ANTIMICROBIAL SCREENING AND METABOLIC FINGERPRINTING OF SOIL *BACILLI* AGAINST URINARY TRACT INFECTIONS (UTIs) CAUSING *E. coli*

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ABSTRACT: *Urinary tract infections (UTIs) are the most frequent bacterial infections that result in the high morbidity and mortality worldwide. E. coli is the main causative agent of the urinary tract infections, affecting the people of all the ages, causing community acquired as well as hospital acquired infections. The increased resistance of the UTI E. coli against the wide range of drugs emphasis the need to introduce novel antibiotics to combat these infections. This study aimed to screen the soil bacilli for antimicrobial activity and compounds against UTI E. coli. In total 20 Bacillus strains were recovered from the soil samples collected from various locations at Lahore, Pakistan. The selected strains were identified by morphological, microscopic, biochemical and physiological characterization. The strains showed potent antimicrobial activity against UTI E. coli in agar diffusion assay and in most of the cases a zone of inhibition in the range of 12 to 23 mm was observed. The methanolic crude extracts of these bioactive strains were chemically analyzed by thin layer chromatography (TLC) and HPLC-UV and the so called metabolic finger prints were documented in each case. The TLC analysis showed different colored bands representative of various structural classes of the antibiotics. Likewise the HPLC-UV chromatogram of each of the isolate exhibited peaks at different retention times (t_R) highlighting the diversity of secondary metabolites produced by the selected strains. Overall the study reveals that these bioactive Bacillus strains could be a valuable source for the antimicrobial compounds which could be useful for the treatment of urinary tract infections.*

Key words: UTI *E. coli*, Bioactive soil *Bacilli*, TLC, HPLC-UV, Secondary metabolites, Metabolic fingerprinting

INTRODUCTION

Urinary tract infections (UTIs) have become the second most common type of infections, caused by the gram negative bacterial family of Enterobacteriaceae that include *E. coli*, *Enterobacters*, *Proteus* and *Klebsiella* [1]. *E. coli* is considered as one of the most common causative agent of urinary tract infections in humans [2, 3]. Urinary tract infections caused by *E.coli* is an old problem but the *E. coli*, worldwide, is becoming resistant to antimicrobial agents and the trend is increasing both in outpatients and hospitalized patients. In Pakistan like in various other parts of the world, the resistance to commonly used antibiotics is becoming a challenge and there are several reports of UTI *E.coli* which are resistant to the available drugs or are at least loosing their susceptibility[4].The major factor for the antimicrobial resistance is the misuse of the antimicrobials by the population that results in the change of the microbial profile of the urinary tract isolates [5]. Because of its ability to produce a large number of antimicrobial peptide, the genus *Bacillus* is becoming an interesting source to search for inhibitory substance [6,7] and the member *B. subtilis* is one of the crucial producers of these substances [8,7]. The genus *Bacillus* includes aerobic endospore-forming bacteria and is one of the largest sources of bioactive natural products [9, 10]. Various research reports confirm that the *Bacillus* species have a wide range of antimicrobial activities since they are used as antifungal, antibacterial, antiviral, antiameobocytic and antimycoplasma agents having a great potential for biopharmaceuticals and biotechnological applications [11].

The use of chemical screening using thin layer chromatography (TLC) and high performance liquid chromatography such as HPLC-UV/RI, HPLC-MS etc provides the opportunity to create a nearly complete picture of the metabolic profile of the secondary metabolites or the so called metabolic finger-prints of each of the isolate [12].These lab scale chromatographic techniques (TLC and HPLC) are frequently used for the detection, isolation and purification of the secondary metabolites as well as biomolecules [13].The major purpose of this study was to screen the *Bacillus* strain from the competent microbiota from the nature (Soil) whose antibacterial compounds can antagonize the growth of UTI *E. coli*. The metabolic fingerprinting and cytotoxicity assay were performed to have an idea about the presence of different constituents present in the crude extracts of the selected strain sand as well as to measure their cytotoxic effect on the living organism. The information about the metabolic profile, antimicrobial activity and cytotoxicity of the selected soil *Bacillus* strains could be valuable in the search for new antibiotics and commercially useful therapeutic agents.

MATERIALS AND METHODS

Isolation of bacterial strains

Soil samples were collected from the Gulshan-e-Iqbal Park, Lahore and were processed for the isolation of *Bacillus* strains by crowded plate technique on Lauria-Bertani agar [14].The suspected strains were selected from the crowding and the cultures were purified by repeated sub-culturing. The strains were identified by morphological, microscopic, biochemical and physiological characterization. In

morphological characterization colony characteristics including, colony shape, size, color, pigment production, margins, elevation were determined. In microscopic, characterization, gram staining was performed and the slides were visualized under microscope (100X) using oil immersion [15]. In case of biochemical and physiological characterization following tests were performed; catalase, oxidase, MR-VP, starch and casein hydrolysis, nitrate reduction, indole test, hydrogen sulphide production and urease test.

Preliminary biological screening

As originally a large number of *Bacillus* strains were obtained by crowded plate technique, so in order to select only the bioactive strains, the whole strain collection was passed through a preliminary antimicrobial activity test against two representative gram positive and gram negative bacteria *Staphylococcus aureus*, and *Pseudomonas*, including *Enterobacter* and *Salmonella* by cross-streak method and solid media bioassay. In cross streaked method the isolated *Bacillus* strains were streaked in the middle of LB agar plate and incubated at 37 ^{o}C for 48 hours and after incubation the test organisms were cross streaked along the main streak and the plates were again incubated for 24 hours at 37 ^{o}C, and growth inhibition of the indicator strains was observed and recorded. In solid media bioassay the isolated *Bacillus* strains were grown for 48 hours at 37 ^{o}C on agar plates and agar discs were recuperated and placed upright down on the test plates containing the indicator organisms, the test plates were incubated for 24 hours at 37 ^{o}C and zone of growth inhibition around the agar discs was observed.

Cultivation of strains and solvent extractions

The selected bioactive *Bacillus* strains were grown as shaking culture in 200ml LB broth for 48 hours at 37 °C. After harvesting, the broth was mixed with ethyl acetate (1:1) and was sonicated for 15 min in a sonicating bath (UltrasonsMedi-II), the mixture was shifted to a separating funnel and was mixed by shaking for few minutes, the separating funnel was allowed to stand until the two layers separated. After the settling down of layers, the upper organic layer containing ethyl acetate was recovered, the extraction was repeated three times and the ethyl acetate was collected in a separate flask. The ethyl acetate containing the active compounds was recycled in the rotary evaporator (Heidolph Laborata 4000) and the resulting crude extract was obtained in methanol and was collected in the glass vials. These methanolic crude extracts were used for determining antimicrobial activity against UTI *E. coli* for chemical screening by TLC and HPLC-UV.

Determination of antimicrobial activity against UTI *E. coli*

The methanolic crude extracts of the selected *Bacillus* strains were screened for activity against the urinary tract infections causing (UTI) *E. coli* by agar diffusion method. The test plates were prepared by the standard plate overlaying method as described by Sajid et al. 2009 [12]. The 24 hours fresh culture of UTI *E. coli* was obtained and 100 µl cell suspension was mixed in 4ml molten agar in a glass tube and was overlaid on the surface of agar plates already poured with 16ml of the LB agar. With the help of sterile cork borer four wells were made in each test plate, the wells were loaded with 60µl of the methanolic crude extracts, blank methanol was used as negative control, and the plates were labelled accordingly and were incubated for 16-24 hours at 37 ^{o}C. The diameter of the zone of inhibition was measured in millimetre and was recorded.

Brine shrimp microwell cytotoxicity assay

The cytotoxicity of the methanolic crude extracts of the bioactive *Bacillus* strains was determined by microwell cytotoxicity assay against the brine shrimp larvae *(Artemia salina*) [16]. In this method the dried eggs of *Artemia salina* (0.5 g) were added to a 500 ml separating funnel containing 400ml of artificial sea water and air was bubbled through the funnel for aeration, the funnel was kept for 24–48 h at room temperature. Later the aeration was stopped, for the settling down of the remaining eggs and the suspension was kept undisturbed for 1 hour. For the selection of active larvae one side of the funnel was covered with aluminium foil and the other was illuminated with a lamp, as a result active phototropic larvae gathered at the illuminated side. About 30-40 shrimp larvae were transferred to a 96 wells microtiter plate, the wells were filled with 0.2 ml of artificial sea water. The numbers of dead larvae (N) were counted, then a solution of 20µg of the crude extract in DMSO was added to the wells, three replicates were used for each sample, the plate was kept at room temperature in the dark. After 24 hours of incubation, the dead larvae number (A) was counted under a stereomicroscope (Swift Instruments, INC SM80). The survived larvae were killed by the addition of 0.5 ml methanol to count the total number (G) of the larvae. DMSO without extract was used as negative control; while Actinomycin D was used as positive control for 100% mortality .The percentage mortality (M) was calculated by the following formula:

$$M= [A-B-N/G-N]$$

M = percentage mortality of larvae after 24 h. A = Number of the dead larvae after 24 h. B = Average number of the dead larvae in the blind samples after 24 h.

Chemical screening

The methanolic crude extracts were analyzed by thin layer chromatography (TLC) and HPLC-UV for metabolic finger printing of the bioactive secondary metabolites produced by the selected strains.

***Thin Layer Chromatography*:** A line was drawn about one inch from the base of TLC plate and sample numbers were marked on it. With the help of a capillary tube each sample was spotted on the TLC plate drop wise, the spots were superimposed with allowing the former spot to dry before super imposing it. The plate was air dried after loading the samples, and developed with 10% CH_2Cl_2/MeOH solvent system. The TLC plate was placed in the solvent tank and it was made sure that spots were not dipped in the solvent. The solvent was allowed to rise one third of the plate, and then allowed to air dry and visualized under UV at 254nm and 366nm. The components showing UV absorbance and fluorescence were marked and scanned. The TLC plates were stained by spraying with Anisaldehyde/H_2SO_4 and Eherlisch's reagents separately. The plates were dried under

a hot air drier and were observed for color bands. The clear bands of different colors were visible in each sample.

***HPLC-UV* analysis:** The crude extracts were analyzed by HPLC-UV on HPLC system (Sykum) using the software clarity. The column used was C18 from Phenomenex with 30cm length. Mobile phase used was methanol and water (95:5) and the flow rate was adjusted to 1 ml/min. The crude extract was dissolved in methanol and 50µl was injected through a micro-syringe. The sample was run for 15 min and UV absorbance was determined at 254 nm. The peaks of each sample were analyzed and were compared at different retention times with standard UV absorption data of secondary metabolites.

RESULTS

A total of 20 strains were isolated by crowded plate method based on the preliminary antimicrobial activity, the strains exhibited distinctive colony characteristics including, regular, irregular, spindle, filamentous, circular shape colonies; small, medium, large colonies; most of the isolates have sticky texture but the strains, A-6, A-11, A-16 exhibit dry texture; the strains A-1, A-7, A-12, A-14, A-15 have convex elevation, A-2, A-4, A-5, A-8 A-9, A-10, A-13, A-17, A-18, A-19, A-20 have flat and A-6, A-11, A-16 showed umbonate elevations. Mostly the colonies have entire margins except the colonies of strains A-4, A-7, A-8, A-9, A-10, A-13, A-18 having erase margins, while the strains A-5, A-6, A-11, A-16 have undulate margins. Most of the strains were non pigment producers, except the strain A-5 that produce yellow colored pigment. The gram staining results showed the gram positive rods in single, pairs and in chains under the light microscope. In biochemical and physiological characterization the isolates also showed variable results; most of the strains were catalase positive except the isolates A-5, A-11 A-17, A-18, and A-19 which showed negative catalase test. Maximum strains were oxidase positive except A-8, A-9 and A-20, which shows these three strains don't have cytochrome oxidase enzyme in the electron transport chain. The ability of the isolates to have mixed acid fermentation; MR-VP test was performed, the strains A-1, A-2, A-6, A-8, A-11, A-12, A-13, A-15, A-16 A,-17 A,-18 A-19 showed negative MR test, the strain A-3, A-8, A-18, A-19, A-20 showed negative VP test, while in all the strains indole and H_2S production was negative. Maximum strains utilize casein as a carbon source showing clear zone when grown on the media plates supplemented with casein except the isolates A-5, A-8, A-11, A-12, A-13, A-19 and A-20. Starch hydrolysis test was negative for A-1, A-5, A-8 and A-12 that did not show clear zone around the streaks on adding the iodine solution in the starch plates while rest of the strains showed clear area of hydrolysis around the growth streaks on adding iodine solution.

The preliminary antimicrobial screening of these selected isolates was done by conventional cross-streaked method (Table 1; Fig 1 A and B) and by agar plug method (Solid media bio-assay) against the test organisms including; *Staphylococcus aureus*, *Pseudomonas* (X_4) and *Enterobacters* (M_9, S_2) (Table 2). The strains A-1, A-3, A-6, A-9, A-11, A-16, A-17, A-18, A-19 among all the isolated strains exhibited significant activity against *S.aureus* and *Pseudomonas* in initial screening. These active strains were selected to investigate their antimicrobial potential against the urinary tract infection (UTI) causing *E. coli* test strains by agar diffusion method. The methanolic crude extracts obtained from the culture broth of the selected *Bacillus* strains showed significant zone of inhibition by agar diffusion method against UTI *E. coli* strains. The maximum zone of inhibition against *E. coli* test strain 1 was 17mm exhibited by isolate A-3, 15mm exhibited by the isolates A-18 and A-19 while against *E. coli* test strain 2; the *Bacillus* isolates showed larger zone of inhibition up to 20mm by the isolates A-6 and A-9, up to 22mm by the isolate A-18 and zone of inhibition up to 23mm by the isolate A-19 (Table 3; Fig 2-A, B, C and D; Fig 3). In micro-well cytotoxicity assay of the crude extracts of the selected bioactive strains against *Artemia salina*; the isolates A-3 and A-16 showed high larval mortality up to 55% and 57.1% respectively, which means they produce some kind of the cytotoxic compounds, while the crude extract of the isolates A-9 and A-17 showed no cytotoxicity i.e. 0% larval mortality. The crude extracts of the isolate A-1 and A-11 showed moderate larval mortality up to 21%, while the crude extracts of the isolates A-6 and A-18 showed minor cytotoxicity i.e. of 5.5% and 4.76 % larval mortality (Table 4, Fig 4).

In the metabolic fingerprinting, methanolic crude extracts of the active *Bacillus* strains were analyzed by thin layer chromatography (TLC) using the Anisaldehyde/H_2SO_4 and Eherlisch's reagent and by HPLC-UV. The TLC analysis showed bands of various chemical constituents in each of the crud extract under short and long UV (254nm, 366nm). The bands pattern under UV at 366 nm was as; the extract of isolate A-1 two prominent bands and the rest of bands were diffused, isolate A-3 two bands, A-6 four band, A-9 three bands and rest were diffused, A-11 two bands, A-16 four prominent band, A-17 six bands, A-18 one prominent band and other was diffused, and the extract of the isolateA-19 had only one band (Fig 5A). The extracts of the isolate A-1 showed 8 prominent bands, A-3, A-6, A-9, A-11 two bands, A-16 only one diffused band, A-17, A-18 one band and A-19 two bands under UV at 254 nm (Fig 5B).

The bands pattern of the TLC plates after staining with Eherlisch's reagent; the extract of the isolate A-1exhibited two purple bands and a yellow diffused band, the isolate A-3 showed one prominent purple band at the bottom, A-6 exhibited two purple bands, A-9 exhibited four purple bands and a few yellow diffused bands, A-11 showed one prominent purple band and a light diffused yellow bands and rest of bands were diffused, A-16 showed two purple bands and the rest were diffused, A-17 showed 6 purple bands, A-18 showed 4 purple bands and a yellow diffused

Tables and figures

Table1 Preliminary activity of selected *Bacillus* isolates against test organisms by cross streak method

S. No.	Selected Strains	Preliminary antimicrobial activity					
		S.aureus	**M9**	**X4**	***Bacillus***	**S_2**	**E_4**
1	A-1	+++	-	++	+	-	-
2	A-2	-	-	-	-	-	-
3	A-3	+	-	-	-	-	-
4	A-4	-	-	-	-	-	-
5	A-5	+++	-	+	+	-	-
6	A-6	++	-	-	-	-	-
7	A-7	-	-	-	-	-	-
8	A-8	-	-	-	-	-	-
9	A-9	+	-	-	-	-	-
10	A-10	-	-	-	-	-	-
11	A-11	+++	-	++	-	-	-
12	A-12	-	-	-	-	-	-
13	A-13	-	-	-	-	-	-
14	A-14	-	-	-	-	-	-
15	A-15	-	-	-	-	-	-
16	A-16	+++	-	+	-	-	-
17	A-17	+	-	-	-	-	-
18	A-18	+	-	-	-	-	-
19	A-19	+	-	-	-	-	-
20	A-20	-	-	-	-	-	-

Key: M9,S_2=*Enterobacter*, X4 = *Pseudomonas*, E4 =*Salmonella*

Table 2 Preliminary antimicrobial activity of the selected isolates against test organisms by agar plug method

S. No.	Strains	Preliminary antimicrobial activity Zone of inhibition(mm)					
		S.aureus	**M9**	**X4**	***Bacillus***	**S_2**	**E_4**
1	A-1	15	-	9	-	-	-
2	A-2	-	-	-	-	-	-
3	A-3	8	-	-	-	-	-
4	A-4	-	-	-	-	-	-
5	A-5	16	-	10	-	-	-
6	A-6	10	-	-	-	-	-
7	A-7	-	-	-	-	-	-
8	A-8	-	-	-	-	-	-
9	A-9	-	-	-	-	-	-
10	A-10	-	-	-	-	-	-
11	A-11	15	-	9	-	-	-
12	A-12	-	-	-	-	-	-
13	A-13	-	-	-	-	-	-
14	A-14	-	-	-	-	-	-
15	A-15	-	-	-	-	-	-
16	A-16	14	-	10	-	-	-
17	A-17	9	-	-	-	-	-
18	A-18	9	-	-	-	-	-
19	A-19	8	-	-	-	-	-
20	A-20	-	-	-	-	-	-

Key: M9, S_2 = *Enterobacter*, X4 = *Pseudomonas*, E4 = *Salmonella*

Table 3 Antimicrobial activity of the crude extracts of selected *Bacillus* strains against UTI *E. coli*

***Selected* Isolates**	**Antimicrobial activity (Zone of inhibition in mm)**	
	E. coli 1	***E. coli 2***
A-1	10	10
A-3	17	15
A-6	14	20
A-9	12	20
A-11	13	17
A-16	12	11
A-17	11	19
A-18	15	22
A-19	15	23

Table 4 Cytotoxicity of the crude extracts of the selected *Bacillus* strains against brine shrimp (*Artemia salina*)

Bacillus isolates	**Wells**	**N**	**A**	**T**	**M=[(A-B-N)/G-N]100**
A-1	1A	0	15	38	21%
A-3	2A	1	23	28	55%
A-6	3A	0	9	36	5.5%
A-9	4A	1	7	20	0%
A-11	5A	1	12	20	21%
A-16	6A	2	25	30	57.1%
A-17	7A	0	7	19	0%
A-18	8A	0	8	21	4.76%
A-19	9A	1	8	17	0%
Blind	10A	0	7	36	19.4%
Positive control	11A	1	20	24	52.17%

Where M = percent of the dead larvae after 24 h. A = Number of the dead larvae after 24 h. B = Average number of the dead larvae in the blind samples after 24 h.

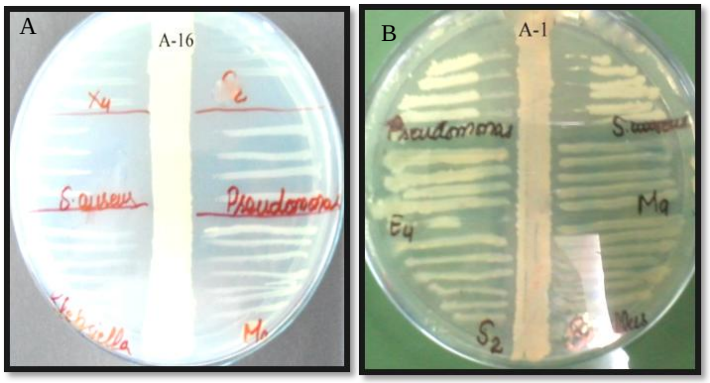

Fig.1 Activity of selected *Bacillus* strains against *S.aureus, Pseudomonas, Enterobacters* (E4, M_9, S_2) by cross streaked method, **A**=A-16, **B**=A-1

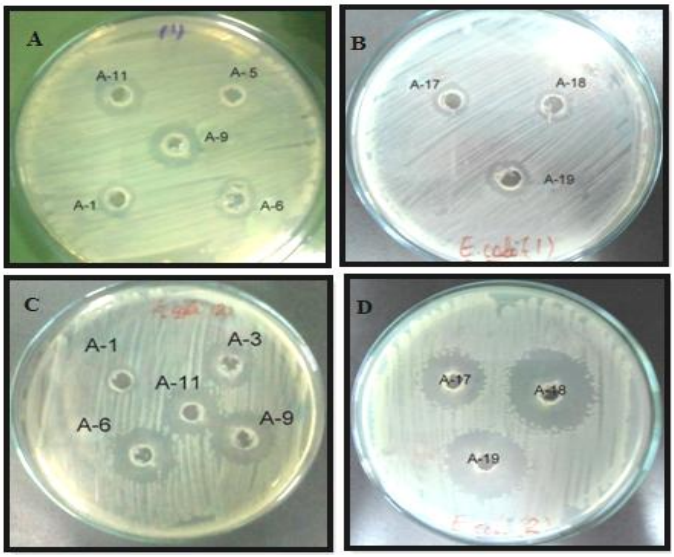

Fig.2 Antimicrobial activity of the crude extracts of the selected *Bacillus* strains against urinary tract infection causing *E. coli*.**A**= Activity of strains A-1, A-6, A-9, A-11 against UTI *E. coli* 1, **B**= Activity of strain A-17,A-18,A-19 against UTI causing *E. coli* 2, **C**= Activity of strains A-1, A-3, A-6, A-9, A-11 against UTI *E. coli* 2, **D**= Activity of strains A-17, A-18, A-19 against UTI *E. coli* 2

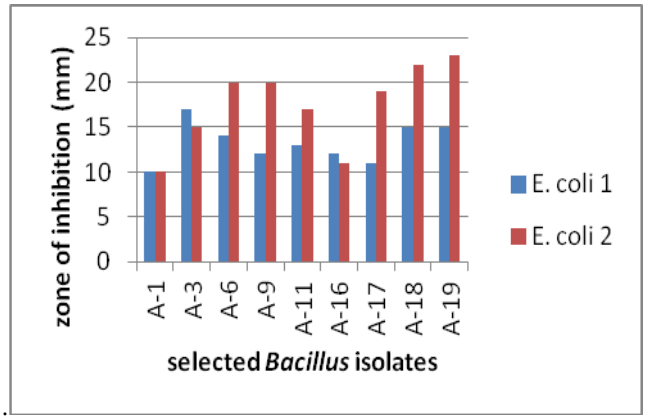

Fig 3: Comparative antimicrobial activity of selected *Bacillus* strains determined with the crude extract by agar diffusion method against UTI *E. coli*

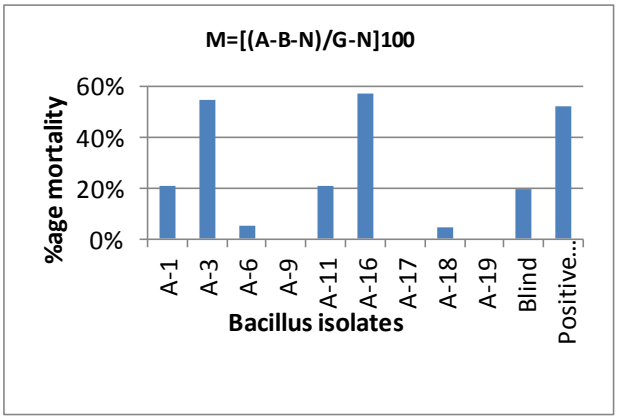

Fig 4: %age mortality of the larvae of *Artemia salina* determined by microwell cytotoxicity assay with methanolic extracts of the selected *Bacillus* strains

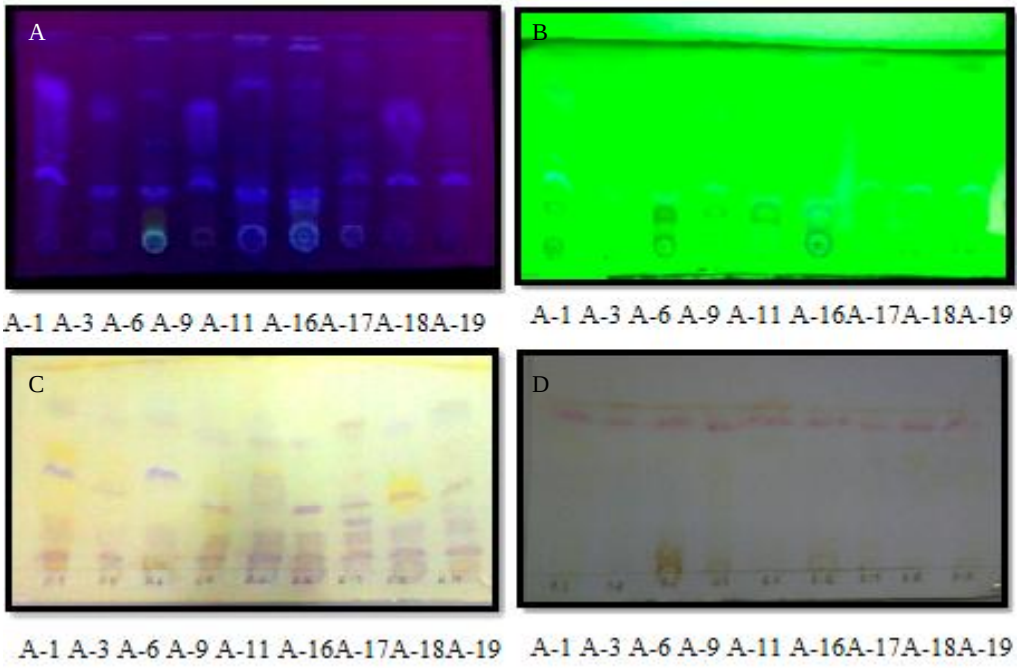

Fig.5 A=TLC plate under 366 nm UV radiation**, B**=TLC plate under 254 nm UV radiation, **C**=TLC plate after staining with Eherlisch's reagent, **D**= TLC plate after staining with Anisaldehyde /H_2SO_4,.

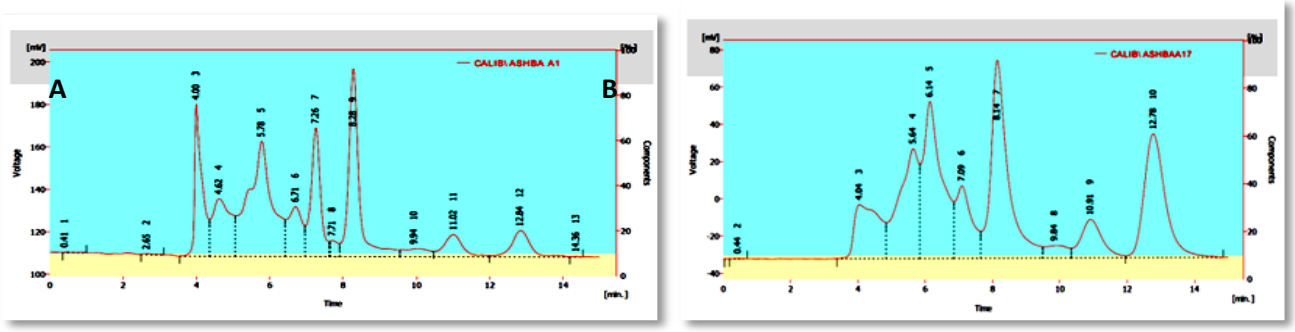

Fig.6 HPLC-UV chromatogram of crude extracts of selected *Bacillus* strains **A**=A-1, **B**=A-17

band, while the crude extract of the isolate A-19 exhibited two purple bands and the rest were diffused (Fig 5C). The TLC plates after staining with Anisaldehyde/H_2SO_4 showed one pink colored band in the crude extract of most of the strains at different *Rf* values (Fig 5D).The HPLC-UV analysis was performed with crude extracts of the selected bioactive strains in order to further investigate the selected strains for the active metabolites produced by them. The HPLC-UV chromatogram of the crude extract showed various peaks at different retention times (t_R) for each strain. In case of the crude extract of isolate A-1 major peak appeared at $t_{R;}$ 8.289min with peak area of 1777.818 [mV.s] (Fig 6A), isolate A-17 at $t_{R;}$ 8.140 min with peak area of 3777.352 [mV.s] (Fig 6B), and in case of extract A-19 at $t_{R;}$ 8.156 min with peak area of 6116.323 [mV.s].

DISCUSSION

The intentions of the present study was to screen the active *Bacillus* species which are now famous for their ability to produced wide range of antimicrobial compounds from many years. The genus *Bacillus* included the gram positive spore forming rod shaped bacteria and is found to be very effective in producing antimicrobial compounds that can have inhibitory effect on the other bacteria. Al-Ajlani and Hasnain in 2010 isolated 118 morphologically different *Bacillus* isolates from soil and found 50 active strains against at least two test organisms [17]. Mendo et al. 2004 isolated a strain of *Bacillus subtilis* from sugar cane fermentation, which produced a polypeptide antibiotic, bacitracin, which inhibited the growth of *Micrococcus flavus* [18]. So there is strong evidence that different species of the genus *Bacillus* are a valuable source in the search for new and useful antimicrobial agents.

So we aimed to identify any antimicrobial compounds from the *Bacillus* species that could inhibit the growth of UTI *E. coli* strains. As the UTI *E. coli* is emerging as multidrug resistant organism which although a commensal resident of large intestine but can opportunistically cause disease state in the individual. Urinary tract infections caused by *E. coli* are becoming the most complicated infections because of the increased prevalence of the antibiotic resistance among the *E. coli.* There are many reports on different outbreaks of UTI *E. coli* worldwide that includes extended spectrum beta lactamases and multiple drug resistance [19, 20]. According to a recent report of WHO (2014) on the antibiotic resistance worldwide depicted significant UTI *E. coli* resistance against the most commonly used anti-bacterial of the third generation cephalosporin's and florouroquinolones in America, Eastern Meditteranean, South- East Asia and Pacific regions [21] which means that the treatment with these common antibacterial is not possible now and there is need for the new anti-bacterial in the market [22, 23]. In this work the *Bacillus* strains were isolated from the soil and then bioactive strains were selected in prescreening by checking their activity against gram positive and gram negative test organisms as a result 9 active *Bacillus* strains that inhibited the growth of test organisms were selected for further testing their potential activity against UTI *E. coli* by agar diffusion method along with the metabolic finger printing of the crude extracts produced by these isolates was performed by thin layer chromatography (TLC) and HPCL-UV analysis. The metabolic fingerprinting is very useful chemical screening technique which gives the opportunity to visualize almost all the significant components present in an extract. The antimicrobial activity by agar diffusion method showed significant zones of inhibition against UTI *E. coli* strains, having maximum zone of inhibition of 23mm by the isolate A-19 (Fig 2; Fig 3). The TLC analysis showed prominent bands under UV and with staining reagent Anisaldehyde and Eherlisch's which showed the metabolic diversity of the compounds present in the crude extract (Fig 5- A, B, C, D) as well as the HPLC chromatogram showed different peaks which depicted the number of different compounds that may be present in the crude extracts of selected bioactive *Bacillus* strains (Fig 6- A, B). The cytotoxicity screening showed that most of the compounds in the crude extracts of *Bacillus* strains are less toxic except the strains A-3 with 55% and A-16 showing 57.1% of cytotoxicity against *Artemia salina* (Table 4; Fig 4). The promising antimicrobial activity and low cytotoxicity of the extracts means, most of the antimicrobial compounds produced by these selected *Bacillus* strains could be useful agents to control the emerging problem of multidrug resistant UTI *E. coli*.

CONCLUSION

Based on the results of this study, it can be concluded that the soil *Bacillus* species are a significant source for the discovery of new antimicrobial compounds that would be valuable in the management of the emerging drug resistance UTI *E. coli* and could be very useful in bioactive microbial natural products research.

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