ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 1, 2014

ISSN - 0974-2441

Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *PITHECELLOBIUM DULCE* POD PULP EXTRACT

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Received: 5 December 2013, Revised and Accepted: 5 January 2014

ABSTRACT

Objective: Diseases due to pathogenic bacteria and fungi represent a critical problem to human health and they are the major cause of morbidity and mortality worldwide. Plants based antimicrobials are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. In the series of medicinal plants, one such medicinal plant which has been widely used in traditional medicine but lacks scientific scrutiny is *Pithecellobium dulce*. The present study was aimed to investigate the antimicrobial properties of *P.dulce* pod pulp extract against common pathogenic gram positive, gram negative bacteria and fungi.

Methods: Ethanolic extract was used for the study. Phytochemical screening, total phenolic and flavonoid content were determined. The antibacterial and antifungal activity of ethanolic extract of pod pulp was tested against clinically important Gram positive, Gram negative bacteria and pathogenic fungal strains. The inhibitory effect was assessed by well diffusion method. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were also determined by serial dilution method.

Results: Phytochemical analysis of the pulp extract revealed the presence of alkaloids, flavonoids, glycosides, saponins, phytosterols, and triterpenoids. The pulp extract was found to contain significant amounts of total phenols and flavonoids. The pulp extract showed significant zone of inhibition in a dose dependent manner. The MIC and MBC values of the pulp extract against both Gram positive and Gram negative bacterial strains varies from 1mg to 5mg and the results are comparable with chloramphenicol. The MIC and MFC values of pod pulp extract against fungal strains varies from 1 mg to 7 mg and the results are comparable with Amphotericin B.

Conclusion: It can be concluded that the pulp extract possesses potent bactericidal and fungicidal activity which in turn may be due to the presence of biologically active ingredients with antimicrobial activity in the pod pulp.

Keywords: *Pithecellobium dulce; Antibacterial; Antifungal; Minimum Inhibitory concentration*

INTRODUCTION

In recent years, the risk of opportunistic fungal infections has greatly increased in patients who are severely immune-compromised due to cancer chemotherapy, organ or bone marrow transplantation and human immunodeficiency virus infection [1]. Likewise, bacterial diseases accounts for high proportion of health problems in both developed and developing countries. Despite the progress made in the understanding of invasion, pathology and control, the incidence of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease causing microbes, pose immense clinical problem in the treatment of public health concerns [2]. This situation highlights the need for advent of safe, novel and effective antimicrobial agents.

Rational drug design does not always yields effective antimicrobials. In the past, potent enzyme inhibitors have been successfully designed and synthesized but they had only modest antibacterial activity, probably owing to the complex issue of drug uptake by the cells [3]. The necessity to develop new drugs requires varied strategies, among them, the bioprospection of secondary metabolites produced by medicinal plants [4].

Plants are valuable sources of ecologically developed secondary metabolites which are important for normal growth and defense against infection and injury. The earliest drug discoveries were made by presumably random sampling of higher plants. Therefore, it is of great importance to carry out a screening of traditional medicinal plants in order to validate their use in folk medicine and also to reveal the active principle by isolation and characterization of their secondary metabolites.

Approximately 20% of the plants found in the world have been subjected to pharmacological screening and a substantial number of new antibiotics introduced in the clinical use are obtained from natural or semi-synthetic resources [5].

Pithecellobium dulce is one such traditional medicinal plant that lacks scientific scrutiny for its pharmacological properties.

Pithecellobium dulce Bentham is an evergreen medium sized, branched, spiny tree that reaches heights of about 22m. It has vernacular names including Manila tamrind, Madras thorn, Monkeypod, Vilayati babul, Black beard and Kodukkapuli. It belongs to the family *Leguminosae* and subfamily *Mimosoideae.* The generic name refers to the curly pod that mimics an ape's earring (pithekos-ellobium) and the species name "dulce" refers to the sweet pod. *Pithecellobium dulce* is the only species among 100-200 species in the genus and has become widespread outside its origin.

It has been commonly used for fencing and tanning, as fodder for feed and pods for food. It coppices readily and can be managed as a hedge. *P. dulce* is noted for their tolerance of heat, drought, salinity and impoverished soils. The plant is well known for its edible fruits and they have been consumed for various ailments in a traditional manner. The fruits are linear, curved legumes (Pods) that range in length from 10 to 13 cm. The pod splits along both margins. The legumes may contain 5 to 12 seeds which are reddish brown to black in colour. The fruits turn from green to reddish brown when they ripen. The pod fragments can be eaten raw or made in to a drink for its nutritive as well as therapeutic values but still most of the chemical constituents of the pods are remained unexplored and underutilized [6, 7]. Various parts of the tree such as bark, leaves and seeds have been studied for their medicinal properties [8, 9, 10]. Survey of literature shows that antimicrobial studies on *P.dulce* pod pulp have not been carried out in the past. Hence, the present study was carried out to determine the bactericidal and fungicidal effects of P. *dulce* pod pulp using common pathogenic bacteria and fungi.

MATERIALS AND METHODS

Plant Material

The plant was taxonomically identified and authenticated by a qualified taxonomist and a voucher specimen has been deposited in our laboratory for future reference. Mature fruits of *P.dulce* were picked from the trees which are growing in the natural environment at the banks of the river "Thamirabarani" in Tirunelveli district, Tamilnadu.

Preparation of Plant extract

Seeds are removed by hand flailing and pod pulp fragments were dried in shade, pulverized by a mechanical grinder and passed through a 40 mesh size to get a fine powder and stored at 0˚ until further use. Known quantity of pulp powder was extracted with petroleum ether (60-80˚C) to remove wax and then extracted with 80% methanol in a soxhlet apparatus. The solvent was evaporated to dryness in a rotary evaporator at reduced pressure below 40˚C. The extract was used for further experiments (Yield 17.6g)

Preliminary Phytochemical Screening

The ethanolic extract of Pithecellobium dulcee pod pulp were subjected to preliminary phytochemical screening [11, 12].

Determination of total phenolic content

Total polyphenol content in the ethanol extract of *P.dulce* were determined according to the Folin-Ciocalteu colorimetric method [13, 14]. A standard curve was built with gallic acid reference solutions. Aliquots ranging from 2 to 10 mL of standard aqueous gallic acid solution (100 µg/mL) were pipetted in to 100 mL volumetric flasks containing 70 mL of distilled water. Folin-Ciocalteu reagent (5 mL) and 10 mL of saturated sodium bicarbonate solution were added, and the volume was made up to 100 mL with distilled water. The solution was thoroughly mixed. The blank was prepared in the same manner, but without gallic acid. After 1 h of incubation at room temperature, the absorbance was measured at 760 nm. The samples were prepared in triplicates for each analysis and the mean value was calculated. For determination of the total phenolic content of *P.dulce*, aqueous solutions at the final concentration of 20 µg/mL were used; proceeding in the same manner described for the reference solutions and the total polyphenolic content was expressed as mg per g of gallic acid equivalents.

Determination of total flavonoid content

Total flavonoid content in the ethanolic extract of *P.dulce* was determined according to the method of Quettier et al., with minor modifications [15]. A standard curve was built with quercetin reference solutions. Aliquots ranging from 2 to 8 mL of standard quercetin ethanol extract solution (50 µg/mL) were pipetted in to25 mL volumetric flasks containing 1 mL of 2% aluminum chloride dissolved in ethanol and the volume was made up with ethanol. The blank was prepared by diluting 1 mL of 2% aluminum chloride dissolved in ethanol in a 25 mL volumetric flask with ethanol. After 1 h at room temperature, the absorbance was measured at 420 nm. *P.dulce* samples were evaluated at a final concentration of 20 µg/mL, proceeding in the same manner described for the reference solutions and the total flavonoid content was calculated as quercetin equivalents (mg/g) from a calibration curve. The samples were prepared in triplicate for each analysis and the mean value of absorbance was recorded.

Bacterial and Fungal strains and growth medium

The bacterial and fungal strains were all standard laboratory strains obtained from the stock cultures of the Division of Microbiology, CAS in Botany, University of Madras, and Chennai and maintained on slopes of Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) at 28°C.

Four Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Enterococcus Faecalis* and Four Gram negative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Klebsiella pneumonia*, *Salmonella typhi* were used in the present study. Fungal cultures of *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus, Penicillium notatum*, *Penicillium chrysogenum* were included in this study.

Determination of antibacterial and antifungal activity

Preparation of inoculum

The suspension for inoculation was prepared from the broth culture. Few colonies of similar morphology of the respective bacteria were transferred with the help of a sterile inoculating loop to a Muller-Hinton broth and were incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard (10^8 CFU/ml) were obtained.

The fungal strains were subcultured on slants of SDA at 28°C for 7 days and the colonies were suspended in 1 ml of sterile normal saline. The resulting mixture of conidia and hyphal fragments was vortexed and the turbidity of each homogenous suspension was adjusted to match that of a 0.5 McFarland standard, as read at 530 nm. At this turbidity, the fungi density was 3×10^6 to 5×10^6 CFU $ml^{-1.}$

Preparation of the McFarland standard

0.5 ml of 0.048M $BaCl_2$ was added to 99.5 ml of 0.18M H_2SO_4 with constant stirring. The standard was distributed in to screw cap tubes of the same size and with the same volume as those used in growing the broth culture. The tubes were sealed tightly to prevent loss by evaporation. The tubes were stored, protected from light at room temperature. The turbidity standard was agitated vigorously on a vortex mixture before use. Standards may be stored for up to 6 months, after which time they should be discarded.

Antibacterial activity of the ethanolic extract of *P.dulce pod pulp* was evaluated by agar well diffusion method [16]. The inocula with respective test bacteria were homogenously seeded onto the 90mm Petri dishes containing 20 ml of cooled molten MH agar medium using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth following incubation [17]. Wells were dug in the medium with the help of a sterile cork borer. Stock solution of the pod pulp extract (2.5 mg/ml) was prepared in sterile distilled water. Dilutions of the stock solution containing 50, 100, 150, 200 and 250 µg were also prepared in sterile distilled water. 100 µl of each dilution was added to their respective wells with a sterile pipette. Control wells received only 100 µl of sterile distilled water. The plates were kept for 1 h at room temperature for the diffusion of the extract into the agar. Subsequently, all the plates were incubated at 37°C for 18-24 h. Following incubation the plates were examined for signs of microbial growth. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the wells. Chloramphenicol (30 µg/ml) was used as positive control. Each experiment was carried out in triplicates.

Antifungal activity of the ethanolic extract of *P.dulce pod pulp* extract was evaluated by disc diffusion method. The inocula with respective fungi were homogenously seeded onto the 90mm Petri dishes containing 20 ml cooled molten SDA medium using sterile swab in such a way as to ensure thorough coverage of the plates and a uniform lawn of growth following incubation. These inoculated plates were left to dry for at least 15 min. The extract was dissolved in distilled water to obtain the different concentrations of 300, 150, 75, 37.5 and 18.75 mg ml^{-1}. Amphotericin B at concentration 10 µg/disc was used as positive control and was dissolved in dimethyl sulphoxide (DMSO). Sterile filter paper disc (6mm in diameter) were impregnated with 10 µl of each different concentration of pulp extract. The discs were allowed to dry and then placed on the agar surface of each petri dish. DMSO was used as negative control. Zone of inhibitions (in mm) were measured after 48-72 h at 28ºC. The complete antifungal analysis was carried out under strict aseptic conditions. Each assay was repeated three times.

Minimum inhibitory concentration (MIC) Minimum bactericidal concentration (MBC), Minimum fungicidal concentration (MFC) assays

A serial of 2-fold macro-broth dilution method was performed to determine the MICs and MBCs of *P.dulce pod pulp* extract for the

respective tested bacterial suspensions (concentration) as recommended by the Clinical and Laboratory Standards Institute (CLSI) [18]. The minimum inhibitory concentration (MIC) of *P.dulce pod pulp* extract against fungal strains was determined using broth microdilution method as described by the National Committee for clinical laboratory standards for fungi (M27-A2). The stock solutions of *P.dulce pod pulp* extract was diluted suitably as required from stock solution. The ranges should be prepared one step higher than the final dilution range required that if a final dilution range of 0.5, 1, 2, 4, 8, and 16 mg/ml is required then a range of 1, 2, 4, 8, 16 and 32 mg/ml should be prepared to compensate for the addition of an equal volume of inoculums. Two rows of 12 capped test tubes were arranged in the rack. In a sterile 30 ml (universal) screw capped bottle, 8 ml of MH broth (bacteria), 8ml SD broth (fungi) containing the required concentration of *P.dulce pod pulp* extract for the first tube in each row was prepared from the appropriate stock solution already made. The contents of the universal bottle were mixed using a sterile pipette and transferred 2 ml to the first tube in each row. Using a fresh sterile pipette, 4 ml of broth was added to the remaining 4 ml in the universal bottle, mixed well and transferred 2 ml to the second tube in each row. Dilutions were continued in this way to as many as 10 tubes. 2 ml of broth free from pod pulp extract was added to the last tube in each row. The density of the bacterial suspension was adjusted (10^8 CFU/ml) to equal that of the 0.5 McFarland standard by adding sterile distilled water as detailed above. The bacterial suspension was suitably diluted (10^6 CFU/ml) and added to the tubes containing MH broth. The density of the fungal suspension was adjusted (3×10^6 to 5×10^6 CFU ml-1) to equal that of the 0.5 McFarland standard by adding sterile distilled water as detailed above. Chloramphenicol (30 µg) was used as positive control for bacteria. After incubation at 37ºC for 24 h, turbidity of the tubes was assessed visually by comparison to uninoculated control.

Amphotericin B was included in the assays as positive control 10 µg/disc for fungi. After incubation at 28ºC for 42-78 h, turbidity of the tubes was assessed visually by comparison to uninoculated control.

The MIC is expressed as the lowest concentration of the pod pulp extract where bacterial and/or fungal growth with no visible growth after incubation. All assays were carried out in triplicates. The MBC was derived by sub-culturing 100 µl from each tube from the MIC assay onto substance free MH agar plates. The plates were incubated at 37ºC for 24 h and the MBC was defined as the lowest concentration of substance that allows no visible growth on the agar plate.

The MFC was determined by plating a 100 µl volume on SDA from the tubes showing no visible growth. The plates were incubated as described above in MIC. The MFC was defined as the lowest concentration of substance that did not allow any visible growth on the agar plate.

RESULTS

The ethanolic extract of *P.dulce pod pulp* yield was 17.6%. The phytochemical screening of ethanolic extract of *P.dulce pod pulp* is depicted in table 1. Phytochemical analysis of the pod pulp extract revealed the presence of alkaloids, flavonoids, glycosides, saponins, phytosterols, and triterpenoids. The total phenolic content and flavonoid content were found to be 2.52 ± 0.09 mg/g equivalents of gallic acid and 5.13 ± 0.11 mg/g equivalents of quercitin respectively.

Table :1 Phytochemical screening of *P.dulcee pod pulp* extract

PHYTOCONSTITUENTS	INFERENCE
Alkaloids	**+**
Flavonoids	**+**
Glycosides	**+**
Saponins	**+**
Tannins	**+**
Phytosterol	**+**
Triterpenoids	**+**
Anthraquinones	**-**

Table 2 shows the antibacterial activity of ethanolic extract of *P.dulce pod pulp* against four different Gram positive and Gram negative bacterial strains. The antibacterial potency of *P.dulce pod pulp* extract was evaluated by the presence or absence of inhibition zones and zone diameters (mm). The results of the present study indicate that the ethanolic extract of *P.dulce pod pulp* showed a maximum inhibitory zone in a dose dependant manner. However, there was no significant difference between the levels of zone of inhibition at the concentration of 200 µg and 250 µg. Among the Gram positive bacteria, *B. subtilis* showed a larger diameter of clearance than that of other Gram positive bacteria used in this study. Among the Gram negative bacteria, *K.pneumoniae* than that of other Gram negative bacteria. The zone of clearance achieved by *P.dulce pod pulp* extract is comparable to that of standard antibiotic, chloramphenicol.

Table2: Antibacterial activity of *P.dulcee Pod pulp* extract - Zone of inhibition in diameter (mm).

S. No.	Bacterial species	Control	50 µg	100 µg	150 µg	200 µg	250 µg	Chloramphenicol (30 µg)
Gram Positive								
1.	*Staphylococcus aureus*	-	2.2	6.0	11.0	18.0	20.0	24
2	*Staphylococcus epidermidis*	-	3.0	9.0	16.5	19.0	21.0	25
3	*Enterococcus Faecalis*	-	3.2	8.5	16.0	18.0	19.5	22
4	*Bacillus subtilis*	-	4.0	10.0	17.5	25.0	26.0	27
Gram Negative								
5	*Escherichia coli*	-	3.0	8.2	16.0	21.5	22.5	22
6	*Klebsiella pneumoniae*	-	4.0	10.2	17.0	23.5	27.0	26
7.	*Salmonella typhi*	-	1.0	9.2	18.0	21.5	23.0	25
8	*Shigella dysenteriae*	-	-	5.0	9.0	14.0	18.0	20

The minimum inhibitory concentration and minimum bactericidal concentration of *P.dulce pod pulp* extract as well as the standard antibiotic, chloramphenicol is shown in Table 3. The MIC value of *P.dulce pod pulp* extract against both Gram positive and Gram negative bacterial strains varies from 1 mg to 5 mg and the results are comparable with the standard antibiotic, chloramphenicol. The highest MIC values were shown by *Enterococcus faecalis* in Gram positive bacteria and by *Salmonella typhi* in gram negative bacteria. The lowest MIC values were displayed by *Bacillus subtilis* in Gram positive bacteria and *K.pneumoniae* in gram negative.

Table3: MICs and MBCs of *P.dulcee pod pulp* extract on Gram positive and Gram negative bacteria.

Bacterial species	Minimum Inhibitory Concentration (MIC)		Minimum Bactericidal Concentration (MBC)	
	P.dulcee pod pulp extract (mg/ml)	Chloramphenicol (µg/ml)	*P.dulcee* fruit extract (mg/ml)	Chloramphenicol (µg/ml)
Gram positive *Staphylococcus aureus*	4	2	3	2

Staphylococcus epidermidis	4	3	2	4
Enterococcus Faecalis	5	4	5	4
Bacillus subtilis	3	2	2	3
Gram negative				
Escherichia coli	4	4	4	8
Salmonella typhi	3	1	6	2
Shigella dysenteriae	5	2	8	4
Klebsiella pneumoniae	1	2	2	4

Table 4 shows the antifungal activity of ethanolic extract of *P.dulce pod pulp* against eight different fungal species. The antifungal potency of *P.dulce pod pulp* extract was evaluated by the presence or absence of inhibition zones and zone diameters (mm). It is evident that the ethanolic extract of *P.dulce pod pulp* showed a maximum inhibitory zone in a dose dependant manner. However, there was no significant difference between the levels of zone of inhibition at the concentration of 1.5 mg and 3 mg/disc. The antifungal potency of *P.dulce pod pulp* on the *C. albicans* showed a larger diameter of clearance than that of other strains. Moreover, the zone of clearance achieved by *P.dulce pod pulp* extract is comparable to that of standard drug, Amphotericin B.

Table4: Antifungal activity of *P.dulcee pod pulp* extract against fungal species tested by disc diffusion assay.

S. No.	Strains	Control	0.175 mg/disc	0.375 mg/disc	0.75 mg/disc	1.5 mg/disc	3 mg/disc	Amphotericin B
1	*Candida albicans*	-	10.2	12.6	13.5	23	25	26
2	*Saccharomyces cerevisiae*	-	-	8.0	12	15.0	16	19.5
3	*Aspergillus fumigatus*	-	13	16	18	21.5	23	24
4	*Aspergillus flavus*	-	10	13	16.0	19	21	22.5
5	*Aspergillus niger*	-	8	10.0	13	20	22	23
6	*Aspergillus ochraceus*		7	10.0	12	18	20	22
7	*Penicillium chrysogenum*	-	8.0	11	16	20	21	23
8	*Penicillium notatum*	-	11	14	17	21	24	25

The minimum inhibitory concentration and minimum fungicidal concentration of *P.dulce pod pulp* extract as well as the standard antifungal drug, Amphotericin B is depicted in Table 5. The MIC value of *P.dulce pod pulp* extract against fungal strains varies from 1 mg to 7 mg and the results are comparable with the standard antifungal agent, Amphotericin B. The lowest MIC was shown by *Candida albicans* and the highest MIC values by *S. cerevisiae.*

Table5: Antifungal activity of *P.dulcee pod pulp* extract against fungal species tested by MIC and MFC.

Fungal species	MIC		MFC	
	***P.dulcee* (mg ml^{-1})**	**Amphotericin B (µg ml^{-1})**	***P.dulcee* (mg ml^{-1})**	**Amphotericin B (µg ml^{-1})**
Candida albicans	2	1.0	2	2
Saccharomyces cerevisiae	7	3	7	4
Aspergillus fumigatus	2.5	1.2	5	3
Aspergillus flavus	3.2	2	3	2
Aspergillus niger	5	3	5	5
Aspergillus ochraceus	4	2	2	1
Penicillium chrysogenum	2	4	4	2
Penicillium notatum	3	5	3	3

DISCUSSION

The worldwide increase in resistance of pathogenic microorganisms to time-honored antibiotics necessitates the search for alternative strategies preferably from plant origin. Plants produce a variety of secondary metabolites such as flavonoids, alkaloids and tannins which have long been of interest to mankind [19]. However, very little information is available on the pharmacological activity of medicinal plants and of the 4, 00,000 plant species on earth, only a small percentage has been systematically studied for their antimicrobial activities [20]. Although screening of Indian medicinal plants has revealed varying degrees of antimicrobial activity against pathogenic and opportunistic microorganisms, there is still a lack of experimental scientific studies confirming the possible antimicrobial properties of a great number of these remedies.

Antimicrobial resistance is a natural biological phenomenon of response of microbes to the selective pressure of an antimicrobial drug. Since antibiotic use became widespread 50 years ago, microorganisms have relentlessly developed resistance [21]. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient [22]. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents [23]. The use of *P.dulce* in folk medicine suggests that it represent an economic and safe alternative to treat common infectious diseases. Detailed investigations in to the active components responsible for the observed antimicrobial activity may open new avenues for drug development and control of antibiotic resistant pathogenesis [24]. Plant based antimicrobials represent a vast untapped source for medicines and further exploration of their usefulness is necessary.

On a global basis at least 130 drugs, all single chemical entities extracted from higher plants are modified further synthetically are currently in use, though some of them were now being made synthetically for economic reasons [25]. Thus, it was considered worldwide to investigate the antibacterial as well as antifungal activities of *P.dulcee* pod pulp, a common medicinal plant that has been widely used in traditional medicine in one form or the other for its beneficial pharmacological activity.

Phytochemical screening suggests the presence of biologically important phytoconstituents such as alkaloids, flavonoids, glycosides, saponins, phytosterols, and triterpenoids. The total phenolic content and flavonoid content were found to be 2.52 ± 0.09

mg/g equivalents of gallic acid and 5.13 ± 0.11 mg/g equivalents of quercitin respectively. Recent studies indicate that appreciable amounts of total phenolic, flavonoid content were present in fruit peel, seeds and leaves [26, 27, 28].

The results of the study indicated that *P.dulce* pod pulp extract showed effective inhibitory activity against Gram-positive bacteria, *Bacillus subtilis* and gram negative bacteria *Klebsiella pneumonia. B. subtilis* showed a larger diameter of clearance than that of other Gram positive bacteria used in this study. Similarly, *P.dulce pod pulp* extract showed a maximum zone of clearance in the Gram negative bacteria, *K.pneumoniae* than that of other Gram negative bacteria.

Minimum inhibitory concentrations are considered the "gold standard" for determining the susceptibility of microorganisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing [29]. A lower MIC value indicates that less drug is required for inhibiting growth of the organism; therefore, antimicrobials with lower MIC values are more effective antimicrobial agents. The highest MIC and MBC values were shown by *Enterococcus faecalis* in Gram positive bacteria and by *Salmonella typhi* in gram negative bacteria. The lowest MIC and MBC values were displayed by *Bacillus subtilis* in Gram positive bacteria and *K.pneumoniae* in gram negative.

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide. Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries [30]. In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years. Although new drugs have been introduced to combat this problem, the development of resistance to antifungal drugs has become increasingly apparent, especially in patients who require long-term treatment or who are receiving antifungal prophylaxis, and there is growing awareness of shifts of flora to more-resistant species.

The fungal strains used in the present study were selected on the basis of their clinical importance. Agar disc diffusion method was performed in the present study to investigate the antifungal activity of *P.dulcee* pod pulp extract. The highest activity (diameter of zone of inhibition 25 mm) was demonstrated by the ethanolic extract of *P.dulce pod pulp* against *C. albicans* while the lowest activity was observed against *S. cervisiae*. The results of the *in vitro* antifungal assay revealed that the growths of fungal strains were affected by the *P.dulce pod pulp* extract by forming clear inhibition zones.

The MICs and MFCs showed that *S. cerevisiae* has the highest MIC (7mg/ml) and MFC (7mg/ml) while the lowest MIC of 2 mg/ml was demonstrated by *C. albicans*. The fungistatic or fungicidal effect of natural products and the mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes. These biological events could take place separately or concomitantly culminating with mycelium germination inhibition and it is also reported that plant lytic enzyme act in the fungal cell wall causing breakage of β-1,3 glycan, β-1,6, glycan and chitin polymer [31]. The observed antifungal effect of the extract might be due to the presence of biologically important ingredients present in the pod pulp.

CONCLUSION

The remarkable bactericidal, fungicidal effects of *P.dulce pod pulp* extract suggest that the pod pulp may be a useful source for the development of novel antibacterial, antifungal agent against pathogenic bacteria and fungi. This *in vitro* study demonstrated that folk medicine can be as effective as modern allopathic medicine to treat pathogenic microorganism.

Conflict of interest

The authors declare that they have no conflict of interest

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