$$Total\ Phenolic\ Content = \frac{Cons\ sampel\left(\frac{mg}{ml}\right) x vol(ml)}{massa\ sampel\ (g)} x100\% \quad (1)$$

Determination of total flavonoid content

The ethanol extract and fractions of *Litsea angulata* BIume leaves were each weighed at a precision of 80 mg and then dissolved with 10 ml of pro-analysis methanol. A volume of 1.0 ml and 0.5 ml of the test solution was dispensed using a pipette, followed by the addition of 0.1 ml of 10%

$AlCl_3$, 0.1 ml of 1 M sodium acetate, and 2.8 ml of distilled water. The solution was then subjected to incubation according to the designated operating time. Absorbance measurement was obtained through the use of UV-Vis spectrophotometry (Shimadzu UV-1800, USA) at a wavelength of 431.6 nm. The replication process was conducted three times.

$$Total\ flavonoid\ content = \frac{cons\ sampel\ \left(\frac{mg}{ml}\right) x vol(ml)}{massa\ sampel(g)} x100\% \quad (2)$$

Determination of in vitro antioxidant activity using the ABTS assay

A volume of 0.5 ml of each extract and fraction was collected, following which 0.5 ml of ABTS solution was added and the mixture was incubated for a period of six minutes. Absorbance was measured with a UV-Vis spectrophotometer (Shimadzu UV-1800, USA) with a wavelength of 734 nm [16]. Subsequently, the percentage of inhibition, linear regression equation, and IC_{50} were calculated. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{inhibisi} = \frac{(Abs\ blanko - Abs\ sampel)}{Abs\ blanko} x100 \quad (3)$$

The calculation of IC_{50} was performed using the formula y=bx +a, where y=50. Therefore, IC_{50} can be determined by $x = \frac{(50-a)}{b}$.

Statistical analysis

All test treatments were replicated three times. The results obtained are presented in the form of average ± standard deviation (SD). The results obtained were analyzed using SPSS version 23.0. The correlation between total phenolic and total flavonoid content and antioxidant activity is expressed as a Pearson correlation coefficient.

Results and Discussion

Mechanism of antioxidant activity: Total phenolic content and total flavonoid content

The total phenolic content was determined using standard gallic acid with a concentration series of 15, 30, 50, 75, 100 ppm, with a maximum wavelength obtained of 764.80 nm. The determination of total flavonoid content (TFC) was conducted using quercetin as a standard, with a concentration series of 20, 30, 40, 50, 60 ppm, with a maximum wavelength obtained of 431.60 nm. The result of measuring quercetin (standard) absorbance using a concentration series indicated that an increase in concentration resulted in an increase in the measured absorbance. The total phenolic content (TPC) of the extract and each fraction was determined using gallic acid as a standard. A linear regression equation of the calibration curve was obtained, $y = 0.006x-0.0057$, with an R^2 value of 0.998 from the absorbance value of gallic acid standard. The total phenolic content was ascertained quantitatively using the Folin-Ciocalteu method. The resultant total phenolic content is expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g). The determination of total flavonoid content was achieved through the establishment of a linear equation derived from the measured absorbance of several concentration series. This equation, expressed as $y = 0.0064x-0.0194$, yielded an R^2 value of 0.9851.

The quercetin standard curve equation was used as a standard to determine the quantity of total flavonoid compounds present in the extract. The total flavonoid content was determined using the $AlCl_3$ method, and the results are expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g). The principle underlying the $AlCl_3$ method is the formation of a stable $AlCl_3$ complex with the C-3 atom or C-5 atom adjacent to the flavonol and flavone groups [17]. Total flavonoid testing uses an $AlCl_3$ solution that forms a complex, causing a shift in the wavelength toward the visible area that is indicated by a more yellow color. The addition of potassium acetate with the aim of maintaining the wavelength in the visible area is also part of the procedure [18]. Aluminum chloride reacts with the carbonyl group of flavonoids, resulting in a stable complex [19]. The results of the aforementioned measurements are presented in **Table 1**.

Table 1. The results of the measurements of total phenolic and total flavonoid content of the extracts and fractions of leaves of kalangkala (*Litsea angulata* BIume)

Sample	Total Phenolics (mg GAE/g ± SD)	Total Flavonoids (mg QE/g ± SD)
Ethanol extract	51.65± 4.04	22.19± 0.91
n-hexane fraction	64.39± 7.63	20.27± 2.38
Ethyl acetate fraction	97.05± 1.46	88.07± 1.34
Methanol fraction	28.96± 2.22	99.62± 1.97

As presented in **Table 1**, the total phenolic contents (TPC) in the ethanol extract, n-hexane fraction, ethyl acetate fraction, and methanol fraction were 51.65 ± 4.04, 64.39 ± 7.63, 97.05 ± 1.46, and 28.96 ± 2.22 mg GAE/g, respectively. The ethyl acetate fraction demonstrated the highest total phenolic content, measuring 97.05 ± 1.46 mg GAE/g, compared to the ethanol extract and other fractions. The results obtained illustrate that 1 gram of ethyl acetate fraction contains 97.05 ± 1.46 mg of gallic acid., indicating that the majority of the antioxidant compounds found in plants are polyphenol compounds [17]. The total phenolic content was determined in this study using Folin-Ciocalteu reagent, which has the ability to react with phenol and non-phenol compounds, such as reducing agents and ascorbic acid [20]. This study corroborates the findings of a prior study, which reported that the ethyl acetate fraction obtained the highest total phenolic content, namely 156.33 mg GAE/g, in comparison to other fractions [21]. The ethyl acetate fraction is likely to contain a significant amount of ascorbic acid, which could result in a comparatively higher total phenolic content in comparison to other extracts and fractions [22]. The high phenolic content may also be due to the large content of betacyanin (pigment) in the plant, relative to its polyphenol content [23]. The high total phenolic content suggests that a greater quantity of phenolic compound components are released by the solvent. The prolonged extraction time has also been demonstrated to increase the withdrawal of polyphenolic compound components [24]. Phenolic compound components are a group of secondary metabolites that are found to be present in abundance in herbs and have antioxidant and anti-inflammatory properties that promote health [25]. The stability of phenol compound components is greatly influenced by heat, light and oxygen, so it is necessary to exercise discernment in selecting the extraction method to ensure the preservation of phenolic components [26]. Phenolic compounds are composed of aromatic rings with hydroxyl groups, acylated sugars, and organic acids. Phenolic groups have been demonstrated to provide high antioxidant effects that prevent free radicals. Furthermore, there is an established association between phenolic compounds and a reduced risk of neurological, cardiovascular, and cancer diseases [27].

The total flavonoid content obtained was 22.19 ± 0.91 mg GAE/g from the ethanol extract, 20.27 ± 2.38 mg GAE/g from the n-hexane fraction, 88.07 ± 1.34 mg GAE/g from the ethyl acetate fraction, and 99.62 ± 1.97 mg GAE/g from the methanol fraction, as shown in **Table 1**. The use of methanol as a solvent yielded high levels of flavonoid, a consequence of the polar nature of methanol, which exhibits a better ability to separate flavonoid compounds in comparison to ethanol [28]. The total flavonoid content resulting from the use of n-hexane as a solvent was the lowest, with an average value of 20.27 ± 2.38, due to the non-polar nature of n-hexane. The polarity of the solvent used is directly proportional to the compound content obtained [29]. The result of the present study is in line with that reported in a prior study, which obtained a total flavonoid content in the methanol fraction that was greater, namely 22.96 mg GAE/g, compared to other fractions [30]. The present study aligns with the findings of another study, which reported total flavonoid content (TFC) ranging from 22.44 to 61.15 mg QE/g, indicative of significant antioxidant activity [31].

In vitro antioxidant activity and ABTS free radical scavenging activity

The antioxidant activity of extracts and fractions of leaves of kalangkala (*Litsea angulata* BIume) was proven in vitro using the UV-Vis spectrophotometer (Shimadzu UV-1800, USA) by determining total phenolic and free radical inhibition using ABTS [32]. The results of the antioxidant activity obtained using the ABTS assay are presented in **Table 2**.

The IC_{50} values, which are a measure of antioxidant potency, of the ethanol extract, n-hexane fraction, ethyl acetate fraction, methanol fraction, and quercetin of the leaves of kalangkala (*Litsea angulata* Blume) were 51.23 ± 3.86, 159.44 ± 2.93, 136.01 ± 6.47, 93.28 ± 0.66, 1.95 ± 0.178, respectively, as shown in **Table 2**. The ethanol extract and methanol fraction showed considerable antioxidant activity, with IC_{50} values ranging from 50 to 100 ppm [33]. The n-hexane fraction and ethyl acetate fraction exhibited moderate antioxidant activity, with IC_{50} values ranging from 101 to 250 ppm [34]. The ethanol extract and methanol fraction have been found to contain bioactive phenolic and flavonoid compounds that have high polarity, demonstrating a higher antioxidant potency in comparison to the ethyl acetate and n-hexane fractions. N-hexane, a non-polar solvent, and ethyl acetate, a semi-polar solvent, can separate active compounds of varying polarities, including polar, semi-polar, and non-polar compounds [35]. The active compounds responsible for antioxidant activity are easily soluble in polar solvents such as ethanol and methanol, enabling ethanol and methanol extracts to attain a faster incubation time than the ethyl acetate fraction.

Table 2. The results of the evaluation of the ABTS assay for the antioxidant activity of the extract and fractions of leaves of kalangkala (*Litsea angulata* BIume)

Sample	Antioxidant Activity (IC_{50}± SD)
Ethanol extract	51.23 ± 3.86
n-hexane fraction	159.44 ±2.93
Ethyl acetate fraction	136.01 ±6.47
Methanol fraction	93.28 ±0.66
Quercetin	1.95 ± 0.178

Table 3. Correlation between the total phenolic and total flavonoid content of the extract and fractions of the leaves of kalangkala (*Litsea angulata* BIume) and the antioxidant activity of the plant material

		Total Phenolic Content	Total Flavonoid Content	Antioxidants (IC_{50})
Total phenolic content	*Correlation coefficient*	1	-0.280	0.580
	Sig.(2-*tailed*)		0.379	0.048*
Total flavonoid content	*Correlation coefficient*	-0.280	1	-0.224
	Sig.(2-*tailed*	0.379		0.484
Antioxidants (IC50)	*Correlation coefficient*	0.580	-0.224	1
	Sig.(2-*tailed*	0.048	0.484	

Note:*= Significant correlation with the antioxidant IC_{50} (Sig.(2-tailed) <0.05)

Flavonoid compounds are classified as polar polyphenol compounds, which renders them more soluble in polar solvents and less soluble in non-polar and semi-polar solvents [35]. The results of the present study are in line with those of a previosu study, which demonstrated that the antioxidant activity of the ethanol extract was notably strong, with an IC_{50} value of 16.45, in comparison to the IC_{50} values of the ethyl acetate, n-hexane and water fractions, which were 47.69, 57.24, and 76.95, respectively [36]. Flavonoids have been reported to have antioxidant properties that stabilize free radicals through both indirect and direct pathways [37]. These antioxidant properties are attributed to the flavonoid structure. The hydroxyl group, a component of flavonoid compounds, have been observed to donate electrons to free radicals [38]. The antioxidant activity of phenolic compounds is related to hydrogen atom transfer (HAT), single electron transfer (SET), and sequential proton loss electron transfer (SPLET), as well as transition metal chelation (TMC) [39]. A study has shown that the ethanol extract of mangrove leaves exhibits better antioxidant activity in comparison to its polar, semipolar and non-polar fractions, as determined by the DPPH and FRAP assays [40].

The statistical analysis obtained a sig.p value less than the significance level of 0.05 for the extract, n-hexane fraction, ethyl acetate fraction, and methanol fraction of leaves of kalangkala. This finding indicates that there are significant differences between the extract and fractions of leaves of kalangkala (*Litsea angulata* BIume) and the quercetin group. Specifically, the extract and fractions of the leaves exhibited antioxidant activity, yet they were significantly different from quercetin, which has been proven to have an excellent antioxidant capacity.

Antioxidant activity is presumably due to the presence of phenolic and flavonoid compounds, which have the capacity to impede oxidation and capture and donate electrons to ABTS free radicals. This capacity is attributed to the presence of hydroxyl groups within the structural composition of these compounds [41]. A high total phenol and flavonoid content corresponds to a high antioxidant capacity, which is manifested through the donation of electrons and the subsequent reduction of free radicals. The principle underlying the ABTS assay is that a change in color intensity occurs as a consequence of the reaction between antioxidants and ABTS. Antioxidants donate electrons or hydrogen atoms, thereby reducing ABTS free radicals. The ABTS assay is a procedure that is used to observe the ability of extracts and fractions to capture or clean free radicals [42].

The most widely used procedure for determining free radical scavenging activity involves measuring the tendency of phenol compounds to donate hydrogen atoms. ABTS radicals are quenched by antioxidants, leading to a reduction in $ABTS^+$ and the formation of stable radicals [43].

Correlation between total phenolic and total flavonoid content and antioxidants

The results of the Kolmogorov-Smirnov test on the total phenolic and total flavonoid content against antioxidants yielded a sig. value of 0.2, which is greater than 0.05. Therefore, it can be concluded that the data obtained were normally distributed. Subsequently, the correlation test employed Spearman's rank correlation, given that the data were normally distributed [44]. The results of the correlation test on the total phenolic and flavonoid content of the extract and fractions of leaves of *Litsea angulata* Blume and the antioxidant activity of the plant material are presented in **Table 3.**

The correlation coefficient of the total phenolic content of the extract and fractions of kalangkala (*Litsea angulata* BIume) leaves to the IC_{50} value was 0.58, with a Sig.(2-tailed) of 0.048, or less than 0.05, as shown in **Table 3**. This result suggests a positive correlation between total phenolic content and antioxidant activity. The correlation coefficient value of the extract and fractions of leaves of kalangkala (*Litsea angulata* BIume), 0.580, indicates a moderate correlation. The Pearson correlation indicates a medium correlation with a range of 0.41-0.60 [45]. These results provide an overview of the relationship between the total phenolic content of the extract and fractions of kalangkala (*Litsea angulata* BIume) leaves and antioxidant activity, with a positive relationship observed that contributed to 58.0% of the activity. The remaining activity was influenced by the content of other compounds. These results are consistent with those of a preceding study, which reported that the total flavonoid content and phenolic content contributed to antioxidant activity, with the former contributing 58.1% and the latter contributing 57.0% [5]. Another study revealed a correlation between phenolic content and IC_{50}, with a Sig.(2-tailed) of 0.000, or less than 0.05, and a correlation of -0.928. An increase in total phenolic content corresponds to a decrease in IC_{50} value [46]. Phenolics contribute greatly to antioxidants through their function as reducing agents, metal chelators, electron donors, and free radical scavengers, and through their capacity to reduce oxygen, which has high energy [33].

The correlation coefficient between the total flavonoid content of the extract and fractions of the leaves of kalangkala (*Litsea angulata* BIume) and antioxidant activity was obtained at -0.224 with a Sig(2-tailed) value of 0.484, which is more than 0.05. The results indicated that the flavonoid content in the extract and fractions of kalangkala (*Litsea angulata* BIume) leaves had an insignificant negative correlation with antioxidant activity. The correlation coefficients obtained were -0.224, indicating a moderate level of correlation with a negative value. This suggests that an increase in flavonoid content would result in a reduction in the IC_{50} value. A reduction in the IC_{50} value corresponds to an increase in antioxidant activity. These results is in line with those reported in another study, which found a significant value of total flavonoid in the extract and fraction of tamarind leaf (0.073), indicating an insignificant correlation because the sig.p value was less than 0.05 [47]. The results of this study demonstrate that the total phenolic and total flavonoid content is not invariably required to be high to manifest antioxidant activity.

The high total phenolic content observed in various plant species is attributable not only to flavonoids but also to other compounds, such as triterpenoids, tannins, coumarins, alkaloids, saponins, and sugars. The correlation between total phenolic and total flavonoid content is not always positive, as these compounds are also influenced by the bioactive components present in plant material [48]. Antioxidant activity can be influenced by various compounds in addition to phenolic and flavonoid compounds. As demonstrated in the kalangkala leaf extract, there are other compounds and several groups of vitamins, alkaloids, keratinoids that have been reported to contribute to antioxidant activity. These include orientin, echinenone, actinodhapine, and forsythoside E.

The antioxidant activity of the extract and fractions of kalangkala (*Litsea angulata* BIume) leaves as a whole is likely due to a combination of synergistic, antagonistic, or various effects from other compounds contained therein [49]. The correlation results showed that the total phenolic content (TPC) in the extract and fractions exhibited different ABTS quenching properties, ABTS reduction properties, iron chelation properties, and phosphomolybdate reduction properties [50]. It has been demonstrated that phenol and flavonoid are capable of donating electrons to free radicals and thereby inhibiting their production. The presence of hydroxyl groups in flavonoid compounds and their derivatives, including glucose, is a contributing factor to this capability [51]. Several other functional groups also contribute to the antioxidant activity of the compound, including carbonyl, alkane,

methylene, ester and di-substituted alkyl groups [52].

Conclusion

The mechanism of activity of total phenolic and flavonoid content in n-hexane extract and fraction, ethyl acetate fraction, and methanol fraction of kalangkala (*Litsea angulata* BIume) leaves contributes to producing antioxidant activity. The findings of the present study indicate that the ethanol extract and methanol fraction of the leaves have strong antioxidant activity, likely due to the presence of a fairly large total phenolic content. In addition, the ethanol extract and methanol fraction exhibited a significant positive correlation with antioxidant activity (sig.p<0.05). This finding indicates that the objective of the present study, namely, to demonstrate the antioxidant activity of the extract and fractions of *Litsea angulata* BIume leaves, has been sucessfully achieved. The potential of the extract and fractions of *Litsea angulata* BIume leaves as antioxidants warrants further investigation, particularly concerning its capacity to accelerate the healing process of diabetes mellitus in diabetic model test animals.

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