

Antioxidant and antimicrobial activities of isolated constituents from the bark of *Polyalthia longifolia*

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The bark of *Polyalthia longifolia* is traditionally reputed to lower blood pressure, stimulate respiration and help in fever and skin diseases, diabetes, hypertension and vitiated conditions of vata and pitta. Preliminary phytochemical investigation of various extracts of the bark of *Polyalthia longifolia* showed the presence of flavonoids, alkaloids, steroids and carbohydrates. An attempt has been made to isolate flavonoids and perform antioxidant and antimicrobial activity of the same. We carried out DPPH radical scavenging assay, nitric oxide scavenging assay, metal chelating activity and reducing power activity in antioxidant activity. In antimicrobial activity, we used six microorganisms, which included two Gram positive, two Gram negative bacteria and two fungi. Both the isolated flavonoids exhibited a concentration-dependant free radical scavenging capacity. Isolated compounds showed promising results against various micro-organisms in comparison with standard drugs (Penicillin, Gentamicin and Ketoconazol).

Key words: Antioxidant, antimicrobial, isolated compounds

INTRODUCTION

In our country, we have well accepted classical system of treatments like Ayurveda, Unani and Siddha. 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 disorders and health hazards. Research on the ancient forms of treatment from natural substances, followed during Charaka and Shushruta's period, is going on even today. As science advances, people are interested in utilizing only the potential components. Hence scientists started isolation, identification and purification of chemical and natural moiety, which is mainly responsible for pharmacological and therapeutic action.^[1]

Annonaceae plants are a rich source of bioactive substances and certain genera of this family have recently attracted much interest, since they have been found to contain a group of antitumour active tetrahydrofuranic acetogennins.^[2] *Polyalthia longifolia* (commonly known as 'Wipping Devdaru') is a tall handsome tree planted as avenue tree and considered a native of Sri Lanka. It is also planted throughout India.^[2] The bark of *P. longifolia* is traditionally reputed to lower blood pressure, help stimulate respiration, reduce fever and skin disease, diabetes, hypertension and vitiated conditions of vata and pitta.^[3] Many pharmacological activities have been reported and responsible alkaloids and terpenoids are

isolated from various parts of the above said plant. There is no scientific report to isolate flavonoids from the bark of *P. longifolia*.^[4-8] Hence in the present study an attempt has been made to isolate flavonoids from the bark of *P. longifolia* and perform the antioxidant and antimicrobial activity of the isolated compound.

MATERIALS AND METHODS

Plant Material

The bark of *P. longifolia* was collected from Botanical garden, Karnataka University, Dharwar, Karnataka and authenticated by Prof. Tarun Kumar Basu, Professor and Head, Department of Botany, Netajee Mahavidyalaya, Hoogly, West Bengal. A voucher specimen (specimen no. NM/BU/2004/05) has been deposited at the Botany Department, Netajee Mahavidyalaya, Hoogly, West Bengal.

Preparation of Extracts

Fresh bark was shade dried at room temperature, pulverized and 100gm of bark powder was extracted with 95% ethanol in Soxhlet extractor. The extract was concentrated in a rotary flash evaporator. The residue was dried in a desiccator over sodium sulfite. The alcoholic extract (16.5% w/w) was subjected to qualitative chemical tests to identify phytoconstituents. Thin layer chromatographic studies were carried out for the above extract to confirm the presence of phytoconstituents detected in qualitative chemical

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tests. Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27) was determined as the solvent system for the TLC of flavonoids.^[9]

Isolation of Flavonoids

Air-dried bark powder (100gm) was defatted with petroleum ether (60-80). It was then extracted with distilled ethanol in a Soxhlet extractor for 16 hours. The extract was filtered and concentrated in rotary flash evaporator at below 40° C. The concentrated ethanolic extract was poured into excess of distilled water with stirring and filtered. The filtrate that comprises water soluble portion of ethanolic extract was extracted in liquid-liquid extractor with petroleum ether (60-80), benzene and ethyl acetate. Ethyl acetate extract was concentrated to a small volume and refrigerated for two days, to yield yellow crystals. These crystals were dissolved in ethyl acetate and tested for the presence of flavonoids. The crude flavonoids were then separated and purified by column chromatography. The fractions were collected at regular intervals of time, evaporated at temperature less than 40 °C and subjected for evaluation of pharmacological activities.

Antioxidant Activity

DPPH radical-scavenging capacity

We determined the scavenging capacities of isolated flavonoidal fractions (F1 and F2) using DPPH as a stable free radical.^[10] In brief, we added 500 µl of various concentrations of the samples in methanol to 500 µl of 0.12 mM methanol solution of DPPH. The mixture was shaken vigorously and left to stand for 30 minutes in the dark. The absorbance was then measured at 517nm against a blank. Radical scavenging activity was expressed as the percentage of DPPH elimination after 30 min and calculated as follow

$$\text{Scavenging ability (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the test compound. BHA and α -tocopherol were used as positive control.

Nitric oxide (NO) scavenging capacity

The scavenging effects of isolated constituents on NO were measured;^[11] 10mM of sodium nitroprusside solution in phosphate buffered saline (PBS), pH 7.4 was prepared immediately before the experiment. Sodium nitroprusside (final concentration 5mM) in PBS was mixed with samples, diluted in PBS and incubated at 25 °C for 150 minutes. After incubation, samples (0.5 ml) were removed and diluted with 0.5 ml of greiss reagent (1% sulfanilamide, 2% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) and the absorbance was read at 546 nm. The inhibition of NO generation was estimated by

comparing the absorbance values of control with that of treatment. The same reaction mixture without the sample or standard but equivalent amount of PBS served as the control.

Metal chelating capacity

The ferrous ion chelating capacity of crude extract and fractions were estimated.^[12] Briefly, different concentrations (100-1000 µg/ ml) of extracts in 0.4 ml methanol were added to a 50µl solution of FeCl_3 (2mM). The reaction was initiated by the addition of 5nM ferrozine (0.2ml) and total volume was adjusted to 4ml with methanol. The mixture was then shaken vigorously and left standing at room temp for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine- Fe^{2+} complex formation was calculated using the formula:

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

Where, A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the samples and standards. The control contains FeCl_2 and ferrozine, complex formation molecules.

Reducing power determination

The reducing power of samples and standards was determined.^[13] Different amounts of samples (50-200µg/ml) in 1 ml methanol were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 1000g for 10 min. the upper layer of sol (2.5ml) was mixed with distilled water (2.5ml) and FeCl_3 (0.5ml, 1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Antimicrobial Activity

The isolated flavonoidal fractions (F1 and F2) were tested for their possible antibacterial and antifungal activity against *Bacillus subtilis*, *Bacillus thuringiensis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, *Aspergillus niger* respectively. The test was carried out by the standard agar cup-plate method.^[14-20] Three concentrations (50 µg, 75 µg 100 µg) of the isolated flavonoidal fractions were prepared for the antimicrobial screening. Compound F1 and F2 were dissolved in sterile water. Penicillin (50 µg/ml) and Gentamicin (50 µg/ml) were used as control for Gram positive and Gram negative bacteria respectively. Ketoconazole (50 µg/ml) was used as control for the fungus.

The antibacterial and antifungal activity were evaluated

by employing 24-hour and 48-hour cultures respectively of above said bacteria and fungus using nutrient agar medium.

Statistical Analysis

The data are results of triplicate experiments. Microsoft Excel was used to compute means and standard deviation.

RESULTS

The preliminary phytochemical study of the bark of *Polyalthia longifolia* revealed the presence of carbohydrates, phenolic compounds, alkaloids, flavonoids, triterpenoids and sterol in its alcoholic extract. Chromatographic studies on this extract substantiated the presence of chemical constituents detected in the qualitative chemical tests. Two spots of different R_f values (0.91 and 0.61) were observed in the TLC of flavonoids. The yield of first isolated and purified compound (F1) was found to be 0.64%. The melting point of compound F1 was found to be 160-165°C, the yield of the second isolated compound was found 0.92% and the melting point of compound F1 was 140-145°C.

DPPH Radical-scavenging Capacity

As illustrated in Figure 1, both the isolated flavonoids exhibited a concentration-dependant DPPH radical scavenging capacity. In between them F1 exhibited stronger DPPH radical-scavenging capacity than F2 [Table 1].

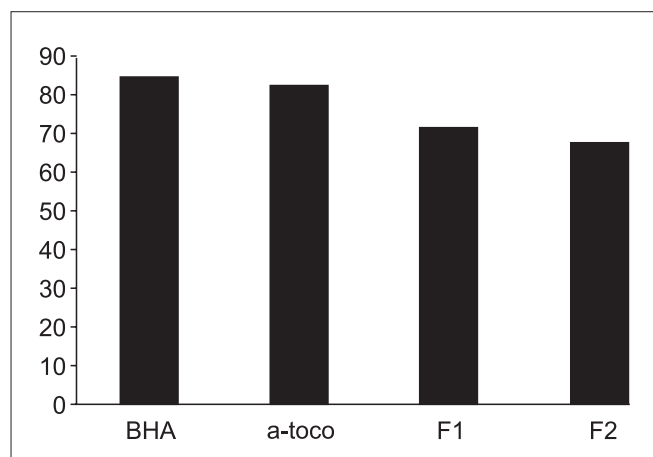


Figure 1: DPPH radical scavenging activity of isolated flavonoids (F1 and F2) The values represent the mean (n=3), BHA= Butylated hydroxyanisole, a-toco= α -tocopherol.

NO Scavenging Capacity

As showed in Table 1, both F1 and F2 exhibited NO scavenging activity [Figure 2].

Metal Chelating Capacity

F1 showed higher chelating activity among both isolated flavonoids. However, the activity was much lower than that of EDTA [Figure 3]. The standard compounds α -tocopherol and BHA did not exhibit any chelating activity at tested concentrations [Table 1]

Reducing Power Determination

In the present study, the reductive capabilities of isolated flavonoids were measured in terms of Fe³⁺ - Fe²⁺ transformation and compared with α -tocopherol, BHA and gallic acid. Both the isolated flavonoids have reduced iron [Figure 4] in a concentration dependant manner [Table 1].

Antimicrobial Activity

In the antimicrobial activity, the compound F1 showed highly significant result against *B.subtilis*. It showed moderately significant result against *B. thuringiensis*, *E. coli* and *P.aeruginosa*. Similarly, compound F2 showed moderately significant results against *B. subtilis*, *B. thuringiensis*, *E. coli* and *P. aeruginosa* [Table 2].

From the present study it can be said that flavonoids are the active constituents in the bark of *Polyalthia longifolia* and

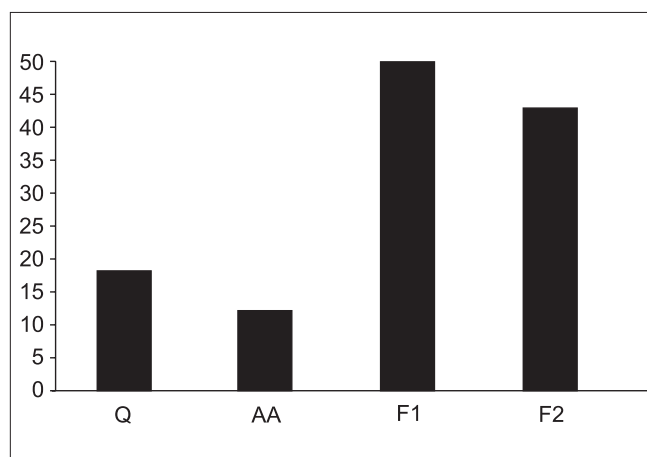


Figure 2: NO scavenging activity of isolated flavonoids (F1 and F2) The values represent the mean (n=3), Q= Quercetin, AA= Ascorbic acid.

Table 1: Free radical scavenging activity of isolated flavonoids (F1 and F2)

Free radicals	% Antioxidant activity						
	BHA	-toco	Q	AA	EDTA	GA	
DPPH							
NO	85± 0.31	83± 0.23	-	-	-	-	72± 1.21 68± 0.41
Ferrous ions	-	-	18± 0.34	12± 0.13	-	-	50± 0.32 43± 1.34
Reducing power	-	-	-	-	87± 0.53	-	42± 0.04 35± 0.43
DPPH	1.3± 0.03	0.43± 0.02	-	-	-	2.2± 0.05	0.81± 0.01 0.32± 0.04

The values represent the mean±SD (n=3), BHA= Butylated hydroxyanisole, α -toco= α -tocopherol, Q= Quercetin, AA= Ascorbic acid, GA= Gallic acid.

are likely to be responsible for its pharmacological actions.

DISCUSSION

This study analyzes the antioxidant and antimicrobial capacity isolated flavonoids from the bark of *Polyalthia longifolia*. 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 scavenging free radicals. The method is based on the reduction of alcoholic DPPH solution into non-radical from DPPH-H in the presence of a hydrogen-donating antioxidant.

NO is an important bio-regulatory molecule, which has a number of physiological effects. Low concentration of NO is sufficient to affect these beneficial functions, however, during infections and inflammation, its formation is elevated and may bring about some deleterious effects. In antimicrobial activity, both the flavonoids are responsible as nonantibiotic antimicrobials.

CONCLUSIONS

The results indicate that both the isolated flavonoid

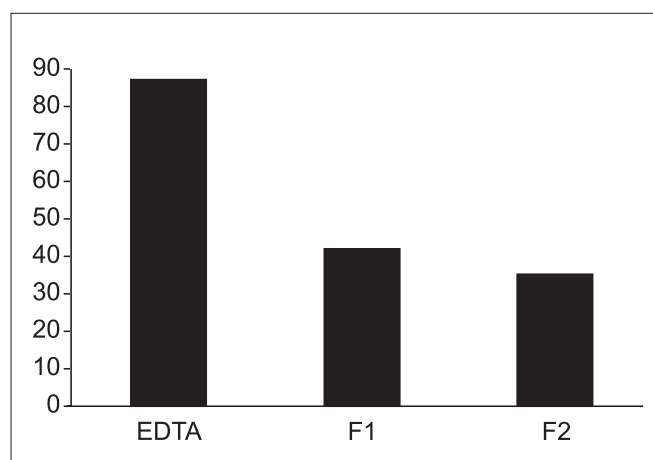


Figure 3: Ferrous ion chelating activity of isolated flavonoids (F1 and F2) The values represent the mean (n=3)

fractions of *P. longifolia* bark inhibited all above said free radicals in a dose-dependent manner and are also active against selected microorganisms. These results clearly indicate that *P. longifolia* is effective against free radical mediated diseases and also as nonantibiotic antimicrobial. Further investigations on their *in vivo* antioxidant activity are necessary to determine the specific mechanisms.

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REFERENCES

1. Dev S. Ethno botanical survey of traditional plants. Curr Sci

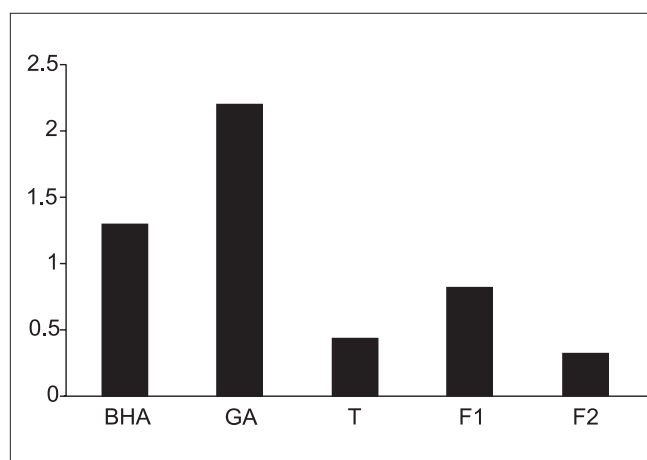


Figure 4: Reducing power of isolated flavonoids (F1 and F2) The values represent the mean (n=3), BHA= Butylated hydroxyanisole, GA= Gallic acid, T= α -tocopherol.

Table 2: Zone of inhibition (Including 6mm bore in the centre) of various microorganisms against various isolated flavonoids (F1 And F2)

Organisms	Compound F ₁			Compound F ₂			P 50µg	G 50µg	K 50µg
	50µg	75µg	100µg	50µg	75µg	100µg			
<i>B.subtilis</i>	20	20	25	15	17	20	27	-	-
<i>B.thuringiensis</i>	25	25	26	16	18	20	35	-	-
<i>E.coli</i>	14	11	12	14	14	16	-	23	-
<i>P.aeruginosa</i>	13	15	15	12	12	12	-	22	-
<i>A.niger</i>	R	R	R	10	10	10	-	-	20
<i>C.albicans</i>	R	R	R	R	R	R	-	-	10

Measurement unit is 'Millimeter', The values represent the mean (n=3), P = Penicillin, G = Gentamicin, K = Ketoconazole, R = Resistant

- 1997;73:909-28.
2. Yoganarsimhan S.N. Medicinal Plants of India-Karnataka. Bangalore, India: Interline Publishing (P).Ltd; 2000.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol 1, Dehradun: Industrial Books Distributer; 1995.
4. Phadnis AP, Patwardhan SA, Dhaneshwar NN, Tavle SG. Clerodane diterpenoids from *Polyalthia longifolia*. Phytochemistry 1998;27:2899-901.
5. Chakrabarty M. 6,7, Dimethoxyonychine and other alkaloids of *Polyalthia longifolia*. Indian J Chem Br 1990;29:394-5.
6. Wu YC, Duh CY, Wang SK, Chen KS, Yang TH. Two new natural azafluorene alkaoids and a cytotoxic aporphine alkaloid from *Polyalthia longifolia*. J Nat Prod 1990;53:1327-32.
7. Rashid MA, Hossain MA, Hassan CM, Reza MS. Antimicrobial diterpenes from *Polyalthia longifolia* var. pendula (Annonaceae) Phytother Res 1996;10:79-81.
8. Sebsibe-Ameha A, Mathur MM. Chemical composition of Asoka (*Polyalthia longifolia*) tree leaves. Indian J Anim Nutr 2000;17:254-5.
9. Males Z, Plazibat M, Vundać VB, Zuntar I. Qualitative and quantitative analysis of flavonoids of the strawberry tree- *Arbutus unedo* L. (Ericaceae). Acta Pharm 2006;56:245-50.
10. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 1995;43:27-37.
11. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide – scavenging properties of *Ginkgo biloba* extract EGB 761. Biochem Biophys Res Commun 1994;201:748-55.
12. Dinis TC, Maderia VM, Almeida LM. Action of phenolic derivatives (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. Arch Biochem Biophys 1994;315:161-9.
13. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986;44:307-15.
14. Mathew J. Antimicrobial activity of selected aromatic plants. Indian Drugs 2005;42:28-33.
15. Kumari T GR, Paul PT, Ayyub S, Iyenger MA, Rao PS. Antimicrobial activity of the essential oil of *Eletharia cardamomum* Maton. Indian Drugs 2004;41:622-3.
16. Kuppuswamy A., Shanmuga P, Veena A. Antifungal activity of newly synthesized piperidin-4-oxime derivatives. Indian Drugs 2000;41:7-11.
17. Talas-Ogras T, Ipekci Z, Bajrovic K, Goziikirmizi N. Antibacterial activity of seed proteins of *Robinia pseudoacacia*. Fitoterapia 2005;76:67-72.
18. Shasrty CS, Aravind MB, Joshi SD, Bheemachari AK. Antibacterial and antifungal activity of *Thespesia populnea* (L). Indian Drugs 2005;42:81-3.
19. Merith K. Antimicrobial activity of fresh plant juice on the growth of bacteria and yeasts. J Qafqaz Univ 1997;1:27-33.
20. Mimica-Dukić N, Bozin B, Soković M, Mihajlović B, Matavulj M. Antimicrobial and antioxidant activities of three mentha species essential oils. Planta Med 2003;69:413-9.

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