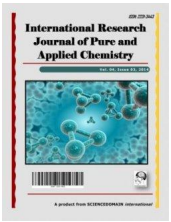

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Determination of Antioxidant Activity of Leave Extracts of *Albizia chevalieri* Using Free Radical Scavenging Activity Assay

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Authors' contributions

This work was carried out in collaboration among all authors. Author AMG performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author HRI designed the study. Author SA managed the analyses of the study and author SJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Dried and powdered leaves of *Albizia chevalieri* were extracted using ethanol. The extract was fractionated to give methanol, chloroform and pet-ether. The four extracts obtained; ethanol, chloroform, methanol and pet-ether were evaluated for antioxidant activity using 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) free radical scavenging activity assay. The results of the DPPH scavenging activity indicated a concentration-dependent antioxidant activity. The DPPH scavenging activity of the ethanol, chloroform and methanol extracts were found to be promising. There is no significant difference in the antioxidant activity between the ethanol, chloroform and methanol extracts with that of standard Ascorbic acid at 10, 25, 250 and 500 µg/ml concentrations. This showed that the ethanol, chloroform and methanol leave extracts of the plant has the potency

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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: Albizia chevalier; antioxidant; free radical; extract; scavenging activity assay; DPPH.

1. INTRODUCTION

The plant *Albizia chevalieri* is a tree or a shrub that grows up to 12m height under harsh conditions of the dry savannah from Senegal, Niger and Nigeria. It has an open and rounded or umbrella-shaped canopy, bark pale-grayish, 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 tennis 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 a remedy for dysentery [1]. There are also reports on the local use of the leaves extract for cancer treatment in Zaria city, Kaduna state [2].

Previous studies on *Albizia chevalieri* have indicated the presence of phenolic compounds from *Albizia amara* with significant antioxidant activity [3] and *Albizia inundata* was reported for effective anti candida activity from Brazilian flora [4]. Liphophlic extracts of *Albizia gummifera* revealed very promising antitrypanosomal activity [5]. The extracts of *Albizia ferruginea* were also reported to have significant antimicrobial activity on selected microorganisms [6] and *Albizia saman* was found to have good antiplasmodial activity [7]. *Albizia lebbeck* was reported to contain 3α, 5-dihydroxy-4β, 7-dimethoxy flavones and N-Benzoyl-L-phenyl alaninol [8]. As the focus of medicine shifts from the 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 [9].

DPPH, known as 2,2-diphenyl-1-picrylhydrazyl, (I.U.P.A.C name, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl with molecular formula $C_{18}H_{12}N_5O_6$ is a stable free radical that is commonly used to evaluate the ability of a compound to act as a free radical scavenger or hydrogen donor and to measure the antioxidant activity of tissue extract [10]. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [11]. Antioxidants offer resistance against oxidative stress by scavenging the free radical and many other mechanisms thus preventing disease progression [12]. The reaction of DPPH with an antioxidant or reducing compound produces the corresponding hydrazine DPPH-H, which can be followed by color change from purple (absorbance at 515-528nm) to yellow. The DPPH method is widely used for the measurement of free radical scavenging ability of antioxidants [13,14]. DPPH is a rapid, simple, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of foods and beverages [15].

This work was designed to investigate the antioxidant properties of ethanol methanol, chloroform and pet-ether leaves extracts of *Albizia chevalieri* with a view to assessing the potential of the plant as a source for antioxidants.

2. MATERIALS AND METHODS

2.1 General

The Ethanol was obtained from Sigma Aldrich, the DPPH and Ascorbic acid was obtained from chemistry laboratory Bayero University Kano. While other reagents and chemicals were of analytical grade supplied by Chemistry Laboratory, Kano University of Science and Technology, Wudil. All glass wares used were washed with detergents and oven dried before use. The leaves of the plant *Albizia chevalieri* were rinsed with clean tap water to remove dust and impurities.

2.2 Collection of Plant Material

The leaves of plant *Albizia chevalieri* were collected on 4th November 2017 from Kududdufawa village Ungogo local government area of Kano State. The plant was authenticated by Baha'uddeen Said Adam from the Department of Plant Biology, Bayero University Kano, with accession number BUKHAN 0378.

2.3 Extraction of Plant Material

The extraction and fractionation of air-dried and ground plant material were carried out according to the method described by Prakash [15]. 150 g of the powdered plant sample was weighed and percolated in 600 ml of 99% Ethanol in 2L conical flask for 14 days at room temperature. It was filtered using Whattman No. 2 filter paper and the solvent (Ethanol) was completely removed using rotary evaporator. The extract obtained was dried under room temperature to get a thick paste form of the plant extract. A dark green glassy and a gummy thick paste were obtained and weighed. It was labelled as AC01.

2.4 Fractionation of Crude Extract

The dried Ethanolic extract was partitioned with chloroform-water in 1:1 ratio of 100ml in a separating funnel. The mixture was shaken for 15 minutes and allowed to settle for 2 hours in a separation funnel, the water and chloroform fractions were separated in glass beakers. The chloroform fraction was concentrated at 35^{o}C using rotary evaporator and drying was completed under room temperature. The chloroform extract obtained was labelled as AC02. While the water fraction was discarded.

Similarly, the dried chloroform extract was further partitioned with Methanol and Pet-ether in 1:1 ratio of 100 ml in a separation funnel. The mixture was shaken for 15 minutes and allowed to settle. The methanol and pet-ether fractions were separated in glass beakers and were concentrated at 45ºC and 35ºC respectively using rotary evaporator. The extracts were completely dried under room temperature and weighed where the methanol and pet ether extracts were obtained and labelled AC03 and AC04 respectively.

2.5 DPPH Free Radical Scavenging Activity Assay

The determination of the radical scavenging activity of the crude extract of *Albizia chevalieri* was carried out using DPPH (1,1-diphenyl-2-picrylhydrazyl) as described by Fatope et al. [16] with slight modification. Various concentrations of 500, 250, 100, 50, 25 and 10 μgml^{-1} of each of the sample extract in methanol were prepared. The commercial known antioxidant, Ascorbic acid (Vitamin C) was used for comparison or as a positive control. The DPPH in the absence of plant extract was used as control and the plant extract in the absence of DPPH was used as blank. DPPH (1 mM, 200 μl) in methanol was added to 100 μl solution of each of the plant extract and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from purple to yellow was then measured at 517 nm on a spectrophotometer. Measurement was performed in triplicate. The percentage of radical scavenging activity was calculated using the following equation:

$$\%Radical\ Scavenging\ activity = 100 - \left\{\frac{Abs\ Sample - Abs\ Blank}{Abs\ Control}\right\} X\ 100$$

Abs sample = Absorbance of plant extract + DPPH
Abs blank = Absorbance of plant extract
Abs control = Absorbance of DPPH

(DPPH*) + H-A ⟶ (DPPH-H) + A*
Purple Yellow

3. RESULTS AND DISCUSSION

3.1 Extraction and Fractionation of Plant Material

The sequence of extraction followed the order ethanol, chloroform, methanol and pet-ether. The extraction of the powdered leaves of *Albizia chevalieri* was carried out using 99%v/v ethanol and the resulting extract was partitioned to give methanol, chloroform and pet-ether fractions (Fig. 1). 5.11% of the plant material went into the ethanol which on partitioning between chloroform/water, about 1.11% of the ethanol fraction went into chloroform. Further partitioning of the chloroform extract resulted in about 0.31% and 0.19% of the extract going into methanol and pet-ether respectively. This showed that the leaves extracts of the plant contain a reasonable amount of medium polar and non-polar compounds (Table 1).

3.2 Antioxidant Activity of *Albizia chevalieri*

Antioxidant properties of leaves extract of *Albizia chevalieri* was evaluated to find a new source of antioxidant. DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay [17]. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds [18]. The principle behind this assay is the colour change of DPPH solution from purple to yellow

as the radical is quenched by the antioxidant [19].

The leaves extracts of *Albizia chevalieri* were screened for DPPH radical scavenging activity according to the method described [20] with slight modification and the result of the screening is shown in (table 2) as compared to Ascorbic acid, a known antioxidant. Four different extracts of *Albizia chevalieri* (AC01, AC02, AC03, and AC04) showed high radical scavenging activity at various concentrations of 10, 25, 50, 100, 250 and 500 µg/ml (Fig. 2).

3.3 Inhibitory concentration at 50% (IC_{50})

The radical scavenging activity of each extract was determined by calculating the inhibitory concentration at 50% (IC_{50}), the IC_{50} of various extract of *Albizia chevalieri* and Ascorbic acid. The lower the IC_{50}, the more potent the extract, this showed that methanol extract has the highest radical scavenging activity (33.08 µg/ml) more than the standard Ascorbic acid (36.85 µg/ml), followed by ethanol extract (52.92 µg/ml), Chloroform extract (54.53 µg/ml) and pet-ether extract (71.59 µg/ml) [Fig. 3].

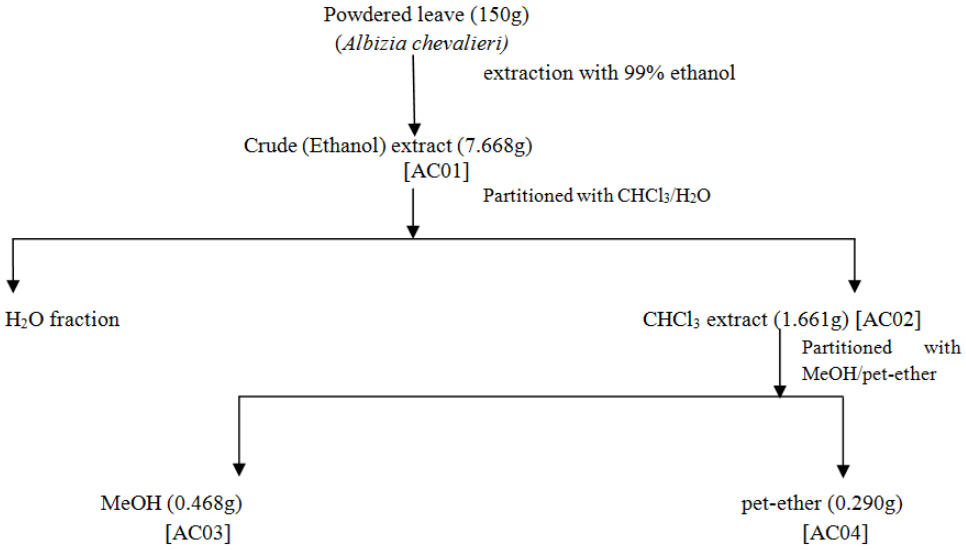

Fig. 1. Extraction and fractionation procedure of the powdered leave of the plant

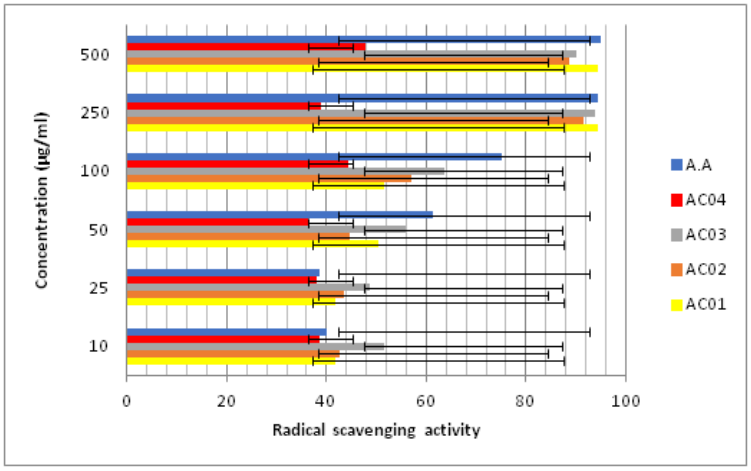

Fig. 2. Free radical scavenging activity of various extract of *Albizia chevalieri* at different concentrations

Table 1. Weights of extracts recovered and their physical properties

Extracts	Colour	Texture	Weight (g)	Weight (%)
AC01 [crude extract]	Dark green	Gummy like	7.668	5.11
AC02 [$CHCl_3$ extract]	Black	Semi-solid	1.661	1.11
AC03 [methanolic extract]	Black	Semi-solid	0.468	0.31
AC04 [pet-ether extract]	Black	Semi-solid	0.290	0.19

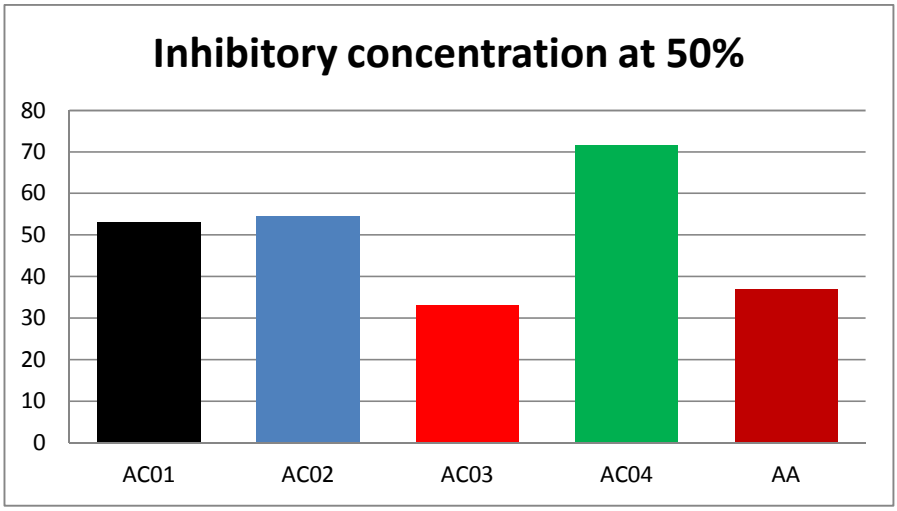

Fig. 3. Inhibitory concentration at 50% (IC_{50}) of various extracts of *Albizia chevalieri*

4. CONCLUSION

These findings revealed the potential of *Albizia chevalieiri* as a source for natural antioxidants. It indicates that the plant could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions. The leaves extracts of *Albizia chevalieri* were found to have high radical scavenging activity as compared with standard Ascorbic acid. The results of the DPPH scavenging activity study indicate a concentration-dependent antioxidant activity which increases with increase in the concentration of the extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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