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Antimicrobial And Radical Scavenging Activity Of Leaf And Bark Of Pimenta Dioica (linn.) Merill. (myrtaceae)- A Comparative Study

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Abstract:The present study aimed at determining and comparing antimicrobial and radical scavenging activity of leaf and bark extract of *Pimenta dioica* (Linn.) Merill (Myrtaceae). Antibacterial activity of extracts was evaluated against six bacteria and two fungi by agar well diffusion assay. Radical scavenging effect of extracts was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. Leaf extract was more effective than bark extract against test microbes and the inhibitory effect was dose dependent. Salmonella typhi and *Crypotococcus neoformans* were highly sensitive to extracts among bacteria and fungi respectively. Extracts have shown dose dependent scavenging of DPPH free radicals. Leaf extract scavenged DPPH radicals more efficiently than bark extract. The bioactivities of leaf and bark extracts observed in this study could be attributed to the presence of bioactive components in the extracts. The plant can be a potent candidate for the development of agents active against pathogens and oxidative stress.

Keywords:*Pimenta dioica*, Antimicrobial, Agar well diffusion, Antioxidant, DPPH

1. INTRODUCTION

Pimenta dioica (Linn.) Merill (family: Myrtaceae) was discovered in Jamaica during the voyages of Christopher Columbus. It is a tree native to West Indies and Central America. The plant is used in perfumery industry, food spice, as a natural pesticide, and folk medicine. It is known as Allspice due to its intricate aroma which is a combination of aroma from spices such as Clove, Nutmeg and Cinnamon. The plant is also known as Pimenta, Pimento, Clove pepper and Jamaica pepper. In India, the leaves are used to flavor rice. Various compounds such as phenylpropanoids, tannins, glycosides and essential oil have been isolated from the plant. Eugenol and Gallic acid, isolated from the plant have selective antiproliferative and anti-tumor properties on human cancer cells and their animal models. The essential oil from leaf and fruit have been used in perfumes, aftershaves and commercial food flavoring [1-5]. The berries are used for flatulent indigestion, as febrifuge and are considered to have anthelmintic properties. The leaves are used as an antihypertensive in Costa Rican folk medicine. In Cuba, the seeds are used in catarrh, stomach pains[2,6].

The crude preparations/extracts, essential oil and their purified fractions from the plant *P. dioica* have shown to possess various biological activities. Aqueous extract from the fresh leaves was found to exhibit hypotensive activity in rats [6]. Aqueous suspension of allspice was found to exhibit antiinflammatory, analgesic, antipyretic, gastric antiulcer, and cytoprotective activities in experimental models [7]. The alcoholic extract of leaves was shown to protect cyclophospamide induced myelosuppression in Swiss mice [8]. The leaf extract showed significant antioxidant activity in reducing power assay, DPPH radical scavenging and Lipid peroxidation assays and hepatoprotective activity in carbon tetrachloride intoxicated Wistar rats[9]. The aqueous, acetone and methanolic extracts of leaves have shown to exhibit antibacterial and ferric reducing activity *in vitro* [10]. The tannins isolated from the air dried leaves have shown to possess anticancer and antioxidant activities [1]. Essential oil and their components are reported to possess biological activities such as anti-termite [11], nematicidal [12], antioxidant [13], anticandidal [14], acaricidal [15] and antibacterial activity[16]. The present study was conducted to determine and compare antimicrobial and radical scavenging activity of leaf and bark extract of *P. dioica.*

MATERIALS AND METHODS

Collection and identification of plant material

Leaves and barks of *P. dioica* were collected at Maragalale, Thirthahalli (taluk), Shivamogga (district), Karnataka during June 2013. The plant materials were washed thoroughly, shade dried and powdered in a blender. The powdered leaf bark materials were stored in air-tight

containers.

Extraction

About 10g of dried and powdered leaf and bark of *P. dioica* were added to 100ml of methanol (HiMedia, Mumbai), sonicated for 30 minutes and left at room temperature overnight. The extracts were filtered through Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in the desiccator[17].

Antibacterial activity of leaf and bark extracts

Agar well diffusion assay was performed in order to determine antimicrobial activity of leaf and bark extracts of *P. dioica* against bacteria viz., *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Entero bacter aerogenes, Shigella flexneri* and *Klebsiella pneumonia and fungi viz., Candida albicans* and *Cryptococcus neoformans.* The test bacteria and fungi were grown in sterile Nutrient broth (HiMedia, Mumbai) and sterile Sabouraud's dextrose broth (HiMedia, Mumbai) respectively. The broth cultures were then aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) and Sabouraud's dextrose agar (HiMedia, Mumbai) using sterile cotton swabs. Wells of 6mm diameter were made in the inoculated plates using sterile cork borer. 100µl of leaf and bark extracts (20mg/ml of 25% dimethyl sulfoxide [DMSO]), standard antibiotic (1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were filled into labeled wells. Fluconazole and Chloramphenicol were used as reference antifungal and antibacterial antibiotic respectively. The plates were incubated at 37oC for 24 hours and the zone of inhibition was recorded[18].

DPPH free radical scavenging assay

The radical scavenging ability of leaf and bark extracts of *P. dioica* was determined on the basis of the radical scavenging effect on the DPPH free radical. Here, 1ml of different concentrations of extracts was mixed with 3ml of DPPH solution (0.004% in methanol) in labeled tubes. The tubes were incubated in dark for 30 minutes at room temperature and the optical density was measured at 517nm. The absorbance of the DPPH control (without extract/standard) was also noted. Ascorbic acid was used as reference standard. The scavenging activity was calculated using the formula: Scavenging activity (%) = [(Ao – Ae) / Ao] x 100, where Ao is absorbance of DPPH control and Ae is absorbance of DPPH in the presence of extract/standard[18]. The IC50 value for each extract was calculated. IC50 denotes the concentration of extract required to scavenge 50% of DPPH free radicals.

RESULTS

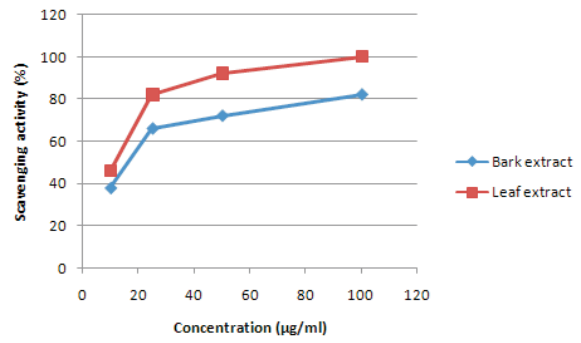
The result of antimicrobial potential of leaf and bark extracts is shown in Table 1. The extracts were able to inhibit test microorganisms dose dependently. All the test bacteria and fungi were found to be susceptible to both extracts but to varied extents. Leaf extract was more effective in inhibiting test microbes than bark extract as revealed by wider zones of inhibition. Among bacteria, high susceptibility was recorded in case of *S. typhi* followed by *E. aerogenes, S. aureus, S. epidermidis, S. flexneri* and *K. pneumoniae.* Among fungi*, C. neoformans* was inhibited to high extent by extracts when compared to *C. albican*s. The test bacteria and fungi were highly sensitive to reference antibiotics when compared with the extracts. No inhibitory activity was displayed by DMSO.

Table 1: Antimicrobial activity of leaf and bark extract of *P. dioica*

Test bacteria	Zone of inhibition in cm				
	Bark extract		Leaf extract		Antibiotic
	25mg/ml	10mg/ml	25mg/ml	10mg/ml	
S. typhi	1.8	1.5	2.0	1.6	4.0
E. aerogenes	1.6	1.4	1.8	1.4	3.5
S. flexneri	1.3	1.0	1.5	1.0	3.8
K. pneumoniae	1.1	0.8	1.4	1.0	3.4
S. epidermidis	1.4	1.0	1.6	1.2	3.5
S. aureus	1.5	1.0	1.7	1.1	4.2
C. albicans	1.0	0.0	1.2	0.8	3.9
C. neoformans	1.2	0.8	1.3	1.0	4.1

The efficacy of leaf and bark extracts of *P. dioica* to scavenge radicals was determined by DPPH radical scavenging model and the result is shown in Figure 1. Here, the extent of bleaching of color of DPPH radicals (purple to yellow) in the presence of varying concentrations of extracts was monitored at 517nm. Scavenging of DPPH free radicals by different concentrations of leaf and bark extract of *P. dioica* was found to be dose dependent. Among extracts, high scavenging potential was displayed by leaf extract (IC50 28.88µg/ml) than bark extract (IC50 35.84 µg/ml). However, the scavenging effects of extracts were lesser than that of ascorbic acid (IC50 2.84 µg/ml).

Figure 1: DPPH radical scavenging activity of leaf and bark extract of *P. dioica*



DISCUSSION

Medicinal plants have found their use since centuries as remedies for various types of diseases of humans and animals. Antimicrobial agents from plant origin have enormous therapeutic potential. During the 2nd half of the 20th century, the wide acceptance of traditional system of

medicine as an alternate form of primary healthcare and the problems associated with the development of drug resistance during antibiotic therapy led the researchers to investigate antimicrobial efficacy of several plants. Antimicrobial agents from plant origin are effective in disease treatment and lack side effects that are commonly associated with synthetic drugs. The plant components are also effective against drug resistant microbes. A number of studies have been carried out on the efficacy of the plants and plant derived agents against infectious microorganisms[19-30]. In the present study, we have evaluated antimicrobial potential of methanol extract of leaf and bark of *P. dioica.* It was observed that leaf extract was more effective in inhibiting test microbes than bark extract. In an earlier study, Khandelwal et al. [10] observed antibacterial activity in acetone and methanol extract of leaves of *P. dioica.* However, these extracts were not inhibitory to fungi. The essential oil was found to exhibit anti-candidal [14] and antibacterial activity[16].

An excessive production of free radicals, particularly reactive oxygen species such as superoxide radical, hydroxyl radical and others, results in a situation called oxidative stress which is implicated in several diseases or disorders. In oxidative stress, macromolecules such as proteins, lipids and nucleic acids suffer severe damage. The harmful effects of free radicals are reduced by substances termed antioxidants. Antioxidants may be endogenous or obtained from external sources such as diet or medicinal plants and are known to act at different levels viz., prevention, interception and repair [31-33]. Synthetic antioxidants such as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT) or propyl gallate (PG) have been widely used but are reported to be carcinogenic and mutagenic on chronic consumption. Thus, discovery of new, safe and effective antioxidants is of considerable interest in preventive medicine. Recent studies have shown that consumption of plants and their products can bring down the risk of chronic diseases produced due to oxidative stress. The protective effect of plants may be attributed to the presence of natural antioxidant compounds[34-37].

A number of methods are available to evaluate free radical scavenging activity of samples. The method of scavenging DPPH radicals is one of the widely used methods to determine radical scavenging activity of a various type of samples including plant extracts [17,28,31,36-42]. DPPH is a stable, organic, nitrogen centred free radical with an absorption maximum band around 515-528nm (517nm). The radical accepts an electron or hydrogen atom and becomes a stable diamagnetic molecule. The effect of antioxidants on scavenging DPPH radical is due to their hydrogen donating ability. The antioxidants reduce the purple colored DPPH radical to a yellow colored compound diphenyl picrylhydrazine and the extent of reaction will depend on the hydrogen donating ability of the antioxidants[38,40,43].

In this study, the decrease in absorption of DPPH in the presence of various concentrations of leaf and bark extracts was measured at 517nm. It was observed that the scavenging potential of extracts increased with increasing concentrations. Leaf extract was able to scavenge radicals to higher extent than bark extract as revealed by lesser IC50 value. Although the scavenging abilities of extracts were lesser than that of ascorbic acid, it was evident that the extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants [38]. In a study, tannins isolated from leaves of P. dioica have shown to possess marked antioxidant activity including DPPH radical scavenging activity [1]. The aqueous extract and the essential oil of P. dioica leaves were found to exhibit DPPH radical scavenging activity[16].

CONCLUSION

In the present study, we have observed marked antimicrobial and radical scavenging activity of leaf extract of P. dioica when compared with the bark extract. The observed biological activities could be attributed to the presence of bioactive components in the crude extracts. The plant can be used as a potent candidate for the development of agents that are active against pathogenic microbes and oxidative stress.

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