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Evaluation of phenol, flavonoid contents and antioxidant activity of *Polyalthia longifolia*

Kavita S. Mundhe, Asha A. Kale, Sucheta A. Gaikwad, Nirmala R.Deshpande, Rajashree V. Kashalkar*

Dr.T.R.Ingle Research Laboratory, Department of Chemistry, S. P. College, Pune, India

ABSTRACT

Polyalthia longifolia has been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension, helminthiasis etc.Now a days; there is growing interest in exploring new sources of potent natural antioxidants for the food industry. In the present study antioxidant activity of ethanolic extracts obtained from the leaves and seeds of P. longifolia along with total phenol and flavonoid content has been evaluated spectrophotometrically.The total phenol content was determined using the Folin – Ciocalteu reagent while total flavonoid content was evaluated with aluminium chloride under basic conditions. The antioxidant activity of ethanol extract of the seeds and leaves was determined by measuring the radical scavenging activity against 2, 2 – Diphenyl– 1-picryl hydrazyl radical (DPPH). .The highest radical scavenging effect was observed in leaves with IC_{50} = 0.5824mg/ml than in seeds with IC_{50} = 1.4677 mg/ml. Phenolic compounds and flavonoid contribute to this activity. The results suggest that P. longifolia is a good source of antioxidant and can be used against various free radical related disorders.

Keywords: Phenol, Flavonoid, Antioxidant activity, *Polyalthia longifolia.*

INTRODUCTION

The use of the plants, plant extracts, and pure isolated compounds from natural sources has always provided a foundation for modern pharmaceutical compounds. Ayurveda, Siddha and Unani are the traditional systems of medicine in India which make ample use of herbal preparations to cure various disorders. India is the largest producer of medicinal herbs. Use of

herbal products as anti-microbial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. The demand of plant based therapeutics is increasing in both developing and developed countries as they are natural products, easily biodegradable, producing minimum environmental hazards, with no adverse side effects and easily available at affordable prices. This prompted to isolate, identify and purify the naturally occurring chemical moieties, which are mainly responsible for pharmacological and therapeutic action [1].

Accumulation of free radicals can cause pathological conditions such as ischemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementia. Antioxidants are radical scavengers which protect the human body against free radical disorders. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are being used as radical scavengers. Natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe, effective and cheap antioxidants. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [2].Flavonoids can induce mechanisms that may kill tumer cells and inhibit its invasion. Multivariant regression analysis has revealed a significant relationship between flavonoid intake and risk of mortality from coronary heart disease after adjustment of age and risk factors. Phenolic compounds serve as defense mechanism against herbivors. *P. longifolia* commonly called, Ashoka (Family: Annonaceae) is a tall evergreen, handsome plant commonly used as an ornamental street tree due to its effectiveness in controlling noise pollution. 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 [3]. 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 [4-6], antifungal [7], anticancer [8], anti-inflammatory and cytotoxic [9], hypotensive [10], fungicides [11] and analgesic [12]. Literature depicts the use of Ashoka (*P. longifolia*) bark as a natural mordant [13] and also as biomonitor of automobile pollution [14]. Antitumour and antioxidant activity of *P. longifolia* stem bark ethanol extract has been reported [15]. The seeds and leaves however have not been studied. The present study attempt extensively to estimate quantitatively phenol and flavonoids and assess the antioxidant activity spectrophotometrically from the said parts.

EXPERIMENTAL SECTION

Plant material

The leaves and seeds of *Polyalthia longifolia* (Sonn.)Thw. were collected from the near by garden in Pune, Maharshtra, India and authenticated at Botanical Survey of India, Pune,India, having a voucher specimen number is POLMK1; BSI/WRC/Tech/2009.

The leaves and seeds of *P. longifolia* were air dried, powdered and the accurately weighed samples were extracted in ethanol (ethanol – water, 8:2) and filtered. The extracts were concentrated to get the solid mass. The extractive value obtained was 9.7% and 12.5% for leaf and seed respectively. Freshly prepared extract was used for the analysis to prevent degradation. UV – Vis S1700 Pharmaspectrophotometer, Schimadzu was used for absorbance measurements.

Chemicals
Folin – Ciocalteau reagent obtained from Merck. All other chemicals were of analytical grade and obtained locally.

Total Phenol Content Determination
The total phenols were determined by Folin – Ciocalteau reagent method described by Malik and Singh, 1980 [16]. The dilute extracts of different concentrations were taken in 10ml. glass tubes and total volume made to 3ml. with distilled water these are then mixed with 0.5ml. Folin – Ciocalteau reagent (1:1 with water) and 2 ml. Na_2CO_3 (20 %). A blue coloured complex, molybdenum blue developed in each tube, as the phenols undergo a complex redox reaction with phosphomolibdic acid in Folin – Ciocalteau reagent in alkaline medium. The tubes containing the blue solutions were warmed for 1 min., cooled and absorbance was measured at 650 nm against the reagent blank. The standard curve [Fig.1] was prepared using known concentrations of catechol at 650 nm. The total phenol content in the test samples was calculated from the standard curve and expressed as mg. catechol equivalent of phenol/ g. sample.

Determination of total flavonoid content
Colourimetric aluminum chloride method was used for flavonoid determination [17]. Aliquots of extract were taken and the volume made to 2ml. with methanol and then mixed with 0.1 ml. $AlCl_3$ (10%), 0.1 ml. Potassium acetate and 2.8 ml. distilled water. The solutions were kept at room temperature for 30 minutes and the absorbance was measured at 415 nm. A standard calibration curve [Fig.2] was prepared using known concentrations of quercetin at 415 nm. The total flavonoid in the test samples were calculated from the standard plot and expressed as mg. quercetin equivalent/ g. sample.

DPPH radical – scavenging activity
The stable 2, 2 – Diphenyl– 1-picryl hydrazyl radical (DPPH) was used for determination of antioxidant activity of the extracts [18, 19, 20]. Different concentrations of each extract were taken and made a volume of 3 ml. with methanol and mixed with 150 µl DPPH. It remained at room temperature for 15 minutes.When DPPH reacts with an antioxidant compound that can donate hydrogen, it is reduced. The changes in colour (from deep – violet to light – yellow) were measured at 517 nm spectrophotometrically. Vitamin C was used as a standard control. Radical scavenging activity was expressed as percentage of DPPH elimination after 30 minutes and calculated as follows,

$\%\ \text{Activity} = [(A_0-A_1) / A_0] \times 100$
Where,
A_0 = Absorbance of the control.
A_1 = Absorbance of the test sample.

RESULTS AND DISCUSSION

Total phenol and flavonoid contents of the leaves and seeds are determined [Table.1]. There is well supported evidence that the phenolic compounds found in various plant materials possess free radical scavenging properties, in addition, it was reported that flavonoids were OH* scavengers [21]. In the present study the phenol content of the leaves and seeds of *P. longifolia* were determined in terms of mg catechol equivalent /g extract using the standard plot of

catechol(y=0.133x, R^2 = 0.9878). The values were found between139 to 302 mg catechol equivalent /g extract. Using the standard plot of quercetin (y = 0.0136x, R^2 = 0.984), the flavonoid contents of leaves and seeds were found ranging from 113 to 339 mg quercetin equivalent /g extract. It was observed that leaves contain less amount of phenol than seeds, where as flavonoid contents was more in leaves than in seeds. The total antioxidant capacities of leaves and seeds of *P. longifolia* [Table 2] indicate that antioxidant capacity is higher for the leaf extract. The radical scavenging activities for leaves and seeds are IC_{50} = 0.5824mg/ml and 1.4677 mg/ml respectively, compared to IC_{50} for ascorbic acid 0.0094mg/ml.

Table.1: Phenol and Flavonoid Contents

Plant Part	Phenol (mg Catechol equivalent /g)	Flavonoid (mg quercetin equivalent/g)
Leaves	255.0	339.2
Seeds	265.2	174.8

Table.2: Antioxidant activity of ethanol extract of leaves and seeds of *Polyalthia longifolia* (DPPH Assay)

Leaf		Seed	
Concentration (mg/ml)	%Activity	Concentration (mg/ml)	%Activity
0.097	72.52	0.2	54.67
0.194	82.82	0.4	58.92
0.388	84.47	0.8	63.18
0.582	91.34	1.2	70.87
0.776	92.03	1.6	79.8
0.97	93.13	2.0	83.65

Fig.1: Calibration plot for phenol determination at 650 nm

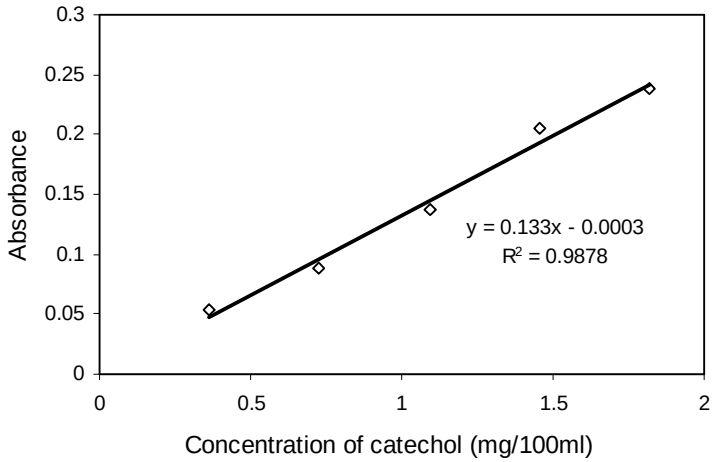
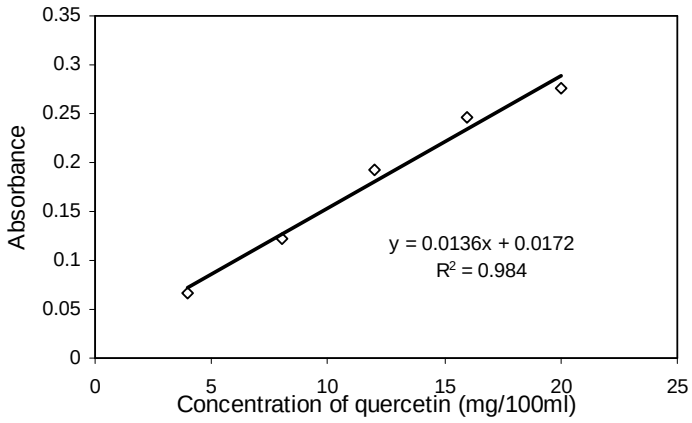


Fig.2: Calibration plot for flavonoid determination at 415 nm



Radical scavenging activities are important due to the deleterious role of free radicals in foods and in biological systems. DPPH assay evaluates the ability of antioxidants to scavenge free radicals. The method is based on the reduction of alcoholic DPPH solution into the non – radical form [DPPH – H] in the presence of hydrogen – donating antioxidant.

CONCLUSION

In conclusion it can be said that the leaves and seeds of *P. longifolia* contain significant amounts of phenols and flavonoid, they also exhibit a good antioxidant activity compared to ascorbic acid. The results clearly indicate that the ethanol extracts of the leaves and seeds are very good source of antioxidants of natural origin. The higher antioxidant activity of the leaves than the seeds can be attributed to their high flavonoid contents compared to seeds.

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