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ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF *PLUMERIA OBTUSA* L.

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Abstract

The present work was carried out to check antibacterial activity of different concentration of leaf extracts of *Plumeria obtusa* L. Extraction was carried out in different solvents (i.e. n-hexane, methanol and chloroform) using Soxhlet's apparatus. Agar well diffusion method was used to check the antibacterial activity of plant extract against two bacterial species i.e. *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Staphylococcus aureus* showed more resistance in all extracts of plant than *Pseudomonas aeruginosa*. Maximum inhibition zones were seen in methanolic plant extracts which were (24.8867 mm ± 0.32501 mm) and (24.6967 mm ± 0.27209 mm) against *S. aureus* and *P. aeruginosa.* Then n-hexane showed clear inhibition zones against *S. aureus* and *P. aeruginosa* i.e. (25.0533 mm ± 0.09074 mm) and (25.0400 mm ± 0.09849 mm). Chloroform extracts also showed clear inhibition zones against both bacterial species but was less resistant than methanol and n-hexane extracts. The diameter was (24.5067 mm ± 0.63129 mm) and (24.0300 mm ± 0.75147 mm) against *S. aureus* and *P. aeruginosa*. While ethanol extracts showed minimum resistance (21.9200 mm ± 0.18520 mm) and (21.9133 mm ± 0.27683 mm) against *S. aureus* and *P. aeruginosa*. All the above results showed that *Plumeria obtusa* could be used for treatment of various diseases and to produce antibiotics.

Key words: Antibacterial activity, Agar well diffusion, Soxhlet's apparatus, Zone of inhibition.

Introduction

Alarge number of medicinal plants are claimed to be useful in various diseases in all traditional system of medicine and folklore. While these plant remedies are being used orally and by local application since ancient, the mechanism whereby such effects elicited has not been looked into. These effects have been brought about by their inherent antibacterial activity by different plants (Deshpande and Chaturvedi, 2014; Devprakash *et al.*, 2011). There are many natural products, which are used as potential antimicrobial agents. Looking to the scope of herbal drug and increasing demand especially in disease of liver, cancer, diabetes, hypertension, renal disease, inflammation, infectious diseases and skin diseases etc (Ali *et al*., 2014; Baghel *et al.*, 2010). The selection of the plant *Plumeria obtusa*
L. was made on the basis of its easy availability, therapeutic value and degree of research work which is not done.

P. obtusa (gulchin) is a class of blossoming plant of family, Apocynaceae. Iridoid glycosides were the primary therapeutically dynamic mixes segregated from the types of *Plumeria*. Along these lines the latex and oil of a portion of these animal categories were found to have other therapeutically dynamic constituents like sterols, sugars, tannins, triterpenoids and alkaloids (Ali *et al*., 2015; Ali *et al*., 2013). Comparative constituents were therefore disengaged from different concentrates of roots and areal pieces of these plants in changed syntheses (Surender *et al*., 2014).

Materials and Methods

Leaves of *P. obtusa* were collected from the campus of Lahore College for Women University Lahore Pakistan. Leaves were washed with tap water to remove the adhering dust particles and dried in shaded area at room temperature for seven days. The dried material was ground in homogenizer to obtained powdered material. The powdered material was used for soxhlet apparatus. The extract was left undisturbed for one day in the apparatus, after which it was removed. The liquid extract was put in a beaker and dried. The same procedure was repeated with other solvents i.e. n-hexane, methanol and chloroform at their suitable temperature. Once the liquid extract had been dried, a hard crust of extract was obtained and the beaker was covered with aluminum foil.

Stock solution was prepared by taking 80 mg plant extract and making the volume up to 10 mL with dimethyl sulfoxide (DMSO). Serial dilutions were made by mixing 5 mL of stock solution and 5 mL of DMSO (4mg/mL). Similarly, 2 mg/mL dilution was prepared by taking 5 mL of 4 mg/mL dilution and 5 mL DMSO, 1 mg/mL dilution was prepared by taking 5 mL of 2 mg/mL dilution and add 5 mL DMSO. 0.5 mg/mL dilution was also prepared by same procedure. So, there were 4 dilutions i.e. 4 mg/mL, 2 mg/mL, 1 mg/mL and 0.5 mg/mL.

Mueller-Hinton Nutrient agar broth was used as media for culturing of bacterial strains. All the test bacterial species were inoculated in the nutrient broth and incubated at 37 °C for 24 hours. Antimicrobial activity of plant extract was checked against different bacterial species by using agar well diffusion method. Bacterial strains were used *Staphylococcus aureus* and *Pseudomonasaeruginosa*.

For media preparation, agar (9.5 g) was added in 250 mL distilled water in a conical flask. It was mixed gently and covered with aluminum foil. Then this media was autoclaved at 15 1b for 20 minutes at 121 °C. After that media was poured into sterilized petri plates then media was allowed to solidify in laminar air flow. Media was inoculated with different cultures of bacterial strains. Using a cork borer, wells were made in solidified nutrient agar medium. Then dilutions of extract were poured in petri plates with the help of micro pipette at four corners and label it with marker. All the petri plates were incubated for 24 hours at 37 °C. After 24 hours antibacterial activity was checked by measuring the diameter of inhibition zone formed as clear region with the help of ruler.

Results

Antibacterial activity of *P. obtusa* leaf extract was determined against two bacterial species *S. aureus* and *P. aeruginosa.* The bacterial strains were grown in already prepared petri plates. Different concentrations of methanolic, ethanolic, chloroform and n-hexane leaf extracts were used for antibacterial activity (table 1). After 24 hours inhibition zones were observed which showed the antibacterial activity with these two strains. Antibacterial activity of stock solution was also checked and inhibition zone was measured. *S. aureus* showed more resistance than *P. aeruginosa.* The diameter of zone increases as the concentration of leaf extract increases as shown in figure 1.

Methanolic and n-hexane extracts had highest inhibition zones i.e. (24.8867 mm ± 0.32501 mm), (24.6967 mm ± 0.27209 mm) and (25.0400 mm ± 0.09849 mm) and (25.0533 mm ± 0.09074 mm) respectively. In the methanolic extracts of *P. obtusa*, the inhibition zones against *P. aeruginosa* and *S. aureus* observed at 4 mg/ml were (20.05 ± 0.15 mm) and (19.86 ± 0.32 mm). At 2 mg/ml the inhibition zones were (19.24 ± 0.38 mm) and (19.32 ± 0.29 mm). Similarly, at the concentration of 1 mg/ml the size of inhibition zone was (17.45 ± 0.47 mm) and (18.21 ± 0.46 mm). At 0.5 mg/ml the inhibition zone was (16.06 ± 0.44 mm) and (15.47 ± 0.46 mm) (table 1b and table 2).

The ethanolic extracts of *Plumeria* also showed antibacterial activity. Against *P. aeruginosa* and *S. aureus* the inhibition zones observed at 4 mg/mL were (18.13 ± 0.14 mm) and (19.87 ± 0.14 mm). At 2 mg/ml the inhibition zones were (17.96 ± 0.05 mm) and (18.07 ± 0.06 mm). Similarly, at the concentration of 1 mg/mL the size of inhibition zones were (16.89 ± 0.16 mm) and (17.55 ± 0.54 mm). At 0.5 mg/mL the inhibition zones observed were (16.01 ± 0.10 mm) and (15.79 ± 0.61 mm) as depicted in table 1c and table 2).

The zones of inhibition of Chloroform extracts at 4 mg/mL (20.59 ± 0.91 mm) and (20.00 ± 0.44 mm) were observed against *P. aeruginosa* and *S. aureus* the inhibition zone. At 2 mg/mL the inhibition zones were (18.16 ± 0.70 mm) and (17.17 ± 0.22 mm). Similarly, at the concentration of 1 mg/mL the size of inhibition zones were (16.95 ± 0.30 mm) and (17.93 ± 0.83 mm). At 0.5 mg/mL the inhibition zones were (16.30 ± 0.45 mm) and (16.10 ± 0.45 mm). The zone of inhibition of stock solution was maximum i.e. (24.0300 ±0.75147 mm) and (24.5067 ± 0.63129 mm) respectively as illustrated in table 1d and table 2.

The n-hexane extract of *P. obtusa* exhibited that against *P. aeruginosa* and *S. aureus* the inhibition zone at 4 mg/ml was (18.83 ± 0.40 mm) and (24.55 ± 0.44 mm). At 2 mg/ml the inhibition zone showed was (19.82 ± 0.42 mm) and (20.11 ± 0.76 mm). Similarly, at the concentration of 1 mg/ml the size of inhibition zone showed was (19.50 ± 0.55 mm) and (17.17 ± 0.75 mm). At 0.5 mg/mL the inhibition zone showed was (19.99 ± 0.08 mm) and (16.10 ± 0.45 mm). The zone of inhibition of stock solution was maximum i.e. (25.0400 ± 0.09849 mm) and (25.0533 ± 0.09074 mm) respectively (table 1e and table 2).

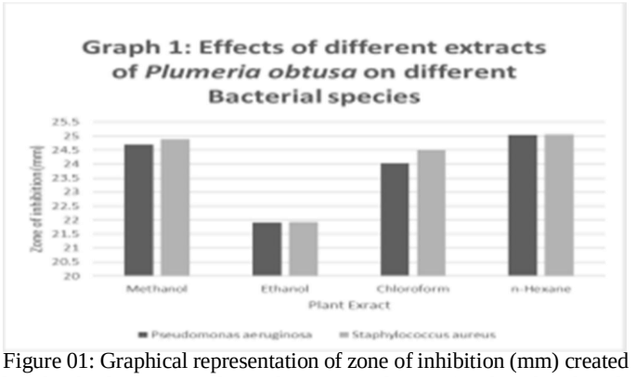


Figure 01: Graphical representation of zone of inhibition (mm) created by the leaf extracts of *P. obtusa* in solvents (methanol, ethanol, chloroform and n-hexane) against *P.auriginosa* and *S. aureus*.

Discussion

Antibacterial activity of *Plumeria alba* (Frangipani) methanolic extracts were evaluated by Syakira and Brenda (2010) against *Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa.* Concentration extracts (80 %) showed the highest inhibition zone towards *Escherichia coli* (14.3 mm). Similarly, in present study the inhibition zones in methanolic extracts of *Plumeria*, against *P. aeruginosa* and *S. aureus* observed at 4 mg/ml were (20.05 ± 0.15 mm) and (19.86 ± 0.32 mm). At 2 mg/mL the inhibition zones were (19.24 ± 0.38 mm) and (19.32 ± 0.29 mm).

Kumar *et al.* (2012) and Farooque *et al.* (2012) revealed the *in vitro* antimicrobial activity of methanol and water extract of the bark of plant *Plumeria alba* Linn. using disc-diffusion method against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* of bacterial strains.

The ethanolic extracts of *Plumeria* also showed antibacterial activity against *P. aeruginosa* and *S. aureus* the inhibition zones observed at 4 mg/mL were (18.13 ± 0.14 mm) and (19.87 ± 0.14 mm). At 2 mg/mL the inhibition zones were (17.96 ± 0.05 mm) and (18.07 ± 0.06 mm). Similar results have been demonstrated by Jaheerunnisa *et al.* (2008) in their study, in which ethanolic extract of *Plumeria acutifolia* stem bark showed antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria. The ethanol extract showed the strong *in vitro* antimicrobial activity against *E. faecalis, B. subtilis, S. aureus, P. aeruginosa, S. typhimurium, A. niger* and *C. albicans* (Choudhary *et al*., 2014).

Muruganantham *et al.* (2015) observed ethanolic extract of *P. rubra* flowers exhibited significant antimicrobial activity. The disc diffusion method result showed the zone of inhibition for 10 mg/mL as 0 mm, 3 mm, 9 mm and 0 mm, for 20 mg/mL as 8 mm,9 mm, 11 mm and 8 mm, for 30 mg/mL showing 10 mm, 11 mm, 12 mm and 10 mm, for 40 mg/mL as 12 mm, 18 mm, 16 mm and 16 mm, for ethanolic extract against *S. typhi, E. coli, E. faecalis* and *B. cereus* respectively. The result indicates that all the test extracts shows good inhibitory activity against all these bacterial strains.

Ahmad *et al.* (2012) showed the antimicrobial (8 bacteria and 1 fungus) activities of different solvent (petroleum ether, ethyl acetate, chloroform, iso-butanol and ethanol) extracted samples from leaves of *Plumeria obtusa* by disc

Table 1: Illustration of antibacterial activity of *P. obtusa* leaves extract in pure and in different concentrations of solvents (i.e., methanol, ethanol, chloroform and n-hexane) against two different pathogenic strains of bacteria i.e., *P. auriginosa* and *S. aureus*.

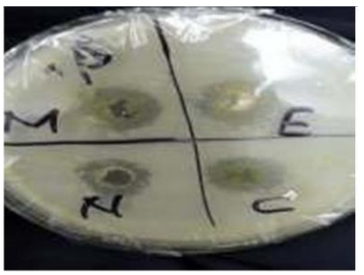
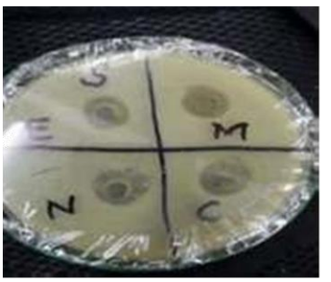
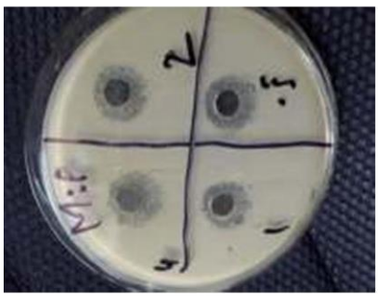
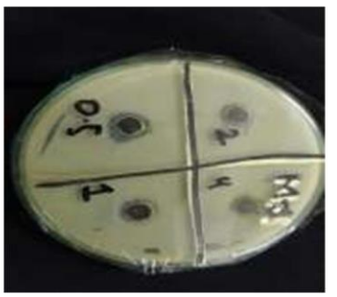
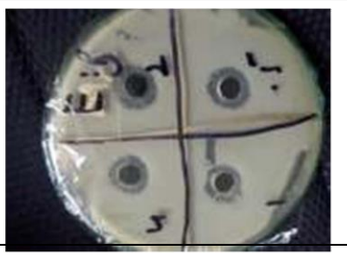
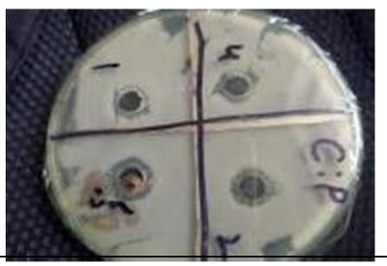
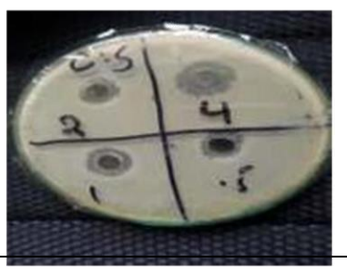
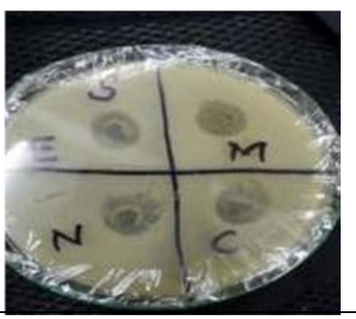
Solvents	*P. auriginosa*	*S. aureus*
a) Pure extract of leaves		
b) Methanol extract		
c) Ethanol extract		
d) Chloroform extract		
e) n-hexane extract		

Table 2: Effects of leaf extracts of *Plumeria obtusa* in different solvents against pathogenic bacterial species.

Sr. No.	Solvents	Concentration of plant extract mg/ml	Inhibition zone (mm)	
			Pseudomonas Aeruginosa	***Staphylococcus aureus***
1.	Methanol	4	19.8600 ± 0.32604	20.0533 ± 0.15177
2.		2	19.2400 ± 0.38000	19.3233 ± 0.29704
3.		1	17.4567 ± 0.47248	18.2133 ± 0.46372
4.		0.5	15.4700 ± 0.46861	16.0600 ± 0.44227
5.	Ethanol	4	18.1333 ± 0.14189	19.8733 ±0.14572
6.		2	17.9667 ± 0.05859	18.0733 ± 0.06888
7.		1	16.8933 ± 0.16166	17.5500 ± 0.54836
8.		0.5	15.7933 ± 0.61011	16.0133 ± 0.10970
9.	Chloroform	4	20.0033 ± 0.44775	20.5900 ± 0.91090
10.		2	17.1600 ± 0.70534	17.1733 ± 0.22189
11.		1	16.9567 ± 0.30989	17.9333 ± 0.83770
12.		0.5	16.3067 ± 0.45786	18.0700 ± 0.07638
13.	n-hexane	4	18.8300 ± 0.40336	24.5500 ± 0.44441
14		2	19.8267 ± 0.42771	20.1167 ± 0.76514
15.		1	17.1767 ± 0.75659	19.5033 ± 0.55537
16.		0.5	16.1033 ± 0.45214	19.9977 ± 0.08021

diffusion method. The data revealed that petroleum ether, iso-butanol and ethyl acetate fractions showed inhibitory activities against all the nine microbial species except *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, respectively (Goyal *et al*., 2015; Manisha and Aher, 2016). The most susceptible gram-positive bacterium was *Bacillus subtilis* while the most resistant gram-positive bacterium was *Staphylococcus aureus***.** Likewise in this study plant extracts of chloroform and n-hexane also exhibited notable antimicrobial activity against the two strains of bacteria.

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