EVALUATION OF 1,1-DIPHENYL-2-PICRYLHYDRAZYL (DPPH) RADICAL SCAVENGING EFFECT OF *POLYALTHIA LONGIFOLIA* LEAF EXTRACTS

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Summary

Polyalthia longifolia (Sonn.) Thwaites (Annonaceae), is a tall, evergreen, ornamental tree indigenous to India and found throughout the Indian subcontinent. The present investigation assessed the different solvent extracts of *P. longifolia* leaf for their *in vitro* free radical scavenging potential by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. All of the extracts exhibited potent *in vitro* free radical scavenging activity that increased with extract concentrations. The methanol extract was found to be the most potent in this regard, followed by the chloroform and petroleum ether extracts. Therefore, the present study confirms marked *in vitro* free radical scavenging activity of *Polyalthia longifolia* leaf.

Key words: Free radical scavenging, leaf, antioxidant, *Polyalthia longifolia.*

Introduction

Antioxidants protect living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage and DNA strand breaking. Current interest is focused on the potential role of antioxidants and antioxidant enzymes in the treatment and prevention of atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus and several others diseases (1).

Antioxidants are added to a variety of foods to prevent free radical induced lipid peroxidation, which is responsible for the development of off-flavors and the undesirable chemical compounds in food (2). These ROS cause destructive and irreversible damage to the components of a cell, such as lipids, proteins DNA and other macromolecules. Although normal cells possess antioxidant defense systems against ROS in the cells induces diseases such as cancer and aging (3).

ROS are formed and degraded by all aerobic organisms. ROS can readily react with most biomolecules including proteins, lipids, lipoproteins and DNA. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive oxygen species, which are capable of oxidizing biological molecules, resulting in tissue damage and cell death. When the mechanism of antioxidant protection becomes unbalanced by exogenous and endogenous factors, it results in inflammation, diabetes, genotoxicity, cancer and accelerating aging (4).

Antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage. The most commonly used antioxidants are BHA, BHT, propyl gallate and *tert*-butyl-hydroquinone (5). However, they have been suspected of being responsible for liver damage and carcinogenesis in laboratory animals. Therefore, the development and use of more effective antioxidants is desired.

Traditional medicine worldwide is being reevaluated by extensive research on different plant species and their therapeutic principles. Plants produce antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

Polyalthia longifolia (Sonn.) Thwaites (Annonaceae), commonly known as *Deodar* in Hindi and *Debdaru* in Bengali, is a tall, evergreen, ornamental tree indigenous to India and found throughout the countries of the Indian subcontinent. It is planted throughout India as an ornamental tree. Traditionally the plant has been used in India for several medicinal purposes. Various parts of *P. longifolia* are used in treatment of fever, skin diseases, mouth ulcers, hypertension, helminthiasis, gonorrhea, uterine ailments, leucorrhoea and menorrhagia (6-9). Several phytochemical and pharmacological investigations are reported on this plant mainly on its stem bark and seeds. However, reports on the experimental studies on its leaf are comparatively scanty. In our earlier study we have reported antileishmanial activity of *P. longifolia* leaf (10). In the present study, we have aimed to evaluate *in vitro* free radical scavenging activity of different extracts from *P. longifolia* leaf against 1, 1-diphenyl-2-picryl-hydrazil.

Materials and methods

Plant material: The mature leaves of *Polyalthia longifolia* (Sonn.) Thwaites (Annonaceae), were collected during November 2010 from Nadia, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen [CNH/I-I/(85)/2010/Tech.II/353] was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts: The dried powdered material (350 g) was defatted with petroleum ether (60-80 °C), the percentage extractive value was 2.99% w/w. The defatted powdered material thus obtained was further extracted with chloroform and methanol for 72 h in a percolator. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue and the percentage extractive values were accordingly 53.21% w/w and 7.09% w/w respectively. The preliminary

phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts (11).

Chemicals: L ascorbic acid (vitamin C) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). All the other chemicals and reagents were of analytical grade obtained commercially.

Free radical scavenging activity measured by 1, 1-diphenyl-2-picryl-hydrazil: The free radical scavenging activity of all of the extracts were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the reported method (12). Briefly, an 0.1 mM solution of DPPH in methanol was prepared, and 1 ml of this solution was added to 3 ml of the all of the extracts solution respectively in petroleum ether, chloroform and methanol at different concentrations (2, 4, 6, 8, 10, 15 μg/ml). The mixture were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Genesys 10 UV: Thermo Electron Corporation). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. Ascorbic acid at same concentrations was used as reference. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1) / A_0) \times 100]$$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance of presence of all of the extract samples and standard. The results are stated in Table 1.

Results and discussion

The petroleum ether extract was found to contain triterpenoides and steroids, the chloroform extract was found to have saponins, phenolic compounds along with triterpenoides and steroids but maximum phtoconstituents were detected in the methanol extract, where it contained alkaloids, carbohydrates, tannins, glycosides along with steroids, saponins, and phenolic compounds.

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progressed, results in the scavenging of the radicals by hydrogen donation. It is visually noticeable that as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity of different antioxidants (13, 14). It has been reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic disease conditions such as arteriosclerosis (15).

Based on the data obtained from the present study, all the extracts were effective free radical inhibitor or scavenger, that reacts with free radicals, which may limit free radical damage occurring in the human body. The results are summarized in the Table 1. Free radical scavenging activity also increased with increasing concentration in the range of 2-15 μg/ml. The methanol extract was found to demonstrate the most active free radical scavenging potential even more

than that of ascorbic acid at the test concentration, followed by the chloroform and petroleum ether extracts. The present preliminary study confirms remarkable *in vitro* free radical scavenging activity of *Polyalthia longifolia* leaf against DPPH.

Table 1. DPPH scavenging activity of the different extracts of *Polyalthia longifolia* leaf.

Name the extracts	Concentration (µg/ml)	% of DPPH scavenging activity respectively	Mean ± SEM
P. longifolia (Petroleum ether)	2, 4, 6, 8, 10, 15	29.21, 31.07, 37.99, 58.51, 68.89, 84.67	49.19 ± 8.78
P. longifolia (Chloroform)	2, 4, 6, 8, 10, 15	29.61, 38.67, 48.28, 59.92, 69.27, 81.55	53.86 ± 7.73
P. longifolia (Methanol)	2, 4, 6, 8, 10, 15	46.89, 58.59, 71.65, 78.62, 84.74, 95.63	74.77 ± 7.47
Ascorbic acid	2, 4, 6, 8, 10, 15	26.70, 33.10, 41.50, 93.20, 97.63	57.94 ± 15.29

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