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Original Article

Antibacterial activity of some local herbs

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Abstract

Aqueous as well as organic extracts of eight herbs were screened for their antibacterial effects against *Staphylococcus (S) aureus, Escherichia (E) coli, Candida (C) albicans* and *Saccharomyces (S) cerevicae*. *C*. *sativum* was found effective antibacterial, excepting the *C*. *albicans*. *A*. *barbadensis* expressed growth inhibition zones against *E. coli*, *S*. *cerevicae*, and *C*. *albicans*. Antibacterial activity of *M*. *azadirachta* against *E. coli* and *S. cerevicae* was also observed. The extracts with positive results were blended in 1:1 ratio for seeking any synergistic activity. Alcoholic extracts of *A. indica* + *A. sativum* proved effective for controlling *S. sureus* and *E. coli*. Whereas n-hexane extracted *Z. officinale* + *A. sativum* were effective against *S. cerevicae* and *S. aureus*. The latter microbe demonstrated resistance to trimethoprim ceftriaxone, cefuroxime sodium and erythromycin. Results of the present study are indicative of promising potential of the herbs the microbial control in otherwise an increasingly antibiotic resistance developing situation.

Key words: *Staphylococcus aureus, Escherichia coli, Candida albicans, Saccharomyces cerevicae*.

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INTRODUCTION

Microorganisms may cause infectious diseases under specific conditions and such microbes are known as opportunistic pathogens. These infections could occur due to disturbance in defensive mechanisms of host (Klainer and Beisel, 1969). Opportunistic pathogens are known to inhabit digestive tract, skin and mucous membranes (Austwick *et al*., 1966). *Staphylococcus aureus* are normally present in human (Loren *et al.,* 2009). *S. aureus* infection is a major source of urinogenital and skin disorders in human beings (Franklin and Lowy, 1998). *Saccharomyces cerevicae*, also known as brewer's or baker's yeast causes opportunistic human infections (Eiriz *et al.,* 2008). *Candida albicans* extremely ubiquitous yeast which colonizes the mucous membranes of the digestive tract and urinogenital tract. It is an opportunistic pathogen, which can cause disease under adverse or abnormal conditions (Enweani *et al*., 1987). Antimicrobial plants extracts may inhibit fungi/bacterial growth (Eloff, 1998). As many herbal products or crude extracts have become an increasing topic for searching alternative of synthetic antimicrobial agents. Local herbs are widely available and may provide an alternative mean of treatment as topical antimicrobial agents. This study focused on antimicrobial potential of aqueous and organic herbal extracts of the plants; *Aloe barbadensis*, *Melia azadirachta*, *Mentha sylvestris*, *Azadirachta indica*, *Zingiber officinale*, *Allium sativum*, *Camellia sinensis* and *Coriandrum sativum* and combination of these herbal extracts against the of *Staphylococcus aureus, Escherichia coli, Saccharomyces cerevisae* and *Candida albicans.*

MATERIALS AND METHODS

Test Microorganisms

Four opportunistic pathogens were selected for testing antimicrobial activities of the plants' extracts. *S. aureus, E. coli, S. cerevicae and C. albicans* were used as test microorganisms. All the microbes were obtained from culture depository of Industrial biotechnology lab., Department of Zoology, University of the Punjab Lahore, except the *S. aureus* which was isolated from facial skin pimple with the help of sterile cotton swab which was swabbed on the mannitol salt agar plates. Golden pigmentation of bacterial colonies

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confirmed the strain. It was passed to nutrient agar, then to mannitol salt agar and then again to nutrient agar to ensure viability. The bacterial as well as yeast isolates were revived and stored on nutrient agar slants at room temperature (29±1°C) until further use. For inocula preparation, fresh growth from isolated colonies was transferred to 20ml nutrient broth and allowed to grow for 18-24 hours at 37°C.

Preparation of herbal extracts:

Eight plants, locally known for their skin health promoting effects were employed in this study (Table I).

Table I: General features of plants materials used in study.

Sr. No.	Botanical;Family (Local) names	Part used	Source
1	*Aloe barbadensis* Asphodelaceae (*Aloe vera*)	Leaves	Collected from Quaid-e-Azam Campus, University of The Punjab, Lahore
2	*Azadirachta indica* Meliaceae (*Neem*)	Leaves	Collected from Quaid-e-Azam Campus, University of The Punjab, Lahore
3	*Melia azadirachta* Meliaceae (*Derek*)	Leaves	Collected from Quaid-e-Azam Campus, University of The Punjab, Lahore
4	*Coriandrum sativum* Apiaceae (*Coriander*)	Seeds	Purchased from medicinal plant market, Lahore
5	*Mentha sylvestris* Lamiaceae (*Mint*)	Leaves	Purchased from medicinal plant market, Lahore
6	*Zingiber officinale* Zingiberaceae (*Ginger*)	Rhizome	Purchased from medicinal plant market, Lahore
7	*Camellia sinensis* Theaceae (*Tea*)	Commercial Kenya tea	Purchased from medicinal plant market, Lahore
8	*Allium sativum* Alliaceae (*Garlic*)	Edible bulb	Purchased from medicinal plant market, Lahore

The given plants/parts were washed with tap water and then with distilled water thoroughly. The herbs *aloe vera, ginger* and *garlic* were peeled off. Thin sections of *aloe vera* were cut with the help of a sharp paper cutter and then dried at room temperature. *Ginger* and *garlic* paste, made with the help of mortar and pestle, was also dried at room temperature.

Leaves of *derek, mint* and *neem* were washed and dried as described above, while *tea* and *coriander* were provided in the dried form. The dried herbs were finely ground into powder with the help of mortar and pestle, and dried in oven at 105ºC till consistent weight. They were kept in air tightened glass jars until extraction.

Extracts of the prepared plants material were made in three different solvents such as distilled water, ethanol, and n-hexane. For aqueous extract 5g of a plant material was dispensed in 100ml of distilled water in a reagent bottle. The suspension was exposed to free percolating steam in autoclave for one hour. This process was repeated for three consecutive days. The solutions were then filtered through pre-sterile Whatman filter paper No.1, into sterile culture bottles. Autoclaved sterilized foam caps were tightly plugged in the culture bottles, while keeping them in the laminar air flow. The bottles were then placed in oven at 105ºC to concentrate the extracts up to 10ml volume. The concentrated extracts were then kept in autoclaved air tightened bottles till further use. Ethanolic extracts were made by suspending 5g of a given material's powder in 100 ml of ethanol in a reagent bottle. The bottles were placed on orbital shaker with agitation speed of 120rpm at room temperature for five days. The material was then filtered through pre sterile Whatman

filter paper No.1. These filtrates were kept in open mouth containers separately for evaporation at the room temperature till 10ml of the extract was left.

These plant extracts were kept in sterile capped tubes. For n-hexane extract 5g powdered of a given plant material was suspended in 100ml of n-hexane in a reagent bottle. The extracts were prepared, filtered and stored according to the protocol described for ethanolic extract preparation.

Evaluation of antimicrobial activity of extracts against microbial isolates

A given plant extract (50µl) was dispensed on discs of 10mm diameter. Test microbe (20µl), after overnight growth, was spread over solidified nutrient agar plates (15ml). The plant extract loaded discs were then dispensed at 4.5cm apart from each other and 15mm from the edge of the nutrient agar plates along with control discs loaded with 50µl of respective solvent. The discs were picked up with the help of sterilized forceps and placed to the described location. After the incubation of 18-24 hours at 37ºC, growth inhibition zones were measured.

For the extracts showing promising growth inhibition for the test microbes, equivalent amounts of different extracts were mixed together and processed for observing their antimicrobial activity. The test organisms were also screened for vancomycin, rifampicin, nalidixic acid, ceftriaxone, streptomycin, trimethoprim, cefuroxime sodium, erythromycin and lincomycin antibiotics susceptibility tests by employing the antibiotics sensitivity discs (Oxoid) against lawning of the microorganisms on nutrient agar Petriplates.

RESULTS

Aqueous extract of *C. sativum* showed antimicrobial activity against *S. aureus*, *E. coli*, and *S. cerevicae* with growth inhibition zones (GIZ) of 17, 11 and 11mm, respectively. While the ethanolic extract of *C. sativum* showed antimicrobial activity only against *S. cerevicae* with a GIZ of 11mm. Its n-hexane extract did not show any antimicrobial activity. Aqueous extract of *M. sylvestris* showed antimicrobial activity only against *S. aureus* with GIZ of 22mm. While ethanolic extract of *M. sylvestris* did not show any antimicrobial activity. The n-hexane extract of *M. sylvestris* showed antimicrobial activity only against *C. albicans* with a GIZ of 11mm. Again the highest antimicrobial activity was shown by aqueous extract against *S. aureus* with GIZ of 22mm. Aqueous and n-hexane extracts of *M. sylvestris* proved to be effective against the selected microbial stains (Table II).

Aqueous extract of *A. barbadensis* manifested antibiotic activity against *E. coli* and *S. cerevicae* with GIZ of 12.5 and 14mm, respectively. Ethanolic extract of the plant did not show any antimicrobial activity. While n-hexane extract of *M. sylvestris* showed antimicrobial activity only against *C. albicans* with GIZ of 11mm. Aqueous extract of *Z. officinale* showed antibiotic activity only against *S. aureus* with a GIZ of 13.5mm (Table II).

Ethanolic extract of the leaves showed antimicrobial activity against *S. aureus*, *E. coli*, *S. cerevicae* and *C. albicans* with GIZ of 14, 12, 12 and 16mm, respectively. This extract against *C. albicans* with GIZ of 16mm for *A. indica* showed highest antifungal activity The n-hexane extract of *A. indica* did not show any antimicrobial activity at all (Table II). Aqueous extract of *C. sinensis* yielded GIZ of 16.5, 13, 17 and 14mm against *S. aureus*, *E. coli*, *S. cerevicae* and *C. albicans,* respectively. While ethanolic extract of *C. sinensis* showed antimicrobial activity against *S. aureus* and *E. coli* with GIZ of 20 and 11mm, respectively.

The n-hexane extract of the plant did not express any antimicrobial activity at all. Aqueous extract of *M. azadirachta* showed antimicrobial activity against *E. coli* and *S. cerevicae* with GIZ of 11.5 and 13mm, respectively. While the ethanolic and n-hexane extracts of the plant leaves did not show any antimicrobial activity. Aqueous extract of *A. sativum* gave antibiotic activity against *S. aureus*, *E. coli*, *S. cerevicae* and *C. albicans* with GIZ of 16.5, 15.5, 16 and 14mm, respectively. While, the alcoholic extract did not show any antimicrobial activity.

The n-hexane extract of *A. sativum* showed antimicrobial activity against *S. aureus*, *S. cerevicae* and *C. albicans* with GIZ of 18, 11.5 and 15mm, respectively (Table II). Among aqueous extracts of all the plants, *M. sylvestris* was most effective against *S. aureus* with growth inhibition zone of 22mm. Against the *E. coli,* the aqueous extract of *A. sativum* showed highest antimicrobial activity with GIZ of 15.5mm, while aqueous extract of *C. sinensis* appeared most effective against *S. cerevicae* with a GIZ of 20mm.

Table II: Antimicrobial activity of aqueous (A), ethanolic (B), n-hexane (C) extracts of the plants against test microorganism.

Test micro-organism	Plant species											
	A. indica			*C. sinensis*			*M. azadirachta*			*A. sativum*		
	A	B	C	A	B	C	A	B	C	A	B	C
S. aureus	12.5[a]	14	R	16.5	20	R	R	R	R	16.5	Rtr	18
E.coli	12	12	R	13	11	R	11.5	R	R	15.5	Rtr	R
S. cerevicae	13.5	12	R	17	R	R	13	R	R	16	R	11.5
C. albicans	13.5	16	Rtr	14	R	R	R	R	R	14	R	15

R: microbe that was found to be resistant to the treatment; **a:** Values express diameter of growth inhibition zones around 10mm disc loaded with 50µl of a given extract; **Rtr:** The microbe showed growth retardation zone against the treatment, evidenced by thin growth

Table III: Antimicrobial activity of aqueous (A), ethanolic (B), n-hexane (C) extracts of the plants against test microorganisms.

Test micro-organism	Plant species											
	C. sativum			*M. sylvestris*			*A. barbadensis*			*Z. officinale*		
	B	C	A	B	B	C	A	B	B	C	A	B
S. aureus	R	R	22	R	R	R	22	R	R	R	22	R
E.colii	R	R	R	R	R	R	R	R	R	R	R	R
S. cerevicae	11	R	R	R	11	R	R	R	11	R	R	R
C. albicans	Rtr	R	R	R	Rtr	R	R	R	Rtr	R	R	R

For abbreviations see table II

Aqueous extract of *C. sinensis* and *A. sativum* were most effective against *C. albicans* with GIZ of 14mm (Table II). Regarding the ethanolic extracts, *C. sinensis* appeared most effective against *S. aureus* with a GIZ of 20mm. Among n-hexane extracts, *A. sativum* showed highest antibacterial activity against *S. aureus* with a GIZ of 18mm. n-Hexane extract of *A. sativum* showed highest antimicrobial activity against *S. cerevicae* and *C. albicans,* with GIZ of 11.5 and 15mm, respectively (Table III).

The above described herbal extracts showing positive results, were mixed in different combinations and then tested for antimicrobial activity. Among the aqueous mixed herbal extracts synergistic effect was shown by *M. sylvestris* + *Z. officinale* against *S. aureus* with GIZ of 24mm (Table IV). While the *A. indica* + *Z. officinale* extracts of these plants separately showed GIZs of 22 and 13.5mm, respectively (Table II). *A. barbadensis* + *M. azadiradita* also showed synergistic effect against *S. cerevicae* with GIZ of 14.5mm (Table IV). While the individually they expressed GIZs of 14 and 13mm, respectively (Table II).

Among the ethanolic mixed herbal extracts, synergistic effect was shown by *A. indica* + *Z. officinale* against *S. aureus* with GIZ of 16.5mm (Table IV). While the *applications* of the extract of these plants separately showed antimicrobial activity against *S. aureus* with GIZ of 14mm and retardation zone, respectively (Table II). Combination of *A. indica* + *A. sativum* again showed synergistic effect against *E. coli* with GIZ of 16.5mm (Table IV), while the applications of these plants separately showed individual antimicrobial activities against *E. coli* with GIZs of 12mm and a growth retardation zone only, respectively (Table II). *C. sativum* + *A. indica* showed synergistic effect against *S. cerevicae* with GIZ of 12.4mm (Table IV), while applications of these plants separately these plants could express GIZ of 11 and 12mm, respectively. Among the n-hexane mixed herbal extracts a slight synergistic effect was shown by *Z. officinale* + *A. sativum* against *S. aureus with* GIZ of 12.5mm in contrast to the corresponding

separate extract GIZ of 10.5 and 11.5mm (Table II). Combination of these plants extract also showed synergistic effect against *S. cerevicae* with GIZ of 12.5mm (Table IV), while the separately yielded GIZ of 10.5 and 11.5mm, respectively (Table II).

Table IV: Antimicrobial effects of combinations of *M. sylvestris* + *Z. officinale* (A), *A. barbadensis* + *M. azadirachta* (B) *A. indica* + *Asativum* (C), *C. sativum* + *A. indica* (D), *Z. officinale* + *A. sativum* (E) extracts (1:1) of the plants against the microorganism

Test microorganism	Aqueous extract		Ethanolic extract		n-Hexane extract
	A	B	C	D	E
S. aureus	24[a]	-	16.5	-	12.5
E. coli	-	-	16.5	-	-
S. cerevicae	-	14.5	-	12.4	12.5
C. albicans	-	-	-	-	-

a: Values express diameter of growth inhibition zones around 10mm disc loaded with 50µl of a given extract;
-: No result was obtained.

Table V: Sensitivity of the test microorganism to the antibodies (A:Vancomycin , B: Rifampicin, C: Nalidixic acid, D:Ceftriaxone, E: Streptomycin, F: Trimethoprim, G: Cefuroxime sodium, H: Erythromycin and I: Lincomycin)

Test micro-organism	Antibiotics								
	A	B	C	D	E	F	G	H	I
S. aureus	27[a]	52	6	R	12	R	R	R	34
E.colii	22.2	18.4	24	8	22	34.6	R	28	17
S. cerevicae	11.2	19.1	38	7.6	16.9	32.3	R	38	17.8
C. albicans	23	21	29	R	18	30	R	32	19

a: Values express diameter of growth inhibition zones around 7mm disc. **R:** The microbe that was found to be resistant to the application

The antibiotics susceptibility tests showed varying GIZs in the range of 6-52mm against the test organisms. Among all the microbes, *S. aureus* was found most resistant against the erythromycin, cefuroxime sodium, trimethoprim and ceftriaxone. Among all the antimicrobial drugs, rifampicin showed highest antimicrobial activity with a GIZ of 52mm, while nalidixic acid showed minimum antimicrobial activity with GIZ of 6mm against *S. aureus. E. coli* and *S. cerevicae* were resistant against cefuroxime sodium.

Trimethoprim showed highest antibacterial activity with a GIZ of 34.6 mm, while ceftriaxone showed minimum antimicrobial activity with a GIZ of 8mm against the *E. coli.* Erythromycin showed highest antimicrobial activity with a GIZ of 38mm, while lincomycin showed minimum antimicrobial activity with a GIZ of 7.6mm against *S. cerevicae. C. albicans* was found resistant against cefuroxime sodium and ceftriaxone. Among all the antimicrobial drugs, erythromycin showed highest antibacterial activity with a GIZ of 32mm, while streptomycin showed minimum antimicrobial activity with GIZ of 18mm against *C. albicans* (Table V).

DISCUSSION

Antimicrobial activities of aqueous, ethanol and n-hexane extracts of the eight plants, employed in this study expressed varying in exerting antimicrobial effects against the test microorganism. *C. sativum* seed aqueous extracts showed antimicrobial activity in the range of 11 to 17 mm of growth inhibition

zone/50 µl to all the microorganisms tested. These results contradict the observations of Ayefer and Turgay (2003) who found that *C. sativum* seed extracts in alcohol and ethyl acetate had no inhibition effects for *S. aureus*. *M. sylvestris* aqueous extract expressed antimicrobial activity only against *S. aureus*. While its n-hexane extract showed minor inhibitory effect against *C. albicans*. *A. barbadensis* aqueous extract inhibited *E. coli* and *S. cerevicae* and did not exhibit antimicrobial activity against *S. aureus* and *C. albicans*. Cock (2008) reported that plant juice showed inhibitory activity against *E. coli* but not against *C. albicans* and *S. aureus*. *Z. officinale* exhibited antimicrobial activities in the range of 10.5-13.5 mm of GIZ/50 µl. The aqueous extract inhibited only *S. aureus,* while the alcoholic extract proved to be effective against *S. cerevicae* and *C. albicans.* n-Hexane extract showed inhibitory effect against *S. aureus, S. cerevicae* and *C. albicans*. Indu *et al.* (2006) found that aqueous extracts of *ginger* showed moderate inhibitory activity against two serogroups of *E. coli.* The results agree with the observations of Ekwenye and Elegalam (2005) who found that aqueous extract of *ginger* had not inhibitory effect on *E. coli*. Jantan *et al*. (2003) mensioned that essential oils of *Z. officinale* exhibited high activity against the yeasts including *C. albicans* and *S. cerevicae*. *C. sinensis* aqueous extract proved to be effective against all the test microorganisms. The ethanolic extract showed inhibitory activity only against *S. aureus* and *E. coli*. Mbata *et al*. (2006) and Toda *et al.* (1989) showed that moderate daily consumption of green tea killed *S. aureus* and other harmful bacteria. Likewise Yam *et al*. (1997) found that aqueous extract of the tea were effective against *E. coli* and *S. aureus*.

The *A. sativum* aqueous extract showed antimicrobial activity against all the test microorganisms. While n-hexane extract proved to be effective against *S. aureus, S. cerevice* and *C. albicans*. Lemar *et al.* (2002) explained the *C. albicans and E. coli* sensitivity to *A. sativum*. Growth inhibition effects of extracts of *A. sativum* against Gram-positive, Gram-negative organisms and fungi are well documented (Watanabe, 1966; Lamar, *et al.,* 2002). Ankri and Mirelman (1999) and Ruddock *et al.* (2005) found to exhibit antibacterial activity allicin, one of the active principles of freshly crushed garlic homogenates, against a wide range of bacteria *E. coli* and *S. aureus* and antifungal activity, particularly against *C. albicans.*

The *A. indica* aqueous and ethanolic extracts proved to be effective against the tested microbial strains. Okemo, *et al.* (2001) pointed out that crude extract of *neem* plant *A. indica* has antibiotic activity against *S. aureus, E. coli* and *C. albicans.* Aqueous and ethanolic extracts of *A. indica* leaves anti-dermatophytic activity (Venugopal, 1994). Bacteria, yeast and fungi have developed resistance to all classes of different antibiotics discovered to date. In addition, use/ misuse of antibiotics have undesirable effects including toxicity and drug-drug interactions (White *et al*., 1998; Alanis, 2005). Use of drugs has led to an increasing prevalence of multiple-drug resistant (MDR) strains. The test microorganism employed in this study were also found resistant to many of the antibiotics. This situation necessitates efforts for searching new effective antimicrobial agents (Cantrell *et al*., 2001). The present study reported antimicrobial effects of crude extracts of the plants. Further work on isolation and characterization of active principles from the plants reported in this study and their knowledge of pharmacodynamics may appreciate their clinical importance in controlling the reported and other opportunistic pathogens. Traditionally in this country several herbal powders are mixed to prepare a medicine. This study stress the importance of synergistic or antagonistic effects of different plants material while formulating a preparation.

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