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Antibacterial and radical scavenging activity of leaf and bark of *Persea macrantha* (Nees) Kosterm. (Lauraceae)

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Abstract:
Persea macrantha (Nees) Kosterm. belonging to the family Lauraceae is found in various states of Karnataka. The plant has got various traditional uses and is reported to exhibit several bioactivities. In the present study, we report antibacterial and radical scavenging potential of leaf and bark of *P. macrantha*. The powdered leaf and bark were extracted using methanol and the extracts were subjected to phytochemical analysis. Antibacterial activity was determined by Agar well diffusion assay. Antioxidant activity was evaluated by DPPH free radical scavenging assay. Total phenolic content of extracts was estimated by FCR method.Preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins and glycosides in both the extracts. Among bacteria tested, *S. aureus* and *S. typhi* were inhibited to higher extent by extracts. The extracts scavenged DPPH free radical dose dependently. Bark extract (IC_{50} 2.44µg/ml) displayed high radical scavenging potential than leaf extract (IC_{50} 8.31µg/ml). Total phenolic content was higher in bark extract than leaf extract. A direct correlation was observed between total phenolic content of extracts and radical scavenging activity. In conclusion, the observed bioactivities of extracts could be ascribed to the presence of phytochemicals in particular phenolics. The plant is shown to be promising for the development of therapeutic agents active against oxidative stress and pathogenic bacteria.

Keywords: *Persea macrantha*, Antibacterial, Agar well diffusion, Antioxidant, DPPH, Total phenolic

Introduction

Persea macrantha (Nees) Kosterm belongs to the family Lauraceae. It is a tree growing up to 30m in length and is mainly distributed in Western peninsula, Ceylon and India etc. In India, the plant is found in various states such as Karnataka, Bihar, Maharashtra and Assam up to an altitude of 2100m. It is called gulmavu in Kannada. The plant is reported to contain several phytochemicals such as norlignans, alkaloids, β-sitosterol and others. The plant in particular leaves and bark has several traditional uses[1,2]. The leaves and bark are traditionally used in the treatment of arthritis, traumatic injury, edema, and wounds[3]. In north-central Western Ghats of India, the paste or poultice made from stem/bark is applied externally to treat bone fracture[4]. The bark is used for treating asthma, tuberculosis and rheumatoid arthritis. In Hallakki Gowda tribe of Western Ghats of Karnataka, India, the powdered bark is mixed with egg-white and applied on the affected part in form of poultice as an anti-inflammatory and anti-rheumatic agent[1,5].The plant *P. macrantha* is shown to exhibit several bioactivities. The leaf and stem bark extracts were shown to exhibit inhibitory activity against bacteria and fungi[3,6-8].Various extracts of stem bark showed anti-inflammatory and anti-arthritic activity in rats[9]. Extracts and fractions of the bark were shown to possess anti-inflammatory, antinociceptive and membrane stabilizing activity[1]. In the present study, we

determined antibacterial and radical scavenging potential of leaf and bark extract of *P. macrantha*.

MATERIALS AND METHODS

Collection and identification of plant material

The plant materials *viz.*, leaf and bark of *P. macrantha* were collected at a place called Haniya, Hosanagara Taluk of Shivamogga district, Karnataka during January 2014. The plant was identified by Dr. Vinayaka K.S, Dept. of Botany, KFGC, Shikaripura, Karnataka.

Extraction and phytochemical analysis

The plant materials were washed thoroughly under clean water and shade dried. The dried leaf and bark were powdered using a blender. 25g of powdered materials was transferred into separate conical flasks containing 100ml of methanol (HiMedia, Mumbai) and left for 2 days with occasional stirring. The contents were filtered through Whatman No. 1 filter paper and concentrated in vacuum under reduced pressure[10]. The concentrated extracts were subjected for preliminary phytochemical analysis to detect phytoconstituents *viz.*, alkaloids, flavnoids, tannins, saponins, steroids and glycosides[11].

Antibacterial activity of leaf and bark extracts

Agar well diffusion assay was carried out to screen antibacterial efficacy of leaf and bark extracts of *P. macrantha*. Here, 24 hours old Nutrient broth (HiMedia, Mumbai) cultures of test bacteria *viz.*, *Staphylococcus aureus* NCIM-2079, *Bacillus cereus* NCIM-2016, *Salmonella typhi* MTCC-734 and *Klebsiella pneumoniae* NCIM-2957were swab inoculated on sterile Nutrient agar (HiMedia, Mumbai) plates. Using a sterile cork borer, wells of 6mm diameter were punched in the inoculated plates. 100µl of extracts (20mg/ml of 25% DMSO (Dimethyl sulfoxide; HiMedia, Mumbai)), standard (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were transferred into labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zones of inhibition formed were measured[11].

Radical scavenging efficacy of leaf and bark extracts

DPPH free radical scavenging assay was carried out to determine radical scavenging potential of leaf and bark extract of *P. macrantha*. In brief, 2ml of different concentrations of extracts (3.125-100µg/ml of methanol) were mixed with 2ml of DPPH solution (0.002% in methanol) in clean and labeled tubes. The tubes were incubated in dark for 30 minutes at room temperature followed by measuring the optical density at 517 nm using UV-Visible spectrophotometer (ELICO, SL159). The absorbance of the DPPH control (2ml of DPPH+2ml of methanol) was also noted. Ascorbic acid was used as reference standard. The scavenging activity of each concentration of both the extracts was calculated using the formula:

Scavenging activity (%) = ((A–B) /A) x100, where A is absorbance of DPPH and B is absorbance of DPPH and extract/standard combination. The IC_{50} value for the extract was calculated. IC_{50} represents the concentration of extract required to scavenge 50% of DPPH free radicals[11].

Total phenolic content of leaf and bark extracts

Folin-Ciocalteu reagent (FCR) method was employed to estimate total phenolic content of leaf and bark extract of *P. macrantha*. Here, a dilute concentration of extract (0.5ml) was mixed with 0.5ml diluted F-C reagent (1:1) and 2 ml of sodium carbonate (7%). The tubes were kept at room temperature for 30 minutes and the

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absorbance was measured at 765nm using UV-Visible spectrophotometer (ELICO, SL159). A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000µg/ml). The concentration of total phenolics in extracts was determined as µg Gallic acid equivalents (GAE) from the graph[11].

Results

Phytoconstituents detected in leaf and bark extracts

Preliminary phytochemical analysis of extracts revealed the presence of all phytoconstituents in bark extract. All except steroids were detected in leaf extract.

Antibacterial activity of leaf and bark extracts

The result of antibacterial efficacy of leaf and bark extracts is shown in Table 1. The extracts were effective against all test bacteria but to a varied extent. Among extracts, marked inhibitory potential was observed in case of bark extract when compared to leaf extract. *S. aureus* and *S. typhi* were susceptible to higher extent among Gram positive and Gram negative bacteria respectively. Reference antibiotic Chloramphenicol inhibited test bacteria to high extent than leaf and bark extracts. DMSO did not cause inhibition of any bacteria.

Table 1: Antibacterial activity of leaf and bark extracts

Test bacteria	Zone of inhibition in cm			
	Leaf extract	Bark extract	Chloramphenicol	DMSO
S. aureus	1.6±0.1	2.1±0.2	2.2±0.1	0.0±0.0
B. cereus	1.2±0.0	1.6±0.1	3.0±0.2	0.0±0.0
S. typhi	1.6±0.2	1.8±0.2	2.6±0.2	0.0±0.0
K. pneumoniae	1.1±0.0	1.7±0.2	2.7±0.1	0.0±0.0

Radical scavenging efficacy of leaf and bark extracts

The scavenging efficacy of different concentrations of leaf and bark extracts against DPPH free radicals is shown in Figure 1. Both extracts scavenged radicals in a dose dependent manner. At 100µg/ml concentration, >90% scavenging of radicals was observed in case of both extracts. Among extracts, the scavenging effect was higher in case of bark extract (IC_{50} = 2.44µg/ml) when compared to leaf extract (IC_{50} =8.31µg/ml) as indicated by low IC_{50} value. However, the scavenging potential of extracts was lower than that of ascorbic acid (IC_{50} =2.20µg/ml).

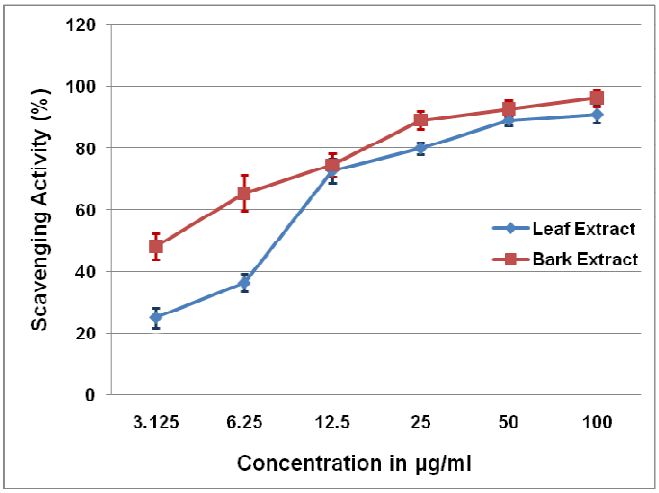

Figure 1: Scavenging of DPPH radicals (%) by leaf and bark extracts

Total phenolic content of leaf and bark extracts

The content of total phenolics was estimated by FCR method and was found to be 112.18 and 185.15µg GAE/mg for leaf and bark extract respectively.

Discussion

Infectious diseases caused by bacteria, fungi, protozoa, helminths and viruses remain an important cause of morbidity and mortality. The

discovery and the use of antibiotics resulted in drastic reduction in mortality. However, the ability of pathogens to adapt and to overcome the challenges of antibiotics made the disease treatment difficult. Bacteria such as *S. aureus*, *Streptococcus pneumoniae*, *Enterococcus* spp., *Acinetobacter baumannii*, *K. pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* are among the important drug-resistant bacteria. The infection caused by these antibiotic resistant pathogens is a global problem and is growing day by day[12-15]. The emergence of antibiotic resistant strains and other drawbacks such as high cost and possible side effects of antibiotics stimulated search for alternatives for disease therapy. Plants have been shown to be promising alternatives for treatment of infections caused by pathogens including drug resistant strains[10,16,17].

In our study, the leaf and bark extracts were shown to inhibit *S. aureus* to high extent when compared to *B. cereus*. Similar result was observed in the earlier study of Shrisha *et al.*[3] where ethyl acetate extract of leaf inhibited *S. aureus* to high extent than *B. cereus*. In the present study, *S. typhi* was shown to be highly susceptible to leaf and bark extracts among Gram negative bacteria. In the study of Shrisha *et al.*[3], ethyl acetate extract of leaf exhibited inhibitory activity against *S. typhi* and other bacteria such as *Xanthomonas oryzae*, *E. coli* etc, however, other solvent extracts were not effective. The aqueous extract of stem bark was shown to exhibit inhibitory activity against *S. aureus*, *P. aeruginosa* and *B. subtilis* but not against *E. coli*[6]. In a recent study, **s**olvent extracts of stem bark exhibited concentration dependent inhibition of Gram positive and Gram negative bacteria and fungi *Candida albicans* and *Aspergillus niger*[7]. In our study, the bark extract caused high inhibition of test bacteria. Similar kind of result was observed in an earlier study where bark extract of *P. macrantha* showed high inhibition of *Colletotrichum capsici* when compared to leaf extract[8].

In a normal healthy individual, a well maintained balance exists between antioxidant defense system and production of free radicals. However, during oxidative stress, this balance shifts towards excessive generation of free radicals or deficit in antioxidant defense. The oxidative stress is implicated in >100 pathophysiological conditions such as diabetes, cardiovascular diseases, neurological disorders, cancer, aging etc. Antioxidant is a substance capable of inhibiting or delaying oxidative processes. Endogenous antioxidants such as ascorbic acid, tocopherols, glutathione, uric acid and thiols and antioxidant enzymes such as superoxide dismutase and catalase protect the body against oxidative stress. However, in pathophysiological conditions, there is extra need for antioxidants from exogenous sources. Strong restrictions have been placed on the use of synthetic antioxidants such as BHT, BHA and PG due to their suspected carcinogenic effect. Hence, natural products such as plants and their metabolites having antioxidant properties are beneficial[18-22].

Various *in vitro* assays are available to screen radical scavenging efficacy of plant extracts. Among these, DPPH free radical scavenging assay is one of the widely used *in vitro* assays. DPPH is a stable, nitrogen centred, organic free radical with absorption maximum at 517nm in methanolic solution. On accepting an electron or hydrogen atom (from antioxidants) it becomes a stable diamagnetic molecule. In this assay, the antioxidants reduce the purple colored DPPH

radical to a yellow colored compound diphenylpicryl hydrazine (DPPHH) and the extent of decolorization depends on the hydrogen donating ability of the antioxidants. This assay has been widely used to screen radical scavenging potential of various kinds of samples including plant extracts[15,18,22-26]. In the present study, the absorption of DPPH in the presence of various concentrations of leaf and bark extracts of *P. macrantha* was measured at 517nm. The extracts exhibited concentration dependent scavenging activity. Bark extract was more effective scavenger than leaf extract as indicated by low IC_{50} value. Although the scavenging abilities of extracts were lesser than that of ascorbic acid, it was evident that the extracts possess hydrogen donating ability and the extracts could serve as free radical scavengers, acting possibly as primary antioxidants[24].

Phenolic compounds represent a large group of naturally occurring phytochemicals and are present in every part of the plants. These compounds are of immense interest in food, pharmacy and medicine as they exhibit wide range of bioactivities including antioxidant activity. The antioxidant activity of these phenolic compounds is mainly because to their redox potential. Phenolics act as reducing agents (free radical terminators), hydrogen donors, metal chelators and singlet oxygen quenchers. As the phenolic compounds are one among the most effective antioxidants, it is important to estimate the phenolic contents of extracts[25,27,28]. A direct correlation was observed between total phenolic content and radical scavenging activity i.e., the bark extract containing high phenolics displayed stronger radical scavenging activity. The result obtained is in justification with the result of earlier studies where a linear correlation between phenolic content and antioxidant activity was observed[15,26,29].

Conclusion

In conclusion, the leaf and bark extracts of *P. macrantha* showed promising antibacterial and radical scavenging potential. The bioactivities of extracts observed in this study could be ascribed to the presence of phytochemicals in particular phenolics as bark extract containing high phenolics exhibited stronger inhibitory and scavenging activity. The plant is shown to be promising for the development of therapeutic agents active against oxidative stress and pathogenic bacteria. Further *in vivo* studies are to be conducted.

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