Antimicrobial activity of different solvent extracted samples from the flowers of medicinally important *Plumeria obstusa*

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Abstract: The present research work was carried out to investigate the antimicrobial (eight bacteria and one fungus) activities of different solvent (ethanol, petroleum ether, chloroform, ethyl acetate and isobutanol) extracted samples from flowers of *P. obstusa* by disc diffusion method. Analysis of the data revealed that all the five extracts from flowers of *P. obstusa* showed different ranges of antimicrobial activities. Petroleum ether fractions showed inhibitory activities against all the nine microbial species except *Klebsiella pneumonia.* Ethyl acetate and isobutanol fractions showed inhibitory effects against all the tested microbial species except *Pseudomonas aeruginosa.* Chloroform and ethanol extracts had varying levels of inhibitions against all of the tested microorganisms. The most susceptible gram positive bacterium was *Bacillus subtilis* which was inhibited by all the five extracts while the most resistant gram positive bacterium was *Staphylococcus aureus*. *Erwinia carotovora* was the most susceptible gram negative bacterium while *Pseudomonas aeruginosa* was highly resistant among the gram negative bacteria.

Keywords: Antimicrobial activity, solvents, fungus, disc diffusion, *Plumeria obstusa*

INTRODUCTION

The steadily increasing bacterial resistance to existing drugs is a serious problem in antibacterial therapy and necessitates continuing research into new classes of antibacterial (Essawi and Srour, 2000; Wood ford, 2003). One way to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). Plants and plant-derived agents have long history to clinical relevance as source of potential chemotherapeutic agents (Cushine and Lamb, 2005). Thousands of plant species have been screened for their antimicrobial activity, but relatively few were found to be sufficiently active (Poyart *et al*., 1990) and non-toxic to humans (Izzo, 2004). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents (Amani *et al*., 1998; Arias *et al*., 2004; Al-Bayati and Sulaiman, 2008; Ordonez *et al*., 2009; Al-Bayati, 2011) with possibly novel mechanisms of action (Hamil *et al*., 2003; Machado *et al*., 2003; Motsei *et al*., 2003; Barbour *et al*., 2004; Bakht *et al*., 2011 a, b, c and d; 2012; 2013 a, b; 2014 a, b, c).

Plumeria belongs to Apocynaceae which is a large family of about 300 genera with more than 1400 species, found predominantly in tropics and sub-tropics (Dassanayake and Fosberg, 1983). *Plumeria* is an introduced ornamental plant commonly known as 'Araliya' or temple tree. The flowers are widely used for religious purposes in Sri Lanka. The *plumeria* plant is mainly grown for its ornamental and fragrant flowers. Methanolic extract of this flower has showed antimicrobial activity against *Bacillus anthracis* and *Pseudomonas aeruginosa* (Amani *et al*., 1998). Species of *Plumeria* include *P. rubra, P. acutifolia, P. obtusa, P. obtusifolia, P. alba, P. bicolor, P. tricolour and P. jamesoni.*

MATERIALS AND METHODS

The present study was conducted at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar KPK Pakistan. Flowers were collected from the University of Agriculture Peshawar KPK Pakistan and PCSIR Lab Complex Peshawar Khyber Pakhtunkhwa. The collected plant materials were shade dried. The dried plant materials were finely powdered by tissue homogenizer (Infinigen™ Tissue Mixer Mill).

Crude extract preparation

Dried powdered flowers were macerated in 97% ethanol for 6 days. During this period the solution was stirred occasionally for thorough mixing. The ethanol soluble compounds were then filtered by Wattman filter paper (Whatman™). Fresh ethanol was added to the remaining plant material and filtered again and this process was repeated thrice. The filtered ethanolic solution was subjected to rotary evaporator for evaporation (Rotavapor ®-R 210/R215; BUCHIL Labortechnik AG). Ethanol was separated at 45 °C under vacuum pressure and semi-solid extract was obtained (crude extract).

Crude extract fractionation

The crude extracts obtained were divided into two portions. One portion was poured into a glass vial to be tested as crude ethanol extract for antimicrobial activity.

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The second portion was further fractionated with different solvents. The second portion was dissolved in 200ml distilled water by glass stirrer, poured into separate funnel and distilled petroleum ether was added into it. Compounds soluble in upper petroleum ether phase were collected and the lower aqueous phase was extracted three times with petroleum ether. All fractions of hexane were combined and semisolid hexane fraction was removed through rotary evaporator. The semisolid petroleum ether fraction was dried in water bath at 45ºC and stored in glass vials until used. The same process of fractionation was carried out for chloroform, ethyl acetate, isobutanol and aqueous phase.

Culture media and its preparation

Nutrient agar media (HiMedia Laboratories Pvt. Ltd.) was used for the culturing and growth and nutrient broth was used for shaking incubation and standardization of different microorganisms. Media was prepared as described in Bakht *et al*. (2011 a, b, c, d; 2012; 2013 a, b).

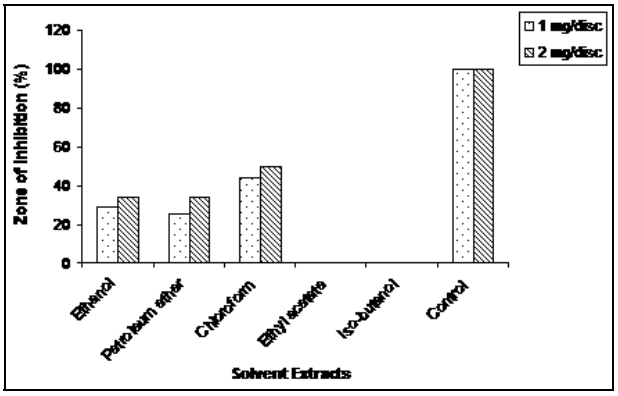

Fig. 1: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *pseudomonas auriginosa* by disc diffusion assay

Microorganisms tested

Antibacterial and anti-fungal activity of different solvent extracted samples from flowers was tested against different bacterial and fungal strains (table 1).

Disc diffusion susceptibility method

The antibacterial activity of different solvent extracted samples of *P. obstusa* was carried by disc diffusion assay as described in Bauer *et al*. (1998) and antifungal activity by Ramdas *et al*. (1998). Antibiotic and anti-fungal drugs used as positive control for Gram positive, Gram negative bacteria and fungus were Erythromycin, Ciprofloxacin and Clotrimazole respectively.

For Gram positive bacteria: Erythromycin 50µg $6\mu l^{-1}$
For Gram negative bacteria: Ciprofloxacin 50µg $6\mu l^{-1}$
For Fungi: Clotrimazole 50µg $6\mu l^{-1}$

RESULTS

Fig. 1 reveals the inhibitory activity of ethanol, petroleum ether, chloroform, ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Pseudumonas auriginosa* by disc diffusion susceptibility assay. Ethyl acetate and isobutanol extracted samples did not inhibit the growth of *Pseudumonas auriginosa* at any concentration when compared with inhibition by azithromycin (positive control). Both of these extracts exhibited zero percent inhibition of *Pseudumonas auriginosa* grown on nutrient agar media.

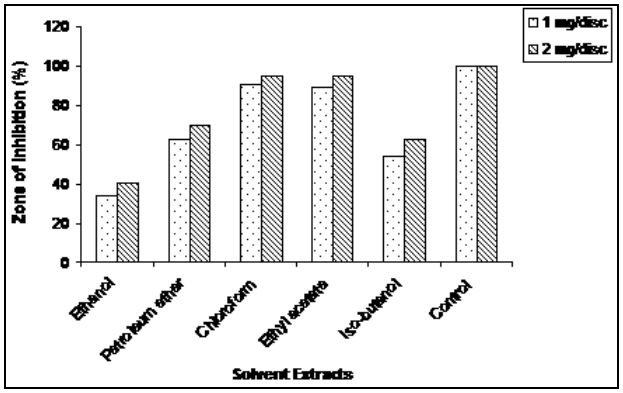

Fig. 2: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Bacillus Subtillis* by disc diffusion assay.

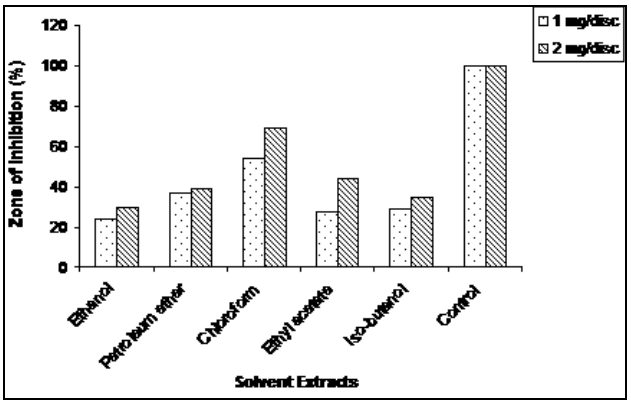

Fig. 3: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Candida aibicans* by disc diffusion assay.

Petroleum ether and ethanol extracts, on the other hand, inhibited the growth of *Pseudumonas auriginosa* effectively at both concentrations. Moreover, chloroform extracted samples were more effective in inhibiting the growth of *Pseudumonas auriginosa* by 44% and 50% ZI (zone of inhibition) at 1 and 2 mg $disc^{-1}$ concentration respectively. It is clear from the data shown in fig. 2 that ethanol extracted samples were found to be less effective against *Bacillus subtilis* at both concentrations. *Bacillus*

subtilis was highly susceptible to chloroform extracted samples followed by ethyl acetate extracted samples. Highest antibacterial activity was shown by chloroform and ethyl acetate extracted samples at high concentration (2mg disc^{-1}) when compared with azithromycin. Chloroform and Ethyl acetate recorded 91%, 95% (ZI) and 89%, 95% at 1 and 2 mg disc^{-1} respectively.

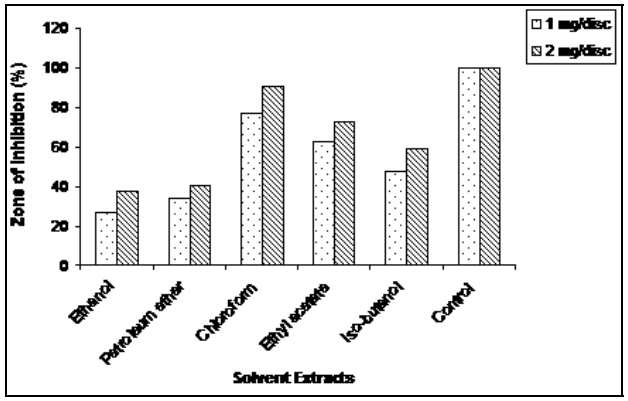

Fig. 4: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Erwinia carotovora* by disc diffusion assay.

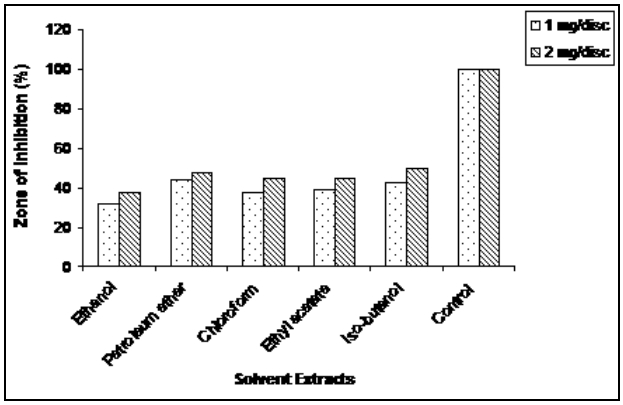

Fig. 5: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Escherichia coli* by disc diffusion assay.

Analysis of the data revealed that ethanol, ethyl acetate, isobutanol and petroleum ether extracted samples were all active against *Candida albicans* at both concentrations (fig. 3). The highest inhibition in *Candida albicans* growth was recorded for chloroform extracted samples (54% and 69% ZI at 1 and 2 mg disc^{-1} respectively) followed by petroleum ether at 2 mg disc^{-1}. The antibacterial activity of all five different solvents extracted samples from flowers of *Plumeria obstusa* against *Erwinia carotovora* by disc diffusion susceptibility assay is indicated in fig. 4. The data indicated all five solvent extracted samples reduced the growth of *Erwinia carotovora* at both concentrations. Chloroform and ethyl acetate extracted samples showed highest inhibition of 77%, 91% and 63%, 73% (ZI) at 1 and 2 mg disc^{-1} concentration respectively. Similar results are also reported by Bakht *et al*. (2011 a, b, c and d; 2012; 2013). Different solvent extracts obtained from flowers of *Plumeria obstusa* showed effective antibacterial activity against *E. coli* is shown in fig. 5. Petroleum ether and iso-butanol extracted samples were more effective against *E. coli* growth when compared with the rest of extracts.

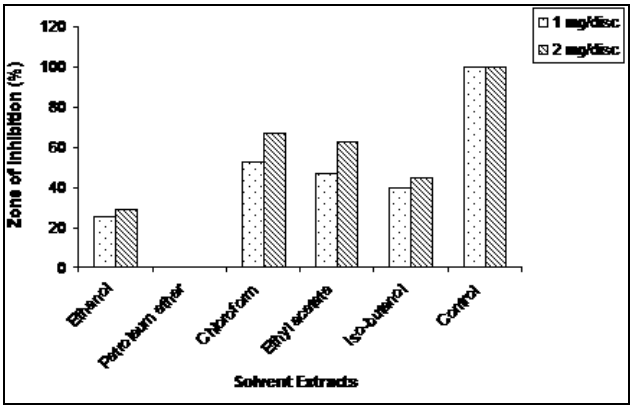

Fig. 6: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Klebsiella Pneumoniae* by disc diffusion assay.

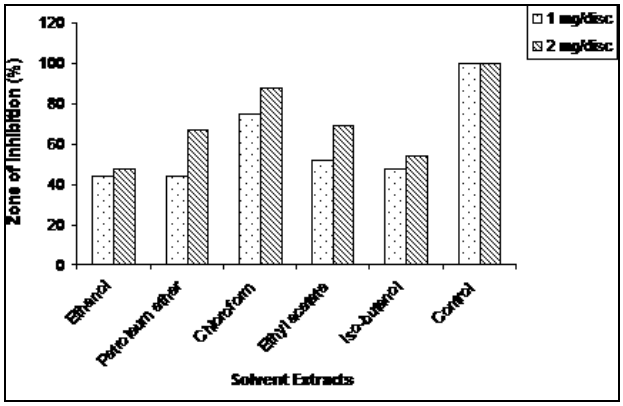

Fig. 7: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Bacillus atrophaeus* by disc diffusion assay.

Isobutanol extracted samples reduced the growth of *E. coli* by 43% and 50% (ZI) at 1 and 2 mg disc^{-1} while petroleum ether fraction inhibited *E. coli* by 44% and 48% at 1 and 2 mg disc^{-1} respectively. *Klebsiella pneumoniae* showed resistance to petroleum ether extracted samples from flowers of *Plumeria obstusa* (fig. 6). Ethyl acetate and chloroform showed maximum activity against *Klebsiella pneumonia.* Chloroform extracted sample showed 53% and 63% inhibitions of *Klebsiella pneumoniae* growth at 1 and 2 mg disc^{-1} respectively. Similarly, ethyl acetate fraction reduced the growth of *Klebsiella pneumoniae* by 47% and 63% at 1

Table 1: Microbial strains used in this research work

Microbial species	Gram Strain type	Details of the microbial strains used
Bacillus subtilis	Positive	Clinical isolate obtained from Microbiology Laboratory of Quaid-e-Azam University Islamabad Pakistan
Candida albicans	Fungus	Clinical isolate obtained from Hayatabad Medical Complex Peshawar, KPK Pakistan
Erwiniacarotovora	Negative	Department of Plant Pathology KPK Agricultural University Peshawar Pakistan
Escherichia coli	Negative	ATCC # 25922
Kleibsiella pneumonia	Negative	Clinical isolate obtained from Microbiology Laboratory of Quaid-e-Azam University Islamabad Pakistan
Pseudomonas aeruginosa	Negative	ATCC # 9721
Salmonella typhi	Negative	Clinical isolate obtained from Microbiology Laboratory of Quaid-e-Azam University Islamabad Pakistan
Staphylococcus aureus	Positive	ATCC # 6538
Bacillus atrophaeus	Positive	Clinical isolate obtained from Microbiology lab. PCSIR, Peshawar
Agrobacterium tumefacien	Negative	Clinical isolate obtained from Microbiology lab. PCSIR, Peshawar

Disc Diffusion Susceptibility Method

and 2 mg disc^{-1} respectively when compared with their positive controls. Analysis of the data also indicated that all the five extracts from flowers of *Plumeria obstusa* showed effective inhibition against the growth of *Bacillus atrophaeus* at both concentrations (fig. 7).

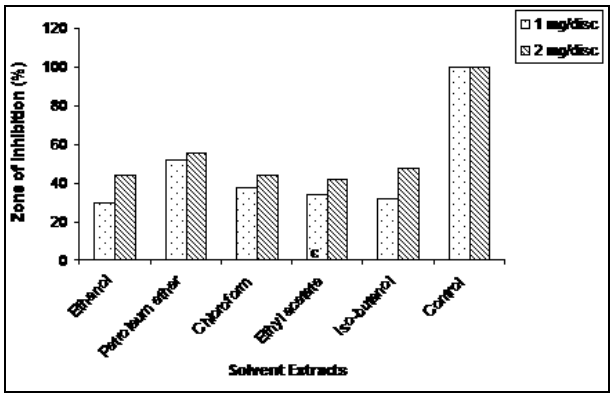

Fig. 8: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Salmonella typhi* by disc diffusion assay.

Chloroform extracted samples had the highest inhibitory activities (75% and 88% zone of inhibition at 1 and 2mg disc^{-1} respectively). Ethyl acetate and iso-butanol extracted samples were also effective against this bacterium reducing its growth 52% and 69% at 1 and 2mg disc^{-1} respectively. Petroleum ether extracted sample recorded growth inhibitions of 44% and 67%, while iso-butanol showed inhibitions of 48% and 54% against *Bacillus atrophaeus* at 1 and 2mg disc^{-1} respectively. These results also agree with Bakht *et al*. (2011 a, b, c and d; 2012; 2013 a, b; 2014).

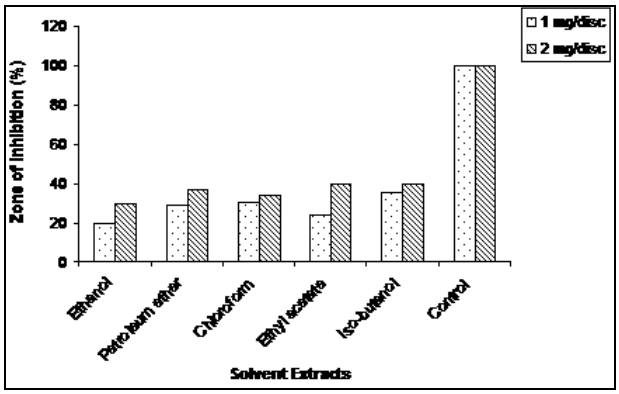

Fig. 9: Antibacterial activity of ethanol, petroleum, chloroform ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa* against *Staphylococcus aureus* by disc diffusion assay.

Fig. 8 shows the antibacterial activities of different flowers extracts of *Plumeria obstusa* against *Salmonella typhi* by disc diffusion susceptibility method. *Salmonella typhi* was susceptible to ethanol, petroleum ether, chloroform, ethyl acetate and isobutanol extracted samples from flowers of *Plumeria obstusa*. Petroleum ether extracted samples showed maximum activity at higher concentration (56% at 2mg disc^{-1}). Ethanol, chloroform and ethyl acetate extracted samples reduced the growth of *Salmonella typhi* by 44%, 44% and 42% at 2mg disc^{-1}. Isobutanol recorded zone of inhibition of 32% and 48% at 1 and 2 mg disc^{-1} respectively. Our results suggested that isobutanol extracted sample was more effective in controlling the growth of *Staphylococcus aureus* when compared with other extracts. Isobutanol extracted samples reduced the growth of *S. aureus* by 36% and 40% at 1 and 2 mg disc^{-1} concentration

respectively. Petroleum ether and ethyl acetate fractions reduced the growth of *Staphylococcus aureus* by 29%, 37% and 24%, 40% at the same concentrations. Similarly, chloroform extracted sample recorded zone of inhibitions of 31% and 34% at the same concentrations (fig. 9).

DISCUSSION

The antimicrobial activity of different solvent extracted samples from flowers of *Plumeria obstusa* was investigated by disc diffusion susceptibility assay. Ethyl acetate and isobutanol extracted samples showed no activity against *Pseudumonas auriginosa* at any concentration when compared with activity recorded by azithromycin (positive control). Contrary to these solvent extracted samples, petroleum ether and ethanol extracts, on the other hand, control the growth of *Pseudumonas auriginosa* effectively at both concentrations. Moreover, chloroform extracted samples were more effective in inhibiting the growth of *Pseudumonas auriginosa* at 1 and 2 mg disc^{-1} concentration. These results agree with Al-Bayati and Sulaiman (2008), Baghel *et al*. (2010) and Bakht *et al*. (2011 a, b, c and d; 2012; 2013 a, b; 2014). Our results revealed that ethanol extracted samples were found to be less effective against *Bacillus subtilis* at both concentrations. *Bacillus subtilis* was highly susceptible to chloroform extracted samples followed by ethyl acetate extracted samples. Maximum antibacterial activity was measured by chloroform and ethyl acetate extracted samples at high concentration (2mg disc^{-1}) when compared with azithromycin. These results agree with those reported by Bhagel *et al*. (2010) and Bakht *et al*. (2011 a, b, c and d; 2012; 2013 a,b; 2014).

The data also indicated that chloroform, ethanol, ethyl acetate, isobutanol and petroleum ether extracted samples were active against *Candida albicans* at both concentrations. The highest inhibition in *Candida albicans* growth was recorded for chloroform extracted samples followed by petroleum ether at 2 mg disc^{-1}. Similar results were also reported by Bakht *et al*. (2011 a, b, c and d; 2012; 2013). The data revealed all five solvent extracted samples reduced the growth of *Erwinia carotovora* at both concentrations. Chloroform and ethyl acetate extracted samples showed highest inhibition at 2 mg disc^{-1} concentration. Similar results are also reported by Bakht *et al*. (2011 a, b, c and d; 2012; 2013). Petroleum ether and iso-butanol extracted samples were more effective against *E. coli* growth when compared with the rest of extracts. Isobutanol extracted samples reduced the growth of *E. coli* at both concentration followed by petroleum ether fraction. These results agree with Baghel *et al*. (2010) and Bakht *et al*. (2011 a, b, c and d; 2012; 2013). *Klebsiella pneumoniae* showed resistance to petroleum ether extracted samples from flowers of *Plumeria obstusa*). Ethyl acetate and chloroform showed maximum activity against *Klebsiella pneumonia* when compared with their positive controls.

Our results also indicated that all the five extracts from flowers of *Plumeria obstusa* effectively control the growth of *Bacillus atrophaeus* at both concentrations. Chloroform extracted samples had the highest inhibitory activities followed by ethyl acetate and iso-butanol extracted samples. Petroleum ether and iso-butanol extracted sample also reduced the activity of *Bacillus atrophaeus* at 1 and 2mg disc^{-1}. These results also agree with Bakht *et al*. (2011 a, b, c and d; 2012; 2013 a, b; 2014). *Salmonella typhi* was susceptible to ethanol, petroleum ether, chloroform, ethyl acetate and isobutanol extracted samples from the flowers of *Plumeria obstusa*. Petroleum ether extracted samples showed maximum activity at higher concentration followed by ethanol, chloroform and ethyl acetate extracted samples. Similar results are also reported by Bakht *et al*. (2011 a, b, c and d; 2012; 2013). Our results also suggested that isobutanol extracted sample was more effective in controlling the growth of *Staphylococcus aureus* at both concentrations followed by petroleum ether and ethyl acetate and chloroform fractions.

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