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*Full Length Research Paper*

# Evaluation of antioxidant activity of leave extract of *Bauhinia rufescens* Lam. (Caesalpiniaceae)

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**Antioxidant evaluation of *Bauhinia rufescens* used in Northern Nigerian traditional medicine, was carried out using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and reducing power assay on the methanolic extract of the leaves. The results of the DPPH scavenging activity indicate a concentration dependent antioxidant activity with no significant difference ($p < 0.05$) at 50, 125 and 250 µgml$^{-1}$ with those of the standard ascorbic and gallic acids. The total phenolic content was determined and found to be 68.40 ± 0.02 mg/g gallic acid equivalent (GAE) and the reducing power of 0.071 ± 0.03 nm was obtained. The phytochemical screening revealed the presence of flavonoids, tannins and saponins whose synergistic effect may be responsible for the strong antioxidant activity. It indicates that the methanolic extract of the leave may have promising antioxidant agents and may also help in the treatment of the diseases caused by free radicals.**

**Key words:** Antioxidants, free radicals, *Bauhinia rufescens,* DPPH, reducing power, total phenolics.

## INTRODUCTION

Reactive oxygen species (ROS) which include the oxygen free radicals; superoxide anion ($O_2^{\cdot -}$), hydroxyl radical (OH·) and some non-radical hydrogen peroxide ($H_2O_2$) derivatives of oxygen are normally produced in living organisms with the potential of reacting with almost all types of molecules in living cells (McCord, 1985). The harmful effects of free radicals are neutralized by the enzymatic antioxidant defenses including the superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). However, overproduction of the ROS arising from either mitochondrial electron transport chain, excessive stimulation of NAD(P)H, or exposure to environmental pollutants, cigarette smoke, ultraviolet-rays, some parasitic infections, radiation and toxic chemicals results in oxidative stress- a phenomenal disturbance in the equilibrium status of pro-oxidant/antioxidants reactions in living systems, which mediates damage to cell structures, including lipids and membranes, proteins, and DNA (Valko et al., 2006). Recently, there has been an increased interest in oxygen containing free-radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic and ageing diseases, including heart disease, stroke, arteriosclerosis, diabetes mellitus, cancer, malaria, rheumatoid arthritis, neurodegenerative diseases (Alzheimer's and Parkinson's diseases) and AIDS (Alho and Leinonen, 1999; Olukemi et al., 2005). Hence, therapy using free-radical scavengers (antioxidants) has potential to prevent, delay or ameliorate many of these disorders (Delanty and Dichter, 2000). Numerous natural antioxidants have already been isolated from different varieties of plant material such as leafy vegetables, fruits, seeds, cereals and algae (Pokorny, 1991). They have been shown to have ROS scavenging and lipid peroxidation preventive effects (Atawodi, 2005; Aqil et al., 2006). The protection can be explained by the capacity of the antioxidants phenolics, flavonoids and polypropanoids in the plants and plant products to scavenge free radicals, due its proton donating ability. *Bauhinia rufes-*

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*cens* is a branched shrub or small tree up to 25 ft high, with white flowers. It is found in the entire Sahel and adjacent Sudan zone, from Senegal and Mauritania across Northern Ghana and Nigeria. Leaves and fruit are applied for the treatment of diarrhea, dysentery, ophthalmic diseases and diabetes mellitus (Kaey et al., 1964). Previous antioxidant activities of plants belonging to *Bauhinia* genus from different part of the world have been reported including *B. galpinii* (Aderogba et al., 2007), *B. monandra* (Argolo et al., 2004; Aderogba et al., 2006), *B. microstachya* (Dasilva et al., 2007) and *B. tarapotensis* (Braca et al., 2001). Furthermore, antitumor activity of *B. racemosa* (Gupta et al., 2004), antidiabetics of *B. forficata* (Pepata et al., 2002) and anti-inflammatory activities of flavonoids and triterpene caffeate isolated from B. variegate (Rao et al., 2008) have also been reported. In our continued investigation on the phytochemical and pharmacological properties of medicinal plants belonging to the Nigerian flora, we evaluated the antioxidants properties of the leaf methanolic extract of *Bauhinia rufescens* with a view to assess its potential use as natural antioxidant as well as understand some molecular basis of its therapeutic properties.

## MATERIALS AND METHODS

### Chemicals and reagents

Deionized water, Folin-Ciocalteu phenol reagent (Fluka, UK) gallic acid (Fluka, UK), 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich Co.), Trichloroacetic acid (Sigma-Aldrich Co.), anhydrous ferric chloride, potassium ferricyanide, anhydrous sodium carbonate, Ascorbic acid and all other chemicals were of analytical grade BDH Chemical Laboratory (England, UK).

### Plant materials

The plant was collected in the month of July, 2008 at Dakace village along Jos road, Zaria. It was taxonomically authenticated at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A Voucher specimen (900230) was deposited there for future reference. The leaves were air-dried for three weeks and grounded to powder using pestle and mortar.

### Extraction and preliminary fractionation

A 350 g of the powdered leave sample was extracted exhaustively with methanol 1.5 L (cold extraction) for two weeks. The extract was filtered using Whatman filter paper no. 2, and concentrated on a Büchi rotary evaporator at 45°C which afforded 42.3 g of the crude extract referred to as *Bauhinia rufescens* methanol extract (BRME), with a percent recovery of 12.08 g.

### Phytochemical screening

Phytochemical screening of the extract and fractions were carried out to identify the constituents, using standard phytochemical methods as described by Sofowora (1993).

### Evaluation of antioxidant activity

The determination of the free radical scavenging activity of each of the crude extract was carried using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay as described by Mensor et al. (2001) with a slight modification. Various concentrations of 250, 125, 50, 25 and 10 $\mu g ml^{-1}$ of sample extracts in methanol were prepared. 1.0 ml of a 0.3 mM DPPH in methanol was added to 2.5 ml solution of the extract or standard, and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 518 nm on a spectrophotometer (Jenway, 6025). The decrease in absorbance was then converted to percentage antioxidant activity (% AA) using the formula:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}\}$$

Blank = Methanol (1.0 ml) plus sample solution (2.0 ml), Negative control=DPPH solution (1.0 ml, 0.25 mM) plus methanol (2.0 ml), ascorbic acid and gallic acid were used as standards.
The scavenging reaction between ($DPPH^{\bullet}$) and an antioxidant (H-A) can be written as:

$$(DPPH^{\bullet}) + (H\text{-}A) \rightarrow DPPH\text{-}H + (A^{\bullet})$$
Purple Yellow

### Reducing power assay

This was determined according the method of Oyaizu (1986). The extract or standard ($100 \mu g ml^{-1}$) was mixed with phosphate buffer ($_P$H 6.6) and potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%, 2.5ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5ml) and the absorbance was measured at 700 nm in a spectrophotometer (Jenway 6025). Higher absorbance of the reaction mixture indicated reductive potential of the extract.

### Total phenolic content

The total phenolic content of the extract was determined using the method of Macdonald et al. (2001) with slight modifications. Calibration curve was prepared by mixing ethanol solution of Gallic acid (1 ml; 0.025 - 0.400 mg/ml) with 5 ml Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 ml, 0.7 M). Absorbance values were measured at 765 nm and the standard curve was drawn. One milliliter of BRME (5 $gL^{-1}$) was also mixed with the reagents above and after 30 min the absorbance was measured to determine the total phenolic contents. All determinations were carried out in triplicate. The total phenolic compound in the extract in gallic acid equivalents (GAE) was calculated by the following formula:

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

Where T=total phenolic contents, milligram per gram of BRME, in GAE; C= the concentration of gallic acid established from the calibration curve, mg/ml; V= the volume of extract, milliliter; M= the weight of BRME (g).

### Statistical analysis

The experiments were done in triplicate. The results are given as mean ±standard deviation (SD) Student's t-test was used for

**Table 1.** Results of phytochemical screening leaf extract of *B. rufescens.*

| Phytochemicals | Results |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Saponins | + |
| Tannins | + |
| Triterpenes | + |
| Anthraquinones | - |
| Cardiac glycosides | - |

+ = present, - = absent.

**Table 2.** Result of Antioxidant activity of leave extract of *B. rufescens.*

| % Antioxidant activity | | | |
|---|---|---|---|
| **Concentration(µg/ml)** | **BRME** | **Ascorbic acid** | **Gallic acid** |
| 10 | 56.21± 0.005 | 71.52 ± 0.002 | 66.26 ± 0.001 |
| 25 | 65.76 ± 0.040 | 81.23 ± 0.002 | 75.27 ± 0.003 |
| 50 | 74.65 ± 0.002* | 85.02 ± 0.001 | 81.81± 0.004 |
| 125 | 83.29 ± 0.001* | 93.66 ± 0.003 | 91.60 ± 0.007 |
| 250 | 83.70 ± 0.003* | 94.81± 0.001 | 92.59 ± 0.003 |

BRME = *Bauhinia rufescens* methanol extract, *($P < 0.05$) no significant difference

comparison between the two means and a one-way analysis of variance (ANOVA) was used for comparison of more than two means. A difference was considered statistically significant when $p < 0.05$.

## RESULTS

Phytochemical screening of BRME revealed the presence of secondary metabolites such as saponins, triterpenes, flavonoids, tannins, and alkaloids (Table 1). The results of the free radical scavenging activity of the DPPH assay showed the percentage antioxidant activity of 83.7, 83.3, 74.7, 65.8 and 56.2 for 250, 125, 50, 25 and 10 µgml$^{-1}$ respectively (Table 2). The reducing power of the extract (0.071 ± 0.003 nm) was found to be lower than the Gallic acid standard (0.096 ± 0.035 nm). The total phenolic content was found to be 68.40 ± 0.02 mg/g expressed as gallic acid equivalent (GAE).

## DISCUSSION

The result of phytochemical screening of methanol extract of *Bauhinia rufescens* showed the presence of saponins, triterpenes, flavonoids and tannins. It is reported that the phenolic compounds constitute a major group of compounds that acts as primary antioxidants (Hatano et al., 1989). The total phenolic content of the *B. rufescens* extract in terms of gallic acid equivalent (GAE) is indicative of high antioxidant potential of the extract, because the phenolic constituents can react with active oxygen radicals such as hydroxyl radical (Hussein et al., 1987), superoxide anion radical (Afanaslev et al., 1989) and lipid peroxy radical (Torel et al., 1986). Literature reports showed that there is high correlation between antioxidant activity and phenolic compounds (Odabasoglu et al., 2004).

The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts (Koleva et al., 2002, Goncalves et al., 2005). The decrease in absorbance by the DPPH radical with increase in concentration of the extract (Figure 1) which manifested in the rapid discolouration of the purple DPPH, suggest that BRME has antioxidant activity due to its proton donating ability (Adesegun et al., 2007). The methanolic extract of its leaves was found to highly scavenge free radicals in that there was no significant difference ($P < 0.05$) between the antioxidant activity of the BRME and those of standard ascorbic and gallic acids at 50, 125 and 250 µgml$^{-1}$ (Table 2).The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir et al., 1995).The higher absorbance at high concentrations indicates the strong reducing power potential of the extract. That the reducing power of the extract was not significantly different ($P < 0.05$) from that of gallic acid, suggest that the leave extract has high redox potentials and can acts as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen et al., 1999).

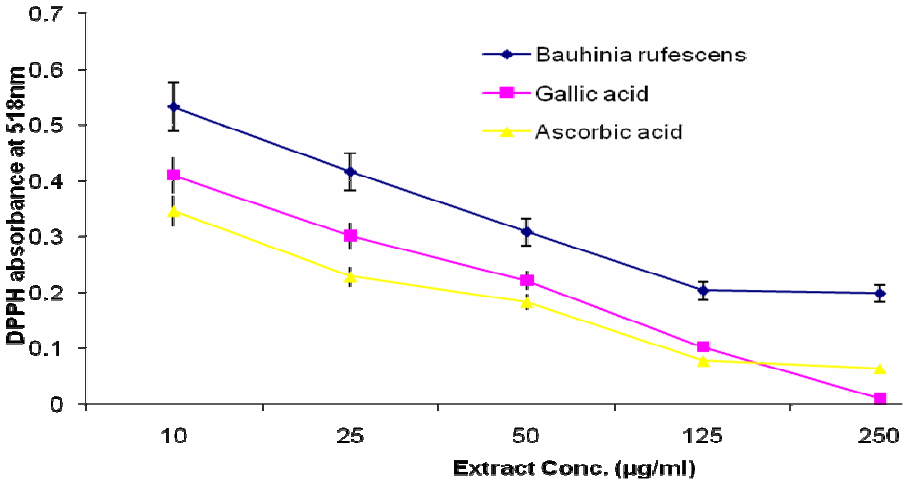


**Figure 1.** Free radical scavenging activity of the methanolic extract of *B. rufescens* leaves.

Considering the phytochemical screening, total phenolics, reducing power and the DPPH radical scavenging activity as indices of antioxidant activity of the extract, these findings revealed the potential of the extract as a source for natural antioxidants. It indicates that the plant could be promising agent in scavenging free radicals and treating diseases related to free radical reactions. This work provides an insight to understanding some molecular basis of therapeutic properties of *Bauhinia rufescens* in traditional medicine. Furthermore, detailed studies on the isolation and characterization of the plant extract as well as *in vivo* assays will be necessary in discovering new biological antioxidants.

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### REFERENCES

Aderogba MA, McGaw LJ, Ogundaini AO, Eloff JN (2007). Antioxidant activity and cytotoxic study of the flavonol glycosides from *Bauhinia galpinii*. Nat. Prod. Res. 21(7):591-599.

Aderogba MA, Ogundaini AO, Eloff JN (2006). Isolation of two flavonoids from *Bauhinia Monandra* (Kurz) leaves and their antioxidative effects. Afr. J. Trad. CAM 3(4):59-65.

Adesegun SA, Fajana A, Orabueze CI, Cooker HAB (2007). Evaluation of antioxidant properties of *Phaulopsis fascisepala* C.BC.I. (Acanthaceae). Evid. Based Complement. Altern. Med. 4:1-5

Afanaslev IB, Dorozhko AI, Bordskii AV (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem. Pharmacol. 38:1763-1769.

Alho H, Leinonen J (1999). Total antioxidant activity measured by chemiluminescence method. Met. Enzymol. 299:3-15.

Aqil F, Ahmad I, Mehmoud Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol. 30:177-183.

Argolo ACC, Sant'Ana AEG, Pletsch M, Coelho L, CBB (2004). Antioxidant activity of leaf extracts from *Bauhinia monandra*. Bioresour. Technol. 95(4):229-233.

Atawodi SE (2005). Antioxidants potential of African medicinal plants. Afr. J. Biotech. 4(2):128-133.

Braca A, De-Tommasi N, Di-Bari L, Pizza C, Politi M, Morelli I (2001). Antioxidant principles from *tarapotensis.* J. Nat. Prod. 64(7):892-895.

Dasilva EG, Behr GA, Zanotto-filho A, Bordignon CL, Da silva FA, Aboy AL, Bassani VL, Henriques AT, Reginatte FH, Dal-pizzol F, Lorenzi R, Pasquali MAB, Ravazolo GL, Moreira JCF (2007). Antioxidant and free radical scavenging potential of *Bauhinia microstachya* extracts linked to their polyphenol content. Biol. Pharm. Bull. 30(8):1488-1496.

Delanty N, Dichter Ma (2000). Antioxidant therapy in neurologic diseases. Arch. Neurol. 57(9):1265-1270.

Gonçalves C, Dinis T, Batista MT (2005). Antioxidant properties of proanthocyanidins of *Uncaria tomentosa* bark decoction: a mechanism for anti-inflammatory activity. Phytochemistry. 66:89-98.

Gupta M, Mazumder UK, Kumar RS, Kumar TS (2004). Antitumour activity and antioxidant role of *Bauhinia racemosa* against erlich ascites carcinoma in swiss albino mice. Acta Pharmacol. Sinica. 25(8):1070-1076.

Hatano T, Edmatsu R, Mori A, Fujita Y, Yasuhara E (1989). Effects of tannins and related polyphenols on superoxide anion radical and on 1, 1-diphenyl-2-picrylhydrazyl radical. Chem. Pharm. Bull. 37:2016-2023.

Hussain SR, Cillard J, Cillard P (1987). Hydroxyl radical scavenging activity of flavonoids. Phytochemistry. 26:2489-2491.

Keay RWJ, Onochie CFA, Stanfield DP (1964). Nigerian Trees. Department of Forestry, Ibadan. 2: 6.

Khakonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kulaja TS, Heinonen M (1999). Antioxidant activity of plants extracts containing phenolic compounds. J. Agric. Food Chem. 47:3954-3962.

Koleva II, van Beek T, Linssen JPH, de Groot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13:8-17.

McCord JM (1985). Oxygen-derived free radicals in post ischemic tissue injury. N. Engl. J. Med. 312:159-163.

McCune LM, Johns T (2002). Antioxidant activity in medicinal plants associated with the symptoms of *diabetes mellitus* used by the Indigenous People of North American boreal forest. J. Ethnopharmacol. 82:197-205.

McDonald S, Prenzler PD, Autolovich M, Robards K (2001). Phenolic content and antioxidant activity of olive oil extracts. Food Chem. 73:73-84.

Meir S, Kanner J, Akiri B, Hada SP (1995). Determination and involvement of aqueous reducing compounds in oxidative defense system

system of various senescing leaves. J. Agric. Food Chem. 43:1813-1819.
Mensor LI, Menezes FS, Leitao GG, Reis AS, dos Santos T, Coube CS, Leitao SG (2001). Screening of Brazilian Plants extracts for antioxidants activity by the use of DPPH free radical method. Phytother. Res. 15:127-130.
Odabasoglu F, Aslan A, Cakir A, Suleyman H, Karagoz Y (2004). Comparison of antioxidant activity and total phenolic content of three lichen species. Phytother. Res. 18:938-941.
Olukemi OA, Olukemi IO, Sofidiya MO, Aniunoh OA, Lawal BM, Tade IO (2005). Antioxidants activity of Nigerian Dietary spices. Electr. J. Environ. Agric. Food Chem. 496):1086-1093.
Oyaizu M (1986). Studies on the product of browning reaction prepared from glucose amine. Jpn J. Nutrition. 44:307-315.
Pepato MT, Keller EH, Baviera AM, Kettelhut IC, Vendramini RC, Brunetti IL (2002). Anti-diabetic activity of *Bauhinia forficata* decoction in streptozotocin-diabetic rats. J. Ethnopharmacol. 81(2):191-197.
Pokorny J (1991). Natural antioxidants for food use. Trends Food Sci. Tech. 2:223-227.
Rao YK, Fang S, Tzeng Y (2008). Antiinflammatory activities of flavonoids and a triterpene caffeate isolated from *Bauhinia variegata*. Phytother. Res. 22(7):957-962.
Sabu MC, Kuttan R (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethnopharmacol. 81:155-160.
Sofowora A (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.
Torel J, Cillard J, Cillard P (1986). Antioxidant activity of flavonoids and reactivity with peroxy radicals. Phytochemistry 25:383-385.
Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2006). Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell. Biol. 7(1):45-78.