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ORIGINAL ARTICLE

Morinda Lucida: Antioxidant and Reducing Activities of Crude Methanolic Stem Bark Extract

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ABSTRACT

The antioxidant activity of *Morinda lucida* (Benth.) stem bark was investigated. Air dried stem bark of *Morinda lucida* was powdered and extracted with 95 % v/v methanol 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. Phenolic composition was calculated to be approximately 80 %. Antioxidant activity of the extract was determined using its ability to prevent the oxidation of β-carotene. Ferric reducing ability of extract was also determined. Antioxidant and ferric reducing activities exhibited by plant extract were concentration dependent. Plant extract achieved a maximum antioxidant activity of 6% within 40minutes. The total phenolic content, antioxidant activity and reducing power of the extract had direct relationship.

Key words: *Morinda lucida*; β-carotene; Phenolic composition; Antioxidant activity; Reducing power.

Introduction

Reactive oxygen species 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). Polyphenolic compounds are secondary plant metabolites found in numerous plant species (Deshpande *et al*., 1984). Polyphenolic compounds have been shown to possess antimutagenic, anticarcinogenic, antiglycemic and antioxidative beneficial properties (Mendel, 1997). 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). They may produce their beneficial effects by scavenging free radicals (Gil *et al*., 2000).

Morinda lucida known as Oruwo in the South-western part of Nigeria is a medium – sized tree at maturity. Stem bark infusion is used as an antimalarial and antidiabetic (Burkill, 1985; 1997). Antimalarial activity (Tona *et al*., 1999; Agomo *et al.* 1992; Asuzu and Chineme, 1990; Makinde and Obih, 1985; Koumaglo *et al.* 1992), anti-Salmonella typhi activity (Akinyemi *et al*., 2005), effect on contractility of isolated uterine smooth muscle of pregnant and non-pregnant mice (Elias *et al*., 2007), toxicity and mutagenic studies (Sowemimo *et al*., 2007; Akinboro and Bakare, 2007; Raji *et al*., 2005) and anti-diabetic property (Olajide *et al*., 1999) of *Morinda lucida* lucida extracts have all been reported.

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We report here on the evaluation of the antioxidant activity and reducing potency of the methanolic stem bark extract of *Morinda lucida*.

Materials and methods

Extraction of plant materials

Morinda lucida stem bark obtained taken from Amina Road, University of Ibadan, Oyo state, Nigeria was identified at the Botany department of same. 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 for 48 hours. 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 obtained after rotary evaporation and drying 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 a refrigerator.

Quantification of total phenolic compound

A modification of the method of Gow Chin Yen and Pin-Der Dur (1994) was used for quantifying total phenolic compound in plant extract. 0.1 ml stock (1 mg/ml) 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. Blue colour measured at 390 nm with a spectrophotometer ensued. A plot of 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 was made. The concentration of total phenolic compound of the methanolic extract was estimated by extrapolation on this standard curve.

Determination of reducing power of plant extract

The method of Oyaizu (1986) was used in the determination of the reducing power of plant extract. Stem bark extract (10-100 µg) in 1 ml distilled water was mixed with 2.5 ml, 0.2 M, pH 6.6 phosphate buffer and 2.5 ml, 1 % Potassium Ferricyanide (K_3Fe (CN) $_6$), after which the mixture was incubated at 50 °C for 20 minutes. 2.5 ml, 10 % Trichloroacetic acid was added to the mixture and mixture was centrifuged at 3000 rpm for 10 minutes. 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), as modified by Lee *et al.,* (1995). 3 ml aliquot of β-carotene-chloroform solution was mixed with 40 mg linoleic acid and 400 mg Tween 40 in a conical flask. 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 aliquot of oxygenated β-carotene emulsion was mixed thoroughly with 40 µl of plant extract in test tubes. 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 minutes 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 7.3465 g of extract after concentration and drying with rotary evaporator. Percentage yield was calculated to be 4.9 %.

A standard plot of absorbance against concentration for catechin is shown in Figure 1. The absorbance value of plant extract at 390nm was 0.368±0.083; this absorbance value corresponds to 0.0016 mg/ml of phenolic compounds in plant extract on the standard catechin graph in Figure 1. Estimated percentage phenolic content of the extract was therefore calculated to be 80 %.

Table 1: Antioxidant activity of *Morinda lucida* extract

Time (minutes)	Absorbance (470nm)*	
	Morinda lucida	Control
0	0.727±0.030	0.690±0.000
10	0.717±0.034	0.682±0.002
20	0.715±0.035	0.676±0.001
30	0.707±0.031	0.668±0.004
40	0.705±0.029	0.663±0.004

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

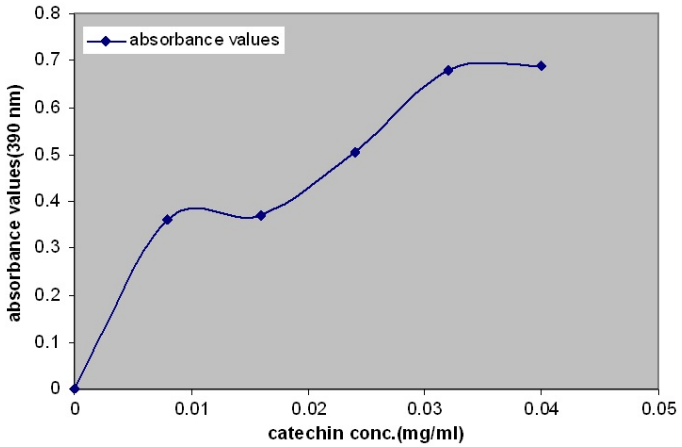

Fig. 1: Quantification of total phenolic compounds in extract. Standard curve with 0.2mg/ml catechin

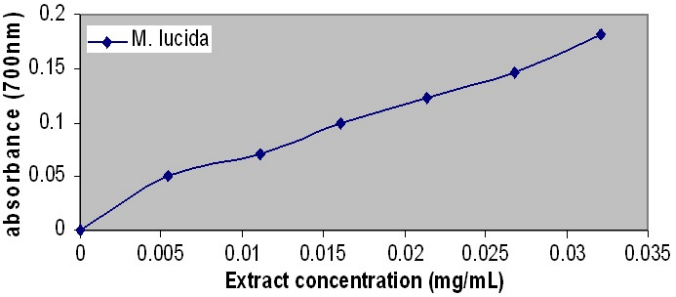

Fig. 2: Reducing power of extract

An analysis of the percentage antioxidant activity exhibited by the extract is shown in Figure 3. 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*

Plant extract exhibited a maximum antioxidant activity of 6.33% at the concentrations used in the experiment.

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. Statistical analysis (T-test: paired two sample for means) of the data for antioxidant activity showed that the antioxidant activity of plant extract, compared to control was not significant ($P < 0.05$) within the 40-minute time frame of the experiment.

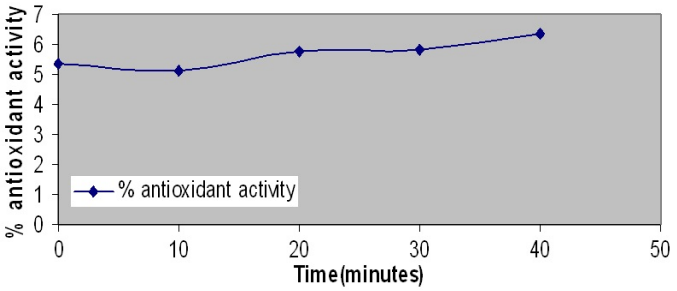

Fig. 3: % antioxidant activity of extract

Discussion

Antioxidant activity has been directly linked to the presence of phenolic moieties present in the molecular structure of natural antioxidants. Many phytochemicals having phenolic moieties have been shown to exhibit antioxidant activity (Kahkonen *et al*., 1999; Frankel *et al*., 1995).

Estimation of the total phenolic component of extract as 80% does not imply that *Morinda lucida* stem bark contains phenolic components estimated from our work as 80%. Indeed, the phenolic estimation done was carried out on only the methanolic extract of the stem bark. The 80% estimated value therefore obtained, is not an approximation of the total phenolic compounds in the stem bark of *Morinda lucida*. 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.

Radical chain reactions could be terminated when substances exhibiting high reducing tendencies donate electrons which can react with free radicals converting them to more stable products in the process (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+} implies 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 correlated directly. The reports of Marja *et al.,* (1999), Frankel, *et al.,* (1995) agreed with our report.

This experiment showed that the methanolic extract of stem bark of *Morinda lucida* exhibits antioxidant activity. The mechanism(s) of antioxidant action of *Morinda lucida* 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 antioxidant activity.

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