

## Original Article

**Evaluation of Antioxidant Properties of *Phaulopsis fascisepala* C.B.Cl. (Acanthaceae)**S. A. Adesegun<sup>1</sup>, A. Fajana<sup>1</sup>, C. I. Orabueze<sup>1</sup> and H. A. B. Coker<sup>2</sup><sup>1</sup>Department of Pharmacognosy and <sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, PMB 12003, Lagos, Nigeria

The antioxidant activities of crude extract of *Phaulopsis fascisepala* leaf were evaluated and compared with  $\alpha$ -tocopherol and BHT as synthetic antioxidants and ascorbic acid as natural-based antioxidant. *In vitro*, we studied its antioxidative activities, radical-scavenging effects,  $\text{Fe}^{2+}$ -chelating ability and reducing power. The total phenolic content was determined and expressed in gallic acid equivalent. The extract showed variable activities in all of these *in vitro* tests. The antioxidant effect of *P. fascisepala* was strongly dose dependent, increased with increasing leaf extract dose and then leveled off with further increase in extract dose. Compared to other antioxidants used in the study,  $\alpha$ -Tocopherol, ascorbic acid and BHT, *P. fascisepala* leaf extract showed less scavenging effect on  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical and less reducing power on  $\text{Fe}^{3+}$ /ferricyanide complex but better  $\text{Fe}^{2+}$ -chelating ability. These results revealed the *in vitro* antioxidant activity of *P. fascisepala*. Further investigations are necessary to verify these activities *in vivo*.

**Keywords:** iron chelating power – phenolic content – radical scavenging – reducing power

**Introduction**

Cancer is a leading cause of death and may result from chronic injury to the epithelium by oxidants and other carcinogens (1–3). Epidemiological and experimental studies also offer strong evidence that implicates oxidative damage in the etiology of brain, heart and nervous system diseases (4–6). Although the body has effective defence systems that protect it against oxidative stress, the capacity of these protective systems decreases with aging (7) creating a need to provide the body with a constant supply of phytochemicals through dietary supplements.

*Phaulopsis fascisepala* C.B.Cl. (Acanthaceae) is an erect or decumbent herb or shrub found throughout the forest zones from Senegal to S. Nigeria and has been used by local indigenous groups for a wide range of medicinal

purposes. Dried and powdered, it is applied to staunch wounds in Nigeria. In Ivory Coast, it is used to treat sores caused by skin-parasites e.g. ringworm and fungal infections and is sometimes put into baths or steam baths to treat fever stiffness and rheumatic pain (8). Since the relationship between oxygen free radicals and damage to skin is well documented (6,9) the ethnopharmacological use of *P. fascisepala* for skin diseases inspired us to investigate its antioxidant properties.

**Methods****Chemicals and Reagents**

Deionized water, 2.0 M Folin-Ciocalteu phenol reagent, gallic acid, anhydrous sodium carbonate, 2,6-Di-tert-butyl-4-methylphenol (BHT),  $\alpha$ -Tocopherol (Vitamin E), 90% 1,1-diphenyl-2-picrylhydrazyl (DPPH),  $\text{FeCl}_2$  tetrahydrate, anhydrous ferric chloride, potassium ferricyanide, [4,4'- [3-(2-pyridinyl-1,2,4-triazine-5,6-diyl)]bis-benzenesulfonic acid] (ferrozine) and trichloroacetic

For reprints and all correspondence: Dr S. A. Adesegun, Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, PMB 12003, Lagos, Nigeria. Tel: 234-1-8035995080; Fax: 234-1-5851432; Email: asegun67@yahoo.com

© 2007 The Author(s).

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/2.0/uk/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

acid were purchased from Sigma Chemical Company (St Louis, MO). Ascorbic acid and all other chemicals were of analytical grade BDH Chemical Laboratory (England, UK).

### Plant Material

The aerial part of *P. fascisepala* collected in Ibadan, Nigerias was identified by Mr Felix Usanga of Forest Research Institute of Nigeria (FRIN). Voucher specimen with FHI number 107231 was deposited at the herbarium. The leaves were plucked from the aerial portion, dried separately at 30°C, crushed into fine particles (~250 µm) and then extracted.

### Extraction

About 500 g of powdered leaves of the plant was extracted with methanol (3l) using Soxhlet apparatus for 48 h. The extract was concentrated under reduced pressure using rotatory evaporator until a semi-solid sticky mass was obtained. The yield was 13.0% w/w.

### Evaluation of Antioxidant Activities

#### Total Phenolic Content

The total phenolic content of the leaf extract was determined separately using the method of Macdonald *et al.* (10) with modifications. Calibration curve was prepared by mixing ethanolic solution of gallic acid (1 ml; 0.025–0.400 mg ml<sup>-1</sup>) with 5 ml Folin-Ciocalteu reagent (diluted ten fold) and sodium carbonate (4 ml, 0.7 M). We measured absorbance at 765 nm and drew the calibration curve. One millimeter of ethanolic plant extract (5 g l<sup>-1</sup>) was also mixed with the reagents above and after 2 h the absorbance was measured to determine total plant phenolic contents. All determinations were carried out in triplicate. The total content of phenolic compounds in the extract in gallic acid equivalents (GAE) was calculated by the following formula:

$$T = \frac{C \cdot V}{M}$$

where  $T$  = total content of phenolic compounds, milligram per gram plant extract, in GAE;  $C$  = the concentration of gallic acid established from the calibration curve, milligram per milliliter;  $V$  = the volume of extract, milliliter;  $M$  = the weight of ethanolic plant extract, gram.

#### Radical Scavenging Activity

We estimated the effect of extract,  $\alpha$ -Tocopherol, ascorbic acid and BHT on the DPPH radical according to the method of Koleva *et al.* (11) with modification.

**Table 1.** Antioxidant activities of extracts,  $\alpha$ -Tocopherol and BHT as expressed by half-effective doses (ED<sub>50</sub>)

Antioxidant reaction	Antioxidant	ED <sub>50</sub> (mg)
DPPH radical	<i>P. fascisepala</i>	0.50
	$\alpha$ -Toc	0.35
	BHT	0.24
	Ascorb	0.10

The plant extract (0.1–2.0 mg), BHT, ascorbic acid or  $\alpha$ -Tocopherol (0.02–1.00 mg), in 4 ml of distilled water was added to a methanolic solution of DPPH (1 mM, 1 ml). The mixture was shaken and allowed to stand at 20°C for 30 min; the absorbance of the resulting solution was measured spectrophotometrically at 517 nm. We then calculated the ability to scavenge DPPH radical using the following equation:

$$\text{Scavenging Effect (\%)} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100$$

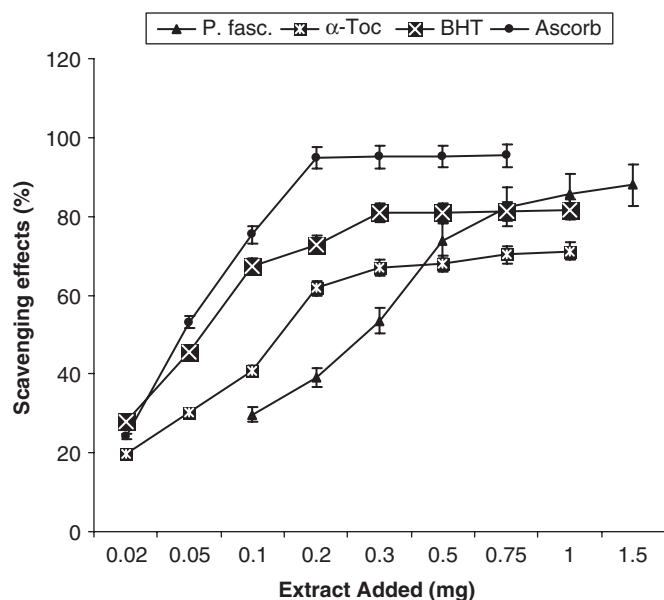
where  $A_0$  was the absorbance of the control and  $A_1$  was the absorbance in the presence of the sample of extract and standard.

#### Reducing Power

Using the modified method of Yen and Chen (12), we determined the reducing power of leaf extract,  $\alpha$ -Tocopherol, ascorbic acid and BHT. The extract (0.1–1.5 mg),  $\alpha$ -Tocopherol, ascorbic acid or BHT (0.1–1.0 mg) in 1 ml of methanol was mixed with phosphate buffer (2.5 ml, 0.2 M, PH 6.6) and potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (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 1000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

#### Determination of Fe<sup>2+</sup>-Chelating Ability

Fe<sup>2+</sup> chelating ability was determined according to the method of Gulcin *et al.* (13). The Fe<sup>2+</sup> was monitored by measuring the formation of ferrous iron-ferrozine complex at 562 nm. The *P. fascisepala* extract (0.1–2.0 mg) in 5 ml of methanol was mixed with FeCl<sub>2</sub> (0.5 ml, 2 mM) and ferrozine (1.0 ml, 5 mM). The resulting mixture was shaken and left to stand for 10 min at room temperature. The absorbance of the resulting solution was measured at 562 nm. The lower the absorbance of the reaction mixture, the higher the Fe<sup>2+</sup>-chelating ability.



**Figure 1.** Scavenging effects of *Phaulopsis fascisepala*,  $\alpha$ -Tocopherol, ascorbic acid and BHT on DPPH radical. Each value represents mean  $\pm$  SD ( $n = 3$ ).

The capability to chelate the ferrous iron was calculated using the following equation:

$$\text{Chelating effect (\%)} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100$$

where  $A_0$  was the absorbance of the control and  $A_1$  was the absorbance in the presence of the sample of extract and standard.

### Statistical Analysis

All data were expressed as mean  $\pm$  SD. Analysis of variance was performed by ANOVA procedures and  $P < 0.05$  was considered to be statistically significant.

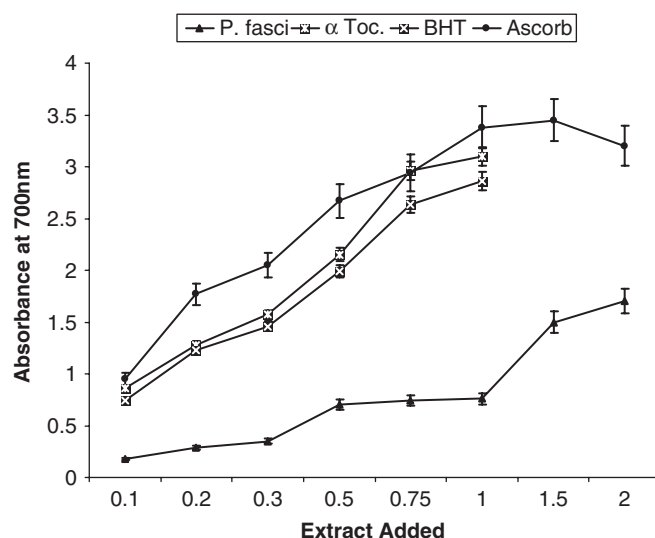
## Results

### Total Phenolic Content

The total phenolic content of the *P. fascisepala* leaf extract measured by Folin Cicalteu reagents in terms of gallic acid equivalent (GAE) was  $29.27 \pm 1.46 \text{ mg g}^{-1}$ .

### Radical Scavenging Increased with Antioxidant Concentration

Figure 1 shows the dose response curve for the radical scavenging effect of *P. fascisepala* extract,  $\alpha$ -Tocopherol, ascorbic acid and BHT by the DPPH coloring method. The radical scavenging effects of extract and synthetic antioxidants used as positive control on DPPH radical were concentration dependent. The scavenging of DPPH radicals increased with increasing antioxidant



**Figure 2.** Reducing power of *Phaulopsis fascisepala*,  $\alpha$ -Tocopherol, ascorbic acid and BHT. Each value represents mean  $\pm$  SD ( $n = 3$ ).

concentration. The radical scavenging activity of the extract and positive controls decreased in the following order: ascorbic acid  $>$  BHT  $>$   $\alpha$ -Tocopherol  $>$  *P. fascisepala*. The scavenging percentage on the DPPH radical was 53.4% for *P. fascisepala* extract at the dose level of 0.3 mg and 61.7, 53.2 and 67.4% for  $\alpha$ -Tocopherol, ascorbic acid and BHT at the dose 0.2, 0.05 and 0.1 mg.

### Reducing Powers Increased with Increasing Concentrations

Figure 2 shows the reducing powers of the extract,  $\alpha$ -Tocopherol, ascorbic acid and BHT. We discovered that the reducing powers of the extract, BHT and natural-based antioxidants like  $\alpha$ -Tocopherol and ascorbic acid on  $\text{Fe}^{3+}$  were concentration dependent. The reducing powers increased with increasing concentration but the reducing powers of  $\alpha$ -Tocopherol, ascorbic acid and BHT were significantly ( $P < 0.05$ ) higher than that of *P. fascisepala* extract. For instance, the absorbances at 700 nm were found to be 0.76, 0.86, 0.95 and 0.74 for *P. fascisepala*,  $\alpha$ -Tocopherol, ascorbic acid and BHT at the doses of 1.0, 0.1, 0.1 and 0.1 mg. This means to reach a similar reducing power, the dose required for *P. fascisepala* was  $\sim 10$ -fold than required for  $\alpha$ -Tocopherol, ascorbic acid and BHT.

### Iron (II) Chelating Ability of the Extract

The extract of *P. fascisepala* leaf showed a moderate ferrous ion chelating ability. This ability increased with increasing *P. fascisepala* leaf extract concentration and reached 34.4% at a concentration of  $2.0 \text{ mg ml}^{-1}$ .

However,  $\alpha$ -Tocopherol, ascorbic acid and BHT showed no such ability.

## Discussion

The phenolic content of *P. fascisepala* leaf extract was  $29.27 \pm 1.46 \text{ mg g}^{-1}$  in terms of GAE. Phytochemicals, especially plant phenolics constitute a major group of compounds that act as primary antioxidants (14). They can react with active oxygen radicals, such as hydroxyl radicals (15), superoxide anion radicals (16) and lipid peroxy radicals (17), and inhibit lipid oxidation at an early stage. They also inhibit cyclooxygenase and lipoxygenase of platelets and macrophages, thus reducing thrombotic tendencies *in vivo* (18).

Proton radical-scavenging action is one mechanism for oxidation. DPPH has a proton free radical and shows characteristic absorption at 517 nm (purple). When it encounters proton radical scavenger, its purple color fades rapidly (19–21), suggesting that antioxidant activity of *P. fascisepala* leaf extract is due to its proton donating ability. *Phaulopsis fascisepala* leaf extract was less able to scavenge radicals than  $\alpha$ -Tocopherol and BHT and these differences were statistically significant ( $P < 0.05$ ). The dose of *P. fascisepala* leaf extract required to reduce the absorbance of DPPH control solution by 50% ( $\text{EC}_{50}$ ) was calculated at 0.50 mg and those of  $\alpha$ -Tocopherol, BHT and ascorbic acid were 0.35, 0.24 and 0.1 mg (Table 1). In other words, *P. fascisepala* leaf extract required a 1.5–5.0-fold dose to scavenge DPPH as well as  $\alpha$ -Tocopherol, BHT and ascorbic acid. Also by simple regression analysis, we found that the total phenolic content of the extract positively correlated ( $r = 0.991$ ) with its DPPH radical scavenging effect, suggesting that phenolic constituents of the plant contributed to the scavenging. Further studies such as ability of the extract to suppress 2,2'-azobis(2-amidinopropane) dihydrochloride-induced plasma oxidation (22) may be necessary to verify these activities.

In this assay, the presence of antioxidants in the sample reduced  $\text{Fe}^{3+}$ /ferricyanide complex to the ferrous form.  $\text{Fe}^{2+}$  can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. This reducing capacity of compounds could serve as an indicator of potential antioxidant properties (23) and increasing absorbance could indicate an increase in reducing power. Although the greatest reducing power was obtained from ascorbic acid,  $\alpha$ -Tocopherol and BHT compared to *P. fascisepala*, our results showed that *P. fascisepala* leaf extract is an electron donor and could react with free radicals, convert them to more stable products and terminate radical chain reaction (24). Also, a strong positive correlation ( $r = 0.980$ ) between total phenolic content and the reducing power of the extract suggests that phenolic compounds in the plant may determine the

ferric ion reducing antioxidant power (FRAP) of the extract. Further investigations such as evaluation of blood total antioxidant capacity (TAC) (25) and antioxidant status in streptozotocin–nicotinamide-induced type 2 diabetic rats (26) may be necessary to verify our findings *in vivo*.

As a transitional metal, the capability of iron to generate free radicals from peroxides by Fenton reactions has been implicated in cardiovascular disease (27). Since  $\text{Fe}^{2+}$  has been shown to induce oxyradical production and lipid peroxidation, reduction of  $\text{Fe}^{2+}$  concentrations in the Fenton reaction protects against oxidative damage (28,29). Thus, *P. fascisepala* leaf extract may offer protection against oxidative damage through its chelating properties. Further work such as deoxyribose degradation assay in the absence of EDTA (22) could also be carried out to further establish these iron chelating properties.

## Conclusion

*Phaulopsis fascisepala* leaf extract demonstrated moderate antioxidant activity, reducing power, as well as scavenging and chelating effects. Purification of the extract may lead to increased activity in its bioactive compounds. The antioxidant activities of *P. fascisepala* leaf extract may be due to its proton donating capability as shown in DPPH radical scavenging results. Acting as an electron donor that can react with free radicals, it converts them to more stable products and terminates radical chain reactions. This mechanism may explain its use for the treatment of wounds and sores.

## Acknowledgements

We acknowledge the contributions of Mr. Elechi N. A. and the technical assistance of Mr. T. I. Adeleke.

## References

- Lai LS, Chou ST, Chao WW. Studies on the antioxidative activities of Hsian-tsoa (*Mesona procumbens* Hems) leaf gum. *J Agric Food Chem* 2001;49:963–8.
- Walaszek Z. Metabolism, uptake and excretion of a D-glucaric acid salt and its potential use in cancer prevention. *Cancer Detect Prev* 1997;21:178–90.
- Murphy GP. Cancer diagnosis, treatment and recovery. *Am Canc Soc* 1997;2:23–45.
- Lee IM, Cook NR, Manson JE. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: Women's Health Study. *J Nat Canc Inst* 1999;91:2102–6.
- Yoshikawa T, Naito Y, Kondo M. Food and diseases 2. In: Hiramatsu M, Yoshikawa T, Inoue M (eds). *Free Radicals and Diseases*. New York: Plenum Press, 1997, 11–9.
- Buyukokuroglu ME, Gulcin I, Oktay M, Kufrevioglu OI. *In-vitro* antioxidant properties of dantrolene sodium. *Pharmacol Res* 2001;44:491–4.
- Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. UK: Oxford University Press, 1999, 60–7.
- Burkill HM. *The useful plants of West Tropical Africa*. Kews: Royal Botanical Garden, 1985, 388–9.

9. Davies KJA. Oxidative stress: the paradox of aerobic life. *Biochem Soc Symp* 1994;61:1–34.
10. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive oil extracts. *Food Chem* 2001;73:73–84.
11. Koleva II, Van Beek TA, Linssen JPH, deGroot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem Ana* 2002;13:8–17.
12. Yen GC, Chen HY. Antioxidant activity of various teas extracts in relation to their antimutagenicity. *J Agric Food Chem* 1995;43:27–32.
13. Gulcin I, Buyukokuroglu ME, Kufrevioglu OI. Metal chelating and hydrogen peroxide scavenging effects of melatonin. *J Pineal Res* 2003;34:1–10.
14. Hatano T, Edamatsu R, Mori A, Fujita Y, Yasuhara E. Effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2-picrylhydrazyl. *Chem Pharm Bull* 1989;37:2016–23.
15. Hussain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* 1987;26:2489–91.
16. Afanasiev IB, Dorozhko AI, Bordskii AV. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol* 1989;38:1763–9.
17. Torel J, Cillard J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radicals. *Phytochemistry* 1986;25:383–5.
18. Moroney MA, Alcaraz MJ, Forder RA. Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J Pharm Pharmacol* 1988;40:787–92.
19. Yamagushi T, Takamura H, Matoba T, Terao J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci Biotechnol Biochem* 1998;62:1201–4.
20. Chang LW, Yen WJ, Huang SC, Duh PD. Antioxidant activities of sesame coat. *Food Chem* 2002;78:347–54.
21. Soares JR, Dinis TCP, Cunha AP, Azeiteiro LM. Antioxidant activities of some extracts of *Thymus zygis*. *Free Radical Res* 1997;26:469–78.
22. Ljubuncic P, Dakwar S, Portnaya I, Coogan U, Azaizeh H, Bomzon A. Aqueous extract of *Teucrium polium* possess remarkable antioxidant activity *in vitro*. *Evid Based Complement Alternat Med* 2006;3:329–38.
23. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defence system of various senescing leaves. *J Agric Food Chem* 1995;43:1813–9.
24. Yoshino M, Murakami K. Interaction of iron with polyphenolic compounds: application of antioxidant characterization. *Anal Biochem* 1998;257:40–4.
25. Ranjbar A, Khorami S, Safarabadi M, Shahmoradi A, Malekiran A, Vakilian K, et al. Antioxidant activity of Iranian *Echium amoenum* Fisch & C. A. Mey flower decoction in humans: A cross-sectional before/after clinical trial. *Evid Based Complement Alternat Med* 2006;3:469–73.
26. Punitha ISR, Rajendran K, Shirwaikar A, Shirwaikar A. Alcohol stem extract of *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. *Evid Based Complement Alternat Med* 2005;2:375–81.
27. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 1990;186:1–85.
28. Halliwell B. Reactive oxygen species in living systems: source, biochemistry and role in human disease. *Am J Med* 1991;91:14–20.
29. Yamagushi F, Ariga T, Yoshimira Y, Nakazawa H. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. *J Agric Food Chem* 2000;48:80–6.

Received September 4, 2006; accepted May 8, 2007