ORIGINAL ARTICLE

Phytochemical quantification and HPLC analysis of *Parkia speciosa* pod extract

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ABSTRACT

Introduction: *Parkia speciosa* Hassk., commonly known as bitter bean or twisted cluster bean, is a tropical leguminous plant species native to Southeast Asia. The plant's edible pods have been traditionally used in various cuisines, particularly in Malaysian, Thai, and Indonesian cooking. Apart from being used as a food ingredient, the pods of *P. speciosa* also have a range of potential applications in other fields, including medicine, agriculture, and industry. The pods are said to have several phytochemicals that hold great therapeutic values such as reducing inflammation, improving digestion, and lowering blood sugar levels. However, there is limited information on the specific phytochemical contents of the pods in the literature. Thus, the aim of this study is to quantify the total phenolic and flavonoid compounds and to determine the concentrations of four selected phytochemical compounds in the *P. speciosa* pod extract (PSPE).

Materials and Methods: Quantification of the total phenolic (TPC) and flavonoid contents (TFC) in PSPE were done via colourimetric methods; and the determination of the concentrations of four specific phytochemicals (gallic acid, caffeic acid, rutin, and quercetin) were done via High-Performance Liquid Chromatography (HPLC).

Results: Colourimetric determination of PSPE showed TPC and TFC values of 84.53±9.40 mg GAE/g and 11.96±4.51 mg QE/g, respectively. Additional analysis of the phytochemicals using HPLC revealed that there were 6.45±3.36 g/kg, 5.91±1.07 g/kg, 0.39±0.84 g/kg, and 0.19±0.47 g/kg of caffeic acid, gallic acid, rutin, and quercetin, respectively.

Conclusion: The findings show that PSPE contains substantial amounts of caffeic acid, gallic acid, rutin, and quercetin, which may indicate its potential as antibacterial, anti-inflammatory, anti-lipid, and antiviral medicines.

KEYWORDS:
Parkia speciosa, ethanol extract, phytochemicals, HPLC

INTRODUCTION

Parkia speciosa Hassk. is a leguminous plant indigenous to Southeast Asia and is abundantly found in tropical countries like Malaysia, Indonesia, Thailand, and the Philippines.[1] It is locally known as 'petai' and has a distinctive pungent odour which has also led to the name "stinky beans". The flat elliptical seeds are encapsulated by green pericarps and are considered a popular ingredient in traditional cuisines but can also be eaten raw. Besides being incorporated into dishes, the beans were also utilised for medicinal purposes. They were reported to relieve stomach pain, liver disease, diabetes, and worm infestations.[2] The beans even harboured anticancer, antibacterial, antioxidant,[3] antiangiogenic,[4] as well as wound healing[5] properties.

Currently, in the food industry, only the beans of *P. speciosa* are consumed while the pods are discarded as waste,[6] and in mature fruits, they make up more than 60% of the biomass.[3] Since the empty pods are considered useless, they are eliminated once the beans are harvested. To date, there have been several studies published on antihypertension, antidiabetic as well as antimicrobial and antioxidant properties of the empty pods of *P. speciosa*.[7-9]

Medicinal properties of plants lie in their bioactive phytochemical constituents. According to Ahmad et al., polyphenols, flavonoids, alkaloids, terpenoids, and tannins were among the phytochemicals found in the extracts of *P. speciosa* beans.[10] These phytochemicals are responsible for the healing properties reported. Similarly, the empty pods of *P. speciosa* harbour multiple bioactive compounds with many therapeutic potentials. Some of the most reported bioactive phytochemical constituents of the pod are quercetin, rutin, kaempferol, epicatechin, gallic acid, catechin, ellagic acid, and caffeic acid.[1,3,5,7,8,11] Despite extensive investigation in identifying the phytochemical constituents of the *P. speciosa* pods, there is not much information on the quantification of the bioactive compounds present. Hence, the present study aims to quantify four selected phytochemical compounds in the ethanolic extract of the *P. speciosa* pods.

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MATERIALS AND METHODS

Plant Material Preparation

P. speciosa fruits were collected from Kuala Krai, Kelantan in late November, and the plant species was verified by the UPM Institute of Bioscience herbarium (Voucher No. KM 0051/23). Approximately 2 kg of pods was sliced into smaller pieces, washed, and oven-dried at 40℃ for 3 days. An electrical grinder was used to finely grind the dried pods and they were kept in airtight containers at room temperature.

P. speciosa Pod Extraction

The exhaustive extraction method adapted from Fithri et al., was applied for the preparation of the extract.[12] The *P. speciosa* pod powder was soaked in 70% ethanol at room temperature and was filtered using Whatman No. 1 filter paper. The solvent was changed every four days until the solution turns colourless. The pooled extract was then concentrated using a rotary evaporator under reduced pressure at 45℃. The resulting extract was further concentrated using a vacuum concentrator for two hours before being subjected to freeze-drying for three days. The collected powder was kept at -20℃ until further use.

The extraction yield (%) was calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{Dry extract weight (g)}}{\text{Dry starting material weight (g)}} \text{ X } 100$$

Measurement of Total Phenolic Content of PSPE

The Folin-Ciocalteu method from Azizan et al., was applied for the determination of the total phenolic content (TPC) of PSPE.[13] Briefly, the reaction mixture was prepared by mixing 20 µL of 10 mg/mL PSPE and 100 µL of Folin-Ciocalteu's reagent in a 96-well plate in triplicates. After incubation for 5 minutes, 80 µL of 0.75% sodium carbonate ($NaCO_3$) was added to the mixture and incubated in the dark for another 20 minutes at room temperature. The absorbance was read at 765 nm using a microplate reader (Tecan Infinite M200, Austria). The procedure was repeated using gallic acid as the standard solution and the standard curve with a range of 31.25–1000 µg/mL was constructed. The content of phenol in the PSPE was expressed in terms of gallic acid equivalent (mg GAE/g).

Measurement of Total Flavonoid Content of PSPE

The total flavonoid content (TFC) of PSPE was measured using the aluminium chloride (AlCl3) method modified from Abd Manan et al.[14] The reaction mixture was prepared by mixing 100 µL 10 mg/mL PSPE and 100 µL of 2% AlCl3 in a 96-well plate. The tests were conducted in triplicates. After incubation for 15 minutes at room temperature, the absorbance was read at 415 nm using a microplate reader (Tecan Infinite M200, Austria). The procedure was repeated using quercetin as the standard solution and the standard curve with a range of 37.50–1200 µg/mL was constructed. The content of flavonoids in the PSPE was expressed in terms of quercetin equivalent (mg QE/g).

HPLC Instrumentation and Chromatographic Conditions

HPLC analysis was performed using Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan), equipped with a reverse-phase C18 column (4.6×250 mm, 5 µm; Agilent Eclipse Plus C18) set at 40°C and a UV/VIS detector that was set at 280 and 356 nm. The mobile phase consists of two different solvents (solvent A: 0.1% acetic acid and solvent B: acetonitrile). All solutions were degassed and filtered. The detection and quantifications of phenolic acids (gallic acid and caffeic acid) were done at 280 nm, using a gradient program that started with 10% B from 0 to 5 min and increased to 30% and back to 10% at 1.0 mL/min. Flavonoids (rutin and quercetin) were detected and quantified at 356 nm, using a gradient starting with 20% B for 5 minutes, increased to 40%, and back to 20% at 1.2 mL/min. All chromatography operations were carried out in triplicates. The PSPE powder was dissolved in HPLC grade methanol (10 mg/mL) and the peaks and retention time obtained were confirmed by comparing them to those of reference standards solutions (100 µg/mL).

RESULTS AND DISCUSSION

Extraction Yield

The dried pod sample was ground into powder to increase the surface area for better contact with the solvent during the extraction process. The solvent of choice was 70% ethanol to retrieve a wider polarity of compounds. While the repetitive maceration technique applied in the exhaustive extraction method ensures the complete removal of active compounds from the plant material.[12]

Both extraction solvents, ethanol and methanol are efficient solvents for phenolic compound extraction, whereby ethanol extracts flavonoids more effectively while methanol easily extracts phenolic acid. However, ethanol is more frequently used for the extraction of antioxidant compounds as it has a lower toxicity level.[15] In the present study, 70% ethanol was the solvent of choice for the maceration technique applied.

A total of 600 g of dried *P. speciosa* pod powder was used in the extraction process. The resulting freeze-dried PSPE acquired was 227 g, making the final extraction yield obtained 37.83%. Thus, our result was substantially higher than those of previous studies. Fithri et al., attained a final yield of 19.66% with the same extraction method[12] while Wonghirundecha et al.,[9] and Gan and Latiff,[6] reported yields of 14.85% and 12.4%, respectively. Despite applying the same method of extraction, we obtained a higher yield compared to Fithri et al., and this is most probably attributed to some changes made. The evaporation temperature used in the current study was 45℃ and it is lower compared to the 70℃ used in the study done by Fithri et al. This might have caused the differences in the yield obtained as flavonoids and phenolic compounds are heat-sensitive and easily oxidized.[16]

Total Phenolic and Flavonoid Contents of PSPE

Phenolics and flavonoids are naturally occurring compounds in plants that possess a broad range of biochemical activities. They account for the majority of the antioxidant activities in plant products. The TPC and TFC of PSPE were determined using the Folin-Ciocalteau and aluminum chloride methods, respectively and the results obtained are tabulated in Table I.

In the present study, the TPC obtained was 84.53±9.40 mg GAE/g and the TFC was 11.96±4.51 mg QE/g. Wonghirundecha et al., studied the ethanolic extract of *P.*

Table I: Quantitative analysis of phytochemicals in ethanolic extract of *P. speciosa*

Sample	Total phenolic content (mg GAE/g±SD)	Total flavonoid content (mg QE/g±SD)
PSPE (10mg/mL)	84.53±9.40	11.96±4.51

P. speciosa pod extract (PSPE)

Table II: Retention time, tr and concentration of phytochemical compounds in 10 mg/mL PSPE

Sample	Gallic acid	Caffeic acid	Rutin	Quercetin
tr standard (min)	3.345	7.666	3.730	8.817
tr PSPE (min)	3.410	7.796	3.640	8.823
Concentration in PSPE (g/kg ± SD)	5.91 ± 1.07	6.45 ± 3.36	0.39 ± 0.84	0.19±0.47

P. speciosa pod extract (PSPE)

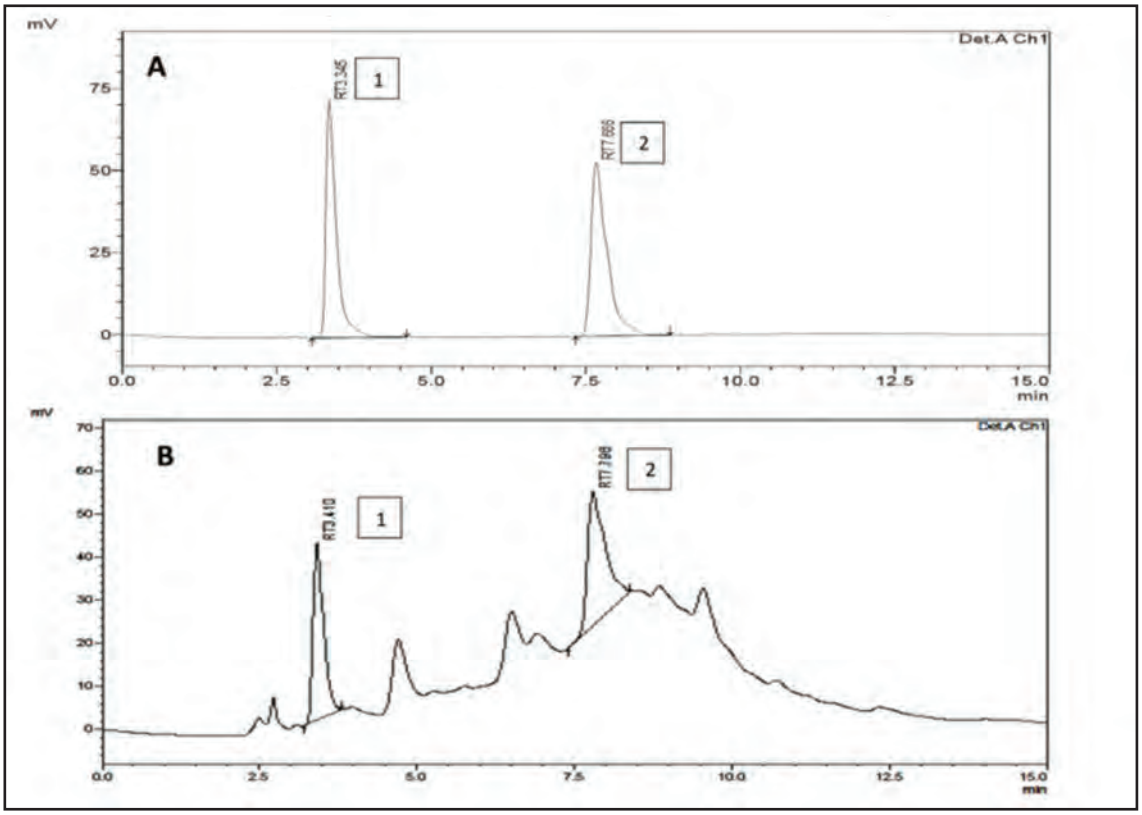

Fig. 1: Chromatogram of reference standard for phenolic acids (A) and PSPE (B) (peak 1: gallic acid; peak 2: caffeic acid) at 280 nm

speciosa pod originating from Thailand and reported TPC value of 71.39±0.08 mg GAE/g, and TFC value of 5.38±0.18 mg CE/g.[9] Another study by Gan & Latiff, reported 21.7±1.0 mg GAE/g sample for TPC and 1.1±0.3 mg PCE/g sample for TFC.[6] Similarly, our results were higher than the previous literature and the difference in results might be due to the variation in collection sites and drying methods of the plant materials. Their study showed that the pods were oven-dried at 70°C[6] and this higher temperature may have degraded some of the bioactive compounds as stated by Le et al.[17] Differences in collection site may also influence the phytochemical constituents in the plant materials since even various soils may have an impact on the phytochemical components of plants, as claimed by Mudau et al.[18]

HPLC Analysis

Using HPLC analysis, four compound standards were compared to PSPE, and multiple peaks were obtained in the sample extract chromatograms as shown in Figure 1 and Figure 2. The retention time (tR) for all standards and compounds detected in the PSPE are shown in Table II.

As recorded in Table II, PSPE contained 5.91±1.07 g gallic acid/kg dry extract and 6.45±3.36 g caffeic acid/kg dry extract. These two phenolic acids have also been reported in a previous study by Ko et al.[3] An almost similar yield of gallic acid was reported (6.58 g/kg) in their ethanolic extract of *P. speciosa* pods while the quantity of caffeic acid detected was not specified. To date, not much information is available regarding these phenolic acid contents in *P. speciosa* pods, unlike the beans. Ghasemzadeh et al., had reported that their *P. speciosa* beans harboured 6.42±0.67 g/kg gallic acid and 1.46±0.67 g/kg caffeic acid.[2] Hence, this shows that the pods and beans of *P. speciosa* possess comparable amounts of these phenolic acids, making the pods as beneficial as the beans.

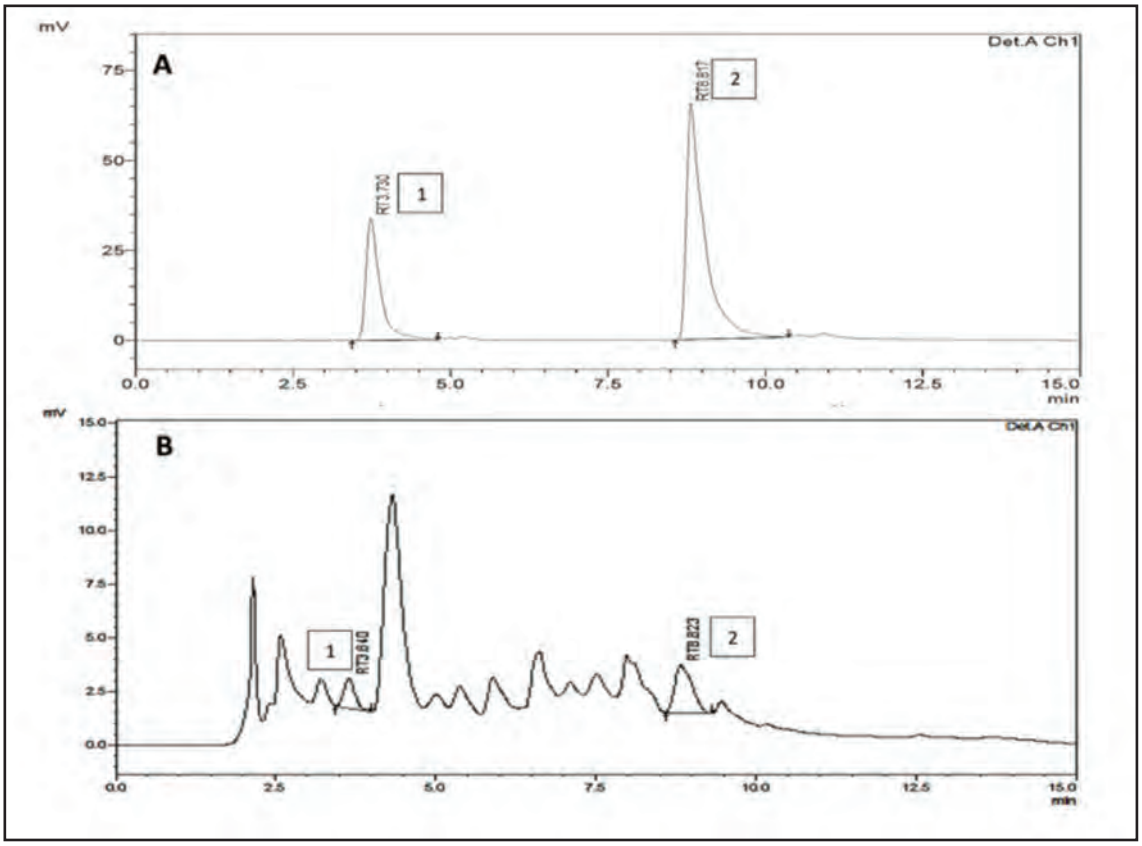

Fig. 2: Chromatogram of reference standard for flavonoids (A) and PSPE (B) (peak 1: rutin; peak 2: quercetin) at 356 nm

The therapeutic propensity of a plant species can be assessed by studying the phytochemicals present as these compounds have been reported to impart various medicinal characteristics. Caffeic acid, which was the most abundant of the four bioactive compounds studied, is usually found in tea, olives, coffee, and propolis. Caffeic acid is well known for its anti-inflammatory, antioxidant, and anticarcinogenic properties. Several studies have demonstrated the ability of caffeic acid to repress inflammation response as well as its potential to be utilised in the treatment of rheumatoid arthritis.[19] On top of that, caffeic acid also demonstrated an antiproliferative effect on cancer cell lines via apoptosis[20], indicating that this compound has the potential as a chemotherapeutic agent.

Gallic acid is frequently reported to have antimicrobial, anti-obesity, and antioxidant properties. This plant-derived hydroxybenzoic acid has a strong antibacterial effect and has the ability to induce irreversible alterations in 61 membrane properties of *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli,* and *Listeria monocytogenes*.[21] Based on a study by Pinho et al., gallic acid acted as an antibacterial agent without causing any damage to the animal cells and this shows that the compound is safe for humans according to ISO 10993- 5:2006.[22] In addition, anti-obesity properties of gallic acid have been observed from multiple reports of inhibition of pre-adipocyte proliferation,[23] regarding inhibition of fat droplet formation and triglyceride accumulation in 3T3-L1 cells,[24] and reduced adipocyte size in mice treated with gallic acid.[25]

As for the flavonoid compounds, rutin and quercetin were quantified by comparing their peaks to the compound standards. As demonstrated in Table II, PSPE contained 0.39±0.84 g rutin/kg dry extract and 0.19±0.47 g quercetin/kg dry extract. Previously, Mustafa et al. reported the presence of 5.84 mg quercetin per 100 g dry extract in the ethyl acetate fraction of ethanolic extract prepared from *P. speciosa* empty pod.[1] A study conducted by Ko et al., revealed a much higher quercetin yield at 4.86 g/kg as compared to the present study.[3] In another study conducted by Siti et al., they reported a 15.5 µg rutin/mg extract and 0.11 µg quercetin/mg extract from *P. speciosa* pods prepared with 95% ethanol.[11] The concentration of their rutin was higher compared to our findings. The discordance in the results obtained can be pinpointed to the different extraction techniques and HPLC settings applied in each study which resulted in the difference in yield for the respective compounds. The polarity index of the solvent used during the preparation of extracts must also be taken into consideration since it has an impact on the type and quantity of output obtained from the extraction process.[18] Use of higher water content will increase the polarity of the solvent[26] and allowed for more compounds to be extracted from the plant material. Additionally, more polar solvents can extract a class of compounds with a broader polarity range. This made it possible for non-phenolic polar molecules like proteins and carbohydrates to dissolve during the extraction process thus increasing the extraction yields. Therefore, in contrast to non-polar solvents, highly polar ones will produce high extract yields but lower phenolic and flavonoid contents.[27]

In the conducted study, the presence and quantities of the two flavonoids were successfully demonstrated in the PSPE. Rutin, a flavonoid prevalent in many fruits, vegetables, and cereals is a member of the vitamin C2 family and is commonly studied for its antimicrobial and anticancer properties.[28] It has previously shown strong antimicrobial activity against *S. aureus, P. aeruginosa*, *E. coli, E. faecalis,* and *K. pneumoniae*.[29] In addition, it inhibits abnormal cell growth by increasing caspase activity, induces cell cycle arrest, and stimulates apoptosis in cancer cells as part of its anticancer effect.[30] The anti-lipid activity of rutin has also been demonstrated by Livingston Raja et al. where the reduction in total cholesterol, triglycerides, low density lipoprotein (LDL) and very low density lipoprotein was seen in rats treated with rutin flavonoid.[31]

Finally, quercetin is a flavonoid with a vital scavenging role in oxidation[32] and is commonly reported as a potential antimicrobial agent.[33] According to Jaisinghani, quercetin was able to inhibit *S. aureus, P. aeruginosa, P. vulgaris*, and *E. coli* at various concentrations.[34] Its antimicrobial activities may result from its ability to disrupt bacterial cell wall and nucleic acid synthesis, inhibition of biofilm formation, and reduction of virulence factor expressions. Next, similar to caffeic acid, quercetin also has a reputation as a potential anti-inflammatory agent.[35] Several groups of researchers have demonstrated this particular property of quercetin, most commonly via inhibition of cytokines synthesis.[36-38] Following that, another attribute of quercetin that is popular among researchers nowadays is the anti-lipid property of this compound. Zhao et al. stated that quercetin exerted positive effects on adipose tissue by means of adipogenesis and lipogenesis inhibition and the suppression of preadipocyte differentiation.[39] Eseberri et al. demonstrated that quercetin was also able to reduce fat accumulation in mature adipocytes.[40] The presence of these compounds, therefore, justifies the therapeutic potential of PSPE to be developed into a beneficial health product from natural sources.

CONCLUSION

The ethanolic extract of *P. speciosa* pods (PSPE) prepared via an exhaustive extraction method has been shown to contain the four targeted phytochemicals namely gallic acid, caffeic acid, rutin, and quercetin. These results suggest it may be a potential natural product for antibacterial, anticancer, anti-inflammatory, anti-lipid, and antiviral purposes. While the results are encouraging, deeper and further investigations are required to completely understand the full potential of the extract.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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