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Research Article

Free Radical Scavenging and A-Amylase Inhibitory Activity of *Swietenia Mahagoni* Seeds Oil

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ABSTRACT
The antioxidant property of *Swietenia mahagoni* seeds oil was evaluated by *in vitro* models such as inhibition of DPPH, hydroxyl and nitric oxide radicals and ferric reducing power. Seeds oil showed free radical scavenging as well as reducing property. Different concentration of oil were used for the study and showed a respective hydroxyl free radical scavenging (23.87, 30.16, 30.3.2, 34.2 and 52.05%), DPPH radical (6.50, 7.32, 8.83, 9.52 and 21.06) and nitric oxide radical (13.89, 15.45, 16.41, 19.52, 35.65 and 51.76) activity and all the results were compared to that of standard drugs BHA. Total phenolic content of seed oil was found to 15µg/ml when assayed by Folin–Ciocalteau reagent. The assessment of the antidiabetic properties of the oil by *in vitro* amylase inhibition assay showed significant activity (86.81, 83.52 and 64.84% µl/ml/min) at respective concentrations of 2, 20 and 200 µl/ml. These results support the use of *Swietenia mahagoni* seeds oil as an antidiabetic as well as antioxidant drug.

Key words: *Swietenia mahagoni,* Seeds oil, antioxidant, amylase inhibition, DPPH.

INTRODUCTION

Oxidative metabolism produces many free radicals having an unpaired or nascent electrons such as reactive oxygen or nitrogen species[1,2]. Reactive oxygen species includes free radicals such as superoxide anion radicals ($0_{2^{\cdot}}$), hydroxyl radicals (OH^{-}) and non-free radicals such as H_20_2 and singlet oxygen (^{102}). These reactive oxygen species cause DNA and chromosomal damage and initiate the peroxidation of membrane lipids[3, 4]. These damages may results in many disorders like cancer, hepatic ailments, cardiovascular diseases, decline of immune system, diabetes mellitus, brain dysfunction and the process of aging [5]. Antioxidants are molecules or compounds that have the ability to act as free radical scavengers. Most antioxidants are electron donors and react with the free radicals to form innocuous end products such as water. Thus antioxidants protect against oxidative stress and prevent damage to cells[6]. The widely used Natural antioxidants such as α-tocopherol and L-ascorbic acid found to be less efficient when compared to synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These synthetic drugs are suspected to cause liver damage[7, 8]. Thus search for new natural antioxidant sources are of great importance.

Diabetes affects about 5% of the global population[9] and the management of diabetes without any side effects is still a challenge to the medical system[10]. It is a chronic disease caused by inherited or acquired deficiency in insulin secretion and decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level and damage many of the body's systems, including blood vessels and nerves[11]. Low levels of plasma antioxidants implicated as a risk factor for the development of the disease hence antioxidants are important in prevention of diseases[12.13]. Plants continue to play an important role in the treatment of diabetes, particularly in developing countries. So there is considerable interest in the development of natural antioxidants and alternative approaches to treat diabetes[14]. Swietenia mahagoni (L.) Jacq, commonly known as "mahogany" is an economically important timber tree belongs to the Meliaceae family. It is native to tropical America, Mexico and South America, usually 40-50 feet in height and 40-60 feet width[15], and has been extensively planted mainly in Southern Asia (India, Sri Lanka and Bangladesh). Traditionally, various parts of this plant have been used in the treatment of fever, diabetes, malaria, hypertension and tuberculosis[16, 17]. The extract of this plant showed ameliorative effects on diabetic mice and antimicrobial properties [18, 19], platelet aggregation inhibitory activity[20], and anti-human immunodeficiency virus (HIV) activities[21]. Seeds oil of *Swietenia mahagoni* shows strong antibacterial activity against three disease causing bacteria viz. *Salmonella typhi*, *Shigella dysenterial* and *Staphyllococcus aureus*[22]. The present investigation was performed to examine the total phenolic

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content, antioxidant and α-amylase inhibitory activities of *S. mahagoni* seeds oil through various *in vitro* models.

MATERIALS AND METHODS

In vitro antioxidant activity: Chemicals: Chemical reagents Nitro blue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Germany, Sodium carbonate, Sodium nitroprusside (10 mM) solution and Trichloro acetic acid (TCA) (S.D-fine chemicals, Mumbai). All other reagents used were of analytical grade.

Plant material:The seeds of *S. mahagoni* were collected in January 2008 from Hooghly District, West Bengal, India. Authentication of the sample was done Department of Botany, Dr. H.S. Gour Central University, Sagar, MP, India.

Preparation of the extract: The air-dried seeds of *S. mahagoni* (35g) were powdered, soaked with 250 ml of petroleum ether and stand for 72 h. The crude extract was filtered and evaporated under reduced pressure and the oil fraction was separated, percentage yield of 5.00% (ml/w).This crude oil was dissolved in 5% DMSO and used for the assessment of antioxidant and antidiabetic activity.

Determination of total phenolic content: Total phenolic content was assessed by Folin-Ciocalteau Phenol reagent using standard curve generated with Catechol. Aliquots of each sample were pipette out in series of test tubes and the volume was made up to 3ml with distilled water. 3ml of the sample solution in distilled water was mixed with 0.5ml of Folin-Ciocalteau reagent, mixed thoroughly and incubated for 3min. at room temperature. To the above reaction mixture 2ml of (20%) Sodium carbonate solution was added and again incubated for 1min. in boiling water bath. Absorbance was measured at 650nm against a reagent blank. Total phenolic content was expressed as µg of Catechol equivalent per mg of sample[23].

Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity: The free radical-scavenging activity of the *S. mahagoni* seeds oil was measured in terms of hydrogen donating ability using the stable radical DPPH. Different concentrations (10, 50, 100 and 250µl) of oil and BHA were taken in separate test tubes and the volume was adjusted to 500µl by methanol. 5ml of methanolic solution of DPPH (0.1 mM) was added to these tubes. After mixing well the tubes were allowed to stand at room temperature for 20 min. A control without the test compound, but with an equivalent amount of methanol was maintained. The absorbance of the samples was measured at 517nm and radical scavenging activities were calculated[24].

$$\%\ \text{radical scavenging activity} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Determination of hydroxyl radical scavenging activity: 250µl of different concentration of oil (10, 50, 100 and 250µl) in 0.1 phosphate buffer was mixed with 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA (0.018%), and 1ml of dimethyl sulphoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) and the reaction was initiated by adding 0.5ml of 0.22% ascorbic acid. These reaction mixtures were then incubated at room temperature for 15min. The reaction was terminated by the addition of 1ml of ice-cold TCA (17.5% w/v). 3ml of Nash reagent (150 g of ammonium acetate, 3ml of glacial acetic acid, and 2ml of acetyl acetone were mixed and raised to 1lit with distilled water) and left at room temperature for 15min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412nm against reagent blank and the percentage of hydroxyl radical scavenging activity was calculated[24].

% hydroxyl radical scavenging activity

$$\%\ \text{hydroxyl radical scavenging activity} = 1 - \frac{(\text{Difference in absorbance of sample})}{\text{Difference in absorbance of blank}} \times 100$$

Determination of nitric oxide radical scavenging activity: 1ml Sodium Nitroprusside (5mM) prepared in buffered saline (pH7.2) was added to 3ml of various concentrations of oil (10, 50, 100 and 250µl) in 0.1M phosphate buffer (pH 7.2). The reaction mixture was incubated for 30 min at room temperature, 1.5ml of the above solution was mixed with 1.5ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1Naphthylethylenediamine dihydrochloride). A control without the test compound, but with an equivalent amount of methanol was maintained. The absorbance of the chromophore formed was measured at 546nm[25]. Nitric oxide radical scavenging activity was calculated

$$\%\ \text{radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD})}{\text{Control OD}} \times 100$$

Determination of ferric reducing scavenging activity: Samples at different concentration (100, 250 and 500µl) were mixed with 2.5ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20min., then 2.5ml of 10% trichloroacetic acid (w/v) were added, 5ml of above solution was mixed with 5 ml of distilled water and 1ml of 0.1% of ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxy anisole (BHA) was used as standard antioxidant[26]

In vitro α- amylase inhibitory activity

Chemicals**:**

1. DNS solution: 1 g of DNS dissolved in 2N NaOH, 30 g of potassium sodium tartarate is added and whole volume is made up to 100ml.
2. 1% starch solution in 50 mM phosphate buffer
3. Amylase

Experimental protocol: Amylase inhibitory activity was perfomed by[27]. Oil were dissolved in ethanol and prepared different concentration (2, 20 and 200 µl).Reaction mixture containing 100µl of oil, 200µl of the porcine pancreatic α-amylase, 100µl of 2 mM phosphate

were mixed and incubated at 37ºC for 10min followed by the addition of 100µl of 1% starch solution. After incubation for 5min, 1ml of DNS solution was added. Reagent solution without test samples was used as control. The tubes were then incubated in boiling water bath for 10min, cooled and the absorbance was measured at 540nm against blank. Concentration of maltose liberated was determined by using standard maltose curve. Enzyme activity was calculated by following formula and expressed as µmoles/min/ml.

$$\text{Activity} = \frac{[\text{Concentration of glucose liberated} \times \text{ml of enzyme used}]}{\text{Mol. wt of glucose} \times \text{incubation time}} \times \text{dilution factor}$$

inhibition at a respective concentration of 10, 50, 100, 250 and 400 µl/ml (Fig.1). The 1-1-diphenyl 2-picryl hydrazyl (DPPH) was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or crude mixtures such as the ethanol extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The oil was able to reduce the stable radical DPPH to yellow colored diphenyl picrylhydrazine which can be quantified by its decrease of absorbance at 517 nm[29].

Hydroxyl radical scavenging activity: The extract

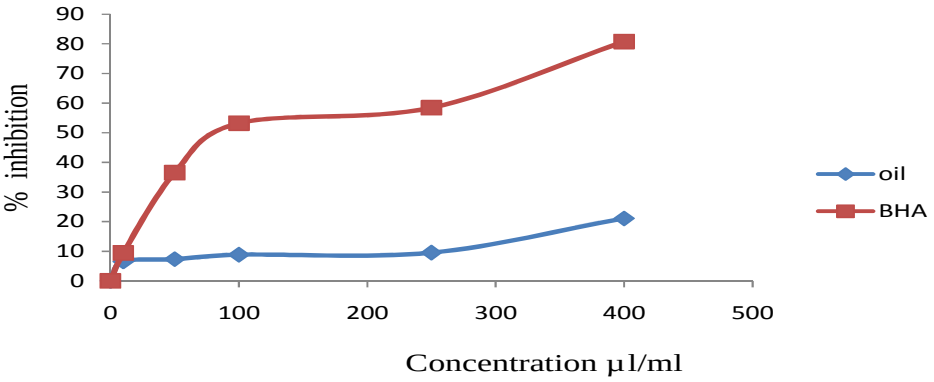

Fig. 1. DPPH radical scavenging activity of the extract of Swietenia mahagoni seeds oil.

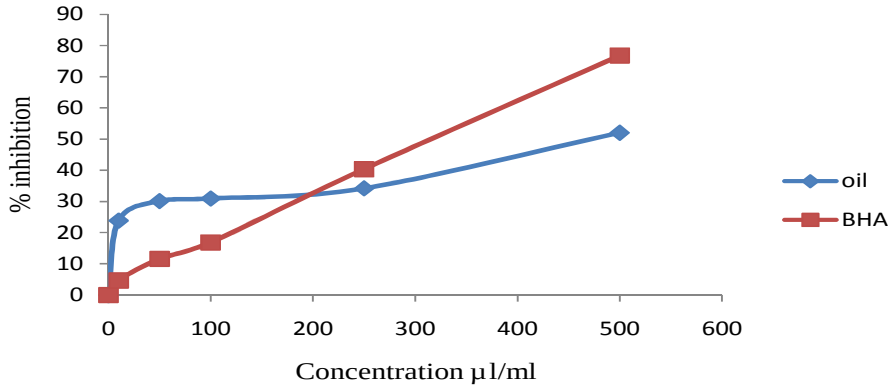

Fig.2. Hydroxyl radical scavenging activity of the extract of Swietenia mahagoni seeds oil

RESULT AND DISCUSSION

Total phenolic content: The total phenolic content in *S. mahagoni* seeds oil was found to 15µg/ml (table: 1) of catechol equivalent. Phenolic compounds could play a major role in antioxidant potentials of foods or serves as natural source of antioxidants. Phenols are very important plant constituents because of their scavenging ability due their redox properties, hydrogen donors and singlet oxygen quenchers[28].

Inhibition of DPPH radical: The extract showed concentrations depended DPPH radical scavenging activity of 6.50, 7.32, 8.83 and 9.52% and 21.06%

displayed potential hydroxyl radical-scavenging activity (Fig. 2). The % of inhibition of extracts at concentrations of 10, 50, 100, 250 and 500µl/ml was 23.87, 30.16, 30.32,

34.2 and 52.05% respectively. All results showed antioxidant activity in dose dependent manner. IC_{50} value for extract and BHA was found to be 455.91µl/ml and 319.93µl/ml respectively. Hydroxyl radical is an extremely reactive free radical formed in biological systems and damaging almost every molecule in living cells[30]. Hydroxyl radical scavenging capacity of an

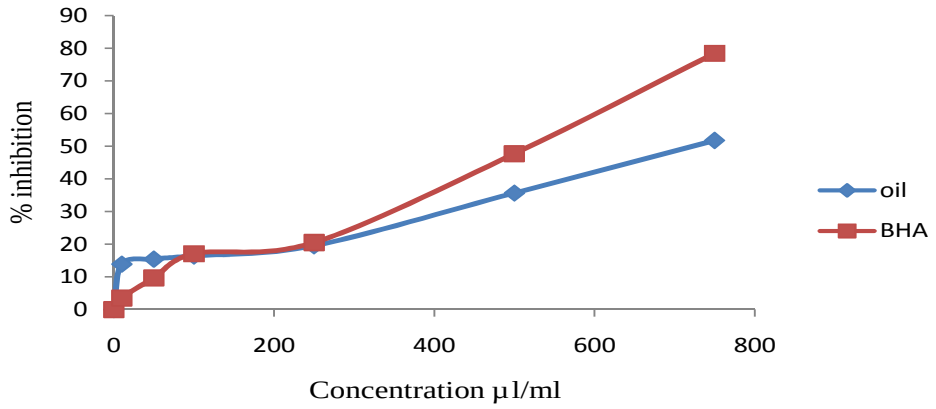

Fig.3. Nitric oxide radical scavenging activity of the extract of Swietenia mahagoni seeds oil

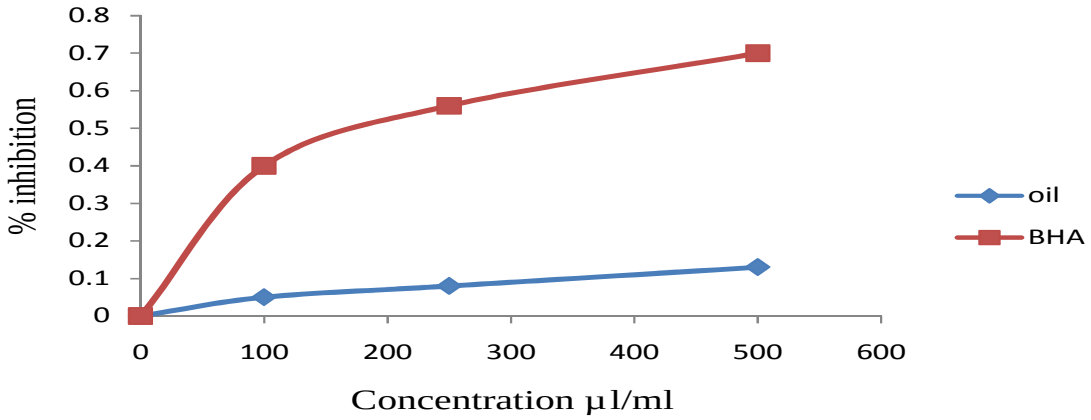

Fig. 4. Ferric oxide radical scavenging activity of the extract of Swietenia mahagoni.;[‘’ seeds oil

Table 1: Activities of amylase at different concentrations of *Swietenia mahagoni* seeds oil

Concentration (µg/ml)	% Activity
2	86.81±0.67
20	83.52±0.77
200	64.84±0.52

extract is directly related to its antioxidant activity[31]. [32] reported that high molecular weight, proximity of many aromatic rings and hydroxyl groups are more important for the free radical-scavenging activity by phenolics than their specific functional groups.

Nitric oxide radical scavenging activity: The extract showed concentration dependent nitric oxide radical scavenging activity of 13.89, 15.45, 16.41, 19.52, 35.65, and 51.76% inhibition at a respective concentration of 10, 50, 100, 250, 500 and 750 µl/ml (Fig. 3). IC_{50} values for extracts and BHA was found to be 742.32µl/ml and 533.01µl/ml respectively. Nitric oxide was generated from sodium nitroprusside in aqueous solution at physiological pH and interacts with oxygen to produce nitrite ions that can be estimated by Greiss reagent[33, 34]. Nitric oxide (NO) is a diffusible free radical that plays effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (Hagerman *et al.*, 1998). The result indicated that the seeds oil might contain compounds able to inhibit nitric oxide and offers scientific evidence for the use of the *Swietenia mahagoni* seeds oil in the indigenous system in inflammatory condition.

Ferric reducing power: The various concentrations of seeds oil (100–500 µg/ml) showed 0.05, 0.08, 0.13% ferric reducing power respectively (Fig.4). The result shows that extract consist of hydrophilic poly phenolic compounds that cause the reducing power.

Amylase inhibitory activity: The seeds oil showed amylase inhibitory activity of 86.81, 83.52 and 64.84 % (µg/ml/min) at respective concentration of 2, 20 and 200µl/ml (Table: 1). Amylase catalyses the hydrolysis of α -1, 4-glucosidic linkage of starch, glycogen and various oligosaccharides and glucosidase further breaks down the

disaccharides into simpler sugars. α- amylase inhibitory activity in the digestive tract of humans is considered to be effective in control of diabetes by diminishing the absorption of glucose[35]. Therefore, the result shows that *Swietenia mahagoni seeds oil* can act as an effective and nontoxic inhibitor of amylase and glucosidase which indirectly posses its antidiabetic potential.

CONCLUTION

In conclusion, the crude seeds oil of *S. mahagoni* has showed antioxidant as well as amylase inhibitory activities in *in vitro* models. The phenolic compounds present in plants can serve as natural sources of antioxidants. The study highlights the significance of the free radical scavenging capacity and the potentials of *S. mahagoni* seeds oil as a source of therapeutic agent.

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