



## PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF LEAVE EXTRACT OF *ALBIZIA CHEVALIERI* HARMS (LEGUMINOSEAE-MIMOSOIDEAE)

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### ABSTRACT

**Antioxidant evaluation of *Albizia chevalieri* used in Northern Nigerian traditional medicine, was carried out using the free radical scavenging activity of the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), total phenolics content and reducing power assay on the methanol leave extract. The results of the DPPH scavenging activity study indicate a concentration dependent antioxidant activity with 59.588, 68.477, 77.240, 85.925 and 94.732% of various concentrations of 10, 25, 50, 125 and 250µg/ml of the extract respectively. The total phenolic content was found to be 60.20 mg/g Gallic acid equivalent (GAE) and the reducing power of 0.071±0.03nm was obtained. The DPPH scavenging activity of the extract was found to be promising. There is no significant difference ( $p < 0.05$ ) in the antioxidant activity between the extract and those of the standard ascorbic and Gallic acids at 50, 125 and 250µgml<sup>-1</sup> concentrations. The phytochemical screening revealed the presence of flavonoids, tannins and saponins. It indicates that the methanol leave extract of the plant has the potency of scavenging free radicals in vitro and may provide leads in the ongoing search for natural antioxidants from Nigerian medicinal plants to be used in treating diseases related to free radical reactions.**

**Keywords:** Antioxidants, Free radicals, *Albizia chevalieri*, DPPH, reducing power, total phenolic content

### INTRODUCTION

Oxidative stress involving enhanced generation of reactive oxygen species (ROS) has been implicated in the etiology of over one hundred human diseases including inflammation, metabolic disorders, cellular aging and atherosclerosis, heart disease, stroke, diabetes mellitus, cancer, malaria, rheumatoid arthritis and HIV/AIDS (Alho and Leinonen, 1999; Olukemi *et al.*, 2005). Antioxidants are molecules that are capable of neutralizing the harmful effects of the ROS through the endogenous enzymatic defense system such as the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) in human system. However, with the increasing damaging environmental factors such as cigarette smoke, uvs, radiation and toxic chemicals; the endogenous defense system is weakened, resulting to a phenomenal disturbance in the equilibrium status of pro-oxidant/antioxidants reactions in living systems. This situation mediates damage to cell structures, including lipids and membranes, proteins, and DNA (Valko *et al.*, 2006). Plants derived antioxidants are regarded as effective in controlling the effects of oxidative damage, and hence have had influence in what people eat and drink (Viana *et al.*, 1996; Sun *et*

*al.*, 2002; Pinder and Sandler, 2004). The antioxidative effect is mainly due to phenolic components, such as flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi *et al.*, 1992). The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

The plant *Albizia chevalieri* is a tree that grows up to 12m high or a shrub under harsher conditions of the dry savannah from Senegal, Niger and Nigeria. It has an open and rounded or umbrella shaped canopy, bark pale-greyish, twigs pubescent with white lenticels, leaves with 8-12 pairs of pinnate and 20-40 pairs of leaflets each. The bark was reported to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and Senegal. It is used in Borno-North eastern Nigeria as purgative, taenicide and also remedy for coughs. A decoction of leaves is used in Northern Nigeria as remedy for dysentery (Burkill, 1995; Le Houèrou, 2009). There are also reports on the local use of the leaves extract for cancer treatment in Zaria city, Kaduna state.

Previous studies on *Albizia* species have indicated the presence of phenolic compounds from *Albizia amara* with significant antioxidant activity (Muchuweti *et al.*, 2006) and *Albizia inundata* was reported for effective anti *candida* activity from Brazilian flora (Tempone *et al.*, 2008). Lipophilic extracts of *Albizia gummifera* revealed very promising antitrypanosomal activity (Freiburghaus *et al.*, 2007). The extracts of *Albizia ferruginea* were also reported to have significant antimicrobial activity on selected microorganisms (Agyare *et al.*, 2006), and *Albizia saman* was found to have good antiplasmodial activity (Kohlera *et al.*, 2002). *Albizia lebbek* was reported to contain 3□, 5 dihydroxy 4□, 7 dimethoxy flavone and N-Benzoyl-L-phenyl alaninol (Rashid *et al.*, 2003). As the focus of medicine shifts from treatment of manifest disease to prevention, increasing awareness on herbal remedies as potential sources of phenolic antioxidants have grown in recent years, and several plants are being screened for their antioxidant properties using different assays (Karou *et al.*, 2006). This work was designed to investigate the phytochemical and antioxidants properties of methanol leave extract of *Albizia chevalieri* with a view to assessing the potentials of the plant as a source for phenolic antioxidants.

## MATERIALS AND METHODS

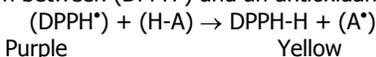
### Plant material

The leaves sample of *Albizia chevalieri* was collected in the month of July, 2008 at Dakace village along Jos road, Zaria. It was taxonomically authenticated at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A Voucher specimen (900247) was deposited there for future reference. The leaves were air-dried for three weeks and grounded to powder using pestle and mortar.

$$AA\% = 100 - \left\{ \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}} \right\}$$

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 (Positive control).

The scavenging reaction between (DPPH\*) and an antioxidant (H-A) can be written as:



### Reducing power assay

This was determined according the method of Oyaizu (1986). The extract or standard (100µgml<sup>-1</sup>) was mixed with phosphate buffer (pH 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<sub>3</sub> (0.1%, 0.5ml) and the absorbance was measured at 700nm in a spectrophotometer (Jenway 6025). Higher absorbance of the reaction mixture indicated reductive potential of the extract.

### Determination of total phenolic content

The total phenolic content of the extract was determined using the method of Macdonald *et al.* (2001) with slight modification. Calibration curve was prepared by mixing ethanol solution of Gallic acid (1

### Extraction of plant material

Powdered leave sample (250g) was extracted exhaustively with methanol (cold extraction) for two weeks (Aliyu *et al.*, 2008). The extract was filtered using Whatman filter paper no. 2, and concentrated on a Büchi rotary evaporator at 45°C, which afforded 48.3 g of the crude methanol extract referred to as ACME.

### Phytochemical screening

Phytochemical screening of the extract was carried out to identify the secondary metabolites such as alkaloids (Mayer's and Dragendorff's test), flavonoids (Shinoda test), terpenoids (Salkowski test), tannins (Ferric chloride test), saponins (Frothing test), cardiac glycosides (Keller-Killiani test) and anthraquinones (Borntrager's test) according to standard phytochemical methods as described by Sofowora (1993).

### Free radical scavenging activity

The determination of the radical scavenging activity of the crude extract was carried out 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 µgml<sup>-1</sup> of sample extract in methanol were prepared. DPPH (0.3 mM, 1.0 ml) in methanol was added to 2.5ml 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:

ml; 0.025-0.400 mg/ml) with 5ml Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 ml, 0.7M). Absorbance values were measured at 765 nm and the standard curve was drawn. One milliliter of crude methanol extract (5g L<sup>-1</sup>) 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:

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

where T=total phenolic contents (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 methanol plant extract (gram).

**Statistical analysis**

The experiments were done in triplicate. The results are given as mean  $\pm$  standard deviation (SD) Student's t-test was used for comparison between the two means and a difference was considered statistically significant when  $P < 0.05$ .

**RESULTS**

Phytochemical screening of methanol extract of *Albizia chevalieri* 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 1, 1-diphenyl-2-picryl-hydrazyl radical (DPPH) assay showed percentage antioxidant activity (%AA) of 59.588, 68.477, 77.240, 85.925 and 94.732% of various concentrations of 10, 25, 50, 125 and 250  $\mu\text{g/ml}$  of the extract respectively (Table 2). The reducing power of the extract ( $0.113 \pm 0.056 \text{nm}$ ) was found to be higher than the Gallic acid standard ( $0.096 \pm 0.035 \text{nm}$ ). The total phenolic content was found to be  $60.20 \pm 0.02 \text{mg/g}$  expressed as gallic acid equivalent (GAE).

**Table 1: Phytochemical screening of leave extract of *A. chevalieri***

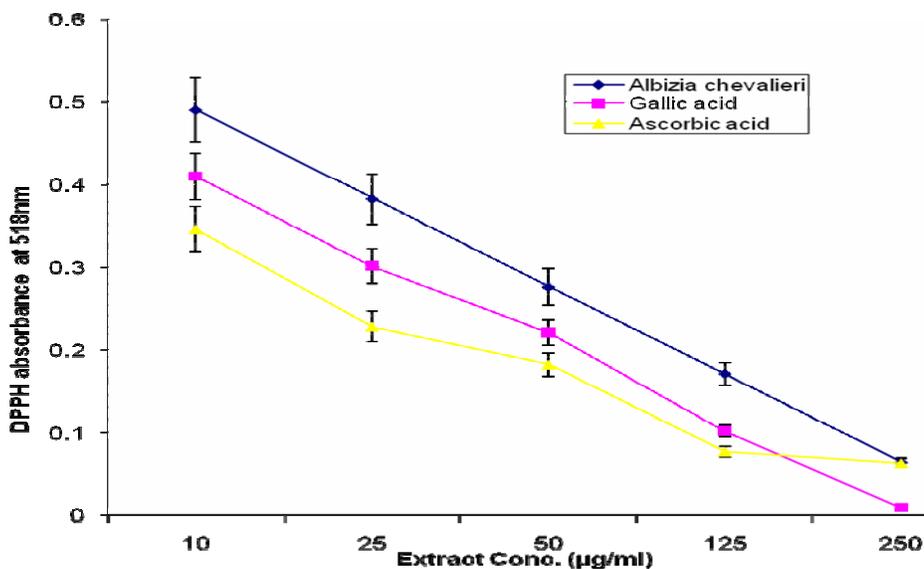
Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Triterpenes	+
Anthraquinones	-
Cardiac glycosides	-

Key: += present, - = absent

**Table 2: Result of Antioxidant activity of leave extract of *A. chevalieri***

Concentration( $\mu\text{g/ml}$ )	% Antioxidant activity		
	ACME	Ascorbic acid	Gallic acid
10	59.588 $\pm$ 0.009	71.52 $\pm$ 0.002	66.26 $\pm$ 0.001
25	68.477 $\pm$ 0.005	81.23 $\pm$ 0.002	75.27 $\pm$ 0.003
50	77.240 $\pm$ 0.008*	85.02 $\pm$ 0.001	81.81 $\pm$ 0.004
125	85.925 $\pm$ 0.003*	93.66 $\pm$ 0.003	91.60 $\pm$ 0.007
250	94.732 $\pm$ 0.001*	94.81 $\pm$ 0.001	92.59 $\pm$ 0.003

ACME= *Albizia chevalieri* methanol extract, \*( $P < 0.05$ ) no significant difference



**Figure 1: Free radical scavenging activity of *A. chevalieri***

## DISCUSSION

The total phenolics content of the *A. chevalieri* extract measured by Folin-Ciocalteu reagent in terms of Gallic Acid Equivalent (GAE) was found to be  $60.20 \pm 0.02 \text{ mg g}^{-1}$ . Phytochemicals especially plant phenolics constitute a major group of compounds that act as primary antioxidants (Hatano *et al.*, 1989). They have high redox potentials which allow them act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen *et al.*, 1999). The delocalization of electrons over the phenolics and stabilization by the resonance effect of the aromatic nucleus prevents the continuation of the free radical chain reaction (Tsao and Akhtar, 2005). The antioxidant effects of the extract may be due to its phenolic content.

The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for 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 the methanol extract of *A. chevalieri* has antioxidant activity due to its proton donating ability. The extract was found to highly scavenge free radicals when compared to standard antioxidants (Table 2). This indicates that there is no significant difference between the antioxidant activity of *A. chevalieri* and those of standard ascorbic and Gallic acids ( $P < 0.05$ ) at 50, 125 and  $250 \mu\text{gml}^{-1}$  concentrations. In the reducing power assay, presence of antioxidants in the extract reduced  $\text{Fe}^{3+}$ /ferricyanide complex to the ferrous form. The reducing

capacity of compounds could serve as indicator of potential antioxidant properties (Meir *et al.*, 1995) and increasing absorbance could indicate an increase in reducing power. Although reducing power of the extract was higher than that of gallic acid, but there is no significant difference ( $P < 0.05$ ) in terms of the reductive capacity. This suggests that the leave extract is an electron donor and could neutralize free radicals (Zhu *et al.*, 2001).

Considering the phytochemical screening, total phenolics, reducing capacity and the DPPH radical scavenging activity as indices of antioxidant activity of the extract, these findings revealed the potential of *A. chevalieri* as a source for natural antioxidants. Although no correlation study was carried out, but literature reports showed that the reduction mechanism of DPPH correlated with presence of hydroxyl groups on the antioxidant molecule (Cotelle *et al.*, 1996; Basnet *et al.*, 1997), which can be inferred that the very good antioxidant activity of this polar extract is probably due to the presence of substances with an available hydroxyl group. This structural requirement could be linked to the presence of flavonols or condensed tannins, which are known to occur in plant species belonging to the Leguminosae-Mimosoideae family (Mensor *et al.*, 2001) to which *Albizia chevalieri* belongs to. It indicates that the plant could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions. Furthermore, detailed studies on the isolation and characterization of the plant extract as well as studies with other models such as lipid peroxidation and *in vivo* assays will be interesting in discovering new biological antioxidants.

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