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Short communication

ANTIBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANT IN TRADITIONAL TREATMENT OF WOUND INFECTION IN SOUTHEAST INDIA

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ABSTRACT

Objective: The wound healing potential of fruit peel of *Pithecellobium dulce* Benth, a plant traditionally used for wound treatment against wound-infecting bacteria, was assessed using disc diffusion and microtitre plate methods.

Methods: *P. dulce* fruit peel was extracted using ethyl acetate, methanol and water and tested for their antimicrobial activities against bacterial isolates from wound infection using disc diffusion and microtitre plate methods.

Results: All the three extracts had antimicrobial activities, among which methanol extract had higher activities against all the tested organisms compared to the ethyl acetate and aqueous extracts. The most susceptible organism to all the extracts was *K. pneumonia* and *S. aureus* while the most resistant were *P. mirabilis* and *P. vulgaris.*

Conclusion: The potential antibacterial activity of *P. dulce* plant against both gram-positive and gram-negative bacteria justifies the use of the plant as the wound healing agent. The isolation of active principle of this plant may serve as source and lead for the synthesis of drugs in pharmaceutical industries.

Keywords: *Pithecellobium dulce*, Wound healing, Antibacterial activity, Herbal extracts

Many infectious microorganisms are resistant or multiresistant to synthetic drugs, which exert side effects to human health. Emergence of new and resistant pathogens, impose the need for permanent search and development of new drugs, which is cost effective and safe. Hence alternative source is very much needed [1]. Medicinal plants can also be a possible source for new potent antibiotics against resistant pathogens.

According to the Wound Healing Society, wounds are physical injuries resulting in the opening or breaking of the skin causing disturbance in the normal skin anatomy and function [2]. Wound healing is a complex mechanism, which can be delayed by several factors such as bacterial infection, lymphatic blockage and diabetes. Rate of healing is increased by external agents. Plants are active healers which promote the repair mechanism in the natural way [3].

In traditional medicines, a wide range of plants are used in the treatment of wounds and other diseases. A number of plants with significant antibacterial activity have been reported in traditional literatures, but most of them are yet to be scientifically explored [4]. Biological activities are essential for safe and effective use of therapeutic agents [5].

Pithecellobium dulce Benth commonly occurs in India. It belongs to *Mimosaceae* family, which comprises 100-200 species, mainly distributed in the tropics. *P. dulce* has got several therapeutic properties. The bark of the plant is astringent and is used for treating dysentery, dermatitis and eye inflammation. Leaves are also astringent, febrifuge, emollient, antidiabetic, and abortifacient and larvicidal and is used for treating dysentery, indigestion, pain, venereal sores, ear aches, leprosy, tooth ache, dermatitis and eye inflammations. The roots are estrogenic and the root bark is a febrifuge and used for treating dysentery [6].

The crude extracts of *P. dulce* fruit peel revealed strong antibacterial activity indicating that *P. dulce* fruit peel can serve as the source of effective drug against wound infecting bacteria. The present study is aimed in the assessment of inhibition potential of *P. dulce* fruit peel against bacteria isolated from wound.

The fruit peels of *P. dulce* was collected from Namakkal District, Tamil Nadu, India, and was washed with distilled water, dried at room temperature for 144 to 168 h and pulverized at room temperature. Ethyl acetate, methanol and aqueous extracts were prepared by adding 10-15 fold volume of corresponding solvent for 2x6 h preferably at 60-70°C and then filtered. The powder of the extract was prepared by removing the solvent under reduced pressure at 40-50°C and stored at 4°C in an air tight container for further use.

Surface wound swabs were collected from 30 patients attending Microbiology Laboratory at Government Hospital, Namakkal, Tamil Nadu, India, and inoculated on Nutrient agar, Mannitol salt agar, Blood agar, MacConky agar and Chocolate agar plates and identified by conventional biochemical methods according to standard microbiological techniques [7,8].

The *in vitro* antibacterial activity of the samples was studied by disc diffusion method, with Polymixin-B and Rifampicin 30 µg antibacterial standard drugs. The control experiments with solvent DMSO were done concurrently [9]. All data regarding antibacterial activity are the average of triplicate analyses. The micro broth dilution method was used to determine the minimum inhibitory concentration (MIC) [10]. MIC was defined as the lowest concentration of the extract that inhibits the visible growth (20% growth inhibition) [11].

S. epidermis, E. coli, K. pneumonia, S. aureus, Enterobater spp, P. aeruginosa, P. mirabilis and *P. vulgaris* are the predominant bacteria isolated from the wound infection. Crude extracts of fruit peel of *P. dulce* showed growth inhibition against the isolates from the wound by disc diffusion method. The extracts inhibited the growth of bacteria with the zone of inhibition ranging from 9 mm to 22 mm diameters.

The ethyl acetate extract showed antibacterial activity against all the isolates in the order of *S. aureus, K. pneumonia, S. epidermis, E. coli, P. aeruginosa, Enterobater spp, P. mirabilis* and *P. vulgaris*. The methanol extract showed antibacterial activity against all the isolates in the order of *K. pneumonia, S. aureus, S. epidermis*, *P.*

aeruginosa, E. coli, Enterobater spp, P. mirabilis and *P. vulgaris*. The aqueous extract showed antibacterial activity against all the isolates in the order of *K. pneumonia, S. aureus, P. aeruginosa, Enterobater spp, E. coli, P. mirabilis*, *S. epidermis* and *P. vulgaris* (Table 1).

Table 1: It shows Antibacterial activity of ethyl acetate, methanol and aqueous extracts of *P. dulce* fruit peel on wound bacterial isolate.

Organisms	Zone of inhibition (mm diameter)				
	Extracts			Standards	
	Ethyl acetate	Methanol	Aqueous	PM	RF
S. epidermis	15	19	12	30	18
E. coli	14	18	14	28	16
K. pneumonia	17	22	19	28	30
S. aureus	18	21	16	38	25
Enterobater spp	10	17	16	35	30
P. aeruginosa	12	19	16	30	18
P. mirabilis	9	14	13	36	10
P. vulgaris	9	14	11	24	12

Values are expressed as mean (*n* = 3); PM, polymyxin-B; RF, rifampicin

The minimum inhibitory concentration (MIC) of ethyl acetate extract against all the isolates was found to be in the order of *K. pneumonia, S. aureus, S. epidermis, E. coli, P. aeruginosa, Enterobater spp, P. mirabilis* and *P. vulgaris.* The MIC of methanol extract against all the isolates was found to be in the order of *K. pneumonia, P. aeruginosa, S. aureus, E. coli, S. epidermis, Enterobater spp, P. mirabilis* and *P. vulgaris.* The MIC of aqueous extract against all the isolates was found to be in the order of *K. pneumonia, S. aureus, P. aeruginosa, Enterobater spp, S. epidermis, E. coli, P. mirabilis* and *P. vulgaris* (Table 2).

Table 2: It shows minimum inhibitory concentration (MIC) ethyl acetate. Methanol and aqueous extracts of *Pithecellobium dulce* fruit peel on wound bacterial isolate.

Microorganisms	Minimum inhibitory concentration (mg/ml) Extracts		
	Ethyl acetate	Methanol	Aqueous
S. epidermis	12.5	12.5	25.0
E. coli	12.5	6.25	25.0
K. pneumonia	6.25	3.125	6.25
S. aureus	6.25	6.25	6.25
Enterobater spp	25.0	12.5	12.5
P. aeruginosa	12.5	6.25	6.25
P. mirabilis	25.0	25.0	25.0
P. vulgaris	25.0	25.0	25.0

Values are expressed as mean (*n* = 3).

Wounds are easy portals for the proliferation of microorganisms. *S. aureus* [12] and *P. aeruginosa* [13] are the most frequently isolated organisms from the wound. These organisms are frequently isolated from the body surface and enter into the patients easily. *E. coli* and *Enterobater spp* are moderately isolated from wound infections. Both gram positive and negative bacteria exert multidrug resistance to commonly used antibiotics. These multidrug resistant strains establish themselves in the hospital environment in areas like sinks, tapes, toilets and thereby spread from one patient to another [14,15].

The wound healing is facilitated by the activation of fibroblast, endothelial cells and macrophages [16]. The recovery of the structure and function of the wound site is mainly due to the fibroblast proliferation [17]. Therapeutic agents stimulate the growth and proliferation of fibroblast and improve the healing of the wound.

Secondary metabolites like flavonoids, alkaloids and terpenoids are used in chemotherapies against wide range of microorganisms [18]. *Quercus* species found to contain tannins which form irreversible complexes with proline rich protein which inhibit cell protein synthesis and can be used as wound healing agent [19,20]. Ethanol extract of *Rubia cordifolia* Linn exhibited wound healing property which may be due to the presence of tannins and anthraquinones [21]. *Aloe vera* leaf contains vitamin-C, vitamin-E and amino acids which are essential for wound healing [22]. *Kigelia pinnata* sausage bark showed significant wound healing activity which may be due to the presence of lapachol, phenyl propanoid, ferulic acid, p-coumaric acid and 6-methoxmelenin [23]. Tannins and saponins in *Gymnema sylvestre* are responsible for its wound healing property [24].

Preliminary phytochemical screening of this plant revealed the presence of saponins, flavanoids, phenolics, steroids, alkaloids and glycosides. Antibacterial activity of extracts of *P. dulce* fruit peel might be due to the presence of these phytochemicals. The methanol extract was found to possess better *in vitro* wound healing property compared to ethyl acetate and aqueous extract. The bioactive compounds in the methanol extract have the high proliferation rate and viability action when compared to ethyl acetate and aqueous extract [25].

Furthermore, MIC values of *P. dulce* fruit peel extracts were found to be very effective against both gram positive and gram negative bacterial isolates from wound, this may be indicative of the presence of broad spectrum antibiotic compounds in this plant. The methanol extract showed significant antibacterial activity compared to standard antibiotics. The lower MIC values of methanol extract against gram-positive bacteria compared to gram-negative bacteria may be due to the difference in their cell wall physiology. The efficacy of the extracts against the bacterial isolates may be due to a single molecule or number of bioactive molecules.

The crude extracts of *P. dulce* fruit peel showed antibacterial activity against isolated bacteria from the wound, suggesting that *P. dulce* fruit peel can be used as potent wound healing agent. However, further works should be performed on the toxicity of the extracts, isolation of active principle to facilitate to use this plant as source and lead for the synthesis of drugs in pharmaceutical industries.

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CONFLICT OF INTEREST

None

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