

Antibacterial and Antibiofilm Activities from Extracts of Selected Cholistani Plants

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Abstract

The Cholistani plants are used as a vital source of medicines by local nomads since long time. Recently, many pharmacological studies like antibacterial, antiviral, antifungal and anti diabetic are done, but very few chemistry-based approaches are available. The current study was designed to evaluate the antibacterial potentials of extracts of Cholistani plants. Aqueous, ethanol, and *n*-hexane extracts were prepared. Extracts are made on the basis of solvent polarity. All the extracts were tested for antibacterial activities through disc diffusion method. The Minimum Inhibitory Concentration (MIC) of each positive extract was measured. The results indicated that the aqueous extract of these plants was more effective than others. The overall trend of antibacterial activity was as follows aqueous >*n*-hexane >ethanol extracts in terms of solvent. In terms of bacteria, almost all extracts were effective against *Klebsiella pneumonia*. The overall antibacterial trend was *Klebsiella pneumonia*>*Proteus vulgaris*>*Staphylococcus aureus*>*Escherichia coli*>*Pseudomonas aeruginosa*. The results indicate that Cholistani plants are a rich source of antibacterial agent(s) and can be used in crude or purified form.

Keywords: Antibacterial activity, Cholistani plants, *E.coli*, *K.pneumonia*.

1. Introduction

Plants are a great source of medicine and food. The history of medicinal plants is as old as the history of humanity. According to World Health Organization, millions of people all around the world use conventional medicines (Arunkumar and Muthuselvam, 2009). In Pakistan, almost 6000 species of flowering plants are reported with medicinal values, and over 700 plants have been confirmed for therapeutic use by providing principal active agent(s) (Shinwari, 2010). The scientific and economic importance of medicinal plants increases and the demand for plant-based products is continuously arising in developing as well as developed countries. Antibacterial potentials of medicinal plants are not only solving the availability of new drugs and but also addressing the issue of multi-drug-resistant strains (Ali and Jahangir, 2001). In recent times, plant-based antimicrobial agents have played an essential role in minimizing the global burden of infectious diseases (Theuretzbacher *et al.*, 2020). Antimicrobial resistance has increased the pressure to find alternative solutions to cope this challenge (Bacha *et al.*, 2016). Antibiotic resistance is mainly caused by inappropriate or over-use of antibiotics and the emergence of a complex extracellular matrix called biofilm (Parrino *et al.*, 2018). The development of

biofilm ability is based on various mechanisms, including; quorum sensing signals, exo polymeric matrix development, antibiotic-resistant cell wall production, beta-lactamase enzymes production, and any other drug-resistant genes expression (Segev-Zarko *et al.*, 2015; Saxena *et al.*, 2019; Gupta *et al.*, 2018). Cholistani plant *Achyranthes asper* (Amaranthaceae) is known as puthkanda in the local language, is well known for its spermicidal and cardiovascular activities (Srivastava *et al.*, 2011). *Haloxylon recurvum* (Chenopodiaceae) is called lani in the local language and is used externally against insect bites and also considered as good source of fuel (Daglaand Shekhawat, 2005). *Neurada procumbens* (Neuradaceae) is commonly known as chapribooti. It is well known for its antiviral activities (Shahzad *et al.*, 2019). *Octhocloa compressa* (Poaceae), commonly known as spider grass, is used as the source of fodder (Ahmed *et al.*, 2014). *Haloxylon silicornicum* (Amaranthaceous) is called khar (Shafi *et al.*, 2002). It is a potential source of firewood, and its extracts are also used to treat diabetes and eye disorders (Naeem *et al.*, 2000). *Oxystelma esculentum* (Asclepiadaceae) is a twiner growing in water logged areas. It is used traditionally as diuretics and anti-ulcer formulations (Shehzad *et al.*, 2020; Pandya and Anand, 2013). *Suaeda fruticose* (Amaranthaceae), known as kali lani. It is a shrubby halophyte and can resist reactive oxygen species (Samiullah *et al.*, 2012). *Sporobolu sicolados* (Poaceae), commonly known as sakham, are mainly used as forage (Gulzar and Khan, 2015). *Solanum surattense* (Solanaceae), widely known as Indian nightshade, has been used to cure fever, cough, asthma and diabetes (Munuswamy *et al.*, 2013). *Salsola bryosoma* (Chenopodiaceae) is commonly called the dandy of *Chenopodiaceae* and is used as fodder, fuel, and fire set. This plant is used in Mauritania for laundering cloth. *Panicum antidotale* (Graminae) and commonly known as bansi ghass. It is used to control soil erosion (Arshadullah *et al.*, 2009). Bacterial diseases are a general cause of morbidity in the human health and livestock industry. *Staphylococcus aureus* is a Gram-positive bacterium, causes carbuncles, skin abscesses, and soft tissue infections (Ooi *et al.*, 2006), and *Klebsiella pneumoniae* is a Gram-negative bacterium and causes liver pneumonia, brain and lung abscess. *Pseudomonas aeruginosa* is a common cause of nosocomial infections and is famous for urinary tract infections (Shaan and Robert, 2013). *Proteus vulgaris* is a Gram-negative bacterium, commonly causes urinary tract infections (Hara, 2005). *Escherichia coli* is a Gram-negative, rod-shaped bacterium commonly found in the large intestine of different species. It causes the hemolytic-uremic syndrome, peritonitis, mastitis, septicemia (Todar, 2007). The emergence of new strains of bacteria and antimicrobial resistance against a current lot of antibiotics poses a serious threat to the future of the currently used regime of antibiotics. There is a need to screen more eco-friendly antibiotics, either made synthetically or isolated from natural sources. Traditionally medicinal plants have more acceptances in public to use. They are cheap, effective, and easy to use. The trends to use medicinal plants against common infections continue to rise world wide (Beulah *et al.*, 2011). The current and other recent studies have supported the natural richness of Cholistani plants against common infections (Aslam *et al.*, 2016; Shahzad *et al.*, 2019). In recent years, the study on anti biofilm potential by various plant extracts and oils have been studied. The antibacterial and anti biofilm possibilities of EtOH extracts of 14 plants were investigated by Wije sundara and Rupasinghe (2019). The significant anti-bacterial and anti-biofilm potential have been reported by MeOH and EtOAc extracts of *Cinnamomum zeylanicum*, *Azadirachta indica* and *Syzygium aromaticum* against *Pseudomonas aeruginosa* MDR strain (Jahan *et al.*, 2018). Similarly, the oil from *Melaleuca alternifolia*, *Syzygium aromaticum*, aerial part explored the anti-bacterial and anti-biofilm potentials.

2. Materials and Methods Collection of specimens

Eleven fresh plants, including *Oxystelm aesculentum* L.F. (Asclepiadaceae), *Haloxylon salicornicum* Moq. (Amaranthaceae), *Haloxylon recurvum* Moq. (Chenopodiaceae), *Achyranthes aspera* ITIS. (Amaranthaceae), *Solanum surattense* Linn. (Solanaceae), *Salsola baryosma* Roem. Et Shult (Chenopodiaceae), *Neuroda procumbens* L. (Neuradaceae), *Panicum antidotale* Retz. (Poaceae), *Sporobolus icolado* sNees ex Trin (Poaceae), *Ochthochloa compressa* CoL (Poaceae), and *Suaeda fruticosa* auct. non-Forsk (Amaranthaceae) were collected from Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur. The plant was identified and voucher numbers were assigned by a taxonomist from the Department of Botany, The Islamia University of Bahawalpur, Pakistan. Taxonomists and vouchers authenticated the plants were saved at CIDS, The Islamia University of Bahawalpur.

Bacterial strains

Five bacterial strains, including *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *K. pneumonia*, and *E. coli*, were obtained from Microbiology lab, Bahawal Victoria Hospital (BVH) and their inoculums were saved at the Department of Biochemistry, The Islamia University of Bahawalpur. All the bacterial strains were confirmed through various biochemical tests, colony morphology, and particular media growth. Reference ATCC strains were also used as positive controls in these tests.

Media preparation

The nutrient broth was made by adding 1.3g in 100mL autoclaved distilled water and agar media was prepared by dissolving 2.8g media in 100mL distilled water. The media were autoclaved for 20 min at 121°C and 15psi.

Solvent Extraction

Aqueous extracts: 10gm powdered plant was mixed with 100mL PBS (pH7.2). The mixture was passed through the freeze and thaw procedure three times. Finally, the mixture was centrifuged at 4000 rpm, and the supernatant was sterilized by passing through 0.22 μ syringe filter.

n-hexane and Ethanol extracts: 10g powdered plants were soaked in 100mL n-hexane. The mixture was kept in an airtight container and stirred for 42hrs. Later, 100mL absolute ethanol was added to the mixture and again stirred for 42hrs. Finally, the layers were separated from each other and passed through a 0.22 μ syringe filter. Each extract was air-dried, and precipitates were dissolved @500mg/ml in each respective solvent.

Phytochemical Evaluation / Preliminary Phytochemical Screening

The phytochemical screening of extracts was performed by standard methods (Banu and Cathrine, 2015; Bhandari *et al.*, 2017).

Total bioactive Contents

The total phenolic content (TPC) of extracts was determined by the Folin-Ciocalteu Reagent protocol with slight modifications of Noreen *et al.* (2017), and results were expressed as equivalent of gallic acid (mg GAE/g extract). The total flavonoid content (TFC) of extracts was determined by the method of Akhtar and Mirza (2015), and TFC were expressed as equivalent of rutin (mg RE/g extract).

Antibacterial assay

Whatman filter paper (qualitative grade gf 1) was used for making discs of 6mm diameter. Sterile discs were soaked in respective plant extract and later used in the antibacterial assay. Solvent controls (negative control) were made by soaking discs in all solvents like PBS, ethanol, and *n*-hexane. The discs soaked in chloramphenicol solution (50mg/mL) were used as positive control.

Bacterial propagation: Bacterial strains were obtained from glycerol stocks. The cultures were incubated at 37°C for 16-18 hrs under constant shaking. Later 0.5 mL culture was spread on agar plates, and the antibacterial assay was performed as the method described by (Morshed *et al.*, 2012). The freshly inoculated culture plates were incubated at 37°C for 1 hr. Then pre-soaked discs were applied against each microbe along with controls. The plates were incubated at 37°C for 24 hrs. The zone of inhibition (ZOI) was measured as the method described by (Hussain *et al.*, 2015). All tests were performed in triplicate, and the minimum inhibitory concentration (MIC) of each positive extract was calculated (Perumal *et al.*, 2012).

Determination of MIC value

For MIC, round bottom 96 well plates were used. 50µL nutrient broth was added to each well. 50µL of extract was added to the first well and mixed gently. Transfer 50µL mixture from first well to second well and similarly serially dilute it till 11th well. The 12th well was used as PBS control. 50µL microbial culture was added to each well, and the plate was incubated at 37°C for 16hrs. The 50 µL of 2mg/ml solution of *p*-Iodonitrotetrazolium Chloride (Sigma, cat # 18377) was added to each well and incubated at 37°C for 1hr. The color change indicates the MIC of the extract.

Anti-biofilm activity

The anti-biofilm activity of extracts was performed according to the protocol described by Sambyal *et al.*, 2017. After calculating MIC of each extract, an equal volume of extract and bacterial culture were added to the microtiter plate and incubated at 37°C for 24 hrs. After incubation, the cells were washed and fixed with PBS and 99 % methanol. Then, cells were stained with crystal violet, resolubilized by 33% glacial acetic acid, OD₆₃₀ was recorded, and % of biofilm inhibition was calculated. Ampicillin and moxifloxacin were used as positive controls.

Scanning Electron Microscopy

The biofilm was developed on the microscopic slide with the procedure mentioned above and visualized first through the light microscope (Schlafer and Meyer, 2017). Later, the surface morphology of biofilm was visualized by scanning electron microscopy at the centralized Laboratory, University of Peshawar, Peshawar, Pakistan (Gomes and Mergulhão, 2017).

Statistical analysis

The obtained data from various studies were interpreted by using one-way analysis of variance (ANOVA) through SPSS 20, IC₅₀ was calculated from Graph Pad Prism software version 8. The graphical figures were operated in Origin pro 8.5 software (Montgomery, 2017).

3. Results

According to the results of this study, ethanol, *n*-hexane, and aqueous extracts from 11 different Cholistani plants have shown remarkable antibiofilm, antibacterial activity against various strains of bacteria.

Phytochemical Investigation

The extract yield and phytochemical extraction from the same plants highly depend on the nature of solvents. The different extracts of the same plants have different levels of activity due to the presence and

abundance of various phytochemicals (Alam *et al.*, 2020). The overall % yield of aerial extract was according to the order Aqu>MeOH>*n*-But >EtOAc>*n*-Hex > DCM. Similarly the order of % yield of floral extracts was Aqu>MeOH>EtOAc>*n*-But> DCM >*n*-Hex. Preliminary phytochemical investigations confirm the max presence of phytochemicals in Aqu, MeOH and DCM extracts, while *n*-Hex extracts were found least effective in phytochemical extraction. Similar results have been reported (Alam *et al.*, 2020). Many plant-based active metabolites have been reported with strong antimicrobial activity (Khan *et al.*, 2014; Stanković *et al.*, 2016; Zuo *et al.*, 2018). The TPC and TFC of MeOH, *n*-But, *n*-Hex and Chloroform extract of *N. procumbens* were reported, and the max TPC and TFC were seen in *n*-But and MeOH extracts, respectively (Khurshid *et al.*, 2019). According to the results of the current study, the TPC and TFC of aerial and floral extracts were ranging from 28.19-127.13 GAE mg/g and 33.2-78.23 RE mg/g of extract, respectively, and these results are slightly different to Khurshid *et al.*, (2019). In the aerial part, the highest amount of TPC was observed in *n*-But extract, while the lowest was exhibited by Aqueous extract. Likewise, the highest TFC was seemed by MeOH extract, while the lowest was observed in the *n*-Hex extract. In the floral part, the MeOH extract attributed the highest, and *n*-Hex attributed the lowest TPC. The DCM extract attributed the max while aqueous extract showed the lowest TFC. Numerous studies also observed the max phenolic in MeOH extracts ((Bazzaza *et al.*, 2011; Gomes *et al.*, 2019).

Biofilm Inhibition

Biofilm formation is a crucial problem in severe infections due to its high resistance to antibiotics. Many medicinal plant base compounds have been reported as anti-bacterial and anti-biofilm agents (Rabin *et al.*, 2015; Pinto *et al.*, 2019). Despite the significant anti-bacterial potential of all extracts, lowest anti-biofilm potential of some extracts have to be found in present study. The biofilm inhibition was evaluated by different concentrations of different extracts (Figure 1 and 2) and IC₅₀ of all extracts was calculated. The aerial MeOH extract exhibited biofilm inhibition > 83% with IC₅₀ < 150 µg/mL against *S. aureus*, *P. aeruginosa*, *S. aureus* MDR and *P. aeruginosa* MDR strains. Similar results was reported from MeOH extract of *H. tiliaceus* bark. The extracted alkaloids and tannins have shown strong anti-biofilm potential against *S. aureus* and *S. apidermis* (Daneshfar *et al.*, 2008). The EtOAc, *n*-Hex and DCM extracts of the aerial part have shown biofilm inhibition > 80% and IC₅₀ < 150 µg/mL against *S. aureus* MDR strain. Though the phytochemicals extracted in *n*-Hex were not too high, the presence of few active metabolites was found active in reducing biofilm formation. This result is in accordance with the findings of Dias *et al.*, (2017). In another study, EtOAc extract of *Orostachys japonicas* was found effective in suppressing *S. aureus* MDR biofilm. The study further confirmed the suppression of *mecA* gene expression in biofilm (Kim *et al.*, 2020). The Aqu extract significantly reduce the biofilm formation by *E. coli* with IC₅₀ > 140 µg/mL. Similar results was reported by Alam *et al.*, (2020). The order of anti-biofilm potential by aerial extracts was MeOH> DCM >EtOAc>Aqu>*n*-But >*n*-Hex. In terms of bacterial strains, the order was: *S. aureus* MDR>*E. coli*>*S. aureus*>*P. aeruginosa* MDR> *K. Pneumoniae*>*P. eruginosa*>*P. vulgaris*. According to the anti-biofilm potential by floral part, the MeOH extract showed max biofilm inhibition > 80% with IC₅₀ < 140 µg/mL against all bacterial strains except *P. aeruginosa*. The DCM extract showed biofilm inhibition with IC₅₀ < 140 µg/mL against *E. coli*, *P. vulgaris*, *K. pneumoniae* *P. aeruginosa* and *P. aeruginosa* MDR. In addition, MeOH and DCM floral extracts exhibited antibiofilm potential better than standard drug (Moxifloxacin) against *S. aureus* MDR and *P. aeruginosa* MDR respectively. These results are supported by previous studies. According to Brambilla *et al.*, (2017) DCM extract exhibited significant anti-bacterial anti-biofilm potentials against MDR strains. In another study, MeOH extract of *Eucalyptus globulus* was found significantly active in anti-biofilm formation against *S. aureus*. The higher

quantity of tannin in MeOH extract was reported as basic anti-biofilm inhibitor (Gomes *et al.*, 2019). The *n*-But and *n*-Hex extracts were also found effective in controlling biofilm formation against *S. aureus* with $IC_{50} < 150 \mu\text{g/mL}$. The *n*-Hex floral extract were low in phenolics and alkaloids contents but exhibited strong anti-biofilm potential. According to Khan *et al.*, (2014) arguments, this may be due to the presence of other secondary metabolites.

Microscopic Visualization

The biofilm formation by *S. aureus* and *S. aureus* MDR strain was studied under light microscope at 40 X magnification. The untreated control biofilm of both strains showed well developed dense network of bacterial cells in biofilm but the treated cells with standard drugs and plant extract (MeOH floral) seems to be scattered and disrupted biofilm (Figure 3). The *S. aureus* MDR biofilm treated by MeOH extract have shown better disruption in biofilm as compared to standard drug (moxifloxacin). The results has supported the fact that extracts were not only reducing the biofilm mass (quantified by 96 wells crystal violet assay) but also changing density of cells. The similar findings were reported by Bazargani & Rohloff, (2016). Several studies have shown the reduction in biofilm density by using plant extracts under light microscopy (Maheshwari *et al.*, 2019; Adnan *et al.*, 2020; Kannappan *et al.*, 2019).

These results were further confirmed through SEM visualization. The untreated controlled biofilm showed well developed amorphous matrix of biofilm while treated cells have shown non-amorphous EPS matrix and crystalline structural change (Figure 4). Several studies have shown the structural morphology change on biofilm by Gram positive and Gram negative bacteria after treatment with different plant extracts (Campos *et al.*, 2009; De Oliveira *et al.*, 2018; Husain *et al.*, 2017). In another study destructive ability of plant extracts on *S. pyogenes* biofilm was confirmed through SEM (Wijesundara and Rupasinghe, 2019)

Antibacterial activity against *S. aureus*

All aqueous (Aq) extract of Cholistani plants was positive against *S. aureus* (Fig.1). The extracts of *S. bryosoma* and *S. icolados* were the best among all and showed maximum activity. Moderate activity was demonstrated by the same extracts of *A. aspera*, *H. recurvum*, *S. fruticosa*, and *P. antidotale*. The least amount of activity was found in the Aq extract of *N. procumbens*, *O. esculentum*, *H. silicornicum*, and *S. surrattense* (Fig 1). According to the *n*-hexane extract results, *S. fruticosa*, *S. surrattense*, and *P. antidotale* have shown the highest activity. Moderate activity was seen from *S. icolados*, *S. bryosoma* extracts. The extracts of *N. procumbens*, *O. compressa*, and *O. esculentum* were the least active. The rest of the *n*-hexane extracts were ineffective in controlling the growth of *S. aureus* (Fig.1). Similarly, in the case of ethanol extract, only four plants were effective. The extract of *S. fruticosa* was highly effective, and extracts of *P. antidotale*, *S. surrattense*, and *O. esculentum* were moderately active (Fig.1). Overall, the order of activity was Aqextract>*n*-hexane > ethanol. The minimum inhibitory concentration (MIC) from each was positive extract was calculated and reported in Table 1.

Table 1. Minimum inhibitory concentrations ($\mu\text{g/ml}$) of different extracts of Cholistani plants

Medicinal plant	Type of Extract	<i>S. aureus</i>	<i>K. pneumonia</i>	<i>P. aeruginosa</i>	<i>P. vulgaris</i>	<i>E. coli</i>
		MIC	MIC	MIC	MIC	MIC
<i>Acyranthesaspera</i>	EtOH *	—*	25	3.12	—	50
	n-Hexane**	—	50	—	25	—
	Aqueous	25	—	—	25	—
<i>Haloxylonrecurvum</i>	EtOH *	—	50	—	—	—
	n-Hexane**	—	50	—	—	—
	Aqueous	—	—	3.12	—	—
<i>Neurodaprocumben</i>	EtOH*	—	50	—	—	25
	n-Hexane**	1.56	50	—	—	50
	Aqueous	—	—	—	25	—
<i>Ochthocloacompressa</i>	EtOH*	—	50	—	—	25
	n-Hexane **	12.5	50	—	—	—
	Aqueous	—	—	—	12.5	—
<i>Haloxylonsilicornicum</i>	EtOH*	—	50	—	12.5	—
	n-Hexane**	—	50	—	50	25
	Aqueous	—	—	3.12	—	—
<i>Oxystelmaesculentum</i>	EtOH*	25	50	1.56	25	50
	n-Hexane**	—	50	—	25	50
	Aqueous	25	25	1.56	25	50
<i>Sauedafruticosa</i>	EtOH *	6.25	50	1.56	25	25
	n-Hexane**	1.56	50	50	50	—
	Aqueous	—	25	—	—	—
<i>Sporobolusicolados</i>	EtOH*	—	25	—	50	25
	n-Hexane**	50	50	3.12	50	—
	Aqueous	—	—	—	—	—
<i>Solanum surrattense</i>	EtOH*	6.25	50	—	25	—
	n-Hexane**	1.56	50	1.56	50	1.56
	Aqueous	3.12	—	—	12.5	—
<i>Salsolabryosoma</i>	EtOH*	—	50	—	50	25
	n-Hexane**	1.56	50	1.56	50	1.56
	Aqueous	6.25	—	—	—	—
<i>Panicumantidotale</i>	EtOH *	12.5	50	1.56	50	25
	n-Hexane**	1.56	50	1.56	50	6.25
	Aqueous	—	—	25	—	—
Chloramphenicol**		25	6.25	1.56	1.56	3.12

*the extract whose antibacterial potential was 0 is represented by _

**the broad-spectrum antibiotics

Figure Legends

Figure 1A. Antibacterial activity against *S. aureus*. 1B. Antibacterial activity against *K. pneumonia*. 1C. Antibacterial activity against *P. aeruginosa*. 1D. Antibacterial activity against *P. vulgaris*. 1E. Antibacterial activity against *E. coli*.

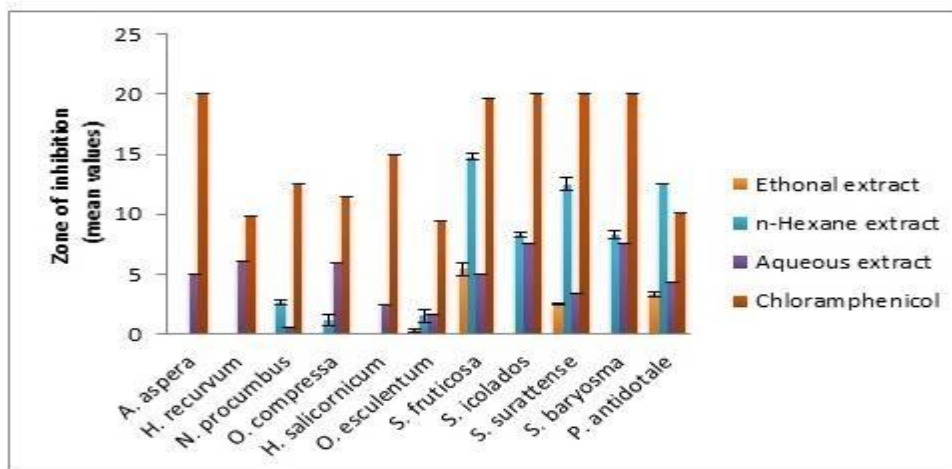


Figure 1 A. Antibacterial activity against *S. aureus*

Antibacterial activity against *K. pneumonia*

In the case of *K. pneumonia* Aq extract of all plants was effective. The extract of *H. silicornicum* has produced the largest zone of inhibition (ZOI), and the extract of *H. recurvum*, *O. compressa*, and *S. fruticosa* has produced the smallest ZOI (Fig.2). According to the results of *n-hexane* extract, eight plants were effective. The extract of *O. esculentum* has shown maximum activity, and the extracts of *S. fruticosa*, *H. recurvum*, and *N. procumbens* were moderately effective. Extracts of *O. compressa*, *A. aspera*, *S. surrattense*, and *S. bryosoma* have shown minimum antibacterial activity (Fig.2). In the case of ethanol extract, again, eight plants were effective. The extracts of *O. compressa* and *O. esculentum* showed maximum antibacterial activity. The extracts of *A. aspera* and *S. surrattense* were moderate in potential, and *S. fruticosa*, *S. icolados*, *H. silicornicum*, and *S. bryosoma* were least effective (Fig.2). Overall, the order of antibacterial potential was Aq extract > n-hexane > ethanol. The MIC from each positive fraction was calculated (Table 1).

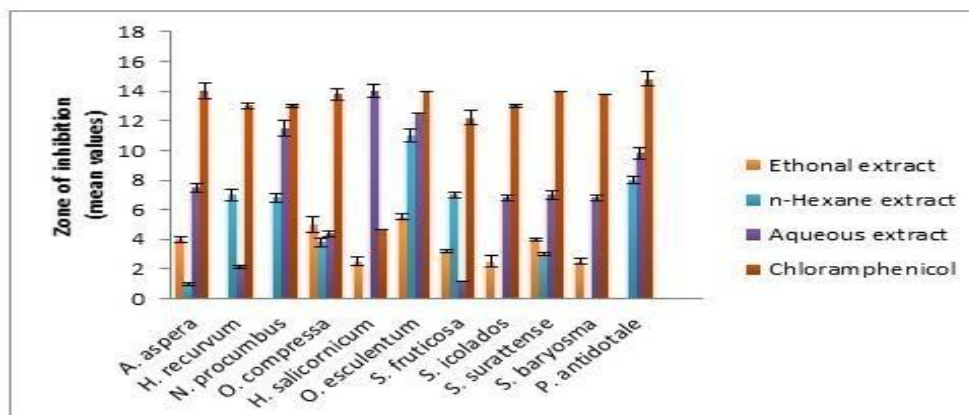


Figure 1B. Antibacterial activity against *K. pneumonia*

Antibacterial activity against *P.aeuriginosa*

Only five plants were influential in their Aq extract against *P. aeuriginosa*(Fig.3). The extract of *H. silicornicum* has shown maximum activity, and extracts of *S. surrattense* and *P. antidotale* have shown minimum activity. The moderate activity was demonstrated by the extract of *O. esculentum* and *H. recurvum*. In the case of *n*-hexane extract, again, five extracts were positive. The extracts of *S. fruticosa* and *S. surrattense* have shown max activity, but extracts of *S. icolados*, *S. bryosoma*, and *P. antidotale* were moderately active against *P. aeuriginosa* (Fig.3). Only four plants were effective in ethanol extract, including *S. fruticosa* with max activity, *A. aspera* and *P. antidotale* with moderate activity, and *O. esculentum* with most minor activity (Fig.3). Overall, the order of potential was Aq extract = *n*-hexane > ethanol.

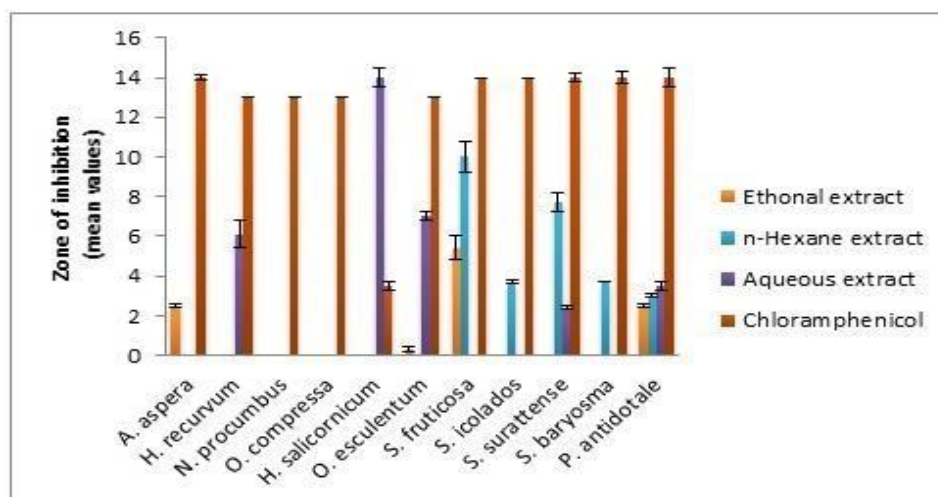


Figure 1C. Antibacterial activity against *P. aeruginosa*

Antibacterial activity against *P. vulgaris*

According to antibacterial activity against *P. vulgaris*, ten cholistani plants were active through their Aq. extract. The extracts of *H. silicornicum* and *N. procumbens* were highly effective, whereas extracts of *O. esculentum*, *S. icolados*, and *S. bryosoma* were moderately effective. The rest of the plants were least effective except *H. recurvum*, which was completely ineffective (Fig.4). In the case of *n*-hexane extract, eight plants were effective. Extracts of *O. esculentum* have shown max activity, while extracts of *S. fruticosa*, *S. icolados*, *S. bryosoma*, and *S. surrattense* were moderately positive. The extracts of *A. aspera*, *H. silicornicum*, and *P. antidotale* were the least effective (Fig.4). In the case of ethanol extract, seven plants were positive. Among these plants, the largest ZoI was produced by *P. antidotale*. While extracts of *S. fruticosa*, *S. icolados*, and *S. bryosoma* were moderately effective. The extracts of *H. silicornicum*, *O. esculentum*, and *S. surrattense* were the least effective (Fig.4). The overall trend was Aq extract > *n*-hexane > ethanol.

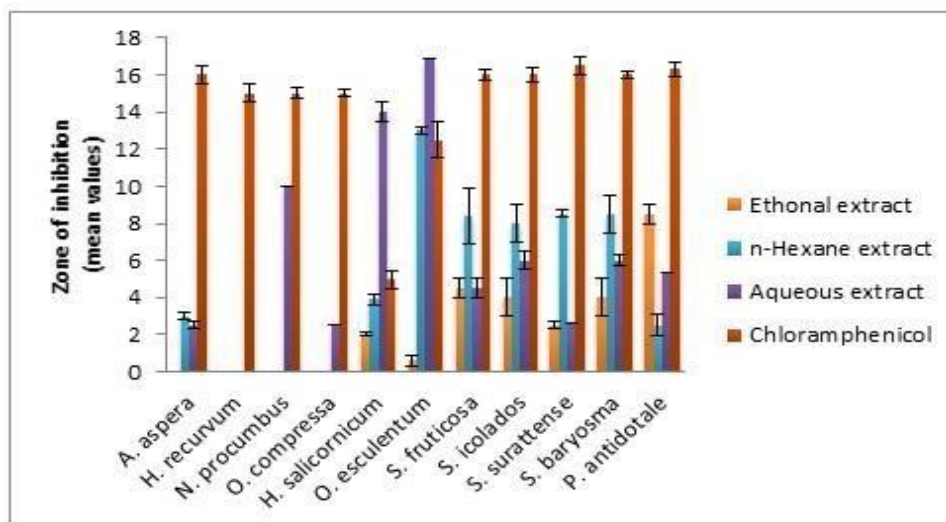


Figure 1D. Antibacterial activity against *P. vulgaris*

Antibacterial activity against *E. coli*

According to the results of this experiment, eight plants have shown activity through their Aq extract. The best activity was reported from extracts of *O. esculentum*, *N. procumben*, and *P. antidotale*. Moderate activity was reported from extracts of *S. fruticosa*, *S. icolados*, and *S. bryosoma*, and minor activity was reported from extracts of *H. recurvum* and *S. surrattense* (Fig.5). Only four plants were effective through *n*-hexane extract, including *N. procumben* with the maxZoI and its MIC value of 50 μ g/ml. *O. esculentum* with moderate activity and *S. surrattense* with most minor activity (Fig.5). The ethanol extract of 7 plants was effective, with the highest activity and its MIC value of 50 μ g/ml from *O. esculentum*. Moderate activity from *S. fruticosa*, *S. icolados*, and *S. bryosoma* and its MIC value 25 μ g/ml and most minor activity from *H. silicornicum*, *H. recurvum*, and *O. compressa* MIC value 0 μ g/ml were observed (Fig.5). The whole trend of activity was Aq extract > ethanol > *n*-hexane extract. The MIC value from each positive extract was calculated and reported (Table 1).

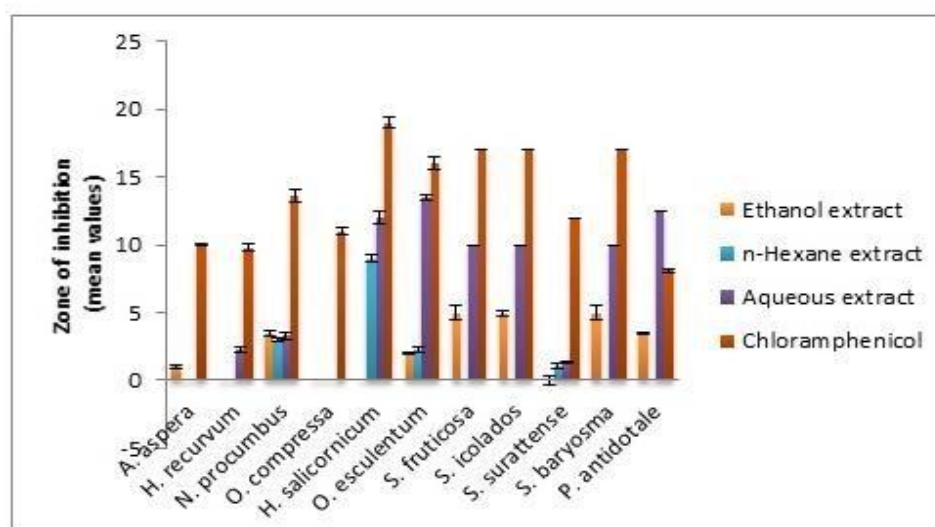


Figure 1E. Antibacterial activity against *E. coli*

4. Discussion

Plants are a natural repository of secondary metabolites like tannins, alkaloids, flavonoids, and glycosides reported for associated antimicrobial activities (Bhalodia and Shukla, 2011). Increasing antibiotic resistance against existing compounds and rising demand for natural products has increased the importance of plants for the medication (Dhanavade *et al.*, 2011). To analyze the significance, the current study was designed. Eleven different Cholistani plants, as mentioned in the methodology, were tested for their antibacterial potential. Three different extracts, namely Aqueous, *n*-hexane, and ethanol extracts, were yielded and used for antibacterial assays. The ZoI was calculated by the disc diffusion method. This study revealed that most of the Aq. extract of tested Cholistani plants are rich source of phytochemicals like reducing sugar, flavonoids, acids and bases (Wadood *et al.*, 2013). According to antibacterial potential against *S. aureus*, all plants were active through Aq. extract, but only 8 and 4 plants were active in *n*-hexane and ethanol extracts. The study indicates that water-soluble antibacterial compounds are more frequently available than *n*-hexane and ethanol.

According to recent studies, the metabolites like alkaloids, tannins, phenolic, flavonoids, steroids and terpenoids have been reported as potent anti-bacterial and anti-biofilm agents (Cock *et al.*, 2018; Mutalib *et al.*, 2015; Tan *et al.*, 2013; Vandeputte *et al.*, 2010). Even the role of catechin and polyphenol fractions have been studied as strong biofilm inhibitors (Zacchino *et al.*, 2017)

Similar studies have been reported (Dabur *et al.*, 2007; Mahesh and Satish, 2008). Different plant extracts were positive against *E. coli* and *S. aureus*, and the disc diffusion method has been performed for antibacterial assay. Similar studies were performed (Mukhtar and Ghor, 2012; Nathiya and Dorcus, 2012). In *K. pneumoniae* again, all plants were effective through Aq extract, but 9 and 8 plants were effective through *n*-hexane and ethanol extracts. Similar results are reported (Sharma *et al.*, 2009). The results of the antibacterial potential of Cholistani plants against *P. aeruginosa* were slightly different from other bacteria. Here *n*-hexane extract was equally effective as compared to Aq extract. Five plants were effective in each case. Only four plants were effective through ethanol extract. Similar studies were reported by (Parekh *et al.*, 2006; Dabur *et al.*, 2007). In *P. vulgaris*, nine plants through Aq extract, eight plants through *n*-hexane extract, and seven plants through ethanol extracts were positive. The results indicate that Cholistani plants are rich source of various antibacterial compounds against *P. vulgaris*. These compounds are frequently available through different extracts of plants. Similar studies were reported by (Narayan *et al.*, 2010; Nathiya and Dorcus, 2012). To check antibacterial activity against *E. coli*, 9 plants through Aq extract, 9 through ethanol extracts, and four plants through *n*-hexane extract were positive. A similar study is reported by (Indu *et al.*, 2006). The microbial diversity is increasing day by day, and resistance against known compounds is also growing. Therefore, the exploitation of plants to search for natural products is increasing (Naidu *et al.*, 2006; Pushpa *et al.*, 2013). Despite precautions and control measures, bacterial diseases such as meningitis, abscess, pneumonia, colitis, and diarrhea are major problems worldwide. Different methods are performed to tackle these diseases, but certain factors cause the emergence of these diseases, such as Changes in society, technology, and the microorganisms themselves (Mitchell and Cohen, 2000). In the current study, the catechin and polyphenols were most significantly observed in MeOH and DCM extracts, which showed max reduction in biofilm formation. The order of floral extracts for anti-biofilm potential was MeOH > DCM > Aq > n-But > n-Hex > EtOAc. In terms of bacteria the order was *E. coli* > *P. aeruginosa* MDR > *S. aureus* MDR > *S. aureus* > *P. vulgaris* > *P. aeruginosa* > *K. pneumoniae*.

As an outcome of this study, it is supported that Cholistani plants can effectively control disease caused by bacteria through their different extracts. Among different types of extracts, Aq extract is best to use

from most plants, and it is worth mentioning that this extract is easy to produce and easy to use compared to other extracts, e.g., such as herbal tea.

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Conflict of Interest

All authors are duly informed and have gone through this manuscript. There is no conflict of interest in this paper.

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