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ORIGINAL ARTICLE

Assessment of the Scavenging Activity of Crude Methanolic Stem Bark Extract of *Newbouldia Laevis* on Selected Free Radicals

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ABSTRACT

From ancient times, the therapeutic application of medicinal plants, also known as alternative medicine has been popular. Today, biological research has shown that this practice of phytotherapy will remain with man for sometime. Some medicinal applications of parts of Newbouldia laevis are known. Air dried leaves of *N. laevis* (Bignoniaceae) were powdered and extracted with 95%v/v methanol by maceration, and the extract concentrated at 40°C using Rotary evaporator. The weight of the extracted plant material was noted for the purpose of calculating yield. In vitro assessment of the ability of the extract to scavenge the Reactive Oxygen Species (ROS), hydrogen peroxide, superoxide anion and the synthetic radical 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was determined. Buthylated hydroxyanisole (BHA), a synthetic antioxidant was used as a positive control. Plant extract showed concentration- dependent scavenging activity on all reactive species used. Scavenging activity of plant extract on hydrogen peroxide and superoxide was more than that of BHA on same. However, BHA was more effective at scavenging DPPH radical than plant extract.

Key words: *Newbouldia laevis*, BHA, DPPH, superoxide, hydrogen peroxide, ROS.

Introduction

Free radicals are chemical entities characterized by a high reactivity. Varying reactivities notwithstanding, radicals, free radicals inclusive have been known to be generally less stable than non-radicals (Paolo *et al*, 1991). Free radical formation during the metabolism of xenobiotics is therefore an important mechanism employed by toxic agents in causing cellular damage. Reactive oxygen species (ROS) capable of damaging DNA, proteins, carbohydrates and lipids are generated in aerobic organisms. These ROS include superoxide anion radical, hydrogen peroxide, hydroxyl radical, and single molecular oxygen. The effects of these ROS are controlled by a system of enzymic and non- enzymic antioxidants. These antioxidants eliminate pro oxidants and scavenge free radicals (Arouma, 1996).

Oxygen is essential and central in free radical pathology due to its physicochemical properties such as water solubility and relatively high electron negativity (Tappel, 1996). Free radicals, especially the oxygen radical, superoxide, when formed could lead to the formation of other radicals. In fact, the toxicity of $O_2^{\cdot}$ in living organisms is due to its conversion into $OH^{\cdot}$ and into reactive radical –metal complexes. The process through which

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superoxide and hydrogen peroxide are converted into $OH^{\cdot}$ and into reactive radical complexes are either referred to as the iron-catalyzed Haber- Weiss reaction or the superoxide driven Fenton reaction (Paolo *et al.,* 1991; Goldstein & Meyerstein, 1993; Fenton, 1894; Koppenol, 1993).

Numerous research have been carried out on various parts of *N. laevis* (Bignoniaceae). *N. laevis,* known more commonly as African Border tree is used for therapeutic purpose against a number of diseases. In Ivory Coast and Nigeria, stem bark decoctions of *N. laevis* is used for the treatment of epilepsy and convulsions in children. After pulping up to a paste, the bark is used for treatment of rheumatism, especially painful arthritis of the knees in Senegal. In Nigeria, decoctions of leaves and roots made from boiling are used as febrifuge. Treatment of breast tumors with bark and leaf decoctions is common in Ghana and Nigeria (Burkill, 1985; Burkill, 1997). Extracts of all parts of *N. laevis*, that is, leaves, stem bark and root have been shown to exhibit antimicrobial activity (Ogunlana & Ramstad, 1975; Kuete *et al*, 2007; Hounzangbe-Adote *et al*, 2005; Gbaessor *et al*, 2006; Eyong *et al*, 2006). Sedative effects of the methanolic leaf extract of *N. laevis* in mice and rats have also been studied and reported (Amos *et al*, 2002).

Materials and methods

Extraction of plant materials

N. laevis stem bark was collected from Amina way, University of Ibadan, Oyo state, Nigeria. Stem bark sample was authenticated at the Herbarium, Botany Department of the institution, and sample portions were deposited at the herbarium. Stem bark was air – dried in shade and powdered. 150g of powdered plant stem bark was extracted in 900ml, 95% v/v methanol by maceration for 48 hours. After decantation of crude extract, filtration and concentration was carried out using Rotary Evaporator, and the weight of concentrated dried stem bark obtained was recorded for the calculation of yield.

Determination of Hydrogen Peroxide H_2O_2 (scavenging activity of plant extract)

Hydrogen Peroxide scavenging activity of plant extract was determined by the method of Ruch *et al* (1989) by Gow - Chin and Hui – Yin (1995). 20 -400µg Buthylated hydroxyanisole (BHA) was added in place of plant extract in 4ml distilled water and the solution was added to 0.6ml H_2O_2 solution in PBS. Absorbance was determined 10 minutes later against a blank solution similar to that above.

Superoxide scavenging activity of plant extract

The effect of plant extract on superoxide generated in a non enzymic system was measured spectrophotometrically using the method of Gow-Chin and Hui-Yin (1995). The reaction mixture consisted of (10 - 1000µg) dilutions of plant extract made to 1ml with distilled water, 1ml, 60µM phenazine methosulphate (PMS), in phosphate buffer (0.1M, pH 7.4) and 150µM, 1ml nitrroblue tetrazolium (NBT) in phosphate buffer. Incubation at ambient temperature followed for 5minutes, and the resultant colour was read spectrophotometrically at 560nm against a blank. The effect of Buthylated Hydroxyanisole (BHA) was also determined by replacing plant extract with 1ml BHA (10 - 1000µg) in methanol in the reaction mixture.

Determination of the effect of plant extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

1mM DPPH solution was prepared by dissolving 31.54 mg DPPH in 95% v/v methanol and made up to 50ml with same. DPPH scavenging activity was assessed using the method of Hatano *et al.,* (1998) as modified by Gow-Chin Yen and Hui-Yin Chen (1995). Absorbance of the resulting solution was measured spectrophotometrically at 517nm. The effect of BHA on DPPH was also assessed for comparism with that of plant extract. Methanolic dilutions (0.2, 0.4, 0.6, 0.8, 1.0ml) of 1mg/ml BHA was made to 4ml with distilled water. 1ml DPPH radical (1nM) was added to each tube, and same procedure as in DPPH scavenging experiment was followed.

Statistical analysis was carried out using T-test (Paired two-sample for means). Values were considered significant for $T_{crit.} < T_{calc.}$ at 95% confidence limit ($P < 0.05$)

Result and Discussion

The extraction of 150g plant material in 900ml (95%v/v) methanol yielded 6.3178g of material after extraction. Percentage yield was therefore 4.2117%. Table 1 show data on the scavenging activity of plant extracts on hydrogen peroxide.

Table 1: Hydrogen Peroxide Scavenging Activity of Plant Extract.

Conc. of plant extract	Absorbance *(230nm)	
(mg/ml)	*N. laevis*	BHA
0.0109	0.039 ± 0.001	0.041 ± 0.001
0.0217	0.036 ± 0.000	0.036 ± 0.002
0.0326	0.032 ± 0.000	0.035 ± 0.001
0.0435	0.019 ± 0.002	0.028 ± 0.001
0.0543	0.017 ± 0.002	0.024 ± 0.003

* Absorbance of control = 0.052 ± 0.001

Percentage free radical scavenging activity was calculated using the formula:

$$\% \text{ SA} = \frac{(A_C - A_E)}{A_C} \text{ Î } 100$$

Where A_C = Absorbance of control
A_E = Absorbance of control
% SA = Percentage scavenging activity

Plant extract scavenged H_2O_2 more effectively than BHA (Figure 1). Both BHA and plant extract scavenged H_2O_2 in concentration – dependent manner. Interestingly, at 0.0217mg/ml, both plant extract and BHA scavenged hydrogen peroxide to the same extent, but at 0.0435mg/ml, plant extract appeared to be about 37.5% more efficient than BHA at scavenging hydrogen peroxide.

Superoxide anion scavenging data shown in Table 2 and corroborated by Figure 2 present an interesting trend. Even at the lowest of the concentrations used (0.05mg/ml), plant extract scavenged 81.48% of superoxide anion generated in the system while BHA only mopped up 5.82% of same, a display of a scavenging efficiency of 1300% over BHA. Figure 3 (percentage DPPH scavenging activity) shows a trend that is removed from that observed for hydrogen peroxide and superoxide anion above. Though both plant extract and BHA scavenged DPPH in concentration –dependent manner, BHA displayed better DPPH scavenging efficiency over plant extract. In fact, at the lowest concentration, BHA scavenges DPPH at twice the efficiency of plant extract. This may have been due to BHA's possession of a methoxy group which increases the accessibility of the radical centre of DPPH to BHA1 Indirect stimulation of lipid oxidation by superoxide as a result of superoxide and hydrogen peroxide act as precursors of singlet oxygen and hydroxyl radical (Cohen & Heikkila, 1974).

Table 2: Superoxide Scavenging Activity of Plant Extract

Conc. of plant extract	Absorbance *(560nm)	
(mg/ml)	N. laevis	BHA
0.0109	0.035 ± 0.002	0.178 ± 0.002
0.0217	0.018 ± 0.002	0.171 ± 0.002
0.0326	0.014 ± 0.003	0.170 ± 0.002
0.0435	0.005 ± 0.001	0.146 ± 0.005
0.0543	0.003 ± 0.000	0.142 ± 0.001

* Absorbance of control = 0.189 ± 0.003

Table 3: Dpph Scavenging Activity of Plant Extract.

Conc. of plant extract	Absorbance *(517nm)	
(mg/ml)	N. laevis	BHA
0.04	0.613 ± 0.003	0.316 ± 0.012
0.08	0.592 ± 0.017	0.302 ± 0.002
0.12	0.548 ± 0.001	0.158 ± 0.031
0.16	0.522 ± 0.013	0.126 ± 0.001

* Absorbance of control = 0.725 ± 0.002

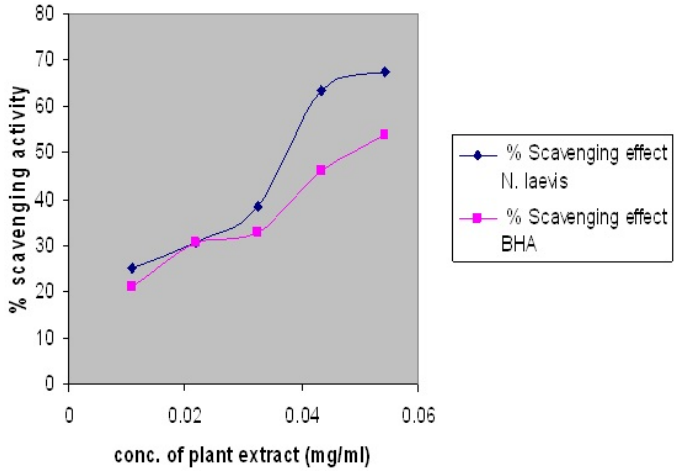

Fig. 1: Percentage scavenging activity of plant extract on hydrogen peroxide

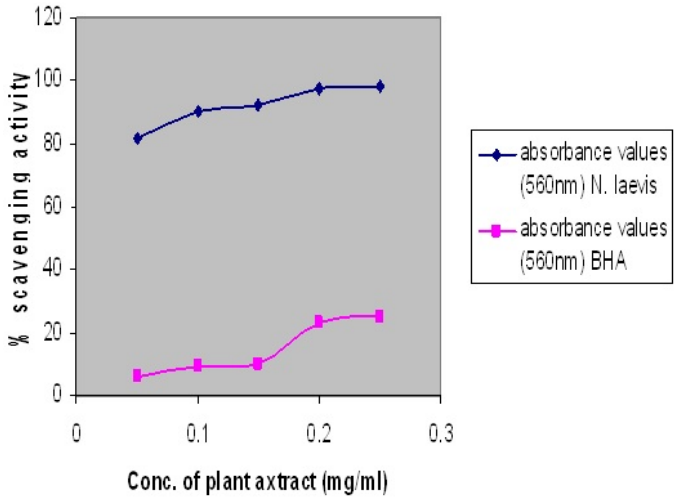

Fig. 2: Percentage scavenging activity of plant extract on superoxide anion radical

Statistical analysis (test of significance) of the data obtained from the free radical scavenging activity of plant extract and BHA using T-test (Paired two sample for means) showed that the difference between the free radical scavenging activities of plant extract and BHA on the natural ROS used, was significant ($P<0.05$). However, a non significant difference in DPPH scavenging activity was seen between the two at same confidence limit. In other words, the plant extract's superoxide radical and hydrogen peroxide scavenging activities were significantly higher than that of BHA. Although BHA's DPPH scavenging activity is higher than that of plant extract, the difference in the DPPH scavenging activity of the two was not significant ($P<0.05$).

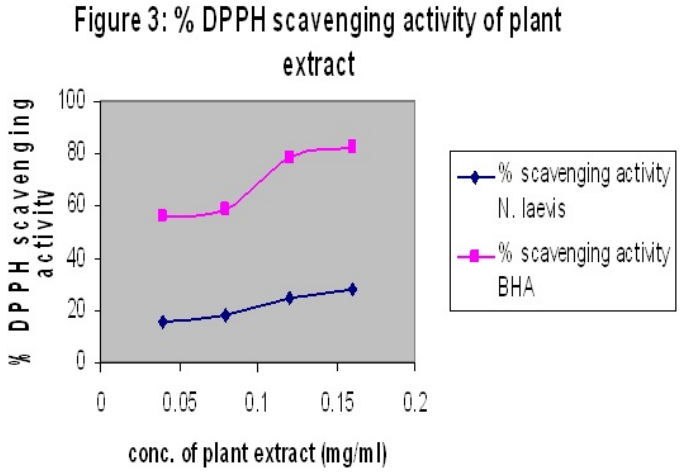

Fig. 3: Percentage scavenging activity of plant extract on DPPH radical

Discussion

The ability of hydrogen peroxide to initiate lipid peroxidation is dependent on its ability to generate hydroxyl radical through the Fenton reaction (Namiki, 1990; Kellog, 1988). The ability of plant extract to scavenge H_2O_2 could also reflect its ability to inhibit the formation of hydroxyl radical in vivo. Harber Weiss reaction explains that both hydrogen peroxide and superoxide radical are required in the presence of metal catalyst for the formation of hydroxyl radical, the oxygen species largely responsible for the damage of macromolecules, the ability of plant extract to scavenge both hydrogen peroxide and superoxide anion to a remarkable extent would culminate in remarkable inhibition of hydroxyl radical formation, hence protection of macromolecules from oxidative damage.

BHA's better efficiency at scavenging DPPH may have been due to its possession of a methoxy group which increases the accessibility of the radical centre of DPPH to BHA (Magassouba *et al*, 2007). From Figures 1 to 3, a few deductions could be made. The IC_{50} of *N. laevis* scavenging activity on hydrogen peroxide is 0.036mg/ml while the extract's IC_{50} for its scavenging activity on both $SO_2^{\cdot-}$ and DPPH could not be determined within the extract concentrations used for the experiment. The minimum plant extract concentration (0.05mg/ml) used for $SO_2^{\cdot-}$ scavenging experiment had above the 50% scavenging effect, required to be measured for IC_{50} determination. Conversely, the maximum plant extract concentration used for DPPH scavenging experiment did not scavenge up to 50% DPPH. IC_{50} for hydrogen peroxide scavenging activity of BHA was 0.055mg/ml, while that of its DPPH scavenging activity could not be determined from the graph because its DPPH scavenging activity at the lowest concentration used in the experiment was more than 50%. Not many publications of research work done on the free radical scavenging activity of *N. laevis* are known yet.

This research provides information which could trigger further research in the direction of partial or full isolation and characterization of the constituents of leaf extract of *N. laevis* in order to decipher the specific phytochemical constituent(s) responsible for the free radical scavenging activity of the plant. When this is done, extracts of N. laevis could find important application in phytotherapy. Experiments confirming these activities of the extract of *N. laevis* in an in vivo system would be necessary. If the results of such experiments agree significantly with our in vitro results, then it would be easy to make the following inferences:

N. laevis extract would prevent oxidative damage to macromolecules by the following mechanisms among others:

- scavenging hydrogen peroxide
- scavenging superoxide anion radical
- inhibiting the formation of hydroxyl radical from the Fenton reaction since it scavenges hydrogen peroxide
- inhibiting the formation of hydroxyl radical from both hydrogen peroxide and superoxide as powered by Harber – Weiss reaction

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