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Full Length Research Paper

In vitro assessment of antioxidant activity of *Newbouldia laevis*

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Poverty, drug resistance and other factors including increasing difficulty in the control of mosquitoes (the vector of the causative organism of malaria), have led to a growing interest in phytochemical research. The antioxidant activity of *Newbouldia laevis* stem bark was investigated. Air dried stem bark of *N. laevis* was powdered and extracted with 95 % v/v methanol by maceration, and the extract concentrated at 40°C using rotary evaporator. The total phenolic composition of methanolic extract of air dried stem bark was estimated using spectrophotometric method. Antioxidant activity of the extract was evaluated on the basis of its ability to prevent the oxidation of β-carotene and the strength of its ferric reducing capacity also determined. Phenolic composition was calculated to be approximately 35%. Plant extract showed concentration - dependent antioxidant activity and ferric reducing power. Plant extract achieved a maximum antioxidant activity of 4% within 40 min. The total phenolic content, antioxidant activity and reducing power of the extract had direct relationship.

Key words: Newbouldia laevis, β-carotene, phenolic composition, antioxidant activity, reducing power.

INTRODUCTION

Reactive oxygen species (ROS) capable of damaging DNA, proteins, carbohydrates and lipids, are characteristic of aerobic organisms, especially those at the higher levels of cellular organization, Davies (1995). A free radical is any chemical species (capable of independent existence), possessing one or more unpaired electron, Cheeseman and Slater (1993), Halliwell (1992). An unpaired electron is one that is alone in an orbital. ROS include superoxide anion, hydrogen peroxide, hydroxyl radical and singlet molecular oxygen, Maged (1999). ROS occur in tissues participating in potentially deleterious reactions controlled by a system of enzymatic and non- enzymatic antioxidants which eliminate pro- oxidants and scavenge free radicals, Paolo et al (1991). Once radicals form they can either react with another radical or another molecule by various interactions, Okezie (1996). The rate and selectivity of reactions of this type occurring depends on high radical concentration, delocalization of the single electron of the radical (thus increasing its lifetime), and the absence of weak bonds in any other molecule present with which the radical could interact, Bensasson et al. (1993), Weiss (1986, 1944). Phenolic compounds have been reported to play key antioxidant roles, especially using the mechanism of delocalization of the single electron of the radical, (Swallow, 1953; Mendel, 1997).

Newbuoldia laevis (Bignoniaceae) commonly known as African Border tree (Hausa-Aduruku or Bareshi, Igbo-Ogirisie, Yoruba-Akoko) 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, Burkill (1985). After pulping up to a paste, the bark is used for treatment of rheumatism, especially painful arthritis of the knees in Senegal, Burkill (1985). In Nigeria, decoctions of leaves and roots made from boiling are used as febrifuge, Burkill (1985), Tor-anyin et al. (2003). Treatment of breast tumors with its bark and leaf decoctions is common in Ghana and Nigeria, Burkill (1985, 1997). Extracts of all parts of *N. laevis*, that is, leaves, stem bark and root have been shown to exhibit antimicrobial activity Ogunlana et al. (1975), Kuete et al. (2007), Hounzangbe-Adote et al. (2005). Leaf and root extracts have been shown to possess antimalarial property, Gbaessor et al. (2006), Eyong et al. (2006). Sedative effects of the methanolic leaf extract of *N. laevis* in mice and rats have

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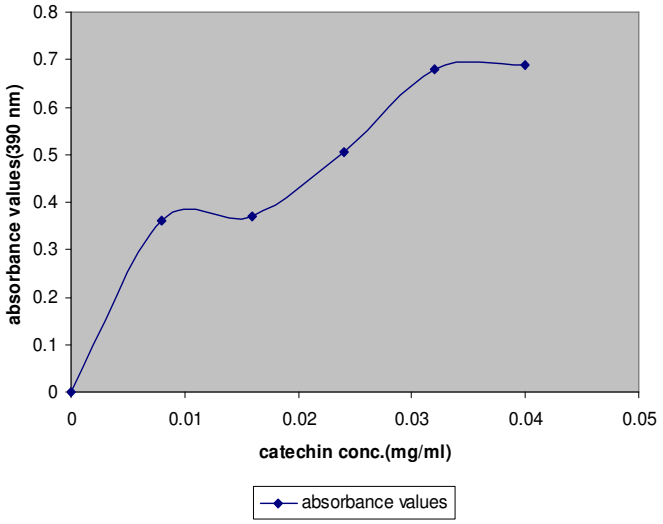

Figure 1. Quantification of total phenolic compounds in extract. Standard curve with 0.2 mg/ml catechin.

also been studied and reported, Amos et al. (2002).

MATERIALS AND METHODS

Extraction of plant materials

N. laevis stem bark was obtained on Amina Road, University of Ibadan, Oyo state, Nigeria. The stem bark was identified at the Botany department, University of Ibadan, Nigeria. Stem bark sample was air dried in shade at room temperature for 14 days and powdered. 150 g of powdered stem bark was extracted in 900 ml (95% v/v) methanol by maceration for 48 h. The crude extract was then decanted, filtered and concentrated using rotary evaporator until methanol was completely removed. Weight of concentrated dry extract was recorded for yield calculations. The solid residue was stored in glass vials in a refrigerator. Portions were taken from the refrigerated portions for each of the experiments, and the remaining extract stored in the refrigerator.

Quantification of total phenolic compound

This was carried out using a modification of Gow Chin Yen and Pin – Der Dur method (1994). 0.1 ml of 1 mg/ml stock solution of the methanolic extract was diluted with 3.25 ml glass distilled water, 0.25 ml Folin Dennis reagent, prepared by slight modification of Official Methods of analysis of the Association of Official Analytical Chemists (1970), was added and the contents of the test tube were mixed thoroughly. After 3 minutes, Na_2CO_3 solution (0.5 ml, 10 g/100 ml) was added and the test tube content was finally quantified to 5 ml with distilled water. The mixture was allowed to stand for 30 minutes with intermittent shaking. The blue colour ensuing was measured with Beckman Du 520 spectrophotometer (Beckman Coulter Inc., USA) at 390 nm. The concentration of total phenolic compound of the methanolic extract was estimated by extrapolation on a standard curve obtained with a plot of various concentrations against corresponding absorbance values for catechin using the same procedure as that used for quantifying total phenols in the plant extract above, and run concurrently with the one for plant extract.

Determination of reducing power of plant extract

The reducing power of the methanolic extract of *N. laevis* stem bark was determined according to the method of Oyaizu (1986). Stem bark extract (10 - 100 µg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml, 1 % Potassium Ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 min. 2.5 ml of 10% Trichloroacetic acid was added to the mixture, and mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml, 0.1 % $FeCl_3$. Absorbance was measured at 700 nm using same spectrophotometer as above.

Determination of antioxidant properties of plant extract

Antioxidant activity of plant extract was determined by the method of Tafa et al. (1984), Lee et al. (1995). A 3 ml aliquot of β-carotene-chloroform solution was added to a conical flask along with 40 mg linoleic acid and 400 mg Tween 40. Chloroform was removed by evaporation at room temperature. 100 ml oxygenated distilled water was added to the β-carotene emulsion and thoroughly mixed. 3 ml aliquots of oxygenated β-carotene emulsion and 40 µl of plant extract was placed in test tubes and mixed thoroughly. The tubes were immediately placed in a water bath and incubated at 50°C. Oxidation of β-carotene emulsion was monitored spectrophotometrically at 470 nm. Absorbance was measured 10, 20, 30 and 40 min after addition of oxygenated water and incubation at 50°C. A control sample consisting of 40 µl methanol instead of plant extract and 3 ml β-carotene emulsion was also prepared.

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 ($P < 0.05$)

RESULTS

150 g of powdered leaves yielded 6.32 g of extract after concentration and drying with rotary evaporator. Percentage yield was calculated to be 4.21%.

The standard curve of catechin on which total phenolic compound was estimated is shown in Figure 1. Absorbance value of plant extract at 390 nm was 0.143 ± 0.036. Extrapolating on the standard catechin curve, this corresponds to 0.007 mg/ml of phenolic compounds in plant extract. The final concentration of plant material in the phenolic content quantification experiment therefore was 0.020 mg/ml. Estimated percentage phenolic content of the extract was calculated to be 35 %.

Figure 3 shows an analysis of the percentage antioxidant activity exhibited by the extract. Percentage antioxidant activity at 10 - minute interval was calculated from Table 1 using the formula:

$$\% \text{ Antioxidant activity} = \frac{(A_{Extract} - A_{Control})}{A_{Control}} \times 100$$

$A_{Extract}$ = *Absorbance in test tube containing plant extract*

$A_{Control}$ = *Absorbance of control*

The maximum antioxidant activity exhibited by the extract at the concentrations used in the experiment was 4%. However, since the extract showed time-dependent anti-

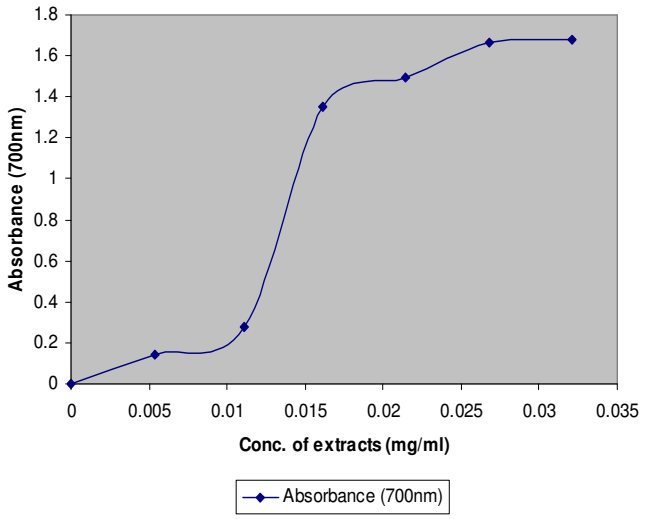

Figure 2. Reducing power of plant extract.

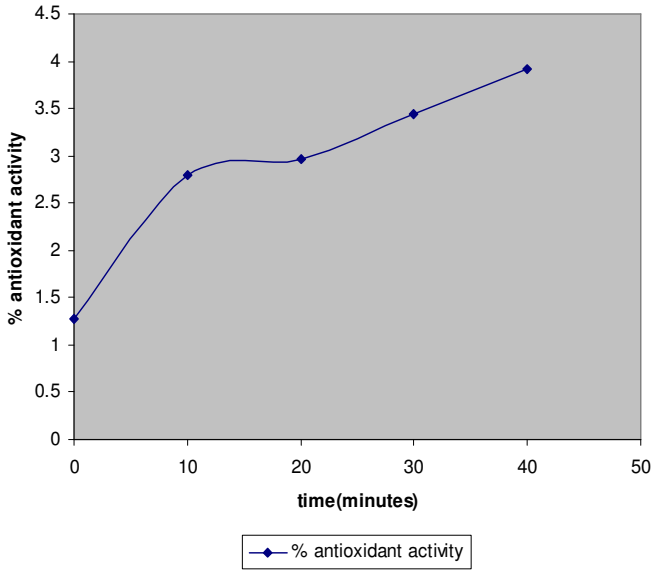

Figure 3. Percentage antioxidant activity of plant extract.

oxidant activity, its antioxidant activity would increase with time. It may also be possible to achieve higher antioxidant activity levels for the plant extract by increasing its concentration.

The result of the experiment for the determination of the reducing power of plant extract (Figure 2) indicated that plant extract exhibited dose-dependent reducing power. A sharp increase in the reducing power of the extract as reflected by the conversion of Fe^{3+} to Fe^{2+} was noticed between concentrations 0.012 and 0.016 mg/ml of plant extract.

Statistical analysis (T-test: paired two sample for means) of the data for antioxidant activity showed that

Table 1. Antioxidant activity of *N. laevis* extract.

Time (minutes)	Absorbance (470nm)*	
	Newbuoldia laevis	**Control**
0	0.710 ± 0.005	0.701 ± 0.000
10	0.701 ± 0.005	0.682 ± 0.002
20	0.696 ± 0.004	0.676 ± 0.001
30	0.691 ± 0.001	0.668 ± 0.004
40	0.689 ± 0.003	0.663 ± 0.004

The values above are presented as mean ± standard deviation of three replicate analysis

the antioxidant activity of plant extract, compared to control was not significant ($P < 0.05$) within the 40 min time frame of the experiment.

DISCUSSION

Phenolic moieties present in the molecular structure of natural antioxidants often help in enhancing their antioxidant activity (Kahkonen et al., 1999; Frankel et al., 1995).

It is important to note that the 35% estimated value for total phenolic content, is not an approximation of the total phenolic compounds in the leaves of *N. laevis*. It only estimates the total phenolic compounds in the methanolic extract that we worked with. β-carotene – linoleic acid emulsion undergoes an oxidation pattern in which β-carotene shields linoleic acid from being oxidized. However, the antioxidant activity of plant extract in the experiment is a measure of the extent of prevention of bleaching of β-carotene by plant extract under comparable oxidation conditions.

When substances exhibiting high reducing tendencies donate electrons which can react with free radicals converting them to more stable products in the process, radical chain reactions could be terminated (Pin-Der, 1998). The data obtained from the experiment for determining the reducing power of plant extract (Figure 2) in which increasing absorbance values implied increased conversion of Fe^{3+} to Fe^{2+}, hence increasing reducing ability of plant extract, showed that the extract exhibited concentration – dependent ferric reducing ability within the range of plant extract concentrations used for the experiment. The total phenolic content, antioxidant activity and reducing power of the extract were observed to relate directly. This agrees with the report from other works done in this direction, Marja et al. (1999), Frankel et al. (1995).

This work among others, laid a foundation for the quest into the free radical scavenging activity, and scavenging mechanism of the extract of *N. laevis*. Our work has shown that the methanolic extract of stem bark of *N. laevis* exhibits antioxidant activity. The mechanism(s) of antioxidant action of *N. laevis* remain(s) open for investigation. Further studies need to be done on the radical

scavenging activity of the extracts from different parts of this plant in order to determine the specific mechanism(s) of antioxidation. *In vivo* studies would also be required to ascertain the possibility of applying *N. laevis* in orthodox medicine.

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