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Antioxidant Activity of the Woody Root Extracts of *Dalbergia saxatilis* Hook F. using UV/Visible Spectrophotometer

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Abstract
The antioxidant potentials of *Dalbergia saxatilis* were investigated using 1,1-diphenyl-2-picarylhydrazyl (DPPH) and Vitamin C. The dried pulverized woody root was extracted with 95% ethanol. It was weighed using Metler H10 into test tubes and various concentrations were made. A known concentration of DPPH was carefully added to all the test tubes and allowed to stand for 15 minutes. The test samples were duplicated summing up to thirty test tubes and each of the test tube samples was analyzed using the UV/Visible double beam spectrophotometer. The average values for each test samples were recorded for the crude 95% ethanol and water extract. Percentage inhibition values was calculated and it was clearly shown from the calculated values of the 95% ethanolic extract of the woody root of *Dalbergia saxatilis* has a high antioxidant activity of (42.9-63.4%) and the water extract has a low antioxidant activity of (26.3-56.2%) when compared with vitamin C (74.2-74.9%). On the basis of the available data in this report, it can be postulated that *Dalbergia saxatilis* woody root could be used as alternative plant for rich antioxidant.

Key Words: Antioxidant, *Dalbergia saxatilis*, UV/Visible Spectrophotometer, Vitamin C.

Introduction

Antioxidants are substances capable of mopping up free radicals and prevent them from causing cell damage (Sies, 1991). Free radicals are responsible for a wide number of health problems such as cancer, aging, heart diseases and gastric problems. Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals. The effectiveness also declines with age (Sies, 1991; Goldfarb, 1993). Increasing the antioxidant intake can prevent diseases and promotes health. Research is increasingly showing that antioxidant rich foods, herbs reap health benefits. Fruits may possibly enhance antioxidant levels because they contain a lot of antioxidant substances. Fruits and vegetables are loaded with key antioxidants such as vitamin A, C, E, beta carotene and important minerals, including selenium and zinc. Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds (Sies *et al*., 1992). Herbs are staging a comeback and

herbal 'renaissance' is happening all over the world. The herbal products today symbolize safety and they are also compatible with human normal physiology. Natural products, mainly obtained from dietary sources provide a large number of antioxidants, and as such, *Dalbergia saxatilis*. It has already been reported that leaf extract of tea tree gives results equivalent to conventional antibiotics in the therapy of impetigo contagiosa (Sharquie *et al*., 2000) while Bassett *et al*. (1990) have reported favorable results with tea-tree oil in the treatment of acne. Oxygen derived free radical reactions have been implicated in the pathogenesis of many human diseases (Agarwal and Prabakaran, 2005; Pourmorad *et al*., 2006; Sen *et al*., 2009). Some of these diseases are:

a. Neurodegenerative disorder like Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, memory loss and depression.
b. Cardiovascular disease like atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock and trauma.
c. Pulmonary disorders like inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease.
d. Diseases associated with premature infants, including bronchopulmonary, dysplasia, periventricular leukomalacia, intraventricularhemorrhage, retinopathy of prematurity and necrotizing enterocolitis.
e. Autoimmune disease like rheumatoid arthritis.
f. Renal disorders like glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria, uremia.
g. Gastrointestinal diseases like peptic ulcer, inflammatory bowel disease and colitis.
h. Tumors and cancer like lung cancer, leukemia, breast, ovary, rectum cancers etcetera.
i. Eye diseases like cataract and age related of retina, maculopathy.
j. Ageing process, Diabetes.
k. Skin lesions Immunodepression.
l. Liver disease, pancreatitis, AIDS, Infertility.

Many species of the genus *Dalbergia* have been reportedly found to possess biological properties (Okwute *et al*., 2009; Misar *et al*., 2005; Gundidga and Gaza, 1993). Butein isolated from D. odorifera was reported to inhibit the iron-induced lipid peroxidation in rat brain with an IC50 value 3.3±0.4μm., which showed it to be as potent as α-tocopherol in reducing the stable free radical (DPPH) with an IC 0.200 value 9.2±1.8μm (Cheng *et al*., 1998).

Recent study on the leaves of *Dalbergia saxatilis*, reportedly focused on the antioxidant activity of the essential oil of the 95% ethanol crude extract which showed reasonably potent antioxidant activity in DPPH, with concentrations of free-radical scavenging assay between 0.2 and 0.5 mg/ml (Okwute and Fatokun, 2014). This paper assesses the antioxidant activity of the woody root extract from *Dalbergia saxatilis* in DPPH compared to vitamin C.

Materials and Methods

Collection and Preparation of Plant Materials

The woody roots of *Dalbergia saxatilis* were collected in October 2011 from the forest of Gumau, Toro Local Government Area of Bauchi State. The plant was authenticated at the National Institute for Pharmaceutical Research and Development. The voucher specimen was deposited at the Herbarium (No.6572). The samples were collected in nylon bags with the mouth left open for aeration and transported to the Chemistry Laboratory of the University of Abuja. The collected woody roots were cut into small pieces and air dried. The dried pieces were pulverized using laboratory hammer mill and stored in a well ventilated environment.

Extraction and Fractionation

A total of 500g of the dried, pulverized woody root was extracted using cold extraction, in an aspirator bottle with 2.5 liters of 95% ethanol for 48 hours after which it was filtered by suction filtration. This process was repeated with a second portion of 2.5 liters of 95% ethanol for another 48 hours and then filtered with a suction pump. The extracts were combined and evaporated to dryness using rotary evaporator at 78^0C to yield a dark brown gum (27.51g representing 5.52%). The crude dry extract (20g) was dissolved in chloroform (300ml) and the solution was transferred into a separating funnel (1L) and fractionated according to standard procedure

(Mitscher *et al*., 1972) which gave various components of the alkaloid fraction (0.47g), the acidic fraction (0.37g), and the non-polar and the polar fraction neutral fractions, (1.85g and 0.35g respectively). The chloroform insoluble ethanol crude residue was completely dissolved in water and taken in a separating funnel and extracted twice with 200ml of isobutyl alcohol, evaporated to give 0.55g of a reddish brown extract. The lower water layer was evaporated to dryness which gave a dark brown water extract (2.13g).

Antioxidant Screening of Extractives

0.1g of the crude extract (sample) was weighed into test tube and dissolved in 10ml of methanol; 5ml of this extract solution was further diluted to concentrations of (0.5, 0.25, 0.125, 0.0625, 0.03125mg/ml) into five test tubes. About 0.1mM of 1,1-Diphenyl-2-Picaryl-hydrazyl(DPPH) in ethanol was prepared and 1ml of this solution was added to 3.0ml of extract solution. This mixture was shaken vigorously and allowed to stand for 30mins, after which the absorbance was measured at 517nm with UV/Visible double beam spectrophotometer (CECIL CE 7500 SHETSCO, Nigeria). The tests were done in duplicate and percentage inhibition of free-radical DPPH (I%) were then calculated using the formula:

$I\ \% = 100 \times (A_{Control} - A_{Sample}) / A_{Control}$

Where, $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

Results and Discussion

The in vitro antioxidant activity of the woody root extracts of *Dalbergia saxatilis* was tested compared to that of Vitamin C (ascorbic acid) in DPPH free radical-scavenger. The Extracts of the plant under study was assessed for possible antioxidant activities by employing DPPH free – radical scavenging. A free radical-scavenging capacity of the plant extract was measured by DPPH assay and the results are shown in Table 1.

It has been observed from the Figure 1 plotted that the 95% ethanol crude extract was reasonably potent having maximum inhibition of 63.4 % at 0.03125mg/ml but was however slightly less potent when compared with the Vitamin C standard. The 95% crude ethanol extract also demonstrated fairly good free radical scavenging activity against DPPH having a maximum percentage inhibition of 63.4% at 0.03125 mg/ml, 60.8% at both 0.0625 mg/ml and 0.125 mg/ml, 54.9% at 0.25mg/ml and lowest percentage inhibition of 42.9% at 0.5 mg/ml. This study showed a reasonable potency of antioxidant at lowest concentration which is in good agreement with the previously reported study on the 95% ethanol crude extract of the leaf fraction (Okwute and Fatokun, 2014). The antioxidant activities of the 95% ethanol crude extracts are those related as % inhibition increases when the concentration decreases while the percentage inhibition of the Water extracts was 56.2% at 0.5mg/ml, 50.9% at 0.25mg/ml, 37.4% at 0.125mg/ml, 26.3% and 27.4% at both lowest concentration of 0.0625mg/ml and 0.03125mg/ml respectively. The comparison is totally opposite of each other in terms of concentrations the higher the concentration of water extract the higher the antioxidant activity while the lower the 95% ethanol crude extract concentration the higher the antioxidant activity. Phenol compounds in herbs act as antioxidants due to their re-dox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators. This may be the reason why the woody root extract has higher percentage inhibition in DPPH. Also, Tannins are a major group of compounds that act as primary antioxidant or free radical scavengers. Hence the potency of the woody root extracts can be due to the presence of tannins and phenols (Okwute and Fatokun, 2014). Thus Results obtained from this study showed that *Dalbergia saxatilis* is a natural source of phenolic compounds and Tannins (Okwute and Fatokun, 2014) which used in food industries as preservatives and in the manufacture of drugs.

Table 1: Antioxidant Activity of 95% Ethanol Crude extracts of the Woody Root of *Dalbergia saxatilis* in DPPH compared to Vitamin C at 517nm

Con. mg/ml	Abs1	Abs 2	Average	% inhibition of *D. Saxatilis*	% inhibition of Vit. C
0.5	0.153	0.152	0.153	42.9	74.2
0.25	0.118	0.123	0.121	54.9	74.7
0.125	0.106	0.108	0.107	60.1	74.8
0.0625	0.107	0.102	0.105	60.8	74.9
0.03125	0.905	0.101	0.908	63.4	74.9

Key: Abs = absorbance; D. *Saxatilis* = *Dalbergia saxatilis*; Vit. C = Vitamin C

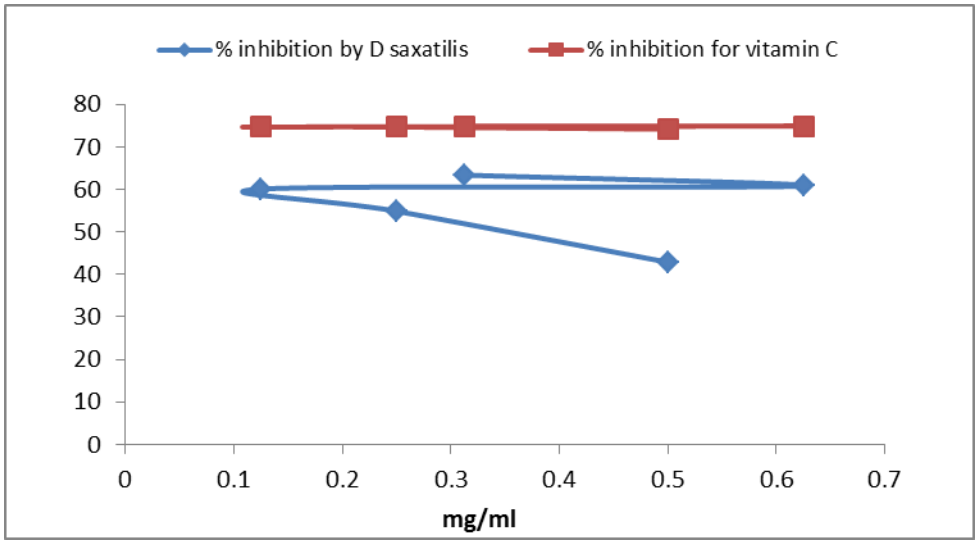

Figure 1: Graph of Antioxidant Activity of 95% Ethanol Crude extracts of the Woody Root of Dalbergia saxatilis in DPPH compared to Vitamin C

Table 2: Antioxidant Activity of Water extracts of the Woody Root of *Dalbergia saxatilis* in DPPH compared to Vitamin C at 517nm

Con. mg/ml	Abs1	Abs 2	Average	% inhibition of *D Saxatilis*	% inhibition of Vit. C
0.5	0.126	0.120	0.123	56.2	74.2
0.25	0.147	0.129	0.138	50.9	74.7
0.125	0.185	0.166	0.176	37.4	74.8
0.0625	0.208	0.206	0.207	26.3	74.9
0.03125	0.198	0.209	0.204	27.4	74.9

Key: Abs = absorbance; D. *Saxatilis* = *Dalbergia saxatilis*; Vit. C = Vitamin C

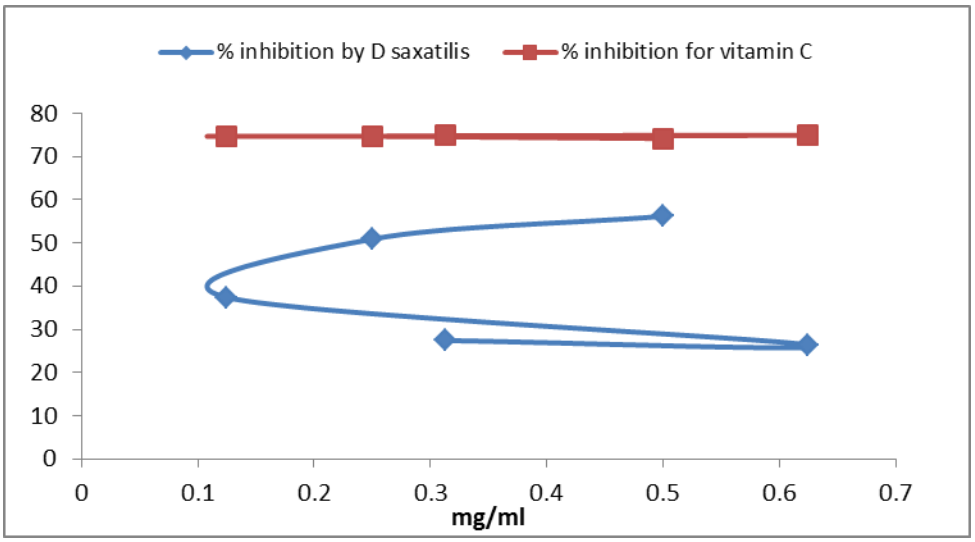

Figure 2: Graph Antioxidant Activity of Water extracts of the Woody Root of *Dalbergia saxatilis* in DPPH compared to Vitamin C.

Conclusions and Recommendation

In this study, antioxidant activities of the 95% ethanol and water extracts of the woody root of *Dalbergia saxatilis* were determined. In general, *Dalbergia saxatilis* extracts exhibited strong activity in DPPH when compared with Vitamin C (ascorbic acid). This could be a good source of antioxidants for human and animals as the plant is edible. Further biological tests, cytotoxicity, isolation and elucidation should be conducted to establish the actual phytochemicals responsible for these antioxidant activities.

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