Tropical Biomedicine 31(4): 607–615 (2014)

Antibacterial and antifungal activity of solvent extracts from *Plumeria obtusa* Linn.

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Received 11 December 2013; received in revised form 8 April 2014; accepted 9 April 2014

Abstract. Extracts of *Plumeria obtusa* are widely used in ethnomedicine and have been investigated for a variety of biological activities; however, the antimicrobial activity of *P. obtusa* flowers is poorly characterized. In this study, the antimicrobial activities of different solvents (petroleum ether, ethyl acetate, chloroform, isobutanol and ethanol) extracts from flowers of *P. obtusa* were investigated by a disc diffusion method against Gram-positive bacteria, Gram-negative bacteria and a fungus. All extracts exhibited growth inhibition of all microorganisms at variable degrees as measured by relative zones of inhibition, however, the petroleum ether extract was ineffective against *Klebsiella pneumonia* and ethyl acetate and isobutanol extracts were ineffective against *Pseudomonas aeruginosa*. The most susceptible Gram-positive bacterium was *Bacillus subtilis* while the most resistant Gram-positive bacterium was *Staphylococcus aureus*. *Erwinia carotovora* was the most susceptible Gram-negative bacterium while *P. aeruginosa* was highly resistant among the Gram-negative bacteria. In this study, for the first time, we investigated the antimicrobial activity of several different solvent extracts from flowers of *P. obtusa* against a broad spectrum of human-pathogenic microorganisms. These compounds warrant further investigation by isolation and structural elucidation with the aim to find novel and affordable bioactive compounds for the treatment of infectious diseases.

INTRODUCTION

The increasing resistance of pathogens against available antimicrobial agents poses a serious threat to human health, and has prompted research efforts to search for novel and more effective antimicrobial agents against resistant pathogens (Essawi *et al.*, 2000; Nascimento *et al.*, 2000; Woodford 2003; Khalafi-Nezhad *et al.*, 2005; Shah 2005; Esterhuizen *et al.*, 2006; Alka *et al.*, 2010; Thaller *et al.*, 2010). Medicinal plant-derived extracts have a wide range of biological activities, including antimicrobial, anti-allergic, antibacterial, anti-diabetic, anti-inflammatory, antiviral, antiproliferative, antipyretic, anti-thrombotic, anti-carcinogenic, hepatoprotective, oestrogenic, insecticidal and antioxidant activities (Middleton *et al.*, 1986; Harborne 1994; Amani *et al.*, 1999; Cowan 1999; Salvat *et al.*, 2001; Tringali 2001; Ordonez *et al.*, 2003; Arias *et al.*, 2004; Kubmarawa *et al.*, 2007).

Major groups of antimicrobial compounds isolated from plants include simple phenols and phenolic acids, quinones, flavones, flavonoids and flavonols, tannins, coumarins, alkaloids, terpenoids and

essential oils, lectins and polypeptides (Middleton *et al*., 1986).

Plumeria obtusa (genus *Plumeria*, family *Apocynaceae*) is one of the medicinally important plants found predominantly in tropics and sub-tropics (Dassanayake *et al*., 1983). The common names of this plant are white frangipani (Australia), champa or chafa (India), melia (Hawaii), araliya (Sri Lanka), temple tree (United Kingdom) and gul cheen (Pakistan). Other species of medical relevance in the *Apocynaceae* family include *Plumeria rubra*, *Plumeria acutifolia*, *Plumeria obtusifolia*, *Plumeria alba*, *Plumeria bicolor*, *Plumeria tricolour and Plumeria jamesoni* (Ali *et al*., 2013).

In traditional medicine, *P. obtusa* is used to treat gastrointestinal ailments, fever, malaria, pain and diabetes. Leaves are used as a purgative, antidote and externally applied to treat headache. Roots are used to treat skin and liver diseases, leprosy, dysentery, worms, ulcers, tumors, and earache. Decoction of leaves of this plant is used for treating wounds and skin diseases. The latex and bark are known to have purgative and diuretic properties. This species also has been reported to possess anticancer properties (Wong *et al*., 2011). The decoction of the bark and roots is traditionally used to treat asthma, ease constipation, promote menstruation and reduce fever. The latex is used to soothe irritation (Wiart 2002). The bioactive phytochemicals extracted from *P. obtusa* have molluscicidal, cytotoxic and anti-bacterial activities. The plant is reported to contain amyrinacetate, amyrins, sitosterol, scopotetin, iridoids, isoplumericin, plumieride, plumieride coumerate and plumieride coumerate glucoside (Edward *et al*., 1994).

Several studies have been conducted for the screening of antimicrobial and antiviral activity of *Plumeria* extracts. An extract of *P. alba* leaves was found useful in the therapy of ulcers, herpes and scabies when used topically (Radha *et al*., 2008). The bark extracts of *P. rubra* showed cytotoxic effects against a number of *in vitro* human cancer cell lines (breast, colon, lung, fibrosarcoma and melanoma) (Kardono *et al*., 1990). The flowers and the latex were found effective in a treatment of vaginal bloodshed (Ruiz-Terán *et al*., 2008). Methanolic extracts of flowers of *P. obtusa* had antimicrobial activity against *Bacillus anthracis* and *P. aeruginosa* (Amani *et al*., 1999). A methanolic extract of *P. alba* exhibited partial inhibition of *Escherichia coli*, *Staphylococcus saprophyticus*, *Proteus vulgaris* and *Serratia marcescens* (Syakira *et al*., 2010). The chemical components of the essential oils obtained from the flowers of three Malaysian species of *Plumeria*, namely *P. rubra* L. (red flower variety), *P. acutifolia Poir*. (Yellow flower variety) and *P. obtusa L*. (white flower variety) were analyzed by GC/MS, and 27 different compounds, including the major components benzyl salicylate, nanodecane and heneicosane, were identified in all the three species. Antimicrobial properties of the essential oils (at 2 ìl per disk) from the three species were determined by agar diffusion method, against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Candida albicans*, *Candida humicola*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum canis*. The essential oil of *P. obtusa* showed the best antimicrobial property (broad spectrum and high level of inhibition) compared to the other two tested species. The oil inhibited all tested microorganisms except *E. coli* (Sulaiman *et al*., 2008). Similarly, the ethanolic extract of *P. acutifolia* stem bark, showed strong *in vitro* antimicrobial activity against *Enterococcus faecalis*, *Bacillus subtilis*, *S. aureus*, *P. aeruginosa*, *Salmonella typhimurium*, *Aspergillus niger* and *C. albicans* (Chitta *et al*., 2008). Different solvent extracts from the *P. obtusa* leaves, recently screened, were found to inhibit the growth of several bacterial strains and *C. albicans* (Ali *et al*., 2013).

Although, different species of *Plumeria* were investigated for a variety of biological activities, the antimicrobial activity of *P. obtusa* flowers is still poorly characterized. Keeping in view the medicinal value of *P. obtusa*, the present study was initiated to investigate the antibacterial and antifungal activity of various extracts from flowers of *P. obtusa*.

MATERIAL AND METHODS

Plant material collection and crude extract preparation

The mature, healthy and fresh flowers of *P. obtusa* were collected from well-grown *P. obtusa* plants grown in the University of Agriculture, Peshawar campus, and Pakistan Council of Scientific and Industrial Research (PCSIR) laboratory complex, Peshawar, Pakistan. To prepare crude extract, the flowers were cut into small pieces, shade-dried for 5 days at room temperature and ground into powder material. The powder was put into separate extraction drums containing ethanol, for about 24 hours at room temperature. The ethanolic extract was filtered through two layers of Whatman® No. 1 filter paper (England) and was dried at 400rpm/30°C under vacuum pressure by using the rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany).

Fractionation of crude extract

The ethanolic crude extract prepared from flowers of *P. obtusa* was divided into two portions; one portion (~10 g) was taken into glass vials to be tested as ethanolic crude fraction for antimicrobial activity while the second portion (~100 g) was taken into a glass beaker for fractionation with different solvents (solvent extraction). Part of the second portion was dissolved in water, poured into a separating funnel and then distilled petroleum ether was added. The separating funnel was shaken to separate the two phases (water and petroleum ether). Compounds soluble in the upper petroleum ether phase were collected and the lower aqueous phase was extracted thrice with petroleum ether. All fractions of petroleum ether were combined and poured into round bottom flask of a rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany) and petroleum ether was evaporated from the fraction leaving behind semisolid petroleum ether fraction. The semisolid petroleum ether fraction was dried in a china dish via water bath at about 45°C and was stored in the air-tight glass vials at 8°C until used. The same extraction methodology was carried out for chloroform, ethyl acetate and isobutanol which resulted in chloroform, ethyl acetate and isobutanol fractions, respectively.

Culture media

Nutrient agar modified (QUELAB QB-39-3504, Montreal, Canada), containing beef extract (1.0 g l^{-1}), yeast extract (2.0 g l^{-1}), gelatin extract (5.0 g l^{-1}), sodium chloride (5.0 g l^{-1}) and agar (15.0 g l^{-1}), was used for the culturing and growth of all microorganisms used in this study. Nutrient broth modified (QUELAB QB-39-3504, Montreal, Canada), containing gelatin peptone (5.0 g l^{-1}), beef extract (1.0 g l^{-1}), yeast extract (2.0 g l^{-1}) and sodium chloride (5.0 g l^{-1}), was used for shaking incubation and standardization of these microorganisms.

Preparation of media

The required quantities of Nutrient Agar Modified (28 g l^{-1}) and Nutrient Broth Modified (13 g l^{-1}) were prepared in distilled water and poured into conical flasks. Some of the Nutrient Broth Modified (approximately 20 ml per test tube) was also poured into the test tubes. All the media flasks and test tubes were plugged with cotton wool and then sterilized in an autoclave at 15 psi pressure and 121°C for 15 min. After sterilization, nutrient agar media was poured aseptically into sterilized Petri plates in a laminar flow hood. The media in the Petri plates was allowed to stand for about 30 minutes to solidify. Then the plates were placed in an incubator at 37°C for 24 hours. After 24 hours, uncontaminated plates were used for culturing of bacteria and fungi. The nutrient broth in the flasks (approximately 20 ml per flask) was used for shaking incubation of the microorganisms while the nutrient broth in test tubes was used for standardization of microbial cultures.

Microorganisms used

Antimicrobial activity of different solvent extracts of flowers of *P. obtusa* was tested against the following bacteria and fungus: Gram-positive bacteria: *Bacillus atrophaeus* (clinical isolate obtained from Microbiology Laboratory, Quaid-I-Azam University, Islamabad, Pakistan), *Bacillus subtilis* (ATCC #26633), *Staphylococcus aureus*

(ATCC # 6538); Gram-negative bacteria: *Erwinia carotovora* (Plant Pathology Department of KPK Agriculture University Peshawar, Pakistan), *Escherichia coli (*ATCC # 25922*)*, *Klebsiella pneumoniae* (clinical isolate obtained from Microbiology Laboratory, Quaid-I-Azam University, Islamabad, Pakistan), *Pseudomonas aeruginosa (*ATCC # 9721*)*, *Salmonella typhi* (clinical isolate obtained from Microbiology Laboratory, Quaid-i-Azam University, Islamabad, Pakistan)*;* and fungus: *Candida albicans* (clinical isolate obtained from Hayatabad Medical Complex, Peshawar, Pakistan).

All microbial stock cultures were freshened by streaking using sterile inoculation loop on nutrient agar media plates in a laminar flow hood and incubated at 37°C for 24 h. The next day, the first day streaked cultures were subcultured on media plates and incubated at 37°C for 24 h. The second streaked cultures were then inoculated into the nutrient broth in flasks and subjected to shaking at 200 rpm for 18 hours at 37°C.

Disc Diffusion Susceptibility Method

The assay was performed as described earlier in the literature (Bauer *et al.*, 1966). The nutrient agar media plates were seeded with 18 to 24 hours cultures of microbial inoculums (standardized inoculums of 1-2 × 10^7 CFU ml^{-1} or 0.5 McFarland Standards). Whatman No. 1 filter paper discs (each of 6 mm diameter) were placed with the help of sterile forceps on the media and then plant extracts in concentrations of 1 and 2 mg $disc^{-1}$ (in 6 and 12 µl volume, respectively) were applied on the discs. Azithromycin (50 µg, ciprofloxacin (30 µg), and clotrimazole (50 µg) were used as positive controls for Gram-positive bacteria, Gram-negative bacteria and for *C. albicans*, respectively. Dimethyl sulfoxide (DMSO; 6 µl $disc^{-1}$) was used as negative control (blank). Inoculated plates were then incubated at 37°C for 18 to 24 hours. The next day, zones of inhibition were measured in millimeter around the discs in each plate. The same procedure was followed three times for each microorganism. The percent measurement of zones of inhibition was calculated as follows:

$$\text{Percent zone of inhibition} = \frac{\text{Average value of replica's}}{\text{Inhibition zone of standard}} \times 100$$

Data were derived from three independent experiments (mean ± SEM).

RESULTS AND DISCUSSION

In the present study, antimicrobial activity of five different extracts of flowers of *P. obtusa* against nine different microbial species was investigated by using the disc-diffusion susceptibility method. Relative zones of inhibition (compared with specific standard antimicrobials) by different extracts against the tested microorganisms are shown in Figure 1.

The highest antibacterial activity was exhibited by chloroform and ethyl acetate fractions at 2 mg $disc^{-1}$. Chloroform and ethyl acetate exhibited inhibitions of (91±7.28% at 1 mg $disc^{-1}$, 95±5.68% at 2 mg $disc^{-1}$) and (89±4.45% at 1 mg $disc^{-1}$, 95±5.7% at 2 mg $disc^{-1}$), respectively. This was followed by petroleum ether extract, which exhibited inhibition of *B. subtilis* by 62±3.1 and 69±3.45% at 1 and 2 mg $disc^{-1}$, respectively. Therefore, isolation of the antibacterial constituents present in the chloroform and ethyl acetate fractions of the plant extract appears to be most interesting at present.

A recent study using extracts from the leaves of *P. obtusa* against *B. subtilis* also reported high inhibition activity in the chloroform (72 and 79% at 1 and 2 mg $disc^{-1}$, respectively) and ethyl acetate (70 and 85% at 1 and 2 mg $disc^{-1}$, respectively) fractions (Ali *et al.*, 2013). However, the petroleum ether extract of *P. obtusa* leaves showed maximum (80 and 97% at 1 and 2 mg $disc^{-1}$, respectively) inhibition against *B. subtilis*. These differences in activity could indicate differences in the composition of *P. obtusa* flowers and leaves extracted by the same solvent. *B. subtilis* was also found to be highly susceptible to isobutanol and petroleum ether fractions. The crude extract

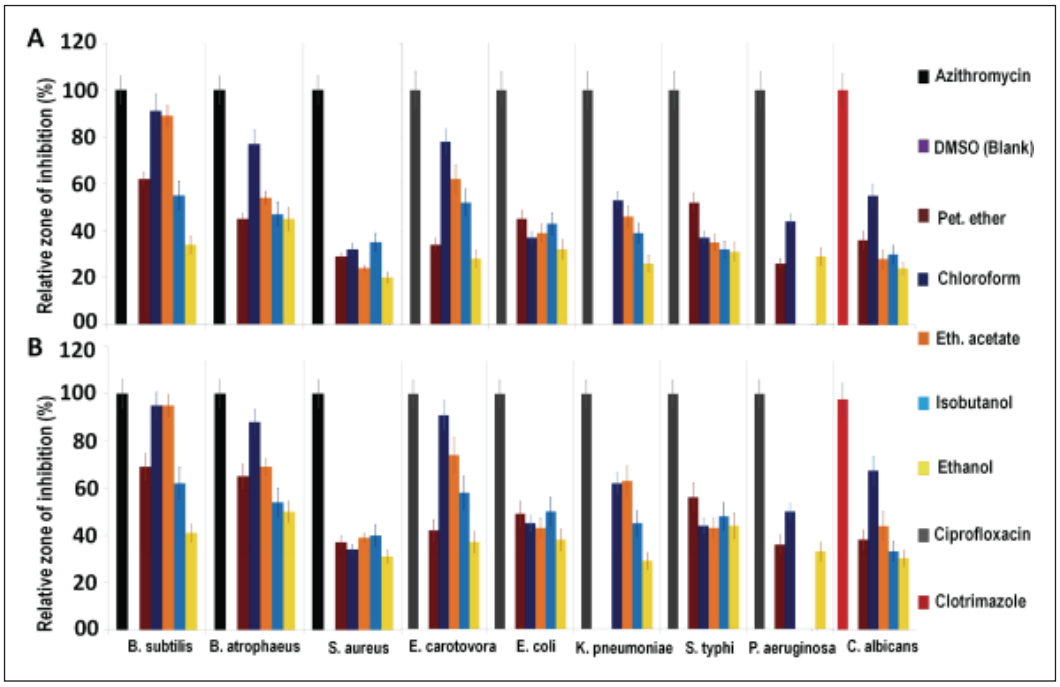

Figure 1. Antibacterial activity of different solvent extracts from flowers of *Plumeria obtusa* against bacteria and fungus by disc diffusion assay. Azithromycin (50 µg per disc), ciprofloxacin (30 µg per disc), and clotrimazole (50 µg per disc) were used as positive controls for Gram-positive bacteria, Gram-negative bacteria and for *C. albicans*, respectively. Dimethyl sulfoxide (DMSO; 6 µl per disc) was used as negative control (blank). Data represent the mean (± SEM) relative zones (%) of three independent experiments. (**A**. 1 mg of extract per disc; **B**. 2 mg of extract per disc).

sample (ethanolic extract) was found to be effective against *B. subtilis* at both concentrations but with minimum inhibition when compared with the inhibition activity of other extracts. *B. subtilis* was found more susceptible to ethanolic extract when compared to other two Gram-positive bacteria *B. atrophaeus* and *S. aureus*. Similar results were reported by Chitta *et al.* (2008).

All five extracts from flowers of *P. obtusa* showed effective inhibition of the growth of *B. atrophaeus* at both 1 and 2 mg disc^{-1}, whereby the chloroform-extracted sample had the highest inhibitory activity (among the five samples) causing 77±% of inhibition at 1 mg disc^{-1} and 88% at 2 mg disc^{-1}. The percentages of inhibition by the ethyl acetate extract against *B. atrophaeus* were 54± 2.7% and 69±3.45% at 1 and 2 mg disc^{-1}, respectively. The petroleum ether extract showed inhibitions of 45±2.25% and 65±5.2% (at 1 and 2 mg disc^{-1}, respectively), while the isobutanol extract exhibited inhibitions of 47±5.17% and 54±5.94% against *B. atrophaeus* (at 1 and 2 mg disc^{-1}, respectively). In our earlier study (Ali *et al.*, 2013), in which extracts from the leaves of *P. obtusa* were used, ethyl acetate and petroleum ether fractions showed the highest and equal inhibition activities (61 and 77% at 1 and 2 mg disc^{-1}, respectively) against *B. atrophaeus;* however, unlike the chloroform extract from *P. obtusa* flowers, the chloroform extract of *P. obtusa* leaves showed the lowest (80 and 97% at 1 and 2 mg disc^{-1}, respectively) inhibition activity against *B. atrophaeus.*

All of ethanolic, petroleum ether, chloroform, ethyl acetate and isobutanol extracts from flowers of *P. obtusa* were effective against Gram-positive *S. aureus.* The data further revealed that isobutanol fraction was more effective in inhibiting the growth of *S. aureus* when compared with the other extracts of flowers of *P. obtusa.* The

isobutanol fraction reduced the growth of *S. aureus* by 35±3.85% and 40±4.4% at 1 and 2 mg disc^{-1} respectively. Petroleum ether and ethyl acetate fractions reduced the growth of *S. aureus* by (29±1.45%, 37±2.96%) and (24±1.2%, 39±1.95%), respectively, at the same above concentrations. Similarly, the chloroform extracted sample exhibited inhibitions of 32±2.56% and 34±2.04% at the same above concentrations. It can be seen that none of the extracts could cause growth inhibition of *S. aureus* by more than 50%. Similar results were found previously in another study wherein leaves extracts of *P. obtusa* were used (Ali *et al.*, 2013).

The antibacterial activity of the five different solvents extracted samples from flowers of *P. obtusa* was also checked against the plant pathogen *E. carotovora*. Isobutanol extracted sample exhibited inhibitory activity against *E. carotovora* at both concentrations used. The highest degree of inhibition was found for chloroform fraction (78±5.46%, 91±6.37% at 1 and 2 mg disc^{-1}, respectively), followed by ethyl acetate fraction (62± %, 74± % at 1 and 2 mg disc^{-1}, respectively) and isobutanol fraction (52±5.2%, 68±6.8% at 1 and 2 mg disc^{-1}, respectively). These results are quite different from those reported previously for the extracts of *P. obtusa* leaves; that is, none of the extracts of *P. obtusa* leaves could inhibit the growth of *E. carotovora* by more than 60% even at 2 mg disc^{-1} concentration (Ali *et al.*, 2013).

The antibacterial activity of crude extract, petroleum ether, chloroform, ethyl acetate and isobutanol extracted samples from flowers of *P. obtusa* against *E. coli* by disc diffusion susceptibility method is also shown in Figure 1. The petroleum ether and isobutanol extracted samples effectively inhibited the growth of *E. coli*. The inhibitions of *E. coli* growth by isobutanol fraction were 43±4.73% (at 1 mg disc^{-1}) and 50±6.0% (at 2 mg disc^{-1}) while the inhibition by petroleum ether fraction was 45±3.6% and 49±5.39% at 1 and 2 mg disc^{-1}, respectively. Comparison of the results in this study with those in our previous study (Ali *et al.*, 2013) indicates that the antibacterial activity of flowers extracts against *E. coli* is very similar to that of leaves extracts of *P. obtusa*.

The antibacterial activity of crude extract, petroleum ether, chloroform, ethyl acetate and isobutanol extracted samples from flowers of *P. obtusa* was also measured against *K. pneumoniae* by disc diffusion method. Ethyl acetate and chloroform exhibited maximum activity against *K. pneumoniae*. Ethyl acetate fraction reduced the growth of *K. pneumoniae* by 47±4.7% and 63±6.3% at 1 and 2 mg disc^{-1}, respectively, when compared with their positive controls. The chloroform extracted sample showed 53±3.71% and 62±4.34% inhibitions of *K. pneumoniae* growth at the above mentioned concentrations. *K. pneumoniae* showed no susceptibility to petroleum ether extracted sample from flowers of *P. obtusa* and no inhibition was observed. Similar result was found for petroleum ether extracted sample from leaves of *P. obtusa* in our previous study (Ali *et al.*, 2013).

All the extracts were effective against *S. typhi*, indicating that this strain was susceptible to the crude extract, petroleum ether, chloroform, ethyl acetate and isobutanol extracted samples from flowers of *P. obtusa*. Petroleum ether fraction showed maximum activity against *S. typhi* (52± 4.16% and 56±6.16% at 1 and 2 mg disc^{-1}, respectively) while the crude extract, chloroform and ethyl acetate extracted samples exhibited (31±4.03%, 44±5.28%), (37±2.69%, 44±3.08%) and (35±3.5%, 43±4.3%) reduction in the growth of *S. typhi* at 1 and 2 mg disc^{-1}, respectively. Isobutanol showed inhibitions of 32±2.53% and 48±5.76% at 1 and 2 mg disc^{-1}, respectively.

The inhibitory activity of crude extract, petroleum ether, chloroform, ethyl acetate and isobutanol extracted samples from flowers of *P. obtusa* against *P. aeruginosa* was also screened by disc diffusion assay. It can be seen that ethyl acetate and isobutanol extracted samples at any of the two concentrations did not inhibit the growth of *P. aeruginosa*. Growth inhibitions of ethyl acetate and isobutanol extracted samples of *P. obtusa* flowers in this study were very different from the inhibitions of ethyl acetate

extracted samples of *P. obtusa* leaves (around 28% and 40% at concentrations of 1 and 2 mg disc^{-1}, respectively) and isobutanol (around 27% and 37% at concentrations of 1 and 2 mg disc^{-1}, respectively) tested in our previous study (Ali *et al.*, 2013). Petroleum ether and crude extracts, on the other hand, inhibited the growth of *P. aeruginosa* significantly. Moreover, chloroform extracted sample was more effective in inhibiting the growth of *P. aeruginosa* by 44 ±3.08% and 50 ±3.5% at concentrations of 1 and 2 mg disc^{-1}, respectively.

The results pertaining to degree of inhibition of *C. albicans* by crude extract, petroleum ether, chloroform, ethyl acetate, isobutanol, extracted samples from flowers of *Plumeria obtusa* revealed that all solvent extracts from flowers of *P. obtusa* showed significant antifungal activities against *C. albicans* at both 1 and 2 mg disc^{-1}. The highest inhibition of *C. albicans* growth was exhibited by chloroform fraction (55± 4.95% and 69±6.21% at 1 and 2 mg disc^{-1}, respectively). These results are similar with the activity results of extracted samples of *P. obtusa* leaves, tested in our previous study (Ali *et al.*, 2013) wherein all of the extracts from *P. obtusa* leaves showed inhibition of *C. albicans* in a range of around 20-50%. Crude ethanol extracts showed effective antifungal activity against *C. albicans* (24±2.64% and 31±3.41% reductions in the growth of *C. albicans* by ethanol extracts from the flowers). Similar results were also reported by Chitta *et al.* (2008).

Overall, among all extracts, the chloroform, ethyl acetate and petroleum ether fractions were found to be the most effective in growth inhibition of most of the microorganisms.

Among the tested Gram-positive bacteria (i.e. *B. atrophaeus*, *B. subtilis* and *S. aureus*), *B. subtilis* was highly susceptible to the chloroform extracts from flowers of *P. obtusa*. This inhibitory activity of chloroform extract against *B. subtilis* was increased with increase in concentrations of the extract. In the case of *B. atrophaeus*, effective inhibition was shown by chloroform extract from flowers, while little inhibition from the same extract of leaves was observed in our previous study (Ali *et al.*, 2013). *S. aureus* was susceptible to chloroform extract from both the leaves and flowers of *P. obtusa*. These results agree with those reported by Baghel *et al.* (2010). Chloroform extracts were strongly effective against the growth of Gram-negative *E. carotovora*, *K. pneumoniae* and *P. aeruginosa* at all concentrations. In the case of *E. coli*, chloroform extracted sample from flowers inhibited the growth by 37±2.59% and 45±3.15% at 1 and 2 mg disc^{-1}, respectively. Similar results were also reported by Sulaiman *et al.* (2008). Chloroform extracted sample from flowers was also highly effective in controlling the growth of *C. albicans* as compared to the same extract from leaves. Similar results were also reported by Sulaiman *et al.* (2008).

Ethyl acetate extracts were highly effective against *B. subtilis* and *B. atrophaeus* by disc diffusion assays. Reduction in the growth of *S. aureus* by ethyl acetate extracts were 27% and 39% at concentration of 1 and 2 mg disc^{-1}, respectively. These results agree with those reported by Sulaiman *et al.* (2008). In contrast, ethyl acetate fraction did not inhibit the growth of *P. aeruginosa*. However, same extract from leaves were more effective against this bacterium (Ali *et al.*, 2013). The Gram-negative bacteria *E. carotovora* and *K. pneumonia* were found to be highly susceptible to the ethyl acetate extracted sample from flowers when tested by disc diffusion susceptibility method. In the case of *E. coli*, ethyl acetate fraction reduced their growth by 39±3.9% and 43±4.3% at 1 and 2 mg disc^{-1}, respectively. This findings agree with those reported by Baghel *et al.* (2010).

The petroleum ether fraction from *P. obtusa* flowers showed inhibitory activity against all the microorganisms tested in this study. *B. subtilis* was the most susceptible to petroleum ether fraction. *B. atrophaeus*, *E. carotovora*, *E. coli*, *S. typhi* and *S. aureus* were also susceptible to petroleum ether fractions while in the case of *K. pneumoniae* no reduction in the growth was caused by the petroleum ether fraction. These results agree with those reported by Sulaiman *et al.* (2008). Similarly, petroleum ether extracted sample

had high level of antifungal activity against *C. albicans* tested by disc diffusion assay suggesting that petroleum ether had effective antifungal activity.

The most susceptible Gram-positive bacterium was *B. subtilis* which was inhibited by all the five extracts while the most resistant Gram-positive bacterium was *S. aureus*. *Erwinia carotovora* was the most susceptible Gram-negative bacterium while *P. aeruginosa* was highly resistant among the Gram-negative bacteria. Chloroform extract had varying levels of inhibition activity against all of the tested microorganisms. Ethyl acetate and isobutanol fractions showed inhibitory effects against all the tested microbial species except *P. aeruginosa;* while petroleum ether fraction showed inhibitory activities against all the nine microbial species, except *K. pneumonia*. The antibacterial and antifungal activities explain traditional uses of *P. obtusa* on scientific grounds. Chloroform, ethyl acetate and petroleum ether extracted samples of flowers of *P. obtusa* should be further studied for their antibacterial and antifungal activities since these extracts showed effective antibacterial and antifungal activities.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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