

Study on phenolic content, flavonoid content, and antioxidant capacity of extracts from *Lansium parasiticum* (Osbeck)

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Abstract: *Lansium parasiticum* (Osbeck) or its synonym *Epicharis parasitica* is from the *Meliaceae* family and has traditionally been used for various medical applications. This study aimed to determine the total phenolic content, total flavonoid content, and antioxidant activity of different duku extracts. A total of four different solvents, 50% ethanol, 100% aqueous, 100% acetone, and 100% chloroform, were used to extract duku flesh. The total phenolic and flavonoid content was determined using the Folin-Ciocaltealuminumminium chloride colorimetric method. The DPPH and ABTS method evaluated the antioxidant activity. The result showed that 50% ethanol presented the highest extraction yield, $10.81\% \pm 0.004$. It is also revealed that aqueous extract exhibited the highest amount of total phenolic content of $152.910 \text{ mg GAE}/100 \text{ g} \pm 22.143$, total flavonoid content of $1669.723 \pm 370.091 \text{ mg QE}/100 \text{ g}$, and antioxidant activity DPPH of $68.51\% \pm 2.730$ and ABTS of $6.063 \text{ U/ml} \pm 0.721$ compared to other extracts. The correlation between total phenolic content and ABTS ($r = 0.719$, $p = 0.029$) showed a statistically significant result. Therefore, the extracts of this fruit have promising potential as cheap sources of future natural antioxidant agents in the food industry. Researchers will further these results for future in-vitro or in-vivo biological studies.

Keywords: Antioxidant; Duku; DPPH; Flavonoid; Phenolic.

1. Introduction

Lansium parasiticum (Osbeck) or its synonym *Epicharis parasitica* have two distinct forms of fruits: duku and langsat¹. These fruits grow like berries with cauliferous habits, which means these fruits grow directly from the tree's stem. Duku and langsat were primarily found in western South-East Asia countries such as Malaysia, Indonesia, Thailand, Vietnam, and the Philippines². In Indonesia, it is identified as langsat, duku, or kokosan; in the Philippines, it is known as lansones³; in Thailand, it is known as long-kong, and in Vietnam, it is known as bon-bon fruits⁴.

Traditionally, each duku plant had been used as a potential medical application. The bark of the duku tree has been used as an astringent, and its decoction has been used for dysentery, anti-fertility and malaria.^{5,6} In Borneo, Dayak community utilized fruit peels as talc powder for sunburn⁷. While the pulverized seeds of duku mixed with water are consumed as a vermifuge and febrifuge for

children⁸. In the Philippines, the pounded seeds of duku decoction are used for deworming and ulcers⁹.

Through considerable previous research, very few reports are available on the antioxidant content ability of phenolic acids and flavonoids of duku fruits^{10,11}. Among these few reports, Lim *et al.*,¹² observed that langsat fruits give the fourth highest total phenolic content among tropical fruits after guava, papaya, and star fruit, which eventually provide the potential as a higher secondary antioxidant that is measured by the iron (II) chelating experiment. It is known that bioactive compounds in plant-based materials could perform as defensive mechanisms against oxidative stress by diligently being consumed as antioxidants by humans¹³. Therefore, this study aims to investigate the phenolic and flavonoid contents and antioxidant capacity of *L. parasiticum* extracts.

2. Experimental

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2.1. Sample preparation of *L. parasiticum*

Fresh *L. parasiticum* fruits were purchased from available sources in Kuala Terengganu, Malaysia. The plant material was authenticated by the Institute of Bioscience, Universiti Putra Malaysia, with a voucher serial number of MFI 0101/19. For the extraction method, the procedure by Mohd Adzim Khalili *et al.*¹⁴ was used in this study with slight modifications. First, the fruits were skin-peeled, carefully washed under running tap water, dried with a soft cloth, and macerated. A total of 30 g of the macerated fruit sample was soaked into four different solvents, which were 100% chloroform, 100% acetone, 50% aqueous ethanol (Analytical Grade Solvents), and 100% aqueous (deionized water) for 24 hours. Subsequently, all the solvent extracts were filtered using a nylon membrane filter (pore size 0.45 µm) and concentrated in a rotary evaporator. Then, the crude extract was dried in the drying oven at 40°C. All the extracts were stored at -20°C before analysis use.

The extraction yields were calculated using the formula described by Dey and Rathod¹⁵ based on the following:

$$\text{Extraction yield} = W2/W1 * 100\%$$

W1= original weight of the sample

W2= weight of the dried extract.

2.2. Proximate analysis

2.2.1. Moisture content

The moisture content was measured using a moisture analyzer machine. Following the instructions of the machine, 5 grams of samples (macerated fruit) were weighed in triplicate and placed in pans. The sample was dried out in the machine, and the percentage moisture of the samples was automatically calculated. This parameter is part of the proximate analysis parameters that need to be discussed regarding its content vs metabolic actions toward antioxidant capacity.

2.2.2. Ash content

The AOAC 900.02 method was used to measure the ash content of the samples¹⁶. Five grams of the *L. parasiticum* samples (macerated fruit) were weighed into the crucible and were dried at 550°C for 12 hours. The crucible's weight was taken after the ash process was completed, and the control was conducted without samples. Similar to moisture content, this parameter is part of the proximate analysis parameters that need to be discussed regarding its content vs metabolic actions toward antioxidant capacity. The ash was expressed as a percentage of the initial fresh sample weight. The percentage was calculated using the following method:

$$\text{Percentage of ash (\%)} = W2/W1 * 100\%$$

Where, W1 = Weight of sample (g)

W2 = Weight of ash (g)

2.2.3. Total phenolic content (TPC) assay

Total phenolic content was determined using the Folin-Ciocalteu method by Alyaqoubi *et al.*,¹⁷ with slight modification. Briefly, 1 mg sample of *L. parasiticum* crude extract was diluted into 1 mL of methanol to obtain 1 mg/mL of sample (stock solution). Then, a 100 µL was obtained from the stock solution and mixed thoroughly with 0.4 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. Then, the sample was left for 5 mins. After 5 mins, 1 mL of 7.5 % sodium carbonate was added, and the samples were allowed to stand in a dark place for 2 hours. The absorbance was measured at 765 nm using a spectrophotometer (Genesys 20). This process was conducted in triplicate for each sample. A calibration curve of Gallic acid was plotted to determine the activity potential of the samples. The findings were expressed as milligrams of Gallic acid equivalents per 100 grams of sample (mg GAE/100 g). Then, the following formulas were used to measure the TPC values:

$$T = cV/M * 100$$

Where,

T = TFC content in mg GAE/100 g of extract

c = Concentration of the Gallic acid from the calibration curve

V = Volume of solvent used to dissolve the extract

M = Weight of extract used in gram (g)

2.2.4. Total flavonoid content (TFC) assay

The total flavonoid content of the crude extract was determined by the aluminum chloride (AlCl₃) colorimetric method. First, 1 mg of *L. parasiticum* crude extract was diluted with 1 mL of methanol to obtain 1mg/mL of sample (stock solution). Then, a 100 µL was obtained from the stock solution and mixed thoroughly 500 µL of distilled water and 100 µL of 5% sodium nitrate. The solution was allowed to stand for 6 mins. Next, 150 µL of 10 % AlCl₃ solution and 200 µL of 1 M (molar) sodium hydroxide were added. Again, it was left for another 5 mins. The absorbance of the spectrophotometer was measured and reported at 510 nm. For each sample, the same procedure was replicated in triplicate. The findings were expressed as milligrams of quercetin equivalents per 100 grams (mg QE/100 g). Then, the following formulas were used to measure the TFC values:

$$T = cV/M * 100$$

Where,

T = TFC content in mg QE/100 g of extract

c = Concentration of the Quercetin from the calibration curve

V = Volume of solvent used to dissolve the extract

M = Weight of extract used in gram (g)

2.3. Antioxidant capacity

2.3.1. DPPH Assay

Using the method defined by Rohin *et al.*,¹⁸ the antioxidant activity was measured using DPPH radical scavenging activity assay. A total of 10 mg *L. parasiticum* extract was dissolved in 1.0 mL methanol, and the solution was applied at room temperature to a 1.0 mL DPPH solution. By using the UV-1601 Shimadzu spectrophotometer, the absorbance was measured at 517 nm. The experiments were done in triplicate, and a standard curve was developed using quercetin, which served as a positive control and was prepared in the same concentration as the crude extract. The findings were represented by test samples as a percentage of the reduction in the initial DPPH absorption as follows:

$$\text{DPPH scavenging effect (\%)} = 100 - [(A_0 - A_1/A_0) \times 100]$$

Where,

A₀ = the absorbance of the control reaction

$$\frac{\text{Abs sample} - \text{Abs control}}{0.01} \div 30 \text{ (min)} \times \frac{\text{Total volume of reaction system (mL)}}{\text{The volume of sample (mL)}} \times \text{df}$$

Where:

Abs = absorbance

df = dilution factor of the sample before evaluated

2.4. Statistical analysis

Data were analyzed using descriptive and inferential statistical analysis, and the values were represented as means and standard deviation (SD). The statistical software SPSS for Social Sciences version 20.0 (IBM, Armonk, NY, US) was employed for all the statistical analyses in this study. Two-tailed tests were performed in this study, and a significant difference at $p < 0.05$.

A₁ = the absorbance of the sample

2.3.2. Total Antioxidant Capacity (TAC)

The antioxidant activity was determined using the antioxidant kit (E-BC-K136) from Elabscience, USA. This kit consisted of five reagents which were reagent 1 (Buffer solution), reagent 2 (Chromogenic agent), reagent 3 (ferric salt stock solution), and reagent 5 (stop solution). First, the reacting mixture consisting of 1 mL buffer (reagent 1), 2 mL chromogenic agent (reagent 2), and 0.5 mL of ferric salt solution (reagent 3) were pipetted into tubes. The solution was then thoroughly mixed and incubated at 37°C for 30 mins. Then, 0.2 mL of reagent 5 was pipetted into the solution. The solution was then fully mixed and left to stand for 10 mins. Lastly, the solution was measured using a spectrophotometer at 520 nm in 1cm cuvette. The procedure was repeated with the control tube, but the samples were put at the last step after reagent 5. The result was expressed as T-AOC activity, U/mL, using the following formula:

3. Results and Discussion

3.1. Extraction yield of *L. parasiticum* extracts

In this study, *L. parasiticum* extracts were attained by applying different polarity solvents of 50% ethanol, 100% acetone, 100% chloroform, and 100% aqueous (deionized water) (Table 1). Based on the findings, the highest extraction yield showed by 50% ethanol extract with 10.81%, followed by acetone, aqueous, and chloroform extracts with 5.59%, 3.01%, and 0.03%, respectively ($p < 0.05$). Thoroughly, chloroform extract as a non-polar solvent gives the lowest extraction yield among the other polar and non-polar solvents for *L. parasiticum* sample.

Table 1. Extraction yield of *L. parasiticum* extracts.

| Samples | Original Weight (g) | Weight of Dried Extract (g) | Extraction Yield (%) | p-value |
|-------------|---------------------|-----------------------------|---------------------------|---------|
| 50% Ethanol | 30 | 3.24 ± 0.01 | 10.81 ± 0.01 ^a | 0.001 |
| Aqueous | 30 | 0.91 ± 0.01 | 3.01 ± 0.01 ^b | |
| Acetone | 30 | 1.68 ± 0.01 | 5.59 ± 0.01 ^c | |
| Chloroform | 30 | 0.01 ± 0.01 | 0.03 ± 0.01 ^d | |

Data are expressed as mean ± standard deviation of 3 independent experiments.

*One-Way ANOVA test, a significant difference ($p < 0.05$).

^{abcd} Variation in the following letters indicates the significance of difference by post-hoc test ($p < 0.05$).

At Present, the solid-liquid extraction method was used to extract the selected compound using four different types of solvent, which were 50% ethanol, 100% aqueous (deionized water), 100% acetone, and 100% chloroform. This study determined that sample

extraction for the 50% ethanol showed the highest, whereas the sample extraction for chloroform was the lowest. Thus, it is indicated that different solvents would give different amounts of extraction yield. In addition, the result showed an

aqueous solvents extract more yield than pure solvents. This could be due to the extraction with aqueous organic solvents of polar and non-polar substances¹⁹.

3.2. Proximate analysis – moisture and ash contents

As depicted in Table 2, the proximate analysis of moisture and ash analysis for *L. parasiticum* extracts were assessed. The result showed *L. parasiticum* sample gives 83.65% amount of moisture and 0.29% amount of ash.

Table 2. Proximate analysis (moisture and ash content) of *L. parasiticum* sample.

| Sample | Analysis | |
|-----------------------|--------------|-------------|
| | Moisture (%) | Ash (%) |
| <i>L. parasiticum</i> | 83.65 ± 0.38 | 0.29 ± 0.09 |

Data are expressed as mean ± standard deviation.

For the analysis, moisture and ash is an analysis that determines the water and mineral content of substances, respectively. This study found that the moisture content (83.65%) of this fruit sample (macerated fruit) is lower than a study reported by Morton²⁰. The high moisture content provides more excellent activity of water-soluble enzymes and co-enzymes needed for the metabolic actions of these plants²¹. Moreover, the high moisture contents indicate that this fruit needs extra maintenance and care for proper protection as it would be susceptible to deterioration²². As for ash content, it is observed that this fruit is less abundant in mineral elements, with less than one percent present. It has been studied that ash content may vary among trees with different growth locations²³. These individual trees may get other fertilizer treatments containing sodium nitrate, calcium, and phosphorus. However, the study observed no definite variation trend in ash content among individual trees. The higher the moisture content, the lower the ash content of a substance, as it is inversely proportional to each other. This relationship is correlated well; the higher the moisture content, the higher the water content, thus

making it more evaporated during the heating process in ash analysis, which leaves lower ash content overall.

3.3. Total phenolic and total flavonoid contents

Findings showed that higher total phenolic content (TPC) is observed in an aqueous extract with 152.91 ± 22.14 mg GAE/100 g, followed by 50% ethanol and acetone extracts with 33.22 ± 2.17 mg GAE/100 g and 1.77 ± 0.38 mg GAE/100 g, respectively (p>0.05). Meanwhile, the higher total flavonoid content (TFC) was observed in an aqueous extract with 1669.72 ± 370.09 mg QE/100g, followed by 50% ethanol and acetone extracts with 1308.61 ± 168.39 mg QE/100g and 1179.72 ± 63.16 mg QE/100g, respectively. By comparing each extract on TPC and TFC, it was observed that aqueous extracted the highest amount while chloroform extracted nothing for the amount. The result was expected as there are more polar than non-polar compounds in the fruit samples. The aqueous solvent extracts more compounds due to its nature as a polar solvent, while chloroform extracts nothing due to its nature as a non-polar solvent.

Table 3. Total phenolic (mg GAE/100g) and flavonoid content (mg QE/100g) of *L. parasiticum* extracts.

| Samples | TPC (mg GAE/100g) | p-value | TFC (mg QE/100g) | p-value |
|-------------|-----------------------------|---------|------------------|---------|
| 50% Ethanol | 33.22 ± 2.17 ^a | 0.001 | 1308.61 ± 168.39 | 0.102 |
| Aqueous | 152.91 ± 22.14 ^b | | 1669.72 ± 370.09 | |
| Acetone | 1.77 ± 0.38 ^a | | 1179.72 ± 63.16 | |
| Chloroform | nil | | Nil | |

Data are expressed as mean ± standard deviation.

*One-Way ANOVA test, a significant difference (p<0.05).

^{ab} Variations in the following letters indicate the significance of difference by post-hoc test (p<0.05).

The TPC and TFC of every plant extract is highly influenced by the solvents selected for extraction and their polarity²⁴, in which the phenolics and flavonoid contents are the main contributors to the antioxidant function of plant materials²⁵. While it is true that

flavonoids are a member of phenolic compounds, it was observed that the lower content of TPC in this study might be due to the presence of different amounts of sugars, carotenoids, ascorbic acid, or methods of extraction²⁶. Therefore, the TPC of the

four solvent extractions was tested using the Folin-Ciocalteu method. This electron transfer-based assay provides a reducing capacity ability, which will be conveyed as phenolic content²⁷. While the TFC was tested using the aluminum chloride colorimetric assay against a flavonoid standard, assuming equal responses from all flavonoids²⁸. In this study, the highest TPC and TFC were observed by an aqueous extract with 152.91 ± 22.14 mg GAE/100 g and 1669.72 ± 370.09 mg QE/100 g.

Previous studies show that the aqueous solvents were more effective than the pure solvents in extracting the phenolic and flavonoid compounds²⁹. For example, a study by Dhar *et al.*,¹⁹ observed the highest TPC from 70% acetone extract compared to pure acetone, ethanol, and methanol solvent extracts. While Boeing *et al.*,³⁰ also showed that 70% acetone extract was the strongest solvent among¹⁹ different pure solvents to extract phenolics from three various berries. Meanwhile, a study by Hismath *et al.*,³¹ reported that the TPC increased accordingly with the addition of ethanol concentration of up to 60%. Still, the phenolic content was reduced when reaching 100% concentration. Similarly, Chan *et al.*,³² also proved that the concentration of ethanol that is over

60% would vividly reduce the number of phenolics extracted.

3.4. Antioxidant capacities (DPPH and TAC assays)

The average readings for DPPH scavenging activity of solvents tested were measured accordingly with values expressed in percentage (%) as illustrated in Table 4. Results showed that DPPH scavenging effect was higher in the aqueous extract at $68.51 \pm 2.73\%$, while the acetone extract demonstrated a lower DPPH scavenging effect at $47.01 \pm 2.24\%$. There is a significant difference between DPPH scavenging effect values between each solvent, $p < 0.05$. Nevertheless, *L. parasiticum* extracts give an increment pattern of DPPH scavenging values as aqueous > 50% ethanol > acetone. While chloroform extract did not have any effect on DPPH scavenging. Based on prior findings, chloroform extracted nothing from the TPC and TFC assay due to its nature to extract non-polar compounds from the fruit sample. Therefore, it corresponds well with non-effect scavenging from DPPH assay, as the non-extracted non-polar compounds could not scavenge the free radical in this assay.

Table 4. DPPH scavenging (%) and T-AOC activity of *L. parasiticum* extracts.

| Samples | DPPH (%) | p-value | T-AOC activity (U/mL) | p-value |
|-------------|--------------------|---------|-----------------------|---------|
| 50% Ethanol | 65.92 ± 5.30^a | 0.003 | 0.20 ± 0.04^a | 0.001 |
| Aqueous | 68.51 ± 2.73 | | 6.06 ± 0.72^b | |
| Acetone | 47.01 ± 2.24^b | | 3.22 ± 0.41^c | |
| Chloroform | nil | | nil | |

Data are expressed as mean \pm standard deviation.

*One-Way ANOVA test, a significant difference ($p < 0.05$).

^{abc} Variation in the following letters indicates the significance of difference by post-hoc test ($p < 0.05$).

Followed by TAC assay, an aqueous extract gives the highest TAC, 6.06 ± 0.72 T-AOC activity, U/mL while 50% ethanol extract gives the lowest TAC value with 0.20 ± 0.04 T-AOC activity, U/mL (Table 4). The mean of T-AOC value (U/mL) in each solvent was significantly different ($p < 0.05$). Nevertheless, the result shows an increment pattern of T-AOC value with 50% ethanol < acetone < aqueous. Also, chloroform extract did not affect T-AOC activity of antioxidant capacity. Based on prior findings, chloroform extracted nothing from the TPC and TFC assay due to its nature to extract non-polar compounds from the fruit sample. Therefore, it corresponds well to the non-effect from T-AOC activity as the non-extracted non-polar compounds could not react with the total antioxidant in this assay.

DPPH assay and ABTS antioxidant capacity were used for the antioxidant assays to evaluate the activity of antioxidants from each solvent sample in

this study. Both DPPH and ABTS assays are based on spectrophotometric techniques that show discoloration and radical scavenging ability when there is a presence of antioxidants in the food extracts^{33,34}. These findings had been hypothesized that the aqueous extract would have a higher content of antioxidants compared to other solvents as water compounds hold the most significant capability to scavenge DPPH radicals like the TPC and TFC assays³⁵. Previous studies also observed that aqueous extract gives the highest DPPH radical scavenging activity on *L. alata* (*Blume*) *Leenh* fruit flesh³⁶ and the highest ABTS reading on Beijing propolis extract³⁷.

However, a different trend was observed by Ashafa *et al.*,³⁸ as the researchers found that aqueous extract gives a minor antioxidant activity compared to other pure solvents. The different obtained results may be because the aqueous extract of duku fruit is more capable of dissolving in a varied array of antioxidant

groups. The researchers proposed that different extracts exhibit dissimilar amounts of scavenging activity according to the compound present in the sample. Additionally, this trend is identical to TPC and TFC, which proposes a correlation between DPPH and (TPC and TFC). It shows that the antioxidant activity of the extracts was intensely reliant on the extraction solvent ³⁹.

3.5. Correlation between TPC, TFC, DPPH, and TAC assays

In Table 5, there is a positive and significant correlation between TPC and TFC with antioxidant capacities (DPPH and T-AOC assays) of the sample tested ($p < 0.05$). In this study, a moderate correlation was found between DPPH with TPC (0.51) and TFC (0.50); T-AOC with TFC (0.54), and a high correlation between T-AOC and TPC (0.72) for *L. parasiticum* sample.

Table 5. The correlation between TPC, TFC, DPPH, and TAC assays.

| | TPC (mg GAE/100g) | TFC (mg QE/100g) |
|--------------|----------------------|---------------------|
| DPPH (%) | 0.51* | 0.50* |
| T-AOC (U/mL) | 0.72* | 0.54* |

*Pearson's correlation test ($p < 0.05$)

Extraction yield, TPC, TFC, and antioxidant capacity can all be affected by the type of solvents used ⁴⁰. The polarity of solvents used has been proven to be an important variable affecting extraction efficiency ⁴¹. A study by Ghasemzadeh *et al.*, ⁴² found that the percentage of solvent beyond 72% will decrease the amount of flavonoid extracted, which might be interrelated to changes in the flavonoid solubility in the solvent. Additionally, it is finely recognized that the antioxidant activity of the plant material usually seems to correlate with the phenolic content ⁴³. Since TPC and TFC showed a correlation with the antioxidant activity of duku flesh, it is thus suggested to conduct a future study to identify bioactive compounds in the flesh of *L. parasiticum*.

4. Conclusion

In conclusion, this study observed that different polarity solvents applied for *L. parasiticum* extraction have other effects on the total yield, TPC, TFC, and antioxidant capacities (DPPH and TAC assays). The results observed 50% ethanol extract of *L. parasiticum* sample gives the highest percentage yield, followed by 100% acetone, aqueous, and 100% chloroform extracts. Results showed *L. parasiticum* sample had 83.65% moisture and 0.29% ash contents. Following, it was observed that aqueous extract gives higher TPC and higher TFC values. Also, the aqueous extract has a higher effect on DPPH scavenging and T-AOC activity. There are no values from chloroform extract in TPC, TFC, DPPH, and T-AOC assays. The relationship then was confirmed with a high and moderate positive correlation between TPC and TFC with DPPH and TAC assays. Therefore, these results can be evidence for further study of *L. parasiticum* samples on phytochemical screening, in-vitro, and in-vivo biological studies.

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