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Research Article

ANTIOXIDANT ACTIVITY OF SEED EXTRACTS OF *POLYALTHIA LONGIFOLIA*

VIJAYA NAGINI DASARI[1], RUPACHANDRA S[1], DINESH MG[2], HANS RAJH CHANDRASEKHARAM [2], RAJA SIDAMBARAM R[3*]

[1]Dept. of Biotechnology, SRM Arts and Science College, Kattankulathur 603203, Chennai, [2]Life Tech Research Centre, Vadapalani, Chennai-600026,[3]Centre for Research in Science and Technology, Stella Maris College (Autonomous), Chennai 600086, India. Email: rajasidambaram@gmail.com

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ABSTRACT

The purpose of our study was to evaluate the antioxidant activity of seed extracts of *Polyalthia longifolia*. Petroleum ether, chloroform, methanol and aqueous extracts of seeds of *P.longifolia* were prepared and evaluated by preliminary phytochemical screening followed by antioxidant activity using DPPH and FRAP assays. The phytochemical analysis showed the major active compound present in all the four extracts as terpenoids. Among the various extracts methanol and petroleum ether extracts showed good antioxidant activity and aqueous extract showed very low activity in both the antioxidant assays. The antioxidant activities were carried out and compared using BHT as the standard. The study confirmed the antioxidant activity of different seed extracts of *P.longifolia* and can be used for further drug formulation studies.

Keywords: DPPH, FRAP, Antioxidant, BHT

INTRODUCTION

Medicinal plants are the local heritage with global importance. Fossil records date human use of plants as medicines at least to the mid Paleolithic age dating back to 60,000 years. From that point the development of traditional medical systems incorporating plants as a means of therapy can be traced back only as far as recorded documents of their likeness. In India, we have well accepted classical systems of treatment like Ayurveda, Siddha and Unani. These are getting dominant over each other at different ages due to many socio-economic and potential reasons. All these utilize potentials of natural products to cure various chronic diseases and health problems. Research on the ancient forms of treatment from natural substances, followed during Charaka and Shushrutha's period, goes on even today. As science advances, people are interested in utilizing only the potential phytocomponents. Hence scientists started isolation, identification and purification of chemical and natural compounds that are mainly responsible for pharmacological action[1].

The goals of using plants as sources of therapeutic agents are *a*) to isolate bioactive compounds for direct use as drugs, e.g., digoxin, digitoxin, morphine, reserpine, taxol, vinblastine and vincristine *b*) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g., metformin, nabilone, oxycodon (and other narcotic analgesics), taxotere, teniposide, verapamil, and amiodarone, which are based respectively on galegine, Δ9 tetrahydrocannabinol, morphine, taxol, podophyllotoxin and khellin, *c*) to use agents as pharmacologic tools, e.g., lysergic acid diethylamide, mescaline and yohimbine and *d*) to use the whole plant or part of it as a herbal remedy, e.g., cranberry, echinacea, feverfew, garlic, ginkgo biloba, St. John's wort and saw palmetto.

It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppression. Currently, there is a growing interest toward natural antioxidants of herbal origin. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems.

Currently, there is great interest in the study of antioxidant substances mainly due to the findings concerning the effects of free radicals in the organism. Plants have been reported to exhibit antioxidant activity due to the presence of antioxidant compounds such as phenolics, proanthocyanidins and flavonoids[2].Phenolic plant compounds have attracted considerable attention for being the main source of antioxidant activity, in spite of not being the only ones. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The antioxidant activities of phenolics play an important role in the adsorption or neutralization of free radicals. Several synthetic antioxidants are commercially accessible but have been reported to be toxic.

Interestingly the body posses defence mechanisms against free radical-induced oxidative stress, which involve preventive mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc. Non-enzymatic antioxidants are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, flavonoids, etc. All these act by one or more of the mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen.

Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic [3, 4]. In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value. Nutraceuticals are supposed to hold the key to a healthy society in future. Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body's ability to use free radicals constructively [5, 6].

Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols and tocotrienols) ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are on the increase[7].

Polyalthia longifolia Benth.commonly called, Ashoka (Family: Annonaceae) is a tall, evergreen and attractive plant commonly grown as ornamental avenue tree. It is planted throughout India.

The various parts of the plant have been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis. A number of biologically active compounds have been isolated from this plant. The plant extract and isolated compounds have been studied for various biological activities such as, antibacterial, antifungal, anti-inflammatory and cytotoxic[8]. Antitumour and antioxidant activity of *P. longifolia* stem bark ethanol extract has been reported[9]. In vitro cytotoxic potential of *P.longifolia* leaves on human cancer cell lines has been reported for the first time in 2008[10] and antioxidant activity of ethanolic extract of seeds have been reported recently[11]. However not much work has been reported on seeds and leaves of *P.longifolia*.

The present study was concerned mainly to evaluate the antioxidant activity of seed extracts (petroleum ether, chloroform, methanol and aqueous) of *P.longifolia* in order to identify active compounds or potential lead compounds.

MATERIALS AND METHODS

Plant material

P.longifolia seeds were collected from the 'Plant Anatomy Research Centre, Chennai' and authenticated. The collected seeds were washed with sterile water and shade dried and powdered and stored in an air tight container.

Preparation of plant extracts

5 g of the dried seed powder of *P.longifolia* was dissolved in 50 ml of different solvents such as petroleum ether, chloroform, methanol and distilled water in separate conical flasks. The suspensions incubated at room temperature for 48 h at 150 rpm in an orbital shaker. The suspension was filtered and concentrated to dryness at 40 ^{o}C in hot air oven. The yield of extracts was calculated and the extracts were dissolved in 0.25% Dimethyl Sulphoxide (DMSO) to a concentration of 100 mg/ml that can be stored as stock solutions[12].

Phytochemical screening

The different extracts were preliminarily screened for phytochemical constituents such as tannins, saponins, flavanoids, alkaloids and terpenoids in accordance with the methods of [13, 14, 15] with little modifications.

Test for tannins

The extracts were diluted to 10 mg/ml concentration. To 1 ml of the extract few drops of 0.1% Ferric chloride reagent added and blue-black colorization indicates the presence of tannins.

Saponins

1 ml of extracts mixed with 2 ml of distilled water and shaken vigorously then observed for foaming (frothing test) and foaming indicates the presence of saponins.

Flavonoids

A pinch of magnesium salt and few drops of conc. Hydrochloric acid added to 1 ml of each extracts and noted for orange red color and it indicates presence of flavonoids.

Alkaloids

1 ml of extract was dissolved in 5ml of 1% Hydrochloric acid on steam bath and 1ml of filtrate was treated with few drops of Draggendoff's reagent and turbidity or precipitation was indicative of presence of alkaloids.

Terpenoids

Salkowski test was done to identify the presence of terpenoids. The formation of reddish brown color at interface when 1 ml of extract was added to 2ml of chloroform and 3ml of conc. sulphuric acid.

DPPH radical scavenging activity

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of extracts[16].100 µl of different extracts (5mg/ml concentration) were added to 2.8 ml of methanol and 200 µl of DPPH (100µM) and shaken and left to stand at room temperature for 30min in the dark. The control consists of only methanol and DPPH solution. Absorbance was read at 517nm and results were compared with the standard BHT. The scavenging effect was calculated using the following formula:

% scavenging effect = (OD of control-OD of sample/OD of control) x 100

Ferric reducing antioxidant power assay

The technique adapted is based on the method described by *Benzie and Strain*[17]. The principle is based on the method of reduction of ferric 2, 4, 6-tris (2-pyridyl)-s-triazine [Fe^{3+}-TPTZ] complex to its ferrous colored form in the presence of antioxidants.1ml of water and 80µl of each extract (5mg/ml) were taken in a standard plastic cuvette to which 600µl of FRAP reagent was added and incubated at 37°C for 4 minutes. Blank was prepared without adding the seed extract and change in absorbance was recorded at 593 nm (the result of Fe^{3+}-TPTZ complex to the blue Fe^{2+}-TPTZ complex at low p^{H}).

Each sample was tested in triplicate to allow a mean absorbance to be calculated. A standard curve was prepared by taking standard solution of 1 mM ferrous sulphate in different concentrations and assay was performed as described above with ferrous sulphate instead of test samples and OD read at 593 nm.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screening of different solvent extracts of seeds showed the presence of tannins, favanoids, saponins, alkaloids and terpenoids. Among the four extracts the methanol extract showed the presence of alkaloids when compared to other extracts and the aqueous and petroleum ether extracts showed the presence of saponins and only the aqueous extract showed the presence of flavanoids. Precipitation was observed only in the chloroform fraction but however all the four extracts showed reddish brown color which indicated the presence of terpenoids. The results are shown in table 1.

Table 1: Phytochemical analysis of different extracts of seeds of *P. longifolia*

S. No	Test	Aqueous extract	Petroleum ether extract	Methanol extract	Chloroform Extract
1.	Tannins	-	-	+	-
2.	Saponins	+	+	-	-
3.	Flavanoids	+	-	-	-
4.	Alkaloids	-	+	-	+
5.	Terpenoids	+	+	+	+

+ = Positive **-** = Negative

DPPH activity

DPPH is a kind of stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity .In the DPPH radical scavenging assay, antioxidants react with the DPPH radicals, which is a stable free radical and exists naturally in deep violet color, to turn into yellow colored, diphenyl-picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant[18].

The different seed extracts of *P.longifolia* showed good antioxidant activity. The methanol and petroleum ether extracts showed almost the same percentage of scavenging effect. But the aqueous extract showed very low antioxidant activity. The assay was repeated thrice and the % scavenging effect compared with standard and was expressed as mean ±SE and shown in table 2. The % scavenging effect for different solvent extracts of *P.longifoila* seeds is shown in figure 1.

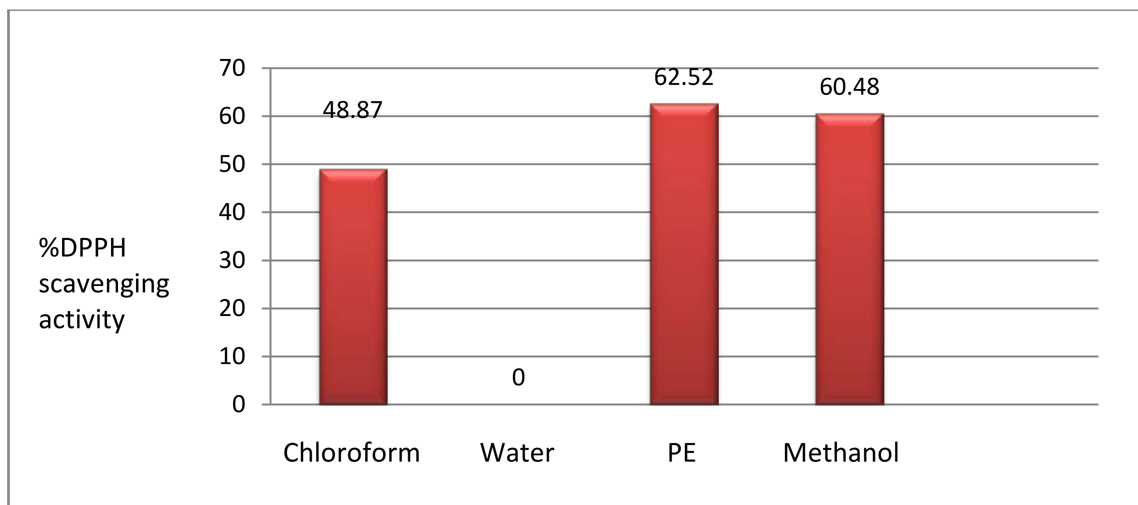

Fig. 1: DPPH activity of different solvent extracts of *P.longifolia* seeds expressed as mean ± SE with significant difference of p<0.01

Ferric Reducing Antioxidant Power Assay

Ferric reducing antioxidant power assay was used to measure the antioxidant potential of different seed extracts of *P.longifolia*. Principally, FRAP assay treats the antioxidants in the sample as reductant in a redox-linked colorimetric reaction.[19]

Antioxidant activity of all the extracts were calculated and expressed in mmol Fe^{2+}/g of extract or fraction and with a linear equation based on a standard curve (figure-2) using $FeSO_4$ (y=0.000x-0.001, R^2=0.997) and shown in table 2.The methanolic extract showed the highest antioxidant activity among the four extracts with the FRAP value of 1.4±0.011547mmol/g.

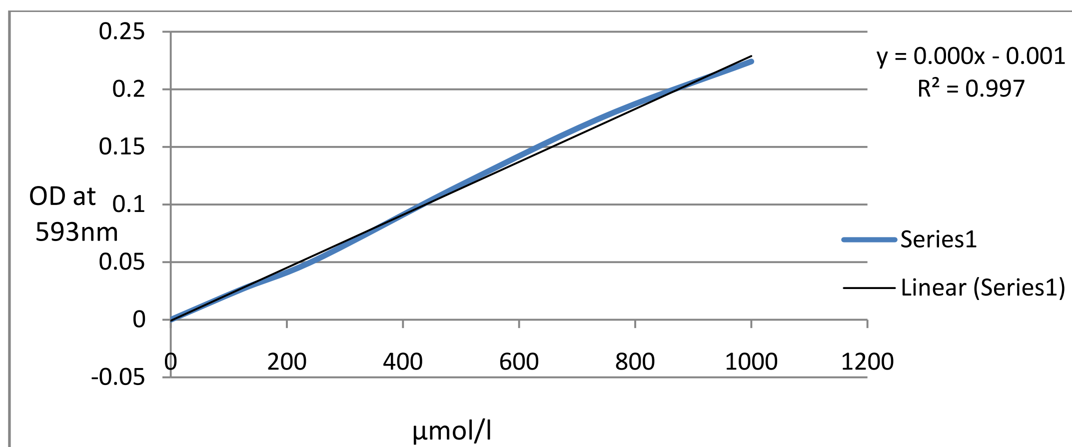

Fig. 2: FRAP Standard Graph

Table 2: DPPH and FRAP mean values

Extracts	DPPH (mean ± SE)	FRAP values (mmol $Fe^{2+}g^{-1}$ ± SE)
Chloroform	48.87±2.07	0.73±0.01
Petroleum ether	62.52±3.81	0.81±0.06
Water	Trace	0.34±0.08
Methanol	98.43±0.26	1.40±0.01

CONCLUSION

The present study has clearly shown that the different seed extracts of *P. longifolia* have shown significant antioxidant activity. Among the four extracts the methanolic extract had good antioxidant activity and can be used for further *in-vivo* investigations. Phytochemical investigations can also be proposed to identify the potential compounds or the active chemical compound to eventually identify the pure drug molecule.

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