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Research Article

PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *SWIETENIA MAHAGONI* SEEDS

SUBHADIP HAJRA*, ARCHANA MEHTA AND PINKEE PANDEY

Lab of Plant Biotechnology, Dept. of Botany, School of Biological and Chemical Sciences, Dr. H. S. Gour Central University, Sagar 470003, Madhya Pradesh, India. Email: dip.microworld@gmail.com

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ABSTRACT

In the present study; evaluate the antioxidant potential of methanolic and aqueous extracts of *Swietenia mahagoni* seeds by various *in vitro* models. Phenolic acids, a major class of phenolic compounds, are widely occurring in the plant kingdom, especially in fruits and vegetables. Typical phenolics that possess antioxidant activity are mainly phenolic acids, flavonoids and tannins. The methanolic extract of *Swietenia mahagoni* seeds showed potent DPPH radical scavenging activity (7.5, 18.4, 27.3, 35 and 58%) when compared to aqueous extract (4.7, 12.8, 18.6, 28.4 and 51.7%) at the concentrations of 10, 50, 100, 250 and 500µg/ml respectively. The methanolic extract also showed significant hydroxyl radical scavenging activity (25.5, 32.2, 33.2, 42.5 and 63.5 %) at respective concentrations. The extract significantly inhibited nitric oxide radical and ferric reducing power in a concentration dependent manner. All the results were compared with that of standard drug Butylated Hydroxyl Anisole (BHA). Total phenolic content was determined using a spectrophotometric technique, based on the Folin-Ciocalteau reagent. Results showed that both extracts possessed significant antioxidant activity. However, methanolic extract showed potent antioxidant activity as compared to aqueous extract. Phytochemical analysis showed the presence of alkaloids saponins, tannins and phenolic compounds which may the active compounds. The results provide justification for the use of the plant in folk medicine to treat various diseases.

Keywords: *Swietenia mahagoni,* Antioxidant, Methanolic, Aqueous and Butylated Hydroxyl Anisole.

INTRODUCTION

In living organisms the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damage to lipids, proteins, enzymes and nucleic acids leading to cell or tissue injury and implicated in more than 100 diseases, including acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis disorders, type II diabetes[1]. In our living system there was a balance between production of free radicals and consumption of antioxidant, when this balance was disrupted the free radicals was increases and cause deleterious effects[2]. The synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate and tertiary butyl hydroquinone has been widely used in industrially to control lipid oxidation in foods. However, the use of these synthetic antioxidants has been questioned due to their potentials health risks and toxicity[3]. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine, in the development of natural antioxidants from plant materials. This situation has forced to search new antioxidant substances in various sources like medicinal plants[4]. The objective of the present study was to assess *in vitro* protective role of extract for the antioxidant activity.

Swietenia mahagoni (Linn.) Jacq. belongs to plant family *Meliaceae,* commonly known as mahogani, is a large, deciduous, and economically important timber tree native to the West Indies, mainly cultivated at tropical zones, such as India, Malaysia, and Southern China[5]. The extract of this plant showed platelet aggregation inhibitory activity[6], and anti-human immunodeficiency virus (HIV) activities[7]. Seeds oil of *Swietenia mahagoni* shows strong antibacterial activity against three disease causing bacteria viz. *Salmonella typhi, Shigella dysenteriae* and *Staphylococcus aureus*[8]. However, there are no scientific references regarding the antioxidant activity of methanolic and aqueous extracts of *S. mahagoni* seeds, therefore, the present investigation was undertaken to examine the total phenolic content and antioxidant activity through various *in vitro* models

MATERIALS AND METHODS

Chemicals

Nitro blue tetrazolium (NBT), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma), Sodium carbonate, Sodium nitroprusside (10mM) solution and Trichloro acetic acid (TCA) (S.D-fine chemicals, Mumbai). Other chemical used were of analytical grade.

Plant material

The seeds of *S. mahagoni* were collected in January 2010 from Hooghly District, West Bengal, India. The authentication of the specimen was done at 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 using mechanical grinder and soaked in 500 ml of 75% methanol and aqueous solvent for 72 h at 35-40°C. The crude extract then filtered and evaporated under reduced pressure (yield was 5.450% w/w and 4.5% w/w). The crude extract was stored at 4°C and dissolved in respective solvent as per the need and used for the assessment of antioxidant activity.

Phytochemical analysis

Phytochemicals screening were performed to detect various compounds such as tannins, flavonoids, alkaloids and steroids etc[9].

Determination of total phenolic content

Total soluble phenolics in the extracts were determined with Folin–Ciocalteau reagent[10]. Aliquots of each sample were pipetted out in series of test tubes and volume was made up to 3 ml with distilled water. 0.5ml of Folin-Ciocalteau reagent was added to each tube, mixed thoroughly and incubated for 3 min. at room temperature. Then 2 ml of Sodium carbonate (20%) solution was added and the mixer was incubated for 1 min. in boiling water bath. Absorbance was measured at 650nm against a reagent blank. The concentration of total phenolic compounds was determined as micrograms of catechol equivalent by using the standard catechol graph.

Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity

The free radical-scavenging activity of the *S. mahagoni* seed extracts was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH[11]. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic

molecule[12]. Different concentration of samples and BHA (10, 50, 100, 250 and 500µg/ml) were taken in different test tubes. The volume was adjusted to 500µl by adding Methanol. 5 ml of a 0.1 mM methanolic solution of DPPH was added to these tubes and mixed well; allowed to stand at room temperature for 20 min. A control without the test compound, but with an equivalent amount of methanol was prepared. The absorbance of the samples was measured at 517 nm and scavenging activity was calculated using the formula:

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

Determination of hydroxyl radical scavenging activity

Various concentration 10, 50, 100, 250 and 500µg of samples were taken in different test tubes and made up to 250µl with 0.1M phosphate buffer. 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of Dimethyl sulphoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) was added to these tubes and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. These reaction mixtures were incubated at room temperature for 15 min. The reaction was terminated by the addition of 1 ml ice-cold TCA (17.5% w/v), 3 ml of Nash reagent (150 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 lit. with distilled water) and left at room temperature for 15 min. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank[11]. The percentage of hydroxyl radical scavenging activity was calculated by the following formula:

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

Determination of nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity were measured[13].Various concentration 10, 50, 100, 250, 500 and 750µg of extracts were taken in different test tubes and made up to 3ml with 0.1M phosphate buffer (pH 7.2). 1 ml of Sodium Nitroprusside (5mM) was prepared in buffered saline (pH7.2) and added to each tube. The reaction mixture was incubated for 30 min at room temperature. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1- Naphthylethylenediamine dihydrochloride). A control without the test compound, but with an equivalent amount of methanol was maintained. The absorbance of the samples was measured at 546 nm. Nitric oxide radical scavenging activity was calculated using the following formula:

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

Determination of ferric reducing scavenging activity

Ferric reducing scavenging activity was determined[14]. Various concentration of samples 10, 50, 100, 250 and 500µg were mixed with 2.5ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide.

The mixture was incubated at 50ºC for 20 min. then 2.5 ml of 10% trichloroacetic acid (w/v) were added, 5 ml of above solution was mixed with 5 ml of distilled water and 1 ml of 0.1% of ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxy anisole (BHA) was used as standard.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Phytochemical analysis of methanolic and water extracts of *Swietenia mahagoni* seeds showed presence of tannins, alkaloids, saponins and terpenoids are main active phytoconstituents.

Total phenolic content

The Total phenolic content (TPC) of methanolic extract of *S. mahagoni* seeds was found to be 18.4µg/mg of catechol equivalent while aqueous extract contain 14.4µg/mg of catechol equivalent.

Inhibition of DPPH radical

DPPH method is widely reported for screening of antioxidants and its comparative effectiveness[15]. The DPPH is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was initiated by the lipid auto-oxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecules[12]. On the DPPH radical, methanolic and aqueous extracts of *S. mahagoni* seeds had significant scavenging effects with increasing concentration in the range of 10-500µg/ml was tabulated in Fig: 1. IC_{50} values for methanolic, aqueous extracts and BHA was found to be 400.79 µg/ml, 474.99µg/ml and 227.10µg/ml respectively. The radical scavenging activity of the extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability[16].

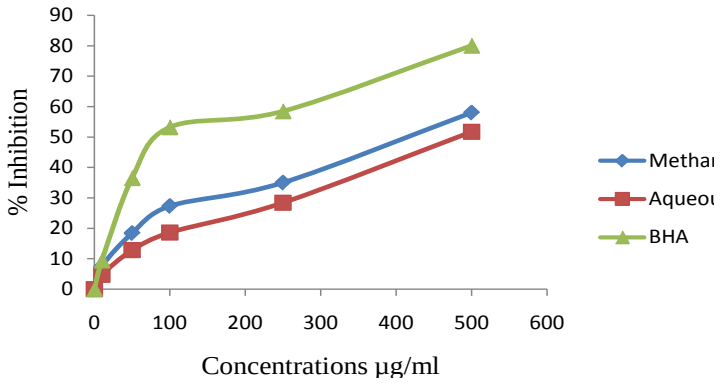

Fig. 1: DPPH radical scavenging activity of the *Swietenia mahagoni* seeds extracts

Hydroxyl radical scavenging activity

The ability of the extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and scavenger of active oxygen species, thus reducing the rate of chain reaction. In the present investigation, both methanolic and aqueous extracts showed antioxidant activity in a concentration dependent manner was tabulated in Fig: 2. IC_{50} values for methanolic, aqueous extracts and BHA was found to be 327.42µg/ml, 421.09µg/ml and 357.72µg/ml. Most notably hydroxyl radicals are produced from the decomposition of hydro-peroxides (ROOH) or in atmospheric chemistry by the reaction of excited atomic oxygen with water. Hydroxyl radical interact with DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity[17]. According to[18] 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. According to[19] methanolic extract of *Ervatamia coronaria* is more capable to inhibit hydroxyl radical generated by Fenton's reaction.

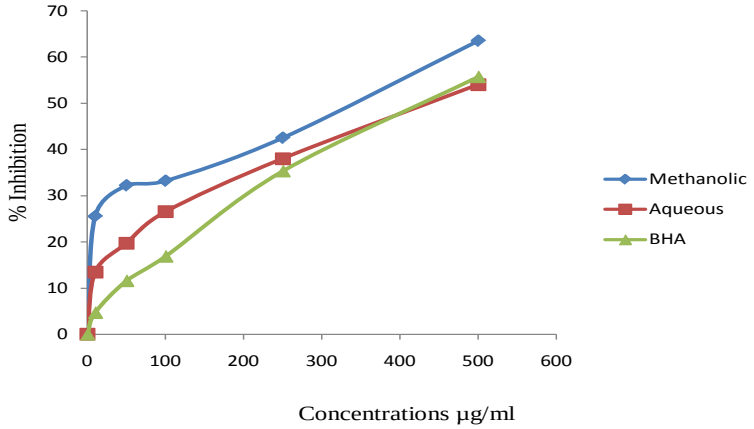

Fig. 2: Hydroxyl radical scavenging activity of the *Swietenia mahagoni* seeds extracts

Nitric oxide radical scavenging

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[20]. The present results showed that both methanolic and aqueous extracts of *S. mahagoni* seeds exhibit significant dose-dependent prevention towards generation of free radicals was tabulated in Fig: 3. IC_{50} values for methanolic, aqueous extracts and BHA was found to be 603.75µg/ml, 609.67µg/ml and 736.7µg/ml respectively. Nitric oxide, a free radical generated by endothelial cells, macrophages and neuron involved in the regulation of various physiological processes[21]. Nitric oxide an essential bio-regulatory molecule required for several physiological processes like neural signal transmission, immune response, control vasodilation and control of blood pressure [22, 23]. The high scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health[22].

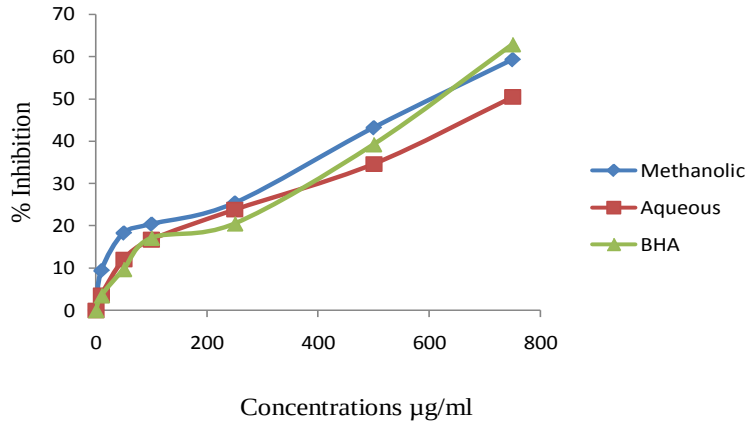

Fig. 3: Nitric oxide radical scavenging activity of the *Swietenia mahagoni* seeds extracts

Ferric reducing scavenging activity

The reducing power of seed extracts (Fe^{3+}– Fe^{2+}) were found to be increased with increasing the concentration. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[24]. The various concentrations of methanolic and aqueous seed extracts (10-500 µg/ml) showed 0.7, 1.3, 1.8, 13 and 30% inhibition respectively; while BHA exhibited 0.4, 0.91, 1.59, 18 and 39% inhibition was tabulated in Fig: 4. The result shows that extract consist of hydrophilic poly phenolic compounds that cause the reducing power.

Methanolic and aquous extracts of *Swietenia mahagoni* seeds showed significant antioxidant activity, which differs in respective extract. This may be due to concentration of active phytochemical present in particular solvent extracts.

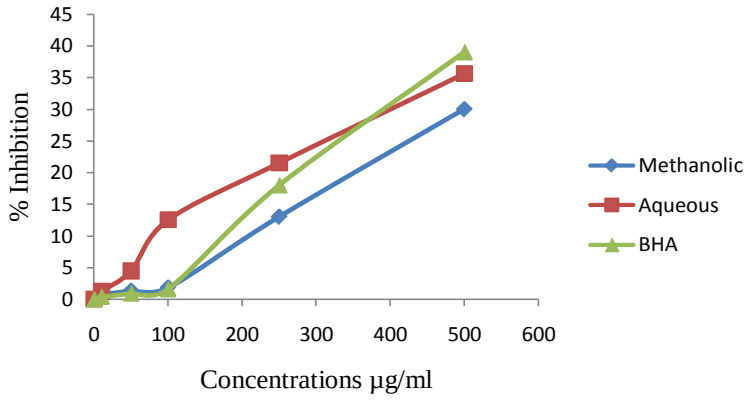

Fig. 4: Ferric reducing power of the *Swietenia mahagoni* seeds extracts

CONCLUSION

The methanolic and aqueous extracts of *S. mahagoni* seeds contained total phenolic compounds and were capable of inhibiting, quenching free radicals to terminate the free radical chain reaction, and acting as reducing agents. In the present study, a significant and linear relationship was found between the antioxidant activity and phenolic content, indicating that phenolic compounds could be major contributors to antioxidant activity. Thus, it can be concluded that methanolic and seed extract of *S. mahagoni* can be used as an accessible source of natural antioxidants with consequent health benefits.

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