

SCREENING OF SOME MEDICINAL PLANTS FOR ANTIBACTERIAL ACTIVITY AGAINST CONJUNCTIVITIS

S. Meher¹, I. Ali*², A. Sami³, M. Ismail², N. Naheed⁴, S. A. Khan⁵ and V. U. Ahmad³

¹Department of Chemistry, Sardar Bahadur Khan Women's University, Brewery Road Quetta, Pakistan

²Department of Chemistry, Karakoram International University, 15100-Gilgit, Gilgit-Baltistan, Pakistan

³HEJ Research Institute of Chemistry, University of Karachi

⁴Department of Chemistry, Federal Urdu University of Science and Technology, Karachi, Pakistan

⁵Department of Chemistry, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah-21589, Saudi Arabia

*Corresponding Authors. e-mail: iftikhar.ali@kiu.edu.pk

ABSTRACT

Antibiotic resistance is a persistently emerging problem all over the world. The constituents of therapeutic plant extracts play a key role in antimicrobial drug discovery. Extracts of 36 medicinal plant species of Pakistan origin that belong to 33 genera and 24 families were tested against the bacterial strains *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In addition the extracts of 23 plant species classified in 21 genera and 17 families were assessed against the bacterial clinical isolates (*S. aureus*, *P. aeruginosa*), using the agar well diffusion method. As a result, 10 plant extracts exhibited different properties against the specific bacterial strains. Some plant extracts were also tested against the clinical *P. aeruginosa* and *S. aureus* isolates. Among the tested samples, Haleej, Anaar and Kali Mirch exhibited the highest relative percentage inhibition against *S. aureus* while Kala Zeera, Abhal and Khobani showed the highest inhibition against *P. aeruginosa*. In addition Anar exhibited the highest inhibition against the clinical *S. aureus* isolate while Pista followed by Zeera showed the highest inhibition against the clinical *P. aeruginosa* isolate. The results obtained in our present study might help explain the ethnobotanical importance of the screened plant species for the treatment of conjunctivitis.

Key words: Plant extracts–Antibacterial activity–Clinical isolates–Conjunctivitis.

INTRODUCTION

Conjunctivitis 'red eye' is a clinical problem (Mahmood and Narang, 2008) caused by infectious pathogens. Other causes of red eye include blepharitis, episcleritis, keratitis etc. (Du Toit and Van Zyl, 2013), while various allergic clinical conditions have been reported (Buckley, 1998). Effective management of such common conditions might be achieved through a stepped care approach that starts with allergen-identification and antigen avoidance (Meyer, 2004). Sublingual immunotherapy has been reported for the treatment of allergic conjunctivitis (Calderon *et al.*, 2012). The inflammation of the conjunctiva, conjunctivitis in general, is due to various infectious and noninfectious agents. Red eye is a form of bacterial conjunctivitis (Tarabishy and Jeng, 2008). Potentially complicated bacterial keratitis is the most visually threatening ocular infectious disease. Corneal perforations have been reported, *P. aeruginosa* and *S. aureus* being the invasive pathogens (Schaefer *et al.*, 2001).

The therapeutic properties of plant species against bacterial infections have been investigated scientifically (Bhattarai *et al.*, 2009). Due to the side effects exhibited by conventional medicines, natural

products are a viable source of alternative therapeutics (Ansari *et al.*, 2006). Sharma and Singh (2002) reviewed the importance of a number of plant species belonging to 66 families to treat or cure conjunctivitis. Since ancient times, plant species have been the best source of medicine. Plants or plant-derived products have been used in traditional and folk therapeutic systems to treat a number of infectious diseases (Kumar *et al.*, 2010). Many edible plants possess beneficial and therapeutic properties to humans, and plant extracts have been used as a source of alternative medication for their antioxidant, antifungal, and anticancer properties (Suwanmanee *et al.*, 2014).

The present study is meant to determine the major bacterial types causing conjunctivitis and to study the efficacy of 36 plant species distributed in 33 genera and 24 families of Pakistan origin. Furthermore the methanolic and some other solvent extracts of 22 species distributed in 21 genera and 17 families were studied against the clinical isolates of *S. aureus* and *P. aeruginosa*.

MATERIALS AND METHODS

Plant Material: All the plant species were collected during 2015 from Sindh province (Pakistan) with the help of the local practitioners and were authenticated by Dr.

Ghulam Rasool. The plant samples were dried in the shade and used for further work.

Preparation of Extracts: The plant materials were dried in the shade and powdered in a grinder. Each sample was soaked in methanol and/or petroleum ether and chloroform for three days and then extracted in a Soxhlet apparatus separately. Each filtrate was concentrated separately and vacuum dried using a rotary evaporator at 40 °C.

Test Microorganisms: Clinical isolates of *S. aureus* (Gram-positive) and *S. aeruginosa* (Gram-negative) were used in this study. The cultures were preserved on nutrient agar plates at a temperature of 4 °C.

Antibacterial assay: Antibacterial activity of the crude plant extracts was studied by the well-known agar-well diffusion method (Jack *et al.*, 1995; Tagg and Dajani, 1976). The test organisms were inoculated into Mueller-Hinton broth (pH 7.4) and incubated for 8 hours. The concentration of the suspension was adjusted to optical density 0.5 using a spectrophotometer. Sterilized cotton swabs were used to seed the isolates onto the Mueller-Hinton agar plates. A sterilized gel borer was used to bore the agar surface to make wells of 6 mm diameter. One hundred µL of the test samples and 100 µL of 10% DMSO (negative control) were poured into the separate wells. The standard antibiotic disc (Imipenem 10 µg/disc) was placed on the agar surface as positive control. Plates were incubated at 37 °C for 48 hours. Triplicate plates were used for each organism.

Determination of Relative percentage Inhibition: The relative percentage inhibition of the test samples was calculated using the following formula with respect to positive control (Ajay *et al.*, 2003; Kumar *et al.*, 2010).

$$\text{Relative percentage inhibition of the test extract} = \frac{100 \times (x-y)}{(z-y)}$$

Where x: total area of inhibition of the test sample

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of inhibition was calculated using $\text{area} = \pi r^2$; where, r = radius of zone of inhibition.

RESULTS

The results of the antibacterial testing of the samples against the bacterial strains *S. aureus* and *P. aeruginosa* are presented in table 1. Among the 36 plant species, 15 extracts exhibited inhibition against *S. aureus* and 20 extracts exhibited inhibition against *P. aeruginosa*. The maximum relative percentage inhibition was exhibited by the methanolic extract of fruit part of Haleej (*Terminalia chebula* Retz.) against *S. aureus* followed by the methanolic extracts of the fruit parts of Anaar (*Punica granatum* L.), Kali Mirch (*Piper nigrum* L.), Kala Zeera (*Bunium bulbocastanum* L.), and the methanolic extract of flower part of Gul e Surkh (*Rosa damascena* Mill.), etc. and the lowest percentage inhibition was manifested by the methanolic extract of the whole plant of Bildi (*Ipomoea hederacea* Jacq.) against the *S. aureus*, while the methanolic extracts of 22 plant species did not exhibit any inhibition against *S. aureus*. Moreover, the maximum percentage inhibition against *P. aeruginosa* was exhibited by the methanolic extracts of Kala Zeera (fruit part), Abhal (seeds) and Khobani (fruit part) etc. The lowest percentage inhibition against *P. aeruginosa* was shown by the methanolic fruit extract of Sufaid Zeera and the methanolic flower extract of Kasini. Seventeen plant species did not show any inhibition against *P. aeruginosa*. Mostly the methanolic extracts of the fruit part of the plant species exhibited the inhibition, followed by the flower part. The root part of Ratanjot, Balcharr and Haldi did not exhibit any inhibition against none of the bacterial strains.

Table 1. Inhibition zone (mm) of different plant extracts against microorganisms

Family	Botanical Name	Local Name	Part Used*	<i>S.aureus</i>	<i>P.aeruginosa</i>
Acanthaceae	<i>Adhatodavastica</i> Nees	Adusa, Bansa	Leaves	-	-
Agaricaceae	<i>Agaricus bisporus</i> Lange.	Botton Mushroom	Fruit	-	-
Apiaceae	<i>Bunium bulbocastanum</i> L.	Kala Zeera	Fruit	12	15
	<i>Centella asiatica</i> (L.) Urb.	Brahmi	Leaves	-	-
	<i>Cuminum cyminum</i> L.	Sufaid Zeera	Fruit	-	8
	<i>Daucus carota</i> L.	Gajar	Fruit	-	-
Asteraceae	<i>Cichorium intybus</i> L.	Kasini	Flower	9	8
Boraginaceae	<i>Onosma achioides</i> (L.) L.	Ratanjot	Root	-	-
Caprifoliaceae	<i>Nardostachys jatamansi</i> (D. Don)	Balcharr	Root	-	-
Combretaceae	<i>Terminalia chebula</i> Retz.	Haleej	Fruit	18	13
Convolvulaceae	<i>Ipomoea hederacea</i> Jacq.	Bildi	Whole plant	7	10
Cupressaceae	<i>Juniperus communis</i> L.	Abhal	Seeds	-	15
Fabaceae	<i>Acacia nilotica</i> (L.) Willd. ex Delile	Sindhi Keekar	Leaves	-	10

	<i>Acacia nilotica</i> (L.) Willd. ex Delile	Sindhi Keekar	Seeds	-	14
Iridaceae	<i>Iris germanica</i> L.	Airsa, Irsa	Leaves	-	13
Lamiaceae	<i>Lavandulastoechas</i> L.	Ustkhuddus	Ground part	10	9
	<i>Origanum majorana</i> L.	Marzanjosh	Leaves	-	-
Linaceae	<i>Linum usitatissimum</i> L.	Alsi	Seeds	10	9
Lythraceae	<i>Punicagranatum</i> L.	Anaar	Fruit coat	15	10
Malvaceae	<i>Hibiscus rosa-sinensis</i> L.	Gul e Gurhal	Flower	-	-
	<i>Myrtus communis</i> L.	Barg e Maurid	Leaves	-	-
	<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	Loang	Fruit	10	11
Piperaceae	<i>Piper nigrum</i> L.	Kali Mirch	Fruit	13	9
Primulaceae	<i>Embelia robusta</i> Roxb.	Baobaranj, Wavarang	Bark	10	12
Pteridaceae	<i>Adiantum capillus-veneris</i> L.	Hansraj	Leaves	9	-
Ranunculaceae	<i>Nigella sativa</i> L.	Kalonji	Seeds	-	-
Rosaceae	<i>Prunus armeniaca</i> L.	Khobani	Fruit	11	15
	<i>Prunus cerasus</i> L.	Surkh Phal	Fruit	-	-
	<i>Prunus domestica</i> L.	Aalu Bukhara	Fruit	-	-
	<i>Rosadamasceana</i> Mill	Gul e Surkh	Flower	12	13
Rutaceae	<i>Aegle marmelos</i> (L.) Corrêa	BelGiri	Fruit	9	9
Solanaceae	<i>Physalis alkekengi</i> L.	Kaknaj	Leaves	-	-
	<i>Physalis minima</i> L.	Rasbhari	Fruit	-	-
	<i>Solanum forskalii</i> Dunal.		Whole plant	-	-
	<i>Withania coagulans</i> (Stocks) Dunal	PaneerDoda	Seeds and Fruit	-	9
Violaceae	<i>Viola odorata</i> L.	Gul e Banafsha	Flower	9	9
Zingiberaceae	<i>Curcuma longa</i> L.	Haldi	Root	-	-

* All samples were extracted in methanol.

The results of the antibacterial tests of certain plant samples in our present study against the clinical isolates are presented in table 2. Among the 23 plant species, only 9 plant species exhibited inhibition of the clinical bacterial isolate *S. aureus*. The highest inhibition was shown by the petroleum ether extract of Anaar (15%) followed by the chloroform extract of Gul e Surkh and methanolic extract of Jhao (13% each). Moreover, 12% inhibition against the clinical isolate (*S. aureus*) was observed by the methanolic extracts of Zeera and Pista. The petroleum ether extract of Gul e Surkh and methanolic extract of Haleej exhibited 11% inhibition each, followed by the methanolic extract of Anjeer (10%) and the chloroform extract of Anaar (7%) exhibited the minimum

inhibition against the clinical bacterial isolate, *S. aureus*. The remaining 16 plant species did not exhibit any inhibition against the clinical isolate, *S. aureus*.

Furthermore, 8 plant species exhibited antagonism against the clinical *P. aeruginosa* isolate while the remaining 17 plant extracts did not show inhibition of the clinical isolate *P. aeruginosa*. The highest inhibition was shown by the methanolic extract of Pista (20%), followed by Zeera (MeOH, 15%), Gul e Surkh (CHCl₃) and Jhao (MeOH, 12% each), Haleej (MeOH, 11%), Anaar (PE, 10%) and Anjeer (MeOH, 9%). The lowest inhibition was shown by the CHCl₃ extract of Anaar (8%) against the clinical isolate of *P. aeruginosa*.

Table 2. Inhibition zone (mm) of different plant extracts against clinical isolates

Family	Botanical Name	Local Name	Part Used	Extractive	<i>S. aureus</i>	<i>P. aeruginosa</i>
Anacardiaceae	<i>Mangifera indica</i> L.	Aam	Fruit	MeOH	-	-
	<i>Pistacia vera</i> L.	Pista	Fruit	MeOH	12	20
Apiaceae	<i>Carum carvi</i> L.	Zeera	Fruit or Seeds	MeOH	12	15
	<i>Coriandrum sativum</i> L.	Dhaniyaa	Seeds	MeOH	-	-
Combretaceae	<i>Terminalia chebula</i> Retz.	Haleej	Fruit	MeOH	11	11
Cucurbitaceae	<i>Citrullus colocynthis</i> (L.) Schrad.	Indrayan	Fruit	MeOH	-	-

Lauraceae	<i>Cinnamomum tamala</i> (Buch.-Ham.) T. Nees & Nees	Tezpat	Leaves	MeOH	-	-
	<i>Cinnamomum zeylanicum</i> Blume	Dar chini	Fruit	MeOH	-	-
	<i>Punicagranatum</i> L.	Anaar	Fruit coat	PE	15	10
	<i>Punicagranatum</i> L.	Anaar	Fruit coat	CHCl ₃	7	8
Moraceae	<i>Ficus carica</i> L.	Anjeer		MeOH	10	9
Papaveraceae	<i>Papaver somniferum</i> L.	Khashkhash	Seeds	MeOH	-	-
Parmeliaceae	<i>Usnea longissima</i> Ach.	-	Aerial part	MeOH	-	-
Polygonaceae	<i>Polygonum bistorta</i> L.	Anjbar	Flowers	MeOH	-	-
	<i>Rheum emodi</i> Wall.	Rewandchini		MeOH	-	-
Primulaceae	<i>Embelia robusta</i> Roxb.	Baobaranj	Bark	MeOH	-	-
Rosaceae	<i>Prunus armeniaca</i> L.	Khobani	Fruit	MeOH	-	-
	<i>Rosadamasceana</i> Mill.	Gul e Surkh	Flower	CHCl ₃	13	12
	<i>Rosadamasceana</i> Mill.	Gul e Surkh	Flower	PE	11	-
Rubiaceae	<i>Rubiacordifolia</i> L.	Majith	Root	MeOH	-	-
Santalaceae	<i>Santalum album</i> L.	Sandal	Fruit	MeOH	-	-
		Safaïd				
Solanaceae	<i>Datura stramonium</i> L.	Kala datura	Seeds	MeOH	-	-
Tamaricaceae	<i>Tamarix dioica</i> Roxb. ex Roth	Jhao	Leaves and flowers	MeOH	13	12
Violaceae	<i>Viola odorata</i> L.	Gul e Banafsha	Flower	MeOH	-	-
Vitaceae	<i>Vitis vinifera</i> L.	Kishmish	Fruit	MeOH	-	-

Table 3. Diameter of Zone of Inhibition (mm) of Standard Drug

Name of Organism	Zone of inhibition (Imipenem 10µg/disc)
<i>S. aureus</i>	43
<i>P. aeruginosa</i>	32

Comparing the results with standard antibiotic i.e. Imipenem, the inhibitory effect of the drug against *S. aureus* was about 43 mm and against *P. aeruginosa* 32 mm (table 3).

DISCUSSION

Natural products are a prime source of antimicrobials. Many efforts have been done to identify compounds that serve as appropriate antimicrobial agents (Ramalivhana *et al.*, 2013). For the production of less toxic and more effective antibiotics, plant products i.e. phyto-chemicals are the eminent resource. The above-mentioned plant extracts showed significant or moderate activities against resistant clinical isolates of *S. aureus* and *P. aeruginosa*. These plant extracts possess great potential as antimicrobials. Furthermore, these plant species can be employed to treat and/or cure infectious diseases like conjunctivitis, usually caused by resistant pathogens. The highest relative percentage inhibition was shown by Haleej against *S. aureus* followed by Anaar. Hogade *et al.* (2011) evaluated the aqueous fruit extract of Haleej against Gram-positive bacteria (e.g. *S. aureus*) and Gram-negative bacteria (e.g. *P. aeruginosa*) by pour plate method in a sterile nutrient agar medium plate. However, in our present study, the methanolic fruit extract of Haleej showed lower activity against *S. aureus* and *P.*

aeruginosa compared to the reported results by the aqueous fruit extract. Similarly, antibacterial screening of the ethanolic fruit extract of Haleej was carried out using the standard disc diffusion test. However, the highest activity was exhibited against *Salmonella typhi*, *Staphylococcus epidermidis* and *Bacillus subtilis* (Kannan *et al.*, 2009). Similarly, a number of reports are available regarding the antimicrobial properties of different varieties of Anaar (Betanzos-Cabrera *et al.*, 2015; Jasim *et al.*, 2014; Kadiet *et al.*, 2011).

Moreover, the maximum percentage inhibition against *P. aeruginosa* was exhibited by the extracts of Kala Zeera, Abhal and Khobani, followed by Sindhi Keekar. The maximum inhibition was shown by the petroleum ether extract of Anaar followed by the chloroform extract of Gul e Surkh and the methanolic extract of Jhao against the clinical isolate of *S. aureus*. However, the methanolic extract of Pista showed the highest inhibition against the clinical *P. aeruginosa* isolate, followed by Zeera, Gul e Surkh and Jhao plant extracts.

According to Khan *et al.* (2013), Kala Zeera (*Buniumbulbocastanum*) was screened for various biological activities and the crude methanolic extract exhibited significant activity against *S. aureus*. However, the Et OAc fraction was inactive against *P.aeruginosa* and *S.aureus* while the aqueous extract exhibited moderate action against *S. aureus* and *E.coli*,but it exhibited low activity against *P.aeruginosa*. Similarly, the antimicrobial properties of Abhal (*J. communis* L.) have been reported (Haziriet *al.*, 2013; Sati and Joshi, 2010; Rezvani *et al.*, 2009). The essential oil of Abhal exhibited activity against *S. aureus* while not against *P. aeruginosa* (Haziri *et al.*, 2013). In a related study Sati and Joshi (2010) reported that the hexane extract of leaves of Abhal showed maximum activity followed by ethanolic, methanolic and chloroform extracts; however, the aqueous extract did not show any activity against any tested organism. Moreover, the essential oil exhibited notable antibacterial activity against *S. aureus* and *P. aeruginosa* (Rezvani *et al.*, 2009). In addition, the reports about the antimicrobial evaluation of Khobani revealed its potential against various organisms including *S. aureus* and *P. aeruginosa* (Sharma *et al.*, 2014; Gomaa, 2013). Similarly, the antibacterial properties of Sindhi Keekar (*Acacia nilotica*) have been determined against various bacteria (Abdallah, 2016; Amjad-ur-Rahman *et al.*, 2014). Furthermore, Banso (2009) found that the bark extract of Sindhi Keekar was active against *Streptococcus viridans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Shigella sonnei*. However, Deshpande (2013) found that the ethanol extract of Sindhi Keekar exhibited better antibacterial activity against the clinical *S. aureus* isolate as compared to the petroleum ether extract and Nagumanthri *et al.* (2012) reported that ethanolic leave and bark extracts of Sindhi Keekar showed activity against the clinical *S. aureus* isolate. Khan *et al.* (2013) reported the antimicrobial potential of Jhao against *S. aureus* and *P. aeruginosa*. The results of the present study agree with the previously reported investigation. While the plant species including *M. indica*, *C.sativum*, *C.colocynthis*, *C.tamala*, *C.zeylanicum*, *P.somniferum*, *U.longissima*, *P.bistorta*, *R.modi*, *E.robusta*, *P.armeniaca*, *R.cordifolia*, *S. album*, *D.stramonium*, *V.odorata* and *V.vinifera* did not exhibit any activity against the tested clinical isolates, the medicinal plant species covered in the present study that exhibited activity against the clinical isolates could be further investigated for the discovery of bioactive natural products.

Conclusions: Among the 36 medicinal plant extracts studied, the highest relative percentage inhibition was exhibited by Haleej (18%) followed by Anaar (15%) and Kali Mirch (13%) against *S. aureus* while the methanolic extracts of Kala Zeera, Abhal and Khobani (15 % each) manifested the highest inhibition against *P.*

aeruginosa. Moreover, maximum inhibition was shown by the petroleum ether extract of Anaar (15%) followed by the chloroform extract of flower part of Gul e Surkh (13%) against the clinical *S. aureus* isolate. Moreover, 12% inhibition against the clinical isolate of *S. aureus* was shown by the methanolic extracts of Zeera and Pista. Furthermore, 8 plant extracts exhibited antagonism against the clinical *P. aeruginosa* isolate. However the maximum inhibition was shown by the methanolic extracts of fruit part of Pista (20%) followed by the fruit and seeds part of Zeera (15%) against the clinical *P. aeruginosa* isolate. The antimicrobial potential of these plant species extracts suggest for their use as alternative therapeutic agents against conjunctivitis. Further insightful research should be carried out to understand its efficacy because it can be used as a potential source for the development of a phyto-medicine to treat or cure conjunctivitis. The development of modern drugs in the form of phyto-medicine from the most active plant extracts should be emphasized for the treatment of conjunctivitis.

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