

ANTIMICROBIAL AND PRELIMINARY
PHYTOCHEMICAL SCREENING OF CRUDE LEAF
EXTRACT OF *PANDANUS ODORATISSIMUS* L.

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Summary

In the current wave of antimicrobial resistance against chemotherapeutic drugs, there is need to search for plants that could be resistance-free and affordable. The objective of this study was to investigate the antimicrobial effects of Petroleum ether, chloroform & hydroalcoholic extracts of *Pandanus odoratissimus* leaf against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. In terms of antimicrobial effects, all the three extracts exhibited effective inhibition zones against Gram-positive bacteria. However, they were ineffective against Gram-negative bacteria and yeast (*Candida albicans*). The minimum inhibitory concentration (MIC) of hydro-alcoholic, chloroform and petroleum ether extracts were found to be 25, 50 and 50 mg/ml respectively against Gram-positive bacteria. Out of three extracts, hydro-alcoholic extract showed good antimicrobial activity. The phytochemical study showed the presence of alkaloids and flavonoids in hydro-alcoholic extract, which might be responsible for its good antimicrobial activity.

Keywords: Antimicrobial, *Pandanus odoratissimus*, MIC

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Introduction

It is well known that infectious diseases account for high proportion of health problems, especially in the developing countries. Microorganisms have developed resistance to many antibiotics in the recent years and this has created clinical problem in the treatment of infectious diseases. Examples of some microorganisms that gained resistance to antimicrobials are: *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella enteritidis*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Candida albicans*. This resistance has increased due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases (1).

Previous studies have shown the presence of several substances such as peptides, unsaturated long chain aldehydes, alkaloids, some essential oils, phenols and water, ethanol, chloroform, ethanol and butanol soluble compounds in plants with potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus (2). The antimicrobial properties of vegetable tannins against various microorganisms such as fungi, yeasts and bacteria have already been reviewed (3).

Medicinal properties of plants are normally dependent on the presence of some phytoconstituents such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and polyphenols which are the bioactive bases responsible for the antimicrobial activity (4). New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants (5). The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains (6).

Pandanus odoratissimus L. (Syn. *P. fascicularis*) belonging to family Pandanaceae, is widely distributed along Indo-Malayan coasts of India and Sri Lanka throughout Southeast Asia to Taiwan, the Ryukyu Islands, Malaysian islands and Australia. The plant is commonly known as 'Kevda' in Hindi, 'Umbrella tree' in English, and 'Kaethakee' in Sanskrit. *P. odoratissimus* is a dioecious shrub,

densely branched with copious aerial roots. Leaves are caudate acuminate, glaucous green, 90–150 cm long, curvaceous margin with ascending spinules (toothed) spadices axillaries, terminal, simple, branched, clothed with leafy spathes. Flowers are small crowded on a catkin like spadix and its branches (7,8,9).

The leaves contain the pyridine alkaloids, pandamarilactone-1(C₁₈H₂₃NO₄), pandamarilactone-31(C₁₉H₂₅NO₄), pandamarilactone-32(C₁₈H₂₁NO₃). The aroma compound 2-acetyl-1-pyrrolidine has been identified from the volatile oil of the leaf (10,11). Lignans and benzofurans have been isolated from roots of *Pandanus odoratissimus* (12).

The leaves of the plant have been mentioned valuable in leprosy, scabies, leucoderma, cephalalgia, coxalgia and otalgia, wounds, ulcers and colic (9,13,14). The oil of the male flowers is considered stimulant, antispasmodic and is administered for headache and rheumatism (15).

The objective of this study was to screen for the presence of antimicrobial activities in petroleum ether, chloroform and hydroalcoholic extracts of *P. odoratissimus* leaves that have been commonly used in Indian folk medicine.

Material and Methods

Procurement and identification of Plant Material:

The leaves of plant were collected from the campus of Kurukshetra University, Kurukshetra during October 2009 and identified as *Pandanus odoratissimus* (Family: Pandanaceae) by Dr. H.B. Singh, Scientist Incharge, Raw Materials and Museum, National Institute of Science Communication And Information Resources, New Delhi where a voucher specimen (No.: NISCAIR/RHM 1381/183) has been deposited.

Preparation of Extracts:

Leaves of *P. odoratissimus* were carefully washed under running tap water and dried in shade for two weeks. Dried leaves were powdered, sieved (#40) and stored in an air tight container at room

temperature. Dried powder was then extracted sequentially with petroleum ether, chloroform, and hydro-alcohol (30:70) by using soxhlation method. The extracts were concentrated to dryness using Rotary evaporator (Heidolph, model-4011, USA). The yield of the extracts was found to be 2.303 % w/w (petroleum ether), 1.985% w/w (chloroform) and 22.092 % w/w (hydro-alcohol). The extracts were preserved in a refrigerator at 4°C.

Test microorganisms

Total five microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121); two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741); one yeast (*Candida albicans* MTCC 227) were selected for evaluation of antimicrobial activity. All the cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were subcultured on nutrient agar whereas yeast on malt yeast agar and incubated aerobically at 37 °C.

Preliminary phytochemical screening:

Phytochemical screening of different extracts was carried out as per reported methods (16, 17).

Screening for antimicrobial activity

Antimicrobial activity of various extracts was determined by agar well diffusion method (18). The density of each microbial suspension was adjusted equal to that of 10^5 - 10^6 cfu/ml (Standardized by 0.5Mc Farland standard) and used as inoculums for performing agar well diffusion method. 20 ml of specific agar media was poured into each petriplate and plates were swabbed with 100 µl inoculum of each test microorganisms and kept for 15 min for adsorption. Using sterile cork borer (diameter, 8 mm), wells were bored into seeded agar plates and loaded with 100 µl volume of different extracts (100 mg/ml) reconstituted in dimethylsulphoxide (DMSO). All plates were incubated at 37 °C for 24 hrs. Ciprofloxacin served as positive control for bacteria and amphotericin-B for fungi, whereas, DMSO used as a negative control.

The antimicrobial activity of extracts was evaluated by measuring the zone of growth inhibition against the test microorganisms with zone reader (Hi antibiotic zone scale). All the experiments were performed in triplicates and the mean value of the diameter of inhibition zones with standard deviation were calculated.

Determination of minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration of a compound/extract that completely inhibits the growth of the microorganisms in 24-48 hrs. MIC was determined by modified agar well diffusion method. Two fold serial dilution of each extract was prepared by first reconstituting the extract in DMSO followed by dilution in sterile distilled water to achieve a concentration range of 50 mg/ml to 0.39mg/ml. A 100 µl volume of each dilution was introduced into wells in the agar plates already seeded with 100 µl of standardized inoculum (10^5 - 10^6 cfu/ml) of the test microbial strains. All test plates were incubated aerobically at 37⁰C for 24 hrs and observed for the inhibition zones. The lowest concentration of each extract exhibiting clear zone of inhibition (considered as MIC) was recorded for each test microorganism (19,20). All the experiments were performed in triplicates.

Results

Preliminary phytochemical screening:

The results of chemical tests of various extracts of *P. odoratissimus* leaves were mentioned in table 1.

Table 1. Preliminary phytochemical screening of *Pandanus odoratissimus* leaves extract

Chemical Constituents	Chemical tests	Petroleum Ether extract	Chloroform extract	Hydro alcoholic extract
Alkaloids	Dragendorff's test	–	–	+
	Mayer's test	-	-	+
	Keller- Killiani	–	–	–

Glycosides	test			
	Borntrager's test	-	-	-
Saponin glycosides	Foam test	-	-	+
Flavonoids	Shinoda test	-	-	+
	Sodium hydroxide test	-	-	+
	Lead-Acetate test	-	-	+
Tannins	Ferric chloride test	-	-	-
	Bromine solution test	-	-	-
Steroids	Salkowaski test	+	+	-
	Liebermann-Burchard test	+	+	-
Carbohydrates	Benedict's test	-	-	+
	Fehling's test	-	-	+
	Molisch test	-	-	+
	Selivnoff's test	-	-	+
	Test for pentoses	-	-	-
	Killer-Killiani test	-	-	+
Mono-sacchacrides	Barfoad's reagent	-	-	+

Screening for antimicrobial activity:

The results of antimicrobial activity of different extracts of *P. odoratissimus* by agar well diffusion method revealed that all the three extracts showed moderate activity against the Gram positive bacteria only *i.e.* *S. aureus*, *B. subtilis* as shown in table 2 & 3. Highest mean of diameter of inhibition zone was produced by the Hydro alcoholic extract (17.3 mm) and a MIC of 25 mg/ml against *S. aureus* and 16.6 mm and a MIC of 25 mg/ml against *B. subtilis* followed by petroleum ether (15.6 mm and a MIC of 50 mg/ml against *S. aureus* and 14 mm and a MIC of 50 mg/ml against *B. subtilis*) and chloroform extract (14.6 mm and a MIC of 50 mg/ml against *S. aureus* and 14.3 mm and a MIC of 50 mg/ml against *B. subtilis*). No antimicrobial inhibitory activity was shown by any of the three extract of *P. odoratissimus* against Gram negative bacteria (*E. coli* and *P. aeruginosa*) and fungi (*C. albicans*).

Table 2. Antimicrobial activity of *Pandanus odoratissimus* leaves extracts using agar well diffusion method

Leaves extract	Diameter of growth of inhibition zones (mm) ^a				
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
Hydro alcoholic (100 mg/ml)	17.3	16.6	-	-	-
Pet ether (100 mg/ml)	15.6	14	-	-	-
Chloroform (100 mg/ml)	14.6	14.3	-	-	-
Ciprofloxacin (20 µg/ml)	27	26.3	26.6	24.3	nt
Amphotericin B (100 µg/ml)	nt	nt	nt	nt	13.6
DMSO	-	-	-	-	-

- No activity, nt = not tested

^a Values, including diameter of the well (8 mm), are means of three replicates, ^b± Standard deviation

Table 3. MIC of *Pandanus odoratissimus* leaves extracts

Leaves extract	Minimum Inhibitory Concentration (mg/ml)	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
Hydro alcoholic	25	25
Pet ether	50	50
Chloroform	50	50

Discussion

Plants are important source for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (21). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (22-28). Some of these observations have helped in identifying the active principle responsible for such activities and in developing the drugs for the therapeutic use in human beings.

The increased frequency of resistance to commonly used antibiotics led to the search for newer, effective, cheap and easily affordable drugs in the management of infectious diseases. Although currently available synthetic drugs are popular, however, herbal medicine continued to be practised due to richness of certain plants in varieties of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids which have been reported to have potent antibacterial activities (29,30).

In the present study, Preliminary phytochemical investigation of *P. odoratissimus* leaves revealed the presence of saponins, alkaloids flavonoids and carbohydrates in the hydro-alcoholic extract, whereas, steroids in the chloroform and petroleum ether extracts respectively (Table 1).

The antimicrobial potency of hydro-alcoholic, chloroform and petroleum ether extracts was determined by agar well diffusion method and minimum inhibitory concentration (MIC) test.

The various extracts were tested on five microbial strains two of which were Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*); two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and one yeast (*Candida albicans*). In terms of antimicrobial effects, all the three extracts exhibited effective inhibition zones against Gram-positive bacterial strains. However, they were ineffective against Gram-negative bacteria and yeast. The MIC of hydro-alcoholic, chloroform and petroleum ether extracts were found to be 25, 50 and 50 mg/ml respectively against Gram-positive bacteria (Table 2 & 3). Out of three extracts, hydro-alcoholic extract showed good antimicrobial activity. The phytochemical study showed the presence of alkaloids and flavonoids in the extract, which might be a reason for the good activity of hydro-alcoholic extract.

However, this is a preliminary work and more work is needed to determine the active ingredients in these extracts which may help in improving management of the different infectious diseases that are developing resistance to commonly used antibiotics. Furthermore, toxicological studies of these extracts can also be carried out to determine the therapeutic use on human beings.

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