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Annals of Biological Research, 2011, 2 (2) :11-18
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library

ISSN 0976-1233
CODEN (USA): ABRNBW

Antioxidant and antibacterial properties of *Pajanelia longifolia* (willd.) K. Schum

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ABSTRACT

A study was conducted on an Ethnomedicine namely Pajanelia longifolia (Willd.) K. Schum, which is commonly used in skin disease in the Dakshina Kannada District of Karnataka State. The antibacterial and antioxidant potential of the bark of the plant has been carried out by using various extracts. The estimation of polyphenols, proteins, carbohydrates and tannins were also carried out. The plant root powder showed remarkable degree of antioxidant activity and antibacterial properties against Staphylococcus aureus, Escherichia coli, Lactobacillus fermentum and Bacillus spp.

Key words: *Pajanelia longifolia* (Willd.) K. Schum, Carbohydrates, Proteins, Polyphenols, Tannins, Antibacterial, Antioxidant.

INTRODUCTION

The nature has bestowed us with herbal wealth for the human health. The Dakshina Kannada (D.K) District of Karnataka State covers the larger area of the foot hills of Western Ghats. This area is rich with the medicinal plants used by many Ayurvedic physicians and Ethnomedical practitioners in the various types of ailments of the human beings and animals[1]. The *Pajanelia longifolia* (Willd.)K. Schum known as Bondubale in Kannada, is one such plant which is widely

used in skin diseases mainly to treat Eczema by the ethno medical practitioners of Kalanjimale Range of Dakshina Kannada District[2].

P. longifolia is a deciduous tree. Leaves pinnately compound, up to 90cm long. Leaflets 13-15cm long, very obliquely ovate, acute or acuminate at apex. Panicle up to 90cm long. Calyx 2.5cm long. Corolla up to 8cm long, while in the tube, crimson-purple on the lobes; lobes wooly ciliate. Capsules up to 50cm long, brown[3].

Scientific research on *P. longifolia* can provide useful information for their exploration in treating diseases as the bark of the plant has been used widely in folklore medicine for treating Eczema, wounds etc. A systematic research has not gone into the bark of the plant in elucidating the medicinal properties of the same and therefore a preliminary screening was done on this plant to record its antioxidant and antibacterial activities.

MATERIALS AND METHODS

Collection of Plant material

The barks of the plant *P. longifolia* were collected from the Kalanjimale Range, Bantwal Taluk, D. K. District of Karnataka and was authenticated by the expert Botanists. A voucher specimen (AAMC/SP/71) of the plant is deposited in the herbarium of the Department of Post Graduate Studies in Dravyaguna, Alva's Ayurveda Medical College, Moodbidri. The barks were shade dried and coarsely powdered and kept in an airtight container.

Preparation of sample extract

The dried coarse powder of the bark was subjected to exhaustive extraction in water, ethanol, methanol, petroleum ether, acetone, ethyl acetate and chloroform separately by using Soxhlet apparatus as explained in the Indian Pharmacopoeia[4]. These extracts were vacuum dried, weighed and preserved at 4 °C in air tight container for further use.

Preliminary phytochemical analysis

The sample extracts were subjected to qualitative analysis to detect the presence of various Phyto constituents like Carbohydrates, Proteins, Starch, Alkaloids, Flavonoids, Triterpenoids, Phenolics, Elagic acid, Tannins, Saponins, Resins and Steroids. The methods given by Sadasivam and Manickam[5].

Quantitative analysis of the Phyto-constituents

Quantitative analysis of some of the key parameters *viz.*, Carbohydrates, Proteins, Tannins and Phenols were carried out. The Carbohydrates were estimated by following the standard Anthrone method[5] and Protein by Lowry's method[5]. The ammount of Phenolic component in the extract were determined using Folin-Ciocalteu (FC) Reagent using pyrocatechol as a standard[5] and Tannins were estimated following Folin-Denis method[6].

Antioxidant activity

For determining antioxidant capacity of the plant material, FRAP assay using standard $FeSO_4.7H_2O$ method has been followed as described by Adedapo[7]. A range of 50µg to 250µg

concentration of water extract, 25mg of methanol and ethanol extract, 25mg ethyl acetate extract dissolved in DMSO were prepared and about 1 ml of each sample was treated with 3ml of FRAP reagent and absorbance was read at 593nm in U.V. Spectrophotometer (Elico.SL159). Iron (II) sulfate ($FeSO_4.7H_2O$) was used as the standard. The antioxidant activity of the extract was compared with standard ascorbic acid (200µM-2000µM).

Antibacterial activity

Antibacterial activity of the sample was analyzed by standard well diffusion assay[8]. The laboratory isolates of *Staphylococcus aureus*, *E. coli*, *Bacillus* spp., *Proteus* spp., *Vibrio para haemolyticus*, *Lactobacillus casei*, *Lactobacillus fermentum* were subcultured in Muller Hinton Broth and were swabbed on sterile Muller–Hinton agar plates. A 5mm wells were bored into each swabbed plates aseptically. A concentration range starting from 500ug to2000ug of each of the extract dissolved in DMSO were loaded on to the wells. A positive control well with penicillin and the negative control well with DMSO were also maintained. All the plates were incubated at 37 °C for 24 hours. The zone of inhibition was recorded.

RESULTS

The percentage yield of plant bark from various extraction procedures are given in table 1 and phytochemical parameters are listed in table 2. The quantitative data of total protein, carbohydrates, phenolics and tannin are given in table 3.

Antioxidant activity of *P. longifolia*

The antioxidant activity of the extracts was found to be in the range of 270 to 2570 mM Fe (II)/g raw material. Among the four extracts, 70% methanol extract showed the highest antioxidant activity (2566 mM Fe (II)/g raw material). However, the Ethyl acetate extract had the lowest (262 mM Fe (II)/g raw material) antioxidant activity. Water extract and ethanol extract gave an antioxidant activity value of 1127 and 630 mM Fe (II)/g raw material respectively. The results are tabulated in table 4 and represented in figure 2.

Antibacterial activity of *P. longifolia*

Figure 3 shows the zone of inhibition recorded for various extracts showing antimicrobial activity for mentioned bacterial isolates. Before well diffusion test was carried out, a broth dilution test was done to see the possible antimicrobial activity of the drug. Ethanolic extract showed the inhibition of microbial growth for *Vibrio parahaemolyticus* and *Bacillus spp.*

DISCUSSION

For use in traditional systems of medicine, various chemical constituents of therapeutic use have been extracted from plants for centuries. Of late, a systematic scientific study on the medicinally important plants which India has harbored, have led to look more scientifically into the use of herbs as medicines. In the last decade or so many therapeutic molecules have been either isolated or designed/synthesized from the knowledge of chemistry and chemical constituents of plants. The present study to understand various chemical constituents of health benefits of the plant

Pajanelia longifolia would throw a light on possible use of the plant in curing some ailments or may hint us at synthesizing new drugs or isolation of the same.

The analysis of various extracts of the bark of *P. longifolia* indicated the presence of phenolics. Polyphenols are major plant compounds with antioxidant activity. Phenol and phenolic compound such as flavonoids have been shown to possess significant antioxidant activities. This activity is believed to be mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Adedapo *et al*., 2008)[7]. Results obtained in the present study revealed that the level of these phenolic compounds in the water and methanolic extracts of the bark of *P. longifolia* was considerable. The results strongly suggest that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

The antioxidant potentials of the extracts of the bark of *P. longifolia* were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the extracts was in the range of 262 – 2566 mM Fe (II)/g. Antioxidant activity increased proportionally with the polyphenol content. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (Adedapo *et al*., 2008)[7]. Plants with antioxidant activities have been reported to possess free radical scavenging activity. Free radicals are known as a major contributor to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism (Adedapo *et al*., 2009)[8].

Adedapo *et al*., (2008), reported that methanol extract of the stem of *Calpurnea aurea* which has higher polyphenol content, also has higher antioxidant activity (3146.98µM Fe (II)/g of dry mass) than that of the leaf extract (111.98µM Fe (Ⅱ)/g of dry mass)[7]. Ara and Nur (2009) recorded antioxidant activity of methanolic extract of *Lippia alba* using DPPH free radical scavenging assay to be 34.4µg/ml. the present study also records highest antioxidant activity in correlation with the highest polyphenol content for methanolic extract[9][10]. Antioxidants therefore play an important role in protecting human body against free oxygen radicals. Therefore, the plant *P. longifolia* showing remarkabely high amount of antioxidant activity could be employed in Ayurveda formulations and further research would isolate the active principle of the antioxidants.

The organic extract of Ethanol when used at a concentration of 5 mg/ml showed antibacterial activity against *Vibrio parahaemolyticus* and *Bacillus spp.* Representing both gram positive and gram negative bacterial groups as well as pathogenic and non pathogenic groups. The growth of the most of the other bacterial species like *Staphylococcus aureus, Escherichia coli, Proteus spp, Lactobacillus casei* and *L. fermentum* could not be retarded by the plant extract at any concentrations tested. Though the inhibitory concentration of 5 mg/ml is very high, nevertheless it showed that the plant extracts under *in vitro* study has antibacterial activity against selected pathogens like Vibrios. It has been shown that the plant extracts were active against both Gram-positive and some Gram-negative strains. Since the plant extract shows some activity against selected microorganisms tested in this study; the use of this plant for medicinal purpose is being

justified. Based on these findings, these extract preparations can be useful in the treatment of infections caused by the microbial organisms.

The present study throws some light on possible antioxidant and antimicrobial activity of the said plant and strengthens the ethno botanical knowledge of the use of the plant with scientific data. The results evidently place in record of antioxidant and antimicrobial activity of *P. longifolia*. Further research on isolation and characterization of chemical constituents of antioxidant and antimicrobial activity would do a long way in development of therapeutics against human ailments.

Table.1. The percentage of extract in various solvents

Solvents	Percentage of the extract
Water	26.44%
Ethanol	5.66%
Methanol	22.12%
Petroleum Ether	3.26%
Acetone	4.44%
Chloroform	6.64%

Table 2. The results of Phyto-chemical analysis

Sl. No.	Phyto-chemical Tests	Observations	Inference
1.	Carbohydrates a. Benedict's test b. Fehling's test c. Molisch's test	 Coloured precipitate Red precipitate Red-violet ring	 Positive Positive Positive
2.	Protein a. Biuret's test b. Millon's test	 Red colour White precipitate	 Positive Positive
3.	Starch	No blue colour formation	Negative
4.	Alkaloids a) Dragendorff's test b) Mayer's test	 Orange-red precipitate Pale yellow precipitate	 Positive Positive
5.	Flavonoids	Brown colour	Positive
6.	Triterpenoids: Liebermann-Burchard's test	 No violet coloured ring	 Negative
7.	Phenolics	Bluish-black colour	Positive
8.	Elagic acid	Canine-red colour	Positive
9.	Tannins	Brown colour	Positive
10.	Saponins	No honey comb like froth.	Negative
11.	Resins	Presence of turbidity	Positive
12.	Steroids a) Liebermann-Burchard's test b) Salkowski Reaction	 Greenish turns to blue colour Formation of red colour	 Positive Positive

Table 3. Quantitative analysis of phytochemicals

Sl. No.	Quantitative Methods	Results
1	Estimation of Carbohydrate	21.9%
2	Estimation of Proteins	11.28%
3	Estimation of Phenols	0.8%
4	Estimation of Tannins	2.7%

Table 4. Antioxidant values for extracts of various solvents

Sl. No.	Solvent used	**Antioxidant activity** mM Fe (II)/g raw material
1	Water	1127
2	Ethanol	630
3	70% Methanol	2566
4	Ethyl Acetate	262
5	Ascorbic acid	2272

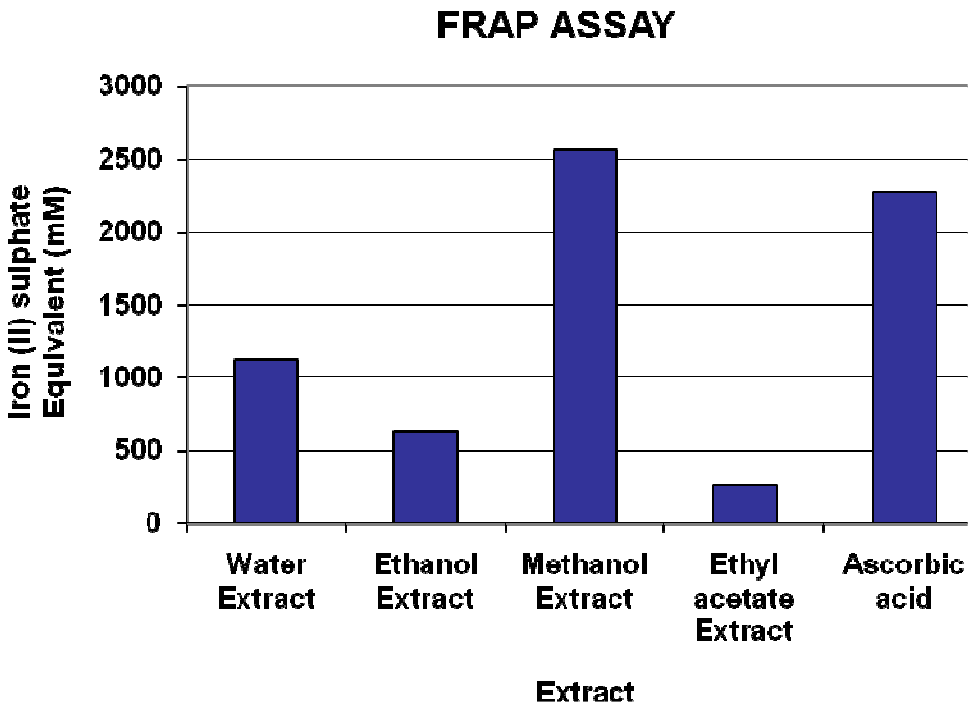
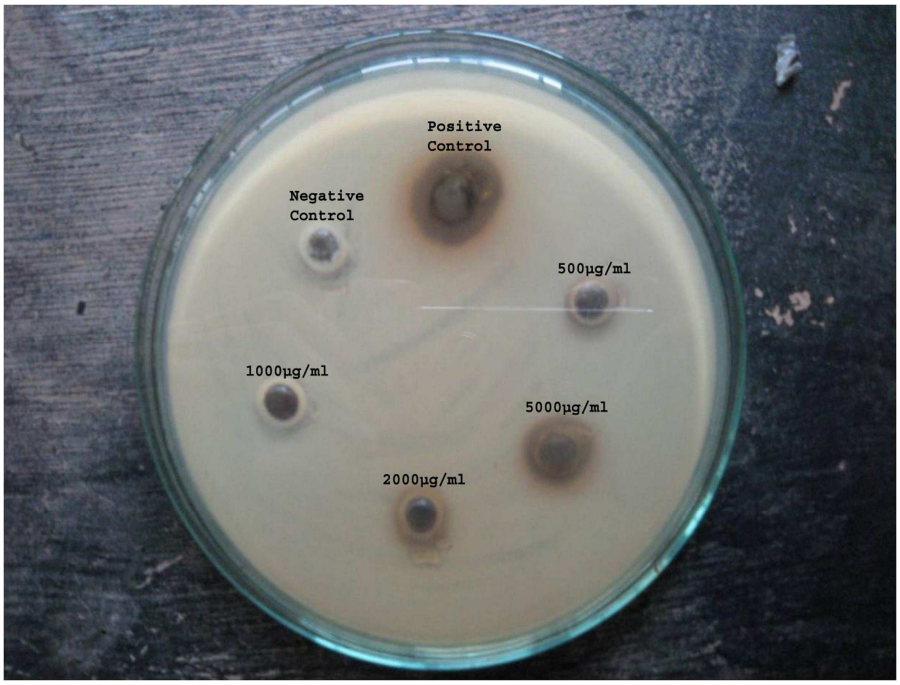


Figure 2: Antioxidant activity of *P. longifolia* (mM Fe (II) per gram raw material).



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