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In vitro antimicrobial activity of plant fractions against major respiratory pathogens

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Abstract: From the ancient times, the use of plants is considered as a cure for many diseases and now, they are being used as a new resource for producing agents that could act as alternative to antibiotics. Current work was carried to investigate the antimicrobial potential of 42 different aqueous plant extracts and oils against *Staphylococcus aureus, Candida albicans and Pseudomonas aeruginosa. In vitro* antimicrobial activity of different concentrations (1.50mg/ml and 0.75mg/ml) of plant fractions was investigated against three clinical isolates: *S. aureus, C. albicans and P. aeruginosa* by agar well diffusion assay. Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) were evaluated by broth dilution and plating method respectively. Among 42 plant fractions, methanol fractions of *Cassia fistula* leaves and flowers were active against *S. aureus*. Hexane fractions of *Ixora coccinea* stalk and leaves, methanol fractions of *C. fistula* flowers and chloroform fraction of *C. fistula* leaves were active against *C. albicans*. Methanol fractions of *I. coccinea* leaves and stalk were active against *P. aeruginosa.* The MICs of active fractions were found to be 0.45mg/ml and 0.6mg/ml for *P. aeruginosa*, *S. aureus* and *C. albicans*. The MBCs were found to be 1.50mg/ml for *P. aeruginosa*, 0.75mg/ml for *S. aureus* and 1.50mg/ml and 0.75mg/ml for *C. albicans*. In antibiotic susceptibility testing, all isolates were found to be sensitive to their respective antibiotics.

Keywords: Antimicrobial activity, MBC, MIC, plant fractions.

INTRODUCTION

In the last few years, due to continuous use of antibiotics, microorganisms have gained multiple drug resistance pathogenicity. Along with this problem, sometimes these commonly used drugs/antibiotics become a cause of adverse effects on host that include depletion of beneficial gut and mucosal microorganism, hypersensitivity and allergic reactions (Lopez A, Hudson JB, Towers GHN, 2001). And the consequence leads to immense clinical problems in the treatment of infectious diseases (Hailu Tadeg., Endris Mohammed., Kaleab Asres., Tsige Gebre-Mariama, 2005). To eliminate or at least reduce the problematic cause, there is a need to develop some alternative antimicrobial drugs for the treatment of infectious diseases, one of the approach to be carried on is to screen local plants for possible antimicrobial properties. The antimicrobial activities of these natural plant materials have been explored in recent years (Yi Xin Seow , Chia Rou Yeo , Hui Ling Chung & Hyun-Gyun Yuk, 2014). Plant materials are still an important resource to encounter serious diseases in the world. According to WHO (2001), herbal medicines serve the health needs of about 80% of the world's population, especially people in rural areas are dependent on these traditional medicines and plant extracts are being used in traditional therapies (World Health Organization, 2001). These plants are popularly known as medicinal plants. These plants are rich in wide assortment of secondary metabolites including alkaloids, tannins, flavonoids, and terpenoids which are considered to have antimicrobial activities in *in vitro* studies (N. Savithramma, M. Linga Rao and D. Suhrulatha, 2011) (Sıdıka Ekren, Oktay Yerlikaya, Hatice Eda Tokul, Aslı Akpınar and Merve Açu, 2013). Therefore, new sources for the production of antibiotic are being investigated, especially the research is being carried on plant sources. Generally anti-infective agents are present in plants. Plants containing picralima type indole alkaloids, protoberberines and garcinia biflavonones are potent producers of antimicrobials (Elgayyar *et al.,* 2001). The aim of the present investigation was to analyze the antimicrobial activities of different plant fractions and to compare these to the effect of the antibiotics on bacteria and since most of the plant fractions have bacteriostatic and bactericidal activity against all the test cultures, they can be effectively used in therapy for infections caused by these microorganisms. As in recent years, pathogenic bacteria has gained the multi-drug resistance against antibiotic therapy so, the medicinal plants can be used as an alternative.

MATERIALS AND METHODS

Plant material

Table 1 exhibits the list of plant fractions used in the study. Plants were collected during May, 2015 from HEJRIC (ICCBS, University of Karachi), identified by plant taxonomist and were deposited in the herbarium of the Department of Botany, with following voucher specimen number.

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Soxhlet extraction

Extraction was performed with the help of hexane followed by chloroform and then methanol for 8 hours in a conventional soxhlet apparatus, filtered and evaporated by using a rotary evaporator. Finally, the dried extract was stored at room temperature for further studies.

Micro-organisms

Human respiratory pathogens (*P. aeruginosa, S. aureus* and *C. albicans*) were used in this study. They were acquired from Reference Culture Collection Laboratory of the Department of Microbiology, University of Karachi. Purity of the cultures was checked as per Bergey's manual of Bacteriology (Holt, 1994).

Plant fractions

The extracts were used in the concentrations of 1.50mg/ml and 0.75mg/ml (these small concentrations were used to carry out the experiment efficiently) 0.2grams of crude extract was diluted in 4 ml Dimethyl sulfoxide to achieve the stock concentration of 50mg/ml, this concentration was then two fold diluted to make 25mg/ml concentration (Kang *et al.,* 2011).

Standardization of microorganisms

0.5 McFarland standard was used to standardize microbial inoculum as described elsewhere (Habeeb *et al.,* 2007; Micklos *et al.,* 2003).

Screening for antimicrobial activity

Following standardization of microorganisms, overnight culture was swabbed onto Mueller Hinton media. Wells were bored in agar and 30µl of different concentrations of extracts were then added into the respective wells. Standard antibiotics were served as positive controls while DMSO was served as a negative control. Plates were incubated at 37°C for 24 hrs and the zone of inhibition around each well was measured. Each assay was run in duplicate (Holder and Boyce, 1994; Athanassiadis *et al.,* 2009).

Determination of MICs and MBCs

Three 96-well plates were used for three different cultures, MICs and MBCs were determined by broth dilution method. In this technique, only those compounds were proceeded that showed significant activity or inhibition in agar well diffusion assay. A total of 0.09ml volume was used, which includes 30µl of plant extract, 30µl of nutrient broth and 30µl of overnight culture suspension. All plates were incubated at 37°C for 24hrs. Each test was run in triplicate. Plates were then examined for bacterial growth by reading plate in ELISA plate reader against 450nm wavelength. The highest dilution without growth was considered as MIC (Elshikh *et al.,* 2016). While, MBCs were evaluated by sub culturing the contents of wells onto MHA plates and incubated at 37°C for 24 hrs and results were recorded as + (positive) for growth and - (negative) for no growth (Chandrasekaran and Venkatesalu, 2004).

RESULTS

The antimicrobial screening of the examined plants showed that they could be used as an alternative for infectious disease treatment. The extracts have shown different range of activities against pathogenic micro-organisms, as previously reported studies on antibacterial activity of plant volatile oils (H.J.D Dorman, 2000). A total of three cultures including one gram negative *P. aeruginosa,* one gram positive *S. aureus* and one fungal isolate *C. albicans* were tested in vitro for anti-microbial activity of different concentrations of plant fractions in the present investigation. Most of the fractions were found to exhibit an excellent anti-microbial activity 1.50mg/ml and 0.75mg/ml. Antibiotic susceptibility testing showed that all microbial strains were sensitive to the respective antibiotic used against them i.e. *C. albicans* was sensitive to fluconazole, *S. aureus* was sensitive to cephalexin and *P. aeruginosa* was sensitive to ciprofloxacin. tables 3,4 and 5 exhibit antimicrobial activity of plant fractions against *C. albicans*, *S. aureus* and *P. aeruginosa,* while the active fractions found in the performed experiment are indicated in tables 6, 7 and 8 respectively.

The results of well diffusion assay showed that plant fractions exhibit strong inhibitory effects against all test organisms. The maximum diameter of zones of inhibition measured ranged between 8.5mm-18mm/30µl of fraction. Among 42 plant fractions, methanol fractions of *C. fistula* leaves and flowers were active against *S. aureus* as also mentioned in previously reported studies on methanol, hexane and water extracts of *C. fistula* against different pathogenic micr-organisms (Duraipandiyan and Ignacimuthu, 2007). Hexane fractions of *I. coccinea* stalk and leaves, methanol fractions of *C. fistula* flowers and chloroform fraction of *C. fistula* leaves were active against *C. albicans*. Methanol fractions of *I. coccinea* leaves and stalk were active against *P. aeruginosa,* also supported by the results revealed in a phytochemical screening that the flower extract of *I. coccinea* possessed flavonoids and tannins which give them the capability of being antimicrobial (Sivaramakrishnan and Surash, 2010). And these constituents act as active antimicrobial agents, as reported by (Ragasa *et al*., 2004).

Active plant fractions against *C. albicans*, *S. aureus* and *P. aeruginosa* are shown in tables 6, 7 and 8 respectively.

In case of MICs and MBCs, fractions showed bacteriostatic and bactericidal effects. The MICs of active fractions were found to be 0.45mg/ml and 0.6mg/ml for *P. aeruginosa*, *S. aureus* and *C. albicans*. The MBCs were found to be, 1.50mg/ml for *P. aeruginosa*, 0.75mg/ml for *S. aureus* and 1.50mg/ml and 0.75mg/ml for *C. albicans*. MICs and MBCs of selected active fractions were recorded as depicted in table 9.

Table 1: Plant Fractions with their voucher specimen number

S. No.	Plant Name	Voucher Number
1	*Ixora coccinea* plant (yellow flowers)	Voucher specimen (KUH GH 91566)
2	*Ixora polyanthus* plant (white flowers)	Voucher specimen (KUH GH 91562)
3	*Ixora coccinea* plant (orange flowers)	Voucher specimen (KUH GH 91565)
4	*Ixora fulgens* plant (red flowers)	Voucher specimen (KUH GH 91567)
5	*Ixora chinensis* plant (pink flowers)	Voucher specimen (KUH GH 91566).

Table 2: Different plant fractions used in the study

S. No.	Plant Fractions	Name of Plant	Part of Plant	Solvent
1	H16	*Ipomoea batata* blackie	Leaves	Hexane
2	C16			Chloroform
3	M16			Methanol
4	H17	*Ipomoea batata* pink frost	Leaves	Hexane
5	M17		Leaves	Methanol
6	H1	*Ixora fulgens*	Flowers	Hexane
7	C1		Flowers	Chloroform
8	M1		Flowers	Methanol
9	H2	*Ixora fulgens*	Leaves	Hexane
10	H5	*Ixora polyanthus*	Leaves	Hexane
11	H8	*Ixora chinensis*	Leaves	Hexane
12	C5	*Ixora polyanthus*	Leaves	Chloroform
13	C2	*Ixora fulgens*	Flowers	Chloroform
14	M2	*Ixora fulgens*	Flowers	Methanol
15	M4	*Ixora polyanthus*	Flowers	Methanol
16	H7	*Ixora chinensis*	Flowers	Hexane
17	C7	*Ixora chinensis*	Flowers	Chloroform
18	M7	*Ixora chinensis*	Flowers	Methanol
19	C8	*Ixora chinensis*	Leaves	Chloroform
20	M8	*Ixora chinensis*	Leaves	Methanol
21	H10	*Ixora coccinea* (yellow flowers)	Flowers	Hexane
22	C10	*Ixora coccinea* (yellow flowers)	Flowers	Chloroform
23	H14	*Ixora coccinea* (orange flowers)	Leaves	Hexane
24	H12	*Ixora coccinea* (yellow flowers)	Stalk	Hexane
25	M12	*Ixora coccinea* (yellow flowers)	Stalk	Methanol
26	M10	*Ixora coccinea* (yellow flowers)	Flowers	Methanol
27	C11	*Ixora coccinea* (yellow flowers)	Leaves	Chloroform
28	M11	*Ixora coccinea* (yellow flowers)	Leaves	Methanol
29	C13	*Ixora coccinea* (orange flowers)	Flowers	Chloroform
30	M13	*Ixora coccinea* (orange flowers)	Flowers	Methanol
31	C14	*Ixora coccinea* (orange flowers)	Leaves	Chloroform
32	M14	*Ixora coccinea* (orange flowers)	Leaves	Methanol
33	H15	*Ixora coccinea* (orange flowers)	Stalk	Hexane
34	M15	*Ixora coccinea* (orange flowers)	Stalk	Methanol
35	H18	*Cassia fistula*	Leaves	Hexane
36	C18	*Cassia fistula*	Leaves	Chloroform
37	M18	*Cassia fistula*	Leaves	Methanol
38	H19	*Cassia fistula*	Flowers	Hexane
39	M19	*Cassia fistula*	Flowers	Methanol
40	M5	*Ixora polyanthus*	Leaves	Methanol
41	H11	*Ixora coccinea* (yellow flowers)	Leaves	Hexane

Table 3: Antimicrobial activity against *Candida albicans*

Zones of Inhibition			
S. no	Fractions	1.5 mg\ml	0.75 mg\ml
1	H16	11 mm	13 mm
2	C16	10.5 mm	12 mm
3	M16	11.5 mm	12 mm
4	H17	9 mm	9 mm
5	M17	10 mm	12 mm
6	H1	12 mm	14 mm
7	C1	11 mm	12 mm
8	M1	NIL	NIL
9	H2	NIL	NIL
10	H5	NIL	NIL
11	H8	11 mm	9.5 mm
12	C5	10.5 mm	11 mm
13	C2	NIL	NIL
14	M2	NIL	NIL
15	M4	NIL	NIL
16	H7	NIL	NIL
17	C7	NIL	NIL
18	M7	NIL	NIL
19	C8	NIL	NIL
20	M8	NIL	NIL
21	H10	NIL	NIL
22	C10	NIL	NIL
23	H14	15 mm	11 mm
24	H12	NIL	NIL
25	M12	NIL	NIL
26	M10	NIL	NIL
27	C11	NIL	NIL
28	M11	NIL	NIL
29	C13	NIL	NIL
30	M13	NIL	NIL
31	C14	10.5 mm	11 mm
32	M14	NIL	NIL
33	H15	13.5 mm	11.5 mm
34	M15	NIL	NIL
35	H18	9 mm	9 mm
36	C18	15 mm	12 mm
37	M18	NIL	NIL
38	H19	NIL	NIL
39	M19	12 mm	10 mm
40	M5	NIL	NIL
41	H11	NIL	NIL

Key:
NIL: No zones
Negative control: DMSO
Positive control: Standard drug

Table 4: Antimicrobial activity against *Staphylococcus aureus*

Zones of Inhibition			
S.no	Fractions	1.5 mg\ml	0.75 mg\ml
1	H16	11mm	13mm
2	C16	NIL	NIL
3	M16	11mm	11mm
4	H17	NIL	NIL
5	M17	NIL	NIL
6	H1	NIL	NIL
7	C1	NIL	NIL
8	M1	NIL	NIL
9	H2	10 mm	10.5 mm
10	H5	14 mm	9.5 mm
11	H8	10 mm	9 mm
12	C5	12 mm	10.5 mm
13	C2	NIL	NIL
14	M2	NIL	NIL
15	M4	NIL	NIL
16	H7	NIL	NIL
17	C7	NIL	NIL
18	M7	NIL	NIL
19	C8	NIL	NIL
20	M8	NIL	NIL
21	H10	NIL	NIL
22	C10	NIL	NIL
23	H14	NIL	NIL
24	H12	NIL	NIL
25	M12	NIL	NIL
26	M10	NIL	NIL
27	C11	13.5 mm	12.5 mm
28	M11	NIL	NIL
29	C13	NIL	NIL
30	M13	13.5 mm	10 mm
31	C14	11.5 mm	10 mm
32	M14	NIL	NIL
33	H15	NIL	NIL
34	M15	NIL	NIL
35	H18	11 mm	10 mm
36	C18	NIL	NIL
37	M18	18 mm	15 mm
38	H19	NIL	NIL
39	M19	17 mm	13 mm
40	M5	NIL	NIL
41	H11	NIL	NIL

Key:
NIL: No zones
Negative control: DMSO
Positive control: Standard drug

Table 5: Antimicrobial activity against *Pseudomonas aeruginosa*

Zones of Inhibition			
S. No.	Fractions	1.5 mg\ml	0.75 mg\ml
1	H16	NIL	NIL
2	C16	NIL	NIL
3	M16	NIL	NIL
4	H17	NIL	NIL
5	M17	NIL	NIL
6	H1	NIL	NIL
7	C1	NIL	NIL
8	M1	NIL	NIL
9	H2	NIL	NIL
10	H5	NIL	NIL
11	H8	NIL	NIL
12	C5	NIL	NIL
13	C2	NIL	NIL
14	M2	NIL	NIL
15	M4	NIL	NIL
16	H7	NIL	NIL
17	C7	NIL	NIL
18	M7	NIL	NIL
19	C8	NIL	NIL
20	M8	13.5 mm	13 mm
21	H10	NIL	NIL
22	C10	NIL	NIL
23	H14	11 mm	10.5 mm
24	H12	NIL	NIL
25	M12	12 mm	11 mm
26	M10	NIL	NIL
27	C11	NIL	NIL
28	M11	12.5 mm	12 mm
29	C13	NIL	NIL
30	M13	NIL	NIL
31	C14	10 mm	9 mm
32	M14	NIL	NIL
33	H15	NIL	NIL
34	M15	NIL	NIL
35	H18	13 mm	10 mm
36	C18	NIL	NIL
37	M18	NIL	NIL
38	H19	NIL	NIL
39	M19	NIL	NIL
40	M5	NIL	NIL
41	H11	NIL	NIL

Key:
NIL: No zones
Negative control: DMSO
Positive control: Standard drug

Table 6: Active plant fractions against *Candida albicans*

S. No.	Fractions	1.5 mg\ml	0.75 mg\ml	Positive control
1	H14	10 mm	9 mm	10.5 mm
		9.5 mm	8.5 mm	10 mm
2	H15	11 mm	10 mm	10 mm
		11.5 mm	9.5 mm	10 mm
3	C18	15 mm	12 mm	10.5 mm
		15 mm	12 mm	10 mm
4	M19	10.5 mm	12 mm	10 mm
		10.5 mm	10.5 mm	10 mm

Table 7: Active plant fractions against *Staphylococcus aureus*

S. No.	Fractions	1.5 mg\ml	0.75 mg\ml	Positive control
1	M18	13.5 mm	11 mm	40 mm
		13.5 mm	11 mm	40 mm
2	M19	13.5 mm	10 mm	26.5 mm
		14 mm	12 mm	29 mm

Table 8: Active plant fractions against *Pseudomonas aeruginosa*

S. No.	Fractions	1.5 mg\ml	0.75 mg\ml	Positive control
1	M12	12.5 mm	9.5 mm	30 mm
		11 mm	9 mm	30 mm
2	M11	12.5 mm	10.5 mm	17 mm
		10 mm	10 mm	15 mm

Table 9: Determination of MICs and MBCs of active plant fractions

Microorganisms	Fractions	MICs (mg/ml)	MBCs (mg/ml)
Candida albicans	H14	0.6	1.5
	H15	0.45	0.75
	C18	0.45	0.75
	M19	0.45	1.5
Staph. aureus	M18	0.6	0.75
	M19	0.45	ND
Pseud. aeruginosa	M12	0.6	ND
	M11	0.45	1.5

Key: ND = Not determined

DISCUSSION

In the present study, major respiratory pathogens *S. aureus*, *P. aeruginosa* and *C. albicans* were included to evaluate the antimicrobial activity of different plant fractions. Results obtained from the investigation shows that plant fractions exhibit strong antimicrobial activity against all test isolates.

The diameters of zones of inhibition that obtained at different concentrations of fractions in well diffusion assay have shown the antimicrobial role of these plant fractions. Fractions showed strong inhibitory effects at all tested concentrations and was found to be bacteriostatic and bactericidal for all three isolates and the assay gives the reliable information regarding the antimicrobial ability of these extracts as also proved earlier in a study (Huang D, Ou B, Prior RL 2005). Current work suggests that *Cassia fistula* flowers and leaf fractions could be used for the treatment of respiratory infections particularly *S. aureus* and *C. albicans*, previously conducted experiments in this field also authenticates its use in traditional medicine for the treatment of diseases caused by *S. aureus* and *C. albicans* (V. Duraipandiyan, S. Ignacimuthu, 2007). Likewise, leaf, stalk and flower fractions of *Ixora coccinea* can be beneficial in case of *C. albicans* and *P. aeruginosa* infections. And the activity of the extract may be attributed to its flavonoids and phenols as mentioned in a study (Carlo *et al*., 1999; Kim *et al*., 1996).

Cowan, 1999 observed in a study that different compounds of plant extracts examined were found to be active against methicillin resistant *S. aureus*. Hence

indicates that these extracts can be effectively used against infections that are currently difficult to treat (Cowan, 1999).

In last recent years, an extensive research has been done on the discovery of the hidden antimicrobial properties of herbs and spices and their components to make their use in medicines. Beneficial health effects of plant extracts are well known and many researches showed that plants have antibacterial and antifungal properties. One of the studies completed in 2010 found that the medicinal impact of plants basically relies upon the secondary products present in the plant and is not usually related to a single compound however it may be a blend of various mixes present in various tissues of plant (Das *et al.,* 2010).

Current work shows that *C. fistula* and *I. coccinea* plant fractions have possessed bacteriostatic and bactericidal potentials, and as they appeared to be effective against major respiratory pathogens which are considered to be among the most resistant, biofilm producing microorganisms, these fractions can be used as a treatment for the infections. Further studies for their therapeutic use must be carried to have a knowledge regarding their administration (Dorman and Deans, 2000).

CONCLUSION

The present investigation indicates that plant fractions have antimicrobial properties against major respiratory pathogens *S. aureus*, *P. aeruginosa* and *C. albicans*. Since, there is a rapid increase in drug resistance among respiratory pathogenic microbes. Therefore, the plant sources for the development of natural antimicrobials are being investigated because, no doubt, natural sources are the superior one.

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