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Physicochemical and Biological Characterization of Petai Pod (*Parkia Speciosa*) as Agrowaste Fibrous Materials and Residues

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Abstract

This study investigates the physicochemical and antioxidant properties of *Parkia speciosa* pod extract, with the aim of assessing the *P. speciosa* pods valuable fibers, physicochemical, antioxidant characteristics and antimicrobial activity. *P. speciosa* pods were gathered, prepared and was extracted using methanol. The work included acquiring *P. speciosa* from the local market and processing the pods. Physicochemical analysis in this study is colour, pH, functional groups, and water solubility. Total phenolic content, total flavonoid content, DPPH and ABTS was assessed for antioxidant analysis. Proximate analyses for moisture, protein, and ash content are carried out as part of the study. Additionally, the antimicrobial activity against *E. Coli* and *B. cereus* was assessed. Proximate analysis for petai pod displayed a moisture content of 16.18%, ash content of 0.05%, and a protein content of 10.84%. Physicochemical analysis revealed a pH of 6.04, a light greenish-yellow color, and 31.39% water solubility. Antioxidant analysis indicated a substantial total phenolic content (TPC), Total Flavanoid content (TFC), and ABTS with result 1.25 mg $GAEg^{-1}$, 3.23 mg $GAEg^{-1}$ and 62.87% showcasing significant antioxidant properties. Meanwhile, the DPPH assay for this study was 25.87%, which was low compared to other antioxidant properties. For antimicrobial activity showed positive results with both Zone of inhibition (ZOI) at gram positive and negative bacteria with 10mm for both bacteria. The study underscores the potential of *P. speciosa* pods as a plant-based protein source and dietary alternative, emphasizing the impact of processing techniques on extract composition and performance. These findings contribute valuable insights for industries seeking diverse and sustainable protein sources. The examination of petai plant byproducts may reveals potential applications in medicine, nutraceuticals, and as an antimicrobial preservative across multiple industries.

1. Introduction

Unique flavour and frequent inclusion in traditional recipes make *Parkia Speciosa* or petai pods have a long cultural past and are particularly popular in Southeast Asian cuisines. These long, green pods are filled with a fruit that resembles a bean and is not only delicious but also has a lot of potential for use in a variety of ways. *P. speciosa* flourishes in primary lowland rainforests in Southeast Asia on podzolic sandy loam and in regions close to riverbanks [38]. The potential health benefits of petai pods have garnered attention, as they possess anti-inflammatory properties [1] that could potentially mitigate inflammation and its associated symptoms.

The study's importance stems from the possibility of using petai pods in a variety of industries, the need to cut waste, and the chance to open up new business opportunities. As a naturally occurring antibacterial [3], petai has potential applications in food preservation. Studies have demonstrated the presence of flavonoids, which are substances with strong antioxidant capabilities, in the methanol extract from the skin of petai seeds [37]. There have also been reports of Parkia speciosa having antioxidant qualities, and its constituents have demonstrated strong anti-inflammatory, anti-tumor, anti-microbial, and antioxidant action [38][21]. Moreover, a study on the antioxidant content of petai pods discovered that they have a very strong antioxidant activity [39]. As a result, petai skin has a high antioxidant content, which is good for health. Furthermore, research demonstrates that it is effective in reducing blood sugar levels, indicating potential therapeutic advantages for individuals with diabetes [2].

Poor waste management of the petai pod causes problems for the environment, even though it is commonly considered agricultural trash. Examining its antioxidant activity and physicochemical characteristics may open up possibilities for sustainable use. Petai pods, which contain a wealth of phenolic chemicals [7], are a valuable but often wasted resource for the environment and economy. Resolving this error generates new opportunities in addition to lowering waste and pollution. Exploring petai pod as a potential future product could reduce environmental impact.

Comprehending the active compounds present in the skin of the petai pod can potentially aid in the development of nutraceuticals, medicinal applications, and functional food ingredients. Further research may reveal possible use of these chemicals, especially their antibacterial properties, as natural preservatives or antimicrobials in a range of industries. By realising petai's full potential, this research could change our understanding and approach to using this plant, paving the way for innovative uses in sustainability, medicine, and nutrition.

The objectives in this study included evaluating the functional fiber production, physicochemical and antioxidant properties and antimicrobial activity of petai pod samples.

2. Material and Method

The chemical used was methanol, Kjhedal catalyst, Nitrate (NH_3), Sulfuric Acid, 2% Ferric chloride ($FeCl_3$), ethyl acetate, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′ -azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid), Hydrochloric Acid (HCl), ethyl acetate. The apparatus that was used (Hirayama HV-85 autoclave, Japan), moisture analyzer, electronic pH metre, FTIR (Perkin Elmer 99365 Spectrophotometer, USA),

2.1 Petai pod Collection and Preparation

P. speciosa was purchased from the local market in Pagoh Muar, Johor. Petai seeds and pods was be separate, after which they were be cleaned, dried, ground and sieve. store for later physicochemical examination. Petai pod was be cut around 1 cm x 1 cm. Peel samples from petai pods was sun-dried for 48 hours [7]. Sample was stored for later physicochemical examination.

2.2 Petai pod extraction

Petai pod was extracted using maceration technique by using methanol as the solvent. The Whatman No. 1 filter paper was used to filter the extracted sample. The sample was kept at -20°C in order to conduct further analysis. To prevent phytochemicals from degrading, the yield was concentrated by evaporating the solvent using a rotary evaporator at 70°C [7].

2.3 Proximate Analysis

In the assessment of total ash content, sun-dried petai pods were blended to achieve a powder texture, weighed at approximately 5 g, and heated gradually to 600±25 ºC. The ash content was determined by the formula [7]:

$$Total\ ash\ content\% = \frac{Weight\ of\ Ash}{Original\ Weight} x\ 100 \quad (2.1)$$

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The moisture content was evaluated using a moisture analyzer machine, where a small sample was weighed and analyzed until completion, with the percentage displayed on the machine's screen [8]. Protein content analysis involved the Kjeldahl method, comprising digestion, distillation, and titration, with a detailed protocol for sample preparation and calculation of protein percentage [9]. The protein content was determined by the formula [25]:

$$Protein\% = \frac{((A - B) \text{ x N x } 14.007 \text{ x } 6.25)}{W} x\ 100 \quad (2.2)$$

A is volume (ml) of 0.2 N HCI used sample titration, B is volume (ml) of 0.2 N HCI used in blank titration N is Normality of HCI, W is weight (g) of sample. Atomic weight of nitrogen equal to 14.007 and the protein-nitrogen conversation factor for fish and its by-products equal to 6.25

2.4 Physicochemical Analysis

The pH of petai pod was determined using an electronic pH meter, calibrated meticulously with standard buffer solutions at pH 4.0 and pH 7.0 for accuracy and precision [4]. Color determination employed a calibrated colorimeter with RGB values, representing darkness to light, green to red, and blue to yellow, respectively [5]. Functional group analysis utilized FTIR spectroscopy, providing insights into chemical interactions and groups within the mid-IR region [5]. Water solubility was assessed by using gravimetric method, combining petai pod pieces with a saturated sodium chloride solution, followed by an incubation period and evaporation to determine solubility characteristics [6].

2.5 Antioxidant Analysis

2.5.1 Total Phenolic Content

The spectrophotometric technique was used to determine the total phenolic content [10]. To summarise, 1 ml of the sample (1 mg/ml) was combined with one ml of Folin-Ciocalteu's phenol reagent. After five minutes, the mixture was stirred well with the addition of 13 ml of deionized distilled water and 10 ml of a 7% Na_2CO_3 solution. The mixture was placed at 23°C in the dark for 90 minutes, and then the absorbance at 750 nm was measured. The calibration curve, which was created by making a gallic acid solution, was extrapolated to yield the TPC. Three separate measurements of the phenolic chemicals were made. A milligram of gallic acid equivalents (GAE) per gramme of dried sample was used to express the TPC.

2.5.2 Total Flavanoid Content

0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$ (0.5 M), and 0.15 ml of $AlCl_3.6H_2O$ (0.3 M) were combined in a 10-milliliter test tube. 1 ml of 1 M NaOH was added after 5 minutes. After the mixture was thoroughly mixed, the absorbance at 506 nm was compared to the reagent blank [10]. Using rutin standard solution (0 to 100 mg/l) and the previously outlined process, a standard curve for total flavonoids was created. The milligrams of rutin equivalents per gram of dried weight were used to express the total flavonoids [34].

2.5.3 DPPH assay

The aqueous extracts' capacity to scavenge free radicals was assessed using a DPPH (2, 2-diphenyl-1-picrylhydrazyl) determined Spectrometric technique against a very stable free radical. The sample extract was added to DPPH aqueous solutions at a concentration of 0.3 mM [7]. Each combination was vigorously whirled for 30 minutes in the dark at room temperature. At 517 nm, the absorbance was measured, and the activity was represented as a percentage. DPPH was determined using formula below [7].

$$DPPH\ essay\ \% = \frac{A0 - A1}{A0} x\ 100 \quad (2.3)$$

A0 is the absorbance of the control reaction and A1 is the absorbance of the presence of each extract sample and the standard.

2.5.4 ABTS assay

By using the ABTS radical cation decolorization assay, it was possible to assessed the free radical scavenging capacity of plant samples. The reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) creates the ABTS+ cation radical, which was then keep at room temperature for 12 to 16 hours in the dark. Methanol was then use to diluted the ABTS+ solution to produce an absorbance of 0.700 at 734 nm. The absorbance was measured 30 minutes after the first mixing of 3.995 ml of diluted ABTS+ solution and 5:l of plant extract [11]. ABTS was determined using formula below [27].

$$ABTS\ assay\ \% = \frac{AB - AA}{AB} x\ 100 \tag{2.5}$$

AB is the absorbance of the ABTS radical with methanol and AA is the absorbance of the ABTS radical with sample extract, was used to compute the percent suppression of absorbance at 734 nm.

2.6 Determination of Antimicrobial Activity

2.6.1 Mueller Hinton Agar Medium

Mueller Hinton Agar with weight of 38 g was suspended in 1 litre of distilled water. The medium was brought to a complete boil to dissolve the medium and the autoclave (Hirayama HV-85 autoclave, Japan) was used to sterilized at 121 °C for 15 minutes [12].

2.6.2 Antimicrobial Activity

The disc diffusion method was used to determine the antibacterial activity of *P. speciosa* seed extracts against strains of both Gram-positive and Gram-negative bacteria [15]. The antibacterial activity was conducted using 24-hour cultures of *E. coli* as gram negative bacteria and *B. cereus* as gram positive bacteria. The spread method was used to ingest the test microorganisms into Mueller Hinton Agar. The petai pods extract was placed discs on the Mueller Hinton Agar surface for incubation. After incubation, zone of inhibition on the plates was observed a circular area that indicates how much the antimicrobial agent was inhibiting the bacteria. A zone of inhibition indicates a degree of sensitivity to the antibiotic, whereas the absence of a zone denotes complete bacterial resistance to the antibiotic. Antibacterial test plates required overnight incubation. The zone of inhibition (ZOI) was seen and its diameter was determined after 24 h of incubation at 37 °C [13].

2.7 Statistical analysis

The results of the experiment were recorded using the Mean ± SD, in Microsoft Excel was used to perform the statistical analysis of the data.

3. Result and Discussion

3.1 Proximate Analysis

Table 1 shows that the petai pods of *P. speciosa* have a moisture content of 16.18%, which is essential for storage, quality, and nutrition. This has an impact on petai pod shelf life and indicates modest level of storage capacity due to enzyme activity and vulnerability to mould growth. The study, utilizing a moisture analyzer, recorded a significantly higher moisture content than Fithri *et al*. (2019) [7] oven drying method (7.97%). Based on this comparison, using moisture analyzer offers speed and ease, directly measuring water content, potentially impacting accuracy for diverse materials like petai pods, while oven drying indirectly evaluates drying loss. In certain case, if the sample has a significant concentration of volatile substances other than water, oven-dry test may be inaccurate for moisture content analysis [35].

The *Parkia speciosa* (petai pod) ash analysis produced a result of 0.05%. The amount of ash in plant materials represents the total amount of minerals after full combustion. The petai pod's low ash level of 0.05% indicates that there isn't much inorganic residue present, pointing to a largely organic makeup. Differences in sample preparation techniques, specifically the divergent approaches of sun-drying in the current investigation and freeze-drying in [14] study, may be the cause of the discrepancy in ash content. Since freeze-drying is known to preserve sample structural integrity, it's possible that this helped explain why the study revealed a reduced ash concentration [17]. The natural drying process and the different climatic circumstances during sun-drying may have contributed to the greater ash concentration in the current study.

P. speciosa pods showed a protein content of 10.84%, suggesting significant implications for nutrition and eating habits in regions where *P. speciosa* is a staple food. A comparative element with Aridi *et al.* (2021) [28] study on *Leucaena leucocephala* pods (7.5% protein) enriches knowledge of protein diversity across plant species. This deeper awareness aids informed dietary decisions, emphasizing the influence of sample preparation methods on nutritional analyses.

Table 1 *Proximate analysis*

Type of analysis	Result
Moisture content dry basis	(16.18 ± 0.55) %
Ash	(0.05%± 0.04) %
protein	(10.84±1.22)%

Values are given as mean ± SD from triplicate

3.2 Physicochemical Analysis

Based on Table 2 *P. speciosa* petai pods pH analysis showed result pH 6.04 indicated that the methanol-based extract was relatively acidic, which may have an impact on its qualities and possible uses. This discovery is significant since acidity affects flavour, enzyme activity, microbial development, and possible health benefits. Research by [29], which employed ethanol as the extraction solvent and presence of ethosome, was showed noticeable lower result with pH 4.74, highlighting the significance of solvents in acidity. Ethosomes with precence of ethanol content can improves the solubility and absorption of drugs [30]. Therefore, the use of ethanol in extraction combined with homogenization using ethosomes, could increase the acidity of the extraction by effectively absorbing phytochemicals.

Colour analysis *P. speciosa* petai pods had a colour that was similar to unripe bananas but less intense L* = 71.71, a* = -22.46, b* = 65.42 which was a light greenish-yellow colour. The L* value (71.71) of the present study appeared lighter than that of the freeze-dried samples from Gan & Latiff (2011) [14] (L* = 71.98), presumably as a result of untreated pods' residual moisture. Study by Gan & Latiff, 2011 [14] give also give difference in the a* value which was 1.02. This comparison may indicate that the chlorophyll pigments had broken down during the freeze-drying process, resulting in a decrease in green tones and an increase in red pigmentation.

The water solubility of petai pods based on Table 2 was 31.39%. The gravimetric water solubility analysis of petai pods revealed a 31.39% solubility, differing from [7] study, which reported 25%. Fithri *et al.* (2019) [7] method involved extracting 1g of macerated pod skin with a chloroform and water solvent over 24 hours. In contrast, this study gravimetric approach offered a comprehensive assessment of water solubility. The variance underscored the impact of extraction methods and solvents on results, emphasizing methodological considerations.

Table 2 *Physicochemical analysis*

Type of analysis	Result
pH	(6.04 ± 0.6) %
Colour	L*=(71.71±0.23)
	a*=(-22.46±0.35)
	b*=(65.42±1.72)
Water solubility	(31.39 ± 0.47) %

Values are given as mean ± SD from triplicate

3.2.1 FTIR analysis

Baseed on Fig. 1, the FTIR examination of the petai pods produced notable peaks were suggestive of several chemicals. The peak at 3300 cm^{-1} indicated the existence of alcohols and phenols, which may have contributed to the compound's anti-inflammatory and antioxidant qualities. Alkane chains served as both a possible energy source and structural stability, resonating at 2900 and 2800 cm-1. A prominent peak at 1700 cm^{-1} suggested the existence of carboxylic acids and ketones, which are necessary precursors to bioactive substances including flavonoids and saponins. At 1600 and 1500 cm^{-1}, fragrant whispers from peaks suggested the presence of aromatic rings.

[31] conducted a different investigation in which she refluxed water-dried petai pod samples to identify distinct peaks in the FTIR spectra. Major peaks at 3292, 2917, 2849, 2112, 1742, 1630, 1420, 1375, 1147, and 1043 cm^{-1} are visible in the BP extract's FT-IR spectrum. There are also smaller peaks located around 1000 cm^{-1}. Both experiments found phenolic peaks, but the functional group peaks varied depending on the extraction solvent between methanol and water and the drying technique sun-dried and air-dried. These variances highlight the various solvents, drying processes, and extraction methods that contribute to the varied chemical compositions and possible bioactivities seen in petai pod extracts.

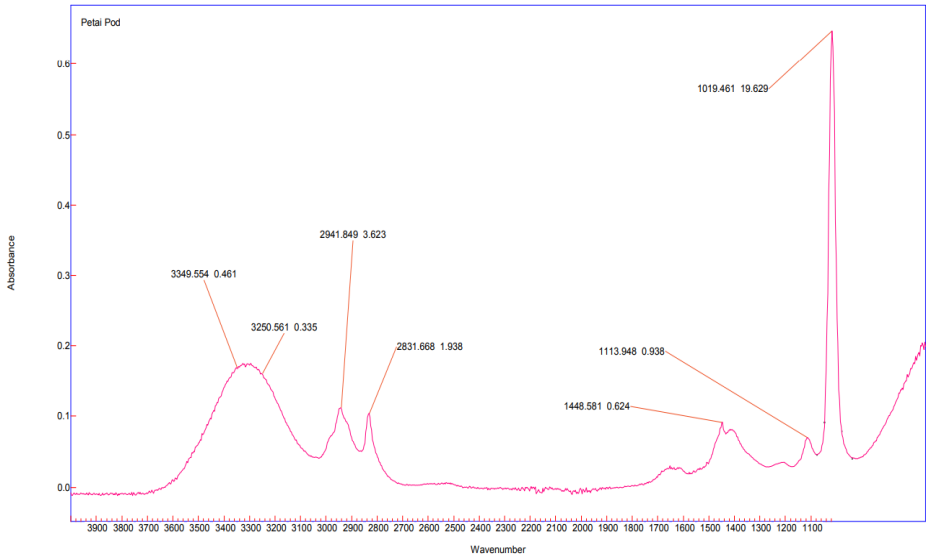

Fig. 1 *FTIR result for petai pod extract*

3.3 Antioxidant Analysis

Based on Table 3, An evaluation of *P. speciosa* pod extract's antioxidant capacity showed that it had significantly low DPPH radical scavenging activity of 25.87%. Even though the result for DPPH was low, petai pod extract may still indicating that it might neutralise radicals linked to cellular damage. This highlights its potential contribution to reducing damage caused by oxidative stress. Petai pods are important for promoting health because of the study, which presents them as natural sources of antioxidants. In contrast, Gan *et al.* (2011) [36] investigated the antioxidant activity of lyophilized *P. speciosa* powders using an alternative methodology, resulting in a 42.8% DPPH assay result at pH 4.5. The difference in antioxidant activity between the two studies could be due to the different extraction solvents used in the two studies whereas Gan *et al.* (2010) [36] focus on may have contributed to different chemical classes influencing the DPPH assay. Furthermore, the pH of the extraction medium was found to be a major effect on Gan *et al.* (2010) [36] experiment by using pH 4.5. Value of pH that was produced can maximum antioxidant activity thus indicating that certain pH values are preferred for the extraction of antioxidant chemicals.

With an ABTS radical scavenging activity of 62.78%, *P. speciosa* pod extract demonstrated significant antioxidant properties. The extract's potential to prevent oxidative cell damage is highlighted by its strong scavenging action against ABTS radicals, which are linked to cellular damage. Further investigation into this extract for potential use in functional foods or pharmaceutical applications is warranted. The study, which concentrated on powdered and sun-dried samples, demonstrated remarkable ABTS scavenging activity, indicating the strong antioxidant content of petai pods. On the other hand, a study conducted in by Muhialdin *et al.* 2020 [33] utilising freeze-dried petai beans and methanol extraction found a greater ABTS value of 92.38%. The discrepancy indicates differences in the antioxidant capabilities of chemicals isolated from beans and petai pods, influenced by the plant part studied, extraction techniques, and drying methods. The choice between sun-drying and freeze-drying may impact the content of bioactive chemicals, raising questions about the effects of processing techniques on ABTS scavenging, with freeze-drying potentially better preserving bioactive chemicals [17]

Further evidence for the antioxidant properties of *P. speciosa* pod extract comes from the measurement of total phenolic content. Based on Table 3, total phenolic content give result at 1.25 mg GAE g^{-1}. The extract's high phenolic concentration indicates that it has the potential to make a substantial contribution to the total antioxidant capacity. There are notable distinctions when comparing this study with the study of Balaji *et*

*al.,*2015 [18], especially in terms of processing methods and TPC content. While Balaji *et al.* (2015) [18] used a Soxhlet extraction procedure with methanol at a higher temperature, this study used a sun-drying and methanol extraction method. Balaji *et al*. (2015) [18] have greater value of 14.16% contrasts sharply with the observed TPC content of 1.25%, raising questions about extraction efficiency and temperature influence. Balaji *et al.* (2015) [18] study's higher extraction temperature might have an effect on the total TPC content by increasing the yields of some phenolic compounds. When it comes to extracting phenolic acids from plant materials, Soxhlet extraction has been compared to other techniques including maceration and ultrasonic-assisted extraction, and it has proven to be a good substitute for better extraction [19].

The evaluation of total flavonoid content in the *P. speciosa* pod extract, measured at 3.23 mg GAE g^{-1} of extract, provides another layer of evidence for its antioxidant properties. Flavonoids, a class of compounds known for their antioxidant properties, contribute to the overall free radical scavenging ability of the extract. Using maceration, the TFC analysis of Petai Pods in the current study produced a TFC of 3.23 mg GAE g^{-1}, whereas Balaji *et al.,* 2015 [18] found a higher TPC of 5.28 mg GAE g^{-1} using a Soxhlet apparatus. This disparity demonstrates how significantly extraction techniques affect the amount of flavonoids extracted from petai pods. The effectiveness of extraction techniques is crucial. Soxhlet extraction can constantly refluxing improves target compound penetration and solubilization, which may account for the higher TPC in Balaji *et al.* (2015) [18] study. These results highlight the significance of taking extraction methods into account, highlight the effectiveness of Soxhlet in removing flavonoids from Petai Pods, and the requirement for method standardisation in flavonoid content research in order to make meaningful comparisons. Soxhlation method give higher yield of extraction compare to maceration technique [20].

Table 3 *Antioxidant analysis*

Type of analysis	**Result ($\bar{x} \pm \sigma$)**
DPPH	(25.87 ± 3.76) %
ABTS	(62.78± 5.15) %
Total Phenolic Content, TPC	(1.25± 0.05) mg GAE g^{-1}
Total Flavonoid Content, TFC	(3.23 ± 1.27) mg GAE g^{-1}

Values are given as mean ± SD from triplicate

3.4 Antimicrobial Activity

Zones of inhibition surrounding the extract discs demonstrated the substantial inhibitory effects of the petai pod extract when tested against *B. cereus* and *E. coli.* Based on Table 4, these zones showed that the extract had the potential to partially inhibit the growth of both bacterial strains. Comparing the petai pod extract discs to the well-known antibacterial medication penicillin, *B. cereus* and *E. coli* displayed larger zones of inhibition surrounding the penicillin discs. Even though the penicillin ZOI was greater than the petai pod extract, it showed that some of the petai pod compounds may have benefits for antimicrobial activity. The polysulfide chemicals found in the petai pod have antibacterial and antimicrobial qualities [21][22].

The zones of inhibition offered quantitative information that shed light on the petai pod extract's comparative effectiveness with penicillin. In contrast to the larger 23 mm inhibition zone surrounding the penicillin disc, *B. cereus* displayed a 10 mm zone of inhibition surrounding the petai pod extract disc. Similarly, the significant 28 mm inhibition zone surrounding the penicillin disc eclipsed the 10 mm inhibition zone surrounding the petai pod extract disc for *E. coli*. These numerical findings highlighted the petai pod extract's significantly lower antibacterial efficacy against the tested bacterial strains compared to penicillin. The current analysis revealed bigger zones of inhibition for both bacteria compared to a prior work by Suchanuch *et al*. (2014) [23] with result 6.87 mm for *E. coli* and 9.50 mm for *B. cereus* by using a different extraction procedure 50% ethanol and hot air oven drying at 45 degrees Celsius. This raised the prospect that method differences have given different results. Methanol is more polar than ethanol, which may lead to better extraction yields for certain compounds [24].

Table 4 *Zone of inhibition for petai pod extract*

Microorganism	Zone of inhibition (ZOI),mm		
	Petai pod extract	Positive control (Penicillin)	Negative control (distill water)
Gram positive (*Bacillus cereus*)	10	23	-
Gram negative (*Escherichia coli*)	10	28	-

4. Conclusion

Research on the petai pod, or *P. speciosa*, has shown that it can be a rich source of antioxidants and flavonoids. The antibacterial investigation proved how effective the extract was against bacteria that were both gram-positive and gram-negative. Because sample preparation methods varied, proximate analysis differed from earlier research in providing important information on protein, ash, and moisture content. A physicochemical analysis revealed the pods' water solubility, acidic character, and light greenish-yellow colour. Additionally suggesting possible health benefits, FTIR examination revealed the presence of phenols, alcohols, alkane chains, ketones, carboxylic acids, and aromatic rings.

Whether used as a functional food or for medicinal purposes, the study emphasises how important it is to have efficient extraction and processing techniques in order to optimise the health advantages of petai pods. Enhancing the overall efficacy and bioavailability of the bioactive components in petai pods requires careful consideration of extraction solvents, drying methods, and processing conditions. Setting careful methods as a top priority enables petai pods to reach their maximum nutritional and therapeutic potential, expanding their use in functional meals and medical application

The use of methanol as the extraction solvent and sun-dried conditions for the petai pod samples are the only two components of the current study. It is necessary to recognise that another constraint that is present in this study is the pH levels, since they are a critical component that can significantly impact the extraction process's overall efficacy. In order to gain a more thorough understanding of the chemical composition and bioactive potential of petai pod extracts, it is imperative that future research investigate alternative extraction solvents, drying methodologies, and carefully address the influence of varying pH levels on the extraction efficiency.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

study conception and design: *Ahmad Fikri bin Ali, Shakila binti Abdullah* ***; data collection:*** *Ahmad Fikri bin Ali;* ***analysis and interpretation of results:*** *Ahmad Fikri bin Ali, Shakila binti Abdullah;* ***draft manuscript preparation:*** *Ahmad Fikri bin Ali, Shakila binti Abdullah. All authors reviewed the results and approved the final version of the manuscript.*

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