

Phytochemical analysis and *in vitro* antioxidant activity of *Parkia speciosa*

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Abstract

Aim: The present research work was performed to evaluate the antioxidant potential and antiradical property of methanolic extract of pods of *Parkia speciosa*. **Materials and Methods:** In the present studies, quantitative estimation of flavonoids content and phenolic content was also carried out by colorimetric methods, using aluminum chloride and Folin–Ciocalteu reagent method, respectively, to establish a relationship between antioxidant activity and total phenolics and flavonoid contents. Antioxidant and radical scavenging activity were determined using different *in vitro* assays including reducing power assay and 2, 2-diphenyl-1-picryl hydrazyl free radical scavenging assay method. **Results and Discussion:** Preliminary phytochemical screening revealed that the extract of *P. speciosa* possesses flavonoids, alkaloids and phenolic materials. The total phenolic and flavonoid contents were found to be 14.16 ± 0.02 mg gallic acid equivalents/g dry weight and 5.28 ± 0.03 mg rutin equivalents/g dry weight, respectively. The extract showed significant antioxidant activity in a dose-dependent manner in both the assays. The IC_{50} values of all parameters were determined. Ascorbic acid and butylated hydroxytoluene were used as a standard. **Conclusion:** The results obtained in the present study indicated that *P. speciosa* pod methanolic extract could be a potential source of natural antioxidant.

Key words: 2, 2-diphenyl-1-picryl hydrazyl, antioxidant, ascorbic acid, hydroxyl radical, *Parkia speciosa*

INTRODUCTION

Reactive oxygen species (ROS), such as singlet oxygen, superoxide anion, hydroxyl radical, and hydrogen peroxide, are often generated as byproducts of biological reactions or from exogenous factors.^[1] These reactive species exert oxidative damaging effects by reacting with nearly every molecules found in living cells^[2] including DNA if excess ROS are not eliminated by the antioxidant system. They play important roles in aging and in the pathogenesis of age-related disorders such as cancer, hypertension, atherogenesis, Alzheimer's disease, and Parkinson's disease.^[3-5] Recent investigations have shown that the antioxidants with free-radical scavenging properties of plant origins could have great importance as therapeutic agents in the ageing process and free radical-mediated diseases including neurodegeneration.^[6,7] Plant extracts and plant products such as flavonoids and other polyphenol constituents have been reported to be effective radical scavengers and inhibitors of lipid peroxidation.^[8,9] Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have stimulated the interest of many investigators to search natural antioxidant.

Parkia speciosa Hassk or stink bean is a plant that is abundantly found in the tropical regions such as Malaysia, Indonesia, Thailand, and Philippines. It is a plant that belongs to the genus *Parkia* and species *speciosa* in the family Fabaceae (also placed in Leguminosae and Mimosaceae). It grows up to 40 m high. It bears green long and flat beans which are called pods in stalks. The stalks are 2-6cm wide and 30-45cm long. The light green stink bean seeds with seed coats are encapsulated in these pods. The seeds have a peculiar smell and can be eaten raw as “ulam” (a Malay word for uncooked) or cooked. The seeds are the most consumed “ulam” in Malaysia, and half-ripe seeds are also usually pickled in brine. The plant seeds have been used by the locals to treat various diseases and symptoms such as diabetes, kidney disorder, and headache.^[10]

In the present investigation, phytochemical screening, quantitative estimation of the total phenolic and total

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flavonoid have been carried out followed by antioxidant activity correlation between antioxidant activity and total phenolic and total flavonoid contents were also investigated to establish if there is a relationship between these groups of phytochemical and antioxidant activity. In view of its wide use and its chemical composition, the pod methanolic extract of *P. speciosa* was evaluated for its *in vitro* anti-oxidative activities. Antioxidant activity of *P. speciosa* was evaluated by the reducing power method and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method.

MATERIALS AND METHODS

Chemicals

Folin–Ciocalteu reagent, DPPH radical, butylatedhydroxy toluene (BHT) and l-ascorbic acid were purchased from R&M Chemicals, Essex, UK. Gallic acid and rutin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade available commercially.

Plant Material

The fresh pods of *P. speciosa* were collected in October 2014 at the local vegetable market, Sungai Petani, Kedah, Malaysia. The pods were dried and coarsely powdered which was stored in an airtight container at room temperature.

Preparation of Extract

The dried powder of pods of *P. speciosa* (100 g) was extracted exhaustively in a Soxhlet apparatus with methanol, (99%, 500 ml) for 6 h at 50°C. The solvent was completely removed by a rotary evaporator (Rotavapor® R-210, BUCHI Corporation). This crude extract was stored at low temperature in the refrigerator and used for phytochemical analysis and evaluation of antioxidant activity.

Phytochemical Screening

Freshly prepared extract of *P. speciosa* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed for alkaloids, glycosides, saponins, tannins, polyphenols, flavonoids, steroids, coumarins, carbohydrates and proteins using standard procedures.^[11,12]

Determination of Total Phenolic Content

Total phenols were determined by Folin–Ciocalteu reagent.^[13] A dilute extract of plant extract (0.5 ml of 1 mg/ml) or gallic acid (standard phenolic compound) was mixed with Folin–Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous

Na₂CO₃ (4 ml, 1 M). The mixtures were allowed to stand for 15 min, and the total phenols were determined by colorimeter at 765 nm (Shimadzu UV-Vis [ultraviolet- visible] 1601). The standard curve was prepared using 1 to 5 µg/ml solutions of gallic acid in methanol. The concentration of total phenolic compounds in the extract was expressed as milligram of gallic acid equivalents (GAE) a gram dry weight of the extract.

Determination of Total Flavonoid Content

The aluminum chloride colorimetric method was used for flavonoids determination.^[14] Plant extract (0.5 ml of 1 mg/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm (Shimadzu UV-Vis 1601). The calibration curve was prepared by preparing rutin solutions at concentrations 10-100 µg/ml in methanol. The concentration of total flavonoids compounds in the extract was expressed as mg of rutin equivalents (RE) per gram dry weight of the extract.

In vitro Antioxidant Activity Reducing Power Assay

The reducing power *P. speciosa* was determined according to the method described.^[15] Different concentrations of *P. speciosa* extract (10-50 µg/ml) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and the absorbance was measured at 700 nm (Shimadzu UV-Vis 1601). Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as a blank solution.

Scavenging Effect on DPPH Radical

The free radical scavenging capacity of the extracts was determined using DPPH.^[16] A DPPH solution (0.004% w/v) was prepared in 95% methanol. Methanolic extract of *P. speciosa* was mixed with 95% methanol to prepare the stock solution (1 mg/ml). The freshly prepared DPPH solution (0.004% w/v) was taken in test tubes then *P. speciosa* extract was added followed by serial dilutions (10 to 50 µg/ml) to every test tube so that the final volume was 3 ml and after 10 min, the absorbance was read at 515 nm using a spectrophotometer (Shimadzu UV-Vis 1601). BHT was used as a reference standard and dissolved in double distilled water to make the stock solution with the same concentration (1 mg/ml) followed by serial dilutions

(10-100 µg/ml). A control sample was prepared containing the same volume without any extract and reference BHT.

$$\text{Inhibition} = [(A_0 - A_1) / A_0 \times 100]$$

Where A₀ was the absorbance of the control (blank, without extract) and A₁ was the absorbance of the extract or standard.

Statistical Analysis

The analyses were performed in triplicate and the experimental results obtained were expressed as mean with 95% confidence interval for IC₅₀ and mean±SD for total phenolic and flavonoid content evaluated by GraphPad Prism 5 software.

RESULTS

Phytochemical Screening

Preliminary phytochemical screening of the extract of *P. speciosa* revealed the presence of various bioactive components of which flavonoids and tannins were the most prominent and the result of the phytochemical test are summarized in Table 1.

Total Phenolic Content

The total phenolic content of *P. speciosa* extract was measured using the Folin–Ciocalteu colorimetric method. In *P. speciosa* extract, the phenolic content was found to be 14.16 ± 0.02 mg GAE/g dry weight [Table 2].

Total Flavonoid Content

Therefore, in the present study, total flavonoid content present in the extract was estimated using the aluminum chloride

colorimetric method. In *P. speciosa* extract, the flavonoid content was found to be 5.28 ± 0.03 mg RE/g dry weight [Table 2].

In Vitro Antioxidant Activity

Reducing power assay

The reducing potential of the *P. speciosa* extract and ascorbic acid was very potent, and the power of the extract was increased with the quantity of sample [Figure 1]. It was determined by using a modified iron (III) to iron (II) reduction assay.

Scavenging effect on DPPH radical

Figure 2 shows the DPPH radical scavenging activity of the *P. speciosa* extract, compared with BHT, as standard. The IC₅₀ values of extract and standard were 74.37 µg/ml and 35.40 µg/ml, respectively.

DISCUSSION

Phenolic compounds are known to be powerful chain breaking antioxidants and are important constituents of plants. Phenolic compounds may contribute directly to antioxidative action. It is suggested that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1.0 g daily from a diet rich in fruits and vegetables.^[17] It has been recognized that flavonoids

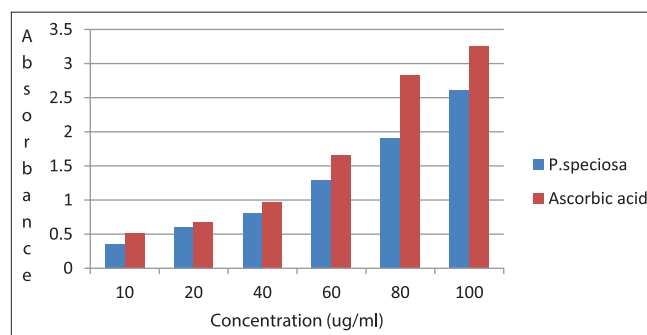


Figure 1: Reducing power assay

Table 1: Phytochemical screening of methanolic extract of *Parkia speciosa*

Phytochemical compound	Result
Alkaloid	+
Glycosides	–
Flavonoids	+
Tannins	+
Phenolic compound	+
Antraquinones	–
Saponin	+
Terpenoids	–
Coumarin	–
Steroids	–

+: Present, –: Absent

Table 2: Total phenolic and flavonoid content of *Parkia speciosa* extract

Total phenolic content (mg GAE/g dry weight)	Mean±SEM	Total flavonoid content (mg RE/g dry weight)	Mean±SEM
14.18	14.16±0.02	5.25	5.28±0.03
14.14		5.30	
14.16		5.28	

GAE: Gallic acid equivalents, SEM: Standard error of mean

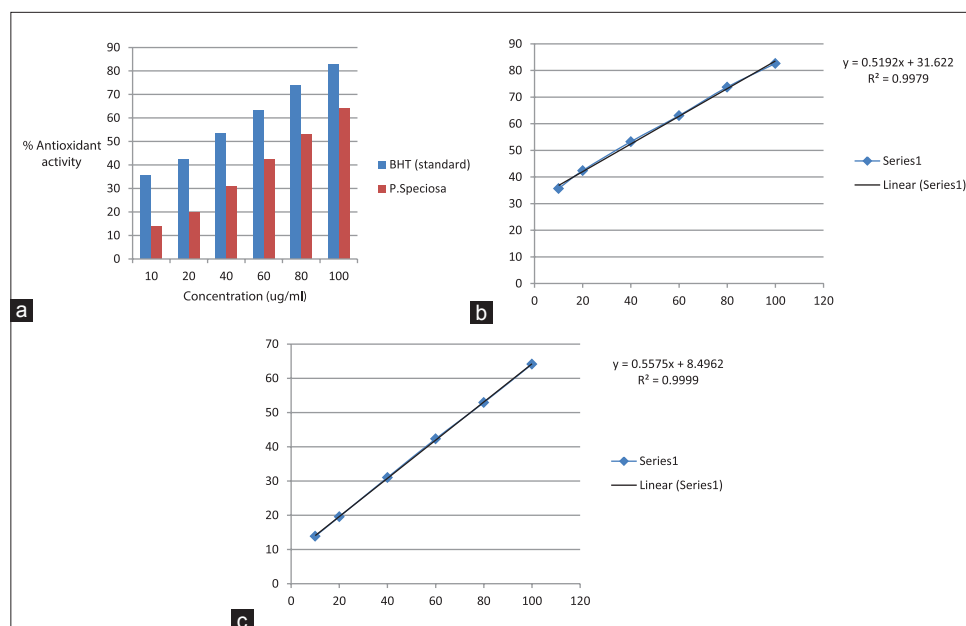


Figure 2: (a) Antioxidant activity of methanolic extract of *Parkia speciosa* (pod), (b) IC50 values of extract at 74.37 µg/ml, (c) IC50 values of extract at 35.40 µg/ml

show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through the scavenging or chelating process.^[18,19] In reducing power assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, we can monitor the Fe^{2+} concentration. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability.^[20] Although the DPPH radical scavenging abilities of the pod methanolic extract were less than that of BHT, the study showed that the extract has the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Phytochemical screening of the *P. speciosa* revealed the presence of phenolic and flavonoid compounds. Flavonoids have been shown to have antioxidant, antibacterial, anti-inflammatory, antiallergic activity, etc.^[21] The presence of phenolic and flavonoids in the drug extract is likely to be responsible for the antioxidant activity. These compounds are reported to be antioxidant or free radical scavengers.^[22]

CONCLUSION

The results obtained in the present study indicate that *P. speciosa* extract exhibits free radical scavenging, reducing power. The overall antioxidant activity of *P. speciosa* extract might be due to its flavonoid, polyphenolic, and other phytochemicals constituents. The findings of the present study suggested that *P. speciosa* could be a potential source of natural antioxidant that could have great importance as

therapeutic agents in preventing or slowing the progress of aging and age-associated oxidative stress-related degenerative diseases. Hence, it is worthwhile to isolate and elucidate the bioactive principle that one responsible for the antioxidant activity that is under process in our laboratory.

REFERENCES

- Pallavi S, Jha AB, Dubey RS, Mohammad P. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J Bot* 2012;2012:1-26.
- Sahoo S, Ghosh G, Das D, Nayak S. Phytochemical investigation and *in vitro* antioxidant activity of an indigenous medicinal plant *Alpinianigra* B. L. Burtt. *Asian Pac J Trop Biomed* 2013;3:871-6.
- Gupta MB, Palit TK, Singh N, Bhargava KP. Pharmacological studies to isolate the active constituents from *Cyperus rotundus* possessing anti-inflammatory, anti-pyretic and analgesic activities. *Indian J Med Res* 1971;59:76-82.
- Radha SS, Ramanujam S, Sundaram ER. An antioxidant inducer in asthma children. *J Ethnopharmacol* 1994;42:88-9.
- Komai K, Ueki K. Chemical properties and behaviour of polyphenolic substances in purple nutsedge. *Weed Res Jpn* 1975;20:66-8.
- Singh SB, Singh PN. A new flavanol glycoside from mature leaves of *Cyperus rotundus*. *J Indian Chem Soc* 1986;63:450-5.
- Singh PN, Singh SB. A new saponin from mature tubes of *Cyperus rotundus*. *Phytochemistry* 1980;19:2056-7.
- Ohira S, Hasegawa T, Hayasah KI, Hoshino T,

- Takaoka D, Nozaki H. Sesquiterpenoids from *Cyperus rotundus*. *Phytochemistry* 1998;47:577-9.
9. Sonwa MM, König WA. Chemical study of the essential oil of *Cyperus rotundus*. *Phytochemistry* 2001;58:799-810.
 10. Kamisah Y, Othman F, Qodriyah HM, Jaarin K. *Parkia speciosa* Hassk.: A potential phytomedicine. *Evid Based Complement Alternat Med* 2013;2013:709028.
 11. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. Germany: Springer; 1998.
 12. Khandelwal KR. Preliminary phytochemical screening. *Practical Pharmacogn* 2005;146-57. Available from: <http://www.allbookez.com/kokate-practical-pharmacognosy/>. [Last accessed on 2014 Jan 21].
 13. Rabeta MS, Nabil Z. Total phenolic compounds and scavenging activity in *Clitoria ternatea* and *Vitex negundo* Linn. *Int Food Res J* 2013;20:495-500.
 14. Atanasova M, Georgieva S, Ivancheva K. Total phenolic and flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *J Univ Chem Technol Metall* 2011;46:81-8.
 15. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J Food Compos Anal* 2006;19:669-75.
 16. Ghasemzadeh A, Jaafar HZ, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules* 2010;15:4324-33.
 17. Patil SM, Kadam VJ, Ghosh R. *In vitro* antioxidant activity of methanolic extract of stem bark of *Gmelina arborea* Roxb. (Verbenaceae). *Int J PharmTech Res* 2009;1:1480-4.
 18. Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J Pharm Pharmacol* 2003;55:131-42.
 19. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *Nutr Biochem* 1996;7:66-76.
 20. Shirwaikar A, Prabhu KS, Punitha IS. *In vitro* antioxidant studies of *Sphaeranthus indicus* (Linn). *Indian J Exp Biol* 2006;44:993-6.
 21. Miller AL. Antioxidant flavonoids: Structure, function and clinical usage. *Alt Med Rev* 1997;2:103-11.
 22. Polterait O. Antioxidant and free radical scavengers of natural origin. *Curr Org Chem* 1997;1:415-40.

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