The Journal of Animal & Plant Sciences, 29(5): 2019, Page: 1433-1441
ISSN: 1018-7081

ACTIVE FRACTIONS FROM *E. COLI* ATCC 35218 WITH ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES

M. Maqsood[1], Z. Mushtaq[1*], Kashif Jilani[1] and U. Khan[1]

[1]Bioactive Molecules Research Lab (BMRL), Department of Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan
Corresponding E-mail and contact: zahidmushtaquaf@uaf.edu.pk, +92-322-6058801

ABSTRACT

Discovery of new therapeutic agents has become crucial because mankind is experiencing issues regarding health and environment. In the current study *Escherichia coli* (ATCC 35218) extracts were evaluated for their antimicrobial and antioxidant potentials against different bacterial and fungal species. Different extracts were prepared following different methods including soxhlet extraction with ethanol using cell mass of *E. coli*. Some other extracts like crude proteins, crude isoprenoid quinine and polar lipids were extracted from the *E. coli* cell mass with different extraction solvents. Another extraction was done using solvents in order of increasing polarity by using methanol as well as *n*-hexane, chloroform, ethyl acetate and water. The antimicrobial potential was evaluated by well diffusion as well as disc diffusion method. The antioxidant effect of *E. coli* extracts were investigated in terms of DPPH free radical scavenging activity. Polar lipids showed maximum antibacterial activity in a range of 5-9 mm against *Bacillus subtilis, Staphylococcus aureus* and *Pasturella multocida*. Extract of Crude proteins exhibited maximum antioxidant activity (83.45%). A significant antifungal activity of about 13 mm against *Fusarium solani* was also shown by crude polar lipids. Finally, results were analyzed statistically by the paired t-test (Tucky's test) using software Statistix, version 8.1.

Keywords: Bioactive compounds, *Escherichia coli,* Antimicrobial potential, Ampicillin, Ciprofloxacine.

INTRODUCTION

The enhanced prevalence and resistance to infectious diseases is becoming a worldwide problem (Barros *et al.,* 2007). Hence, treatment of infectious diseases is becoming a challenge since pathogens have developed resistance against a number of antibiotics (Ferber, 2010). Different pathogenic diseases in humans e.g., ulcerative colitis (UC), Crohn's disease (CD) and human inflammatory bowel diseases (IBD) are the lethal diseases caused by the microbes e.g., *Mycobacterium avium* spp (Frank *et al.,* 2007). Cell aging, carcinogenesis, metabolic disorder and inflammation etc occur due to different oxidants like free radicals and reactive oxygen species (ROS) (Omonhinmin *et al.,* 2015). It is very tough to understand the process by which endogenous and exogenous oxidants interact with different body organs because as we inhale oxidants it starts a number of processes (Ciencewicki *et al.,* 2008).

So, in these alarming conditions there is a need to look for new therapeutic agents. Scientists always paid attention for discovering improved and novel techniques to overcome these harmful challenges (Guo*et al.,* 2008). Because existing important drugs have side effects for humans and are expensive, so there is a need of finding and producing effective novel antibiotics from microorganisms (Bizuye *et al.,* 2013). Microorganisms not only cause infection, but also possess different organic substances to cure infection. A number of natural products with clinical importance are isolated from microorganisms since the discovery of penicillin in 1929 which introduced a new period of antibiotics. From that time, natural products between 30, 000 to 50, 000 from microorganisms have been discovered from which some (more than 8000) are antibiotics and some (more than 10, 000) are biologically active (Fenical and Jensen, 1993). Risk of many diseases like cardiovascular disease, reduces due to these discoveries (Etherton *et al.,* 2004).

Different probiotic bacteria (e.g., *Lactobacillus rhamnosus, Enterococcus faecium, Bacillus clausii* and *Lactobacillus gasseri*) isolated from human milk etc were with antimicrobial characteristics (Villoslada *et al.,* 2007; Nighat and Mushtaq, 2019). *Streptomyces sp*. is a source of lenticulone with antibacterial activity. *Streptomyces* is a common source of secondary metabolites (sugars, terpenes, fatty acids, amino acids etc) with antimicrobial effect (Omura *et al.,* 2001). A number of high and low molecular weight bioactive compounds with antimicrobial activity have been isolated from *pseudo alteromonas* species (Bowman, 2007). An efficient enzymatic (superoxide dismutase, catalase, laccase and peroxidases) and non-enzymatic (polysaccharides or phenolic derivatives) antioxidant system is also possessed by certain microorganisms like *Ganoderma applanatum, Flammulinavelutipes, Ganoderma lucidum, Meripilu sgiganteus,* and Endophytic Fungi (Nimse and Palb, 2015).

Different bioactive compounds are also produced by *Escherichia coli.* Glycosylated polyketides

is a secondary metabolite produced as bioactive compound in *Escherichia coli* possessing antimicrobial activity and extracted by using ammonium acetate and acetonitrile solvents (Peiru *et al.,* 2004). *Escherichia coli* possess a number of compounds with bioactive potential, for example, isoprenoid quinones like Mequinone-8 (MK-8), Ubiquinone-8 (UQ-8) and Ubiquinone-7 (UQ-7) detected in *Escherichia coli* and possess antimicrobial effects (Gao *et al.,* 2004). Some other important bioactive compounds like phospholipids are also present in *Escherichia coli*. These may include phosphatidyl glycerol and phosphatidyl ethanolamine (Yasuhiro *et al.,* 1967). These phospholipids also possess significant antimicrobial activities (Dorosz *et al.,* 2010). *E. coli* cytoplasmic membrane contains lipopolysaccharides and peptidoglycan which are involved in host defense mechanism and provides protection against bacteria so it also imparts considerable antibacterial potential (Inouye *et al.,* 2008; Rosenfeld and Shai, 2006). *Escherichia coli* also contain different antioxidants like two superoxide dismutases (SOD). For all living cells that are exposed to oxygen that is superoxide (O_2^-), SOD serves as an important antioxidant (Beauchamp and Fridovich, 1971).

Keeping the above discussion in mind that *E. coli* could be a source of bioactive agents we planned to extract active fractions from *E. coli* through different methods and then tested them for their antimicrobial and antioxidant potential. Thus, the main objective of this study is to evaluate the bioactive potential of organic and aqueous extracts of *E. coli* by *in vitro* assays related to antimicrobial and antioxidant importance.

MATERIALS AND METHODS

The present study was planned to evaluate the bioactivities of different extracts obtained from selected strain of *E. coli.* The research was conducted in Bioactive Molecules Research Laboratory (BMRL), Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan.

Culturing and cell harvesting of bacterial strain: *E. coli* (ATCC 35218) strain was used in our study. The strain of *Escherichia coli* was confirmed from Institute of Microbiology, University of Agriculture, Faisalabad. Nutrient agar (NA) and nutrient broth (NB) (Merck, UK) were used as basal medium to grow bacterial cultures (Müller *et al.,* 2016). Then the broth culture was centrifuged (Centrifuge H-200 NR, Kokusan, Japan) at 6,702 × g for 10 minutes at 4 °C to obtain bacterial cell mass, which was dried, weighed and used for extraction procedure.

Extraction and fractionation

Protein extraction (PE): Bacterial crude protein was extracted for bioactivities with Protein Extraction Buffer (PEB) by following the procedure reported previously with little modifications (Jamil *et al.,* 2007). 1 g of dry cell mass of organism was resuspended and sonicated (COMPLIANT RoHS) for 10 minutes in 5 mL of PEB [10 mM Na_2HPO_4 (Merck, Germany), 15 mM NaH_2PO_4 (Merck, Germany), 100 mM KCl (Merck, Germany), 2 mM EDTA (BDH), 1.5 % polyvinyl polypyrrolidone (PVPP) (Merck, Germany), 1 mM phenyl methyl sulfonylfloride (PMSF) (Merck, Germany), and 2 mM thiourea (Merck, Germany)]. This mixture was then centrifuged at 6,702× g for 10 minutes stored at 4°C after filteration. The crude protein contents of sample were determined spectrophotometrically (BioTek, Winooski, VT, USA) by Bradford method using bovine serum albumin (BSA) as standard (Bradford, 1976).

Extraction through different organic and aqueous solvents with increasing order of polarity: Solvent extraction method of Emran *et al.,* (2015) was followed with little modifications. 5 g dry cell mass of *E. coli* was used for extraction. Extraction was done by resuspending the dry cell mass of organism in methanol (Merck, Germany) followed by stirring for 48 hours and centrifugation. Then the supernatant containing crude methanol extract (CM) expressed as digit "a" and pellet containing residual cell mass expressed as digit "b" was subjected to fractionation with different aqueous and organic solvents (Merck, Germany) including water (AQ), *n*-hexane (NH), chloroform (CL), ethyl acetate (EA) and methanol (ME) respectively. Depending upon polarity of respective solvent different layers were formed each time and carefully separated. These layers were dried, weighed, dissolved in DMSO (Dimethyl sulfoxide) (Scharlau, Spain E.U) and stored for further bioactivity based studies.

Soxhlet extraction (SE): The Soxhlet (Borosilicate glass) extraction was implemented with 5 g of dry cell mass of *E. coli,* solvent used for extraction was ethanol (b.p. 78.37°C). 12 hours of extraction process was done at 80°C, followed by solvent evaporation. The extract was dried, weighed, dissolved in DMSO and used to evaluate their antimicrobial and antioxidant potential (Li *et al.,* 2014).

Isoprenoid Quinone (IQ) and Polar Lipids (PL) Extraction: Mixtures containing chloroform and methanol have been shown to provide efficient extraction of isoprenoid quinone and polar lipids, and the established procedure of Bligh and Dyer was conveniently used for the extraction of bacterial lipids (Minnikin *et al.,* 1984).

Antimicrobial (antibacterial and antifungal) Assay by disc diffusion/well diffusion method: Antibacterial activity of *E. coli* (ATCC 35218) extracts against gram positive *Staphylococcus aureus*, *Bacillus subtilis* and gram negative *Pasteurella multocida* and local strain of

Escherichia coli and antifungal activity against *Fusarium solani* was assessed. These strains were characterized from the Department of Veterinary Microbiology, University of Agriculture, Faisalabad.

Pure cultures were maintained on nutrient agar for bacterial strains and potato dextrose agar (PDA) medium for fungal strain in the petri plates. The inoculums of nutrient broth (ICN Biomedicals) for bacterial strains and sabrose dextrose liquid media (Merk, UK) for fungal strain with pure cultures strains were grown at 37˚C. The inoculums with 1×10^8 spores/mL were used for antimicrobial assay.

Antimicrobial activity of *E. coli* extracts was determined by well diffusion as well as disc diffusion method (Candan *et al.,* 2003). 100 µL of inoculums were added to each sterile plate aseptically and mixed with media homogenously. The nutrient agar and PDA were then allowed to solidify. After this, wells of 3 mm in diameter were bored into the solidified agar with the help of sterilized borer. 50-70 µL of extracted samples were poured into the wells. Positive controls were Ampicillin (GSK Pakistan Limited F-268-Sindh industries) and Ciprofloxacin (SAMI Pharmaceuticals Pvt. Ltd. F-95, S.I.T.E., Karachi-Pakistan) in antibacterial assay and Terbinafine and Fluconazol (Sigma-Aldrich) in antifungal assay was used. The Petri plates were then incubated at 37^{o}C for 24 hours, for microbial growth. The *E. coli* extracts having antibacterial and antifungal activity, inhibited the bacterial and fungal growth and clear zones were formed around them. The diameters of inhibition zones were measured in millimeters with the help of vernier caliper (Zaidan *et al.,* 2005; Tepea *et al.,* 2004).

Antioxidant assay by DPPH scavenging activity: The antioxidant activity of *E. coli* extracts were assessed by measuring their scavenging ability to 1, 1-diphenyl-2-picrylhydrazyl stable free radicals (DPPH) (Sigma-Aldrich). Previously reported DPPH assay was performed with slight modifications (Roopa *et al.,* 2015). 5 µL of sample solutions were added in 95 µL of methanolic solution of DPPH. After 30 minutes incubation in darkness at room temperature the absorbance was recorded at 517 nm. The experiment was performed for three times. Ascorbic acid (Sigma-Aldrich) was used as standard control. Inhibition of free radical by DPPH was calculated in the following way:

I (%) = ($A_{blank} - A_{sample} / A_{blank}$) x 100: Where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test compounds. Percentage scavenging was calculated as depicted in formula where I (%) is the percentage inhibition of DPPH free radical.

Statistical analysis: Results were analyzed statistically in which the significance of mean zone of inhibition between standards and *E. coli* extracts were tested by the paired t-test (Tucky's test) analysis using software Statistix, version 8.1. Data were expressed as mean ± S.D (Saha *et al.,* 2010).

RESULTS AND DISCUSSION

Extraction of bioactive fractions from *E. coli:* An integrated procedure of Minnikin *et al.,* (1984), was used for the extraction of isoprenoid quinines (IQ) and polar lipids (PL) in which 500 mg of dry cell mass of *E. coli* was used. Crude IQ and PL yields were 20 and 26.6 mg/mL of DMSO respectively. Soxhlet extraction (SE) was performed by using 5 g of dry cell mass of *E. coli* and its ethanol extractable yield was 150 mg/mL of DMSO. For solvent based extraction, *E. coli* dry cell mass of 5 g was subjected to extraction using different aqueous and organic solvents from which CM, NH-a, CL-a, EA-a, MS-a, WS-a, NH-b, CL-b, EA-b, MS-b and WS-b were with yields of 125, 61, 60, 100, 146, 50, 72, 100, 253, 30 and 40 mg/mL of DMSO respectively. Crude protein contents were extracted by using 1 g dry cell mass of *E. coli* with 5 mL of protein extraction buffer and its amount was 163 µg/mL.

Antimicrobial activity of *E. coli* extracts: In the present study, the antimicrobial potential of *E. coli* extracts were determined through well diffusion as well as disc diffusion method (Candan *et al.,* 2003). Results indicated that MS-a and PL showed considerable antibacterial activity when compared with positive controls that are Ampicillin (10 mg/mL) and Ciprofloxacin (400 mg/100mL). PL showed strongest activity against *P. multocida* and *B. subtilis* that is 9 mm ± 0.264 and 6 mm ± 0.251 respectively. MS-a also showed significant activity against *Pasteurella multocida* that is 7 mm ± 0.173. Protein extracts of *E. coli* showed no antibacterial activity against any of the strain. Antibacterial activity of *E. coli* (ATCC 35218) extracts was also checked against another strain of *E. coli*. The EE and CM extracts showed little activity against this *E. coli* strain. In this case only one positive control was used i.e., Ampicillin (10 mg/mL). IQ is the secondary metabolite that also showed significant antibacterial activity when compared with both of the controls (Ampicillin and Ciprofloxacin). Other extracts or fractions showed a little or no activities (Table 1). Results are also represented in graph as shown in Fig 1 and Fig 2 that shows zone of inhibition.

Our PL extract showed strongest antibacterial activity, reason behind this is that as molecular solubility with components of bacterial cell membrane increases it causes interaction and disruption of associated functions (John and Sons, 2011). Our *E. coli* EA extracts showed moderate antibacterial activity against Gram-positive and Gram-negative bacteria. In a study, *Streptomyces* showed significant antimicrobial activity against Gram-negative

and Gram-positive bacteria and against some fungus. 2-hydroxy-9,10-anthraquinone is the bioactive compound isolated by ethyl acetate extract. This EA extract showed good activity when subjected to antimicrobial assay against fungi and bacteria (Balachandrana *et al.,* 2016). The reason for this difference in activity can be attributed to the antibacterial activity of two different bacterial extracts.

In a report, the antibacterial activity of *Debregeasia salicifolia* chloroform, ethyl acetate, methanol and aqueous extracts were evaluated. Their ethyl acetate extracts showed strongest activity against selected bacterial strains i.e., *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli* showed 13.9, 12.3 and 16.2 mm respectively (Emran *et al.,* 2015). While in our study, *E. coli* (ATCC 35218) EA extract showed mild activity against these bacterial strains i.e., 5 mm for *B. subtilis* and *S. aureus* and 0 mm in case of another *E. coil* strain. Our *E. coli* ME also showed good activity against *Staphylococcus aureus* and *Pasturella multocida*. CL also showed good activity against *Pasturella multocida.* This CL, EA and ME activities were due to the presence of compounds like lipids, saponins, alkaloids and amino acids in these fractions (Bhagavathy *et al.,* 2011). Whereas, other extracts showed little or no activity against these bacterial strains as shown in Table 1.

Antifungal activity of the *E. coli* extracts was determined through well diffusion method as used by Serban *et al.,* (2011). We used Terbinafine and Fluconazol as the standard drugs. All the *E. coli* extracts were subjected to antifungal activity against selected strain of *Fusarium solani*. In a study conducted by Yadav *et al.,* (2005), the protein extracts (PE) from various strains of *E. coli* (XL Blue, HB101) showed good anti-Aspergillus activity. But our *E. coli* strain (ATCC 35218) PE does not showed any antifungal activity. This difference may be due to the extraction from two different bacterial strains (CL) of *E. coli. E. coli* (ATCC 35218) showed good antifungal activity (9 mm of ZOI) as described by Islam *et al.,* (2013), for *Spondiasdulcis* leaves CL. EA-a and WS-a of *E. coli* also showed 9 and 10 mm ZOI respectively Fig 3.

All these results were compared with the value of standard drug's ZOI (Figure 2). Fluconazol and Terbinafine with 11.5 and 31.66 mm of ZOI were used as standard drugs (Table 1). Results are also represented graphically as shown in Fig 3.

Antioxidant studies of *E. coli* extracts by DPPH assay: Free radicals cause oxidative damage to biomolecules. Serious consequences causes due to this damage including cancer, atherosclerosis, aging and many other diseases. These disease causing free radicals are efficiently inhibited by antioxidants. Antioxidant potential of *E. coli* extracts was evaluated through DPPH free radical scavenging ability of the extracts. 1, 1-Diphenyl-2-picryl-hydrazyl is an indicator of this activity, is a stable free radical and present in violet color when in its radical form whereas it turns to yellow coloration on reduction.

Antioxidant potential of the extract is directly proportional to its capacity to reduce DPPH free radical (Brand *et al.,* 1999). In the present work the organic and aqueous extracts of *E. coli* were analyzed for their antioxidant potential through DPPH free radical scavenging activity. Table 1 showed significant antioxidant activity of organic and aqueous extracts of *E. coli*. Results are also represented in graph as shown in Fig 4.

Our results showed that all extracts have antioxidant potential but some showed more antioxidant behaviour. For example, protein extract showed strongest antioxidant activity than all others extracts. It showed 83.45% DPPH free radical scavenging activity that is near about of antioxidant activity of standard ascorbic acid (84.86%) used. It is reported that, microbial proteases like *Bacillus licheniformis* alkaline protease (AP), *Aspergillus oryze* validase (Val), *Bacillus subtilis* neutral protease (NP) were reported for producing peptides with antioxidant potential. This hydrolysate showed significant DPPH free radical scavenging activity too (Hogan *et al.,* 2009). In a study conducted by Borquaye *et al.,* (2015), the antioxidant activity of crude peptide extracts from *Galatea paradoxa* (*G. paradoxa*) and *Patella rustica* were evaluated. Antioxidant activity for *G. paradoxa* and *P. rustica* was 56.77 % and 79.77 % respectively.

In our study, other extracts also showed significant activity for example, PL are the more active with 70% inhibition. It is due to its polar nature. As molecule move towards polarity it causes increase in its antioxidant activity (Zhang *et al.,* 2010). Some extracts showed less activity e.g., EA-b and WS-a exhibits 23.03% and 25.32% respectively as shown in Table 1.

Table. 1. Antimicrobial and Antioxidant Studies on some *Escherchia coli* extracts.

Sr #	Sample	Antibacterial activity				Antifungal activity	Antioxidant activity
		Bacillus subtilis	***Staphylococcus aureus***	***Pasteurellamultocida***	***Escherichia coli***	***Fusarium solani***	**Mean ± S.E**
		Mean ZOI (mm) ± S.E	**Mean ZOI (mm) ± S.E**	**Mean ZOI (mm) ± S.E**	**Mean ZOI (mm) ± S.E**	**Mean ZOI (mm) ± S.E**	
1	**IQ**	$5.26^{D} \pm 0.251$	$4.26^{F} \pm 0.251$	$6.06^{E} \pm 0.208$	Nill	$11^{C} \pm 0.17$	$60.62^{GH} \pm 0.10$
2	**PL**	$6.26^{C} \pm 0.251$	$5.26^{E} \pm 0.251$	$9.1^{C} \pm 0.264$	Nill	$13^{B} \pm 0.16$	$70.64^{C} \pm 0.09$
3	**EE**	$4.36^{E} \pm 0.321$	$5.26^{E} \pm 0.251$	$4.03^{G} \pm 0.251$	$3.16^{C} \pm 0.152$	$10^{CD} \pm 0.18$	$65.82^{E} \pm 0.04$
4	**CM**	$3.26^{F} \pm 0.305$	$7^{C} \pm 0.251$	$3.26^{H} \pm 0.251$	$6.3^{B} \pm 0.264$	$5^{F} \pm 0.26$	$42.52^{J} \pm 0.09$
5	**NH-a**	$4.23^{E} \pm 0.251$	$6.2^{D} \pm 0.2$	$4.3^{G} \pm 0.3$	Nill	Nill	$61.71^{G} \pm 0.05$
6	**CL-a**	$4.3^{E} \pm 0.3$	$5.23^{E} \pm 0.208$	$6.1^{E} \pm 0.264$	Nill	$9^{D} \pm 0.19$	$65.22^{E} \pm 0.0001$
7	**EA-a**	$5.3^{D} \pm 0.208$	$5.23^{E} \pm 0.251$	$5.23^{F} \pm 0.251$	Nill	$9^{D} \pm 0.19$	$68.53^{D} \pm 0.04$
8	**MS-a**	$5.23^{D} \pm 0.208$	$5.33^{E} \pm 0.305$	$7.1^{D} \pm 0.173$	Nill	$7^{E} \pm 0.22$	$63.67^{F} \pm 0.09$
9	**WS-a**	$3.4^{F} \pm 0.360$	$5.3^{E} \pm 0.3$	$6.33^{E} \pm 0.305$	Nill	$10^{CD} \pm 0.18$	$25.33^{K} \pm 0.12$
10	**NH-b**	$4.3^{E} \pm 0.3$	$5^{E} \pm 0.2$	$6.06^{E} \pm 0.115$	Nill	$9^{D} \pm 1$	$53.23^{I} \pm 0.08$
11	**CL-b**	$5.3^{D} \pm 0.3$	$5.1^{E} \pm 0.264$	$5.2^{F} \pm 0.2$	Nill	$9^{D} \pm 1$	$60.13^{H} \pm 0.01$
12	**EA-b**	$4.26^{E} \pm 0.251$	$4.03^{F} \pm 0.152$	Nill	Nill	Nill	$23.03^{L} \pm 0.09$
13	**MS-b**	$4.23^{E} \pm 0.251$	$4.03^{F} \pm 0.251$	Nill	Nill	Nill	$61.34^{GH} \pm 0.08$
14	**WS-b**	$4.36^{E} \pm 0.321$	$4.13^{F} \pm 0.152$	Nill	Nill	$9^{D} \pm 1$	$54.54^{I} \pm 0.04$
15	**Protein extract**	Nill	Nill	Nill	Nill	Nill	$83.46^{B} \pm 0.06$
16	**Ampicillin (+)**	$12.5^{B} \pm 0.577$	$12.5^{B} \pm 0.5$	$12.5^{B} \pm 0.5$	$11.5^{A} \pm 0.5$	---	---
17	**Ciprofloxacin (+)**	$30.5^{A} \pm 0.5$	$31.5^{A} \pm 0.5$	$32.33^{A} \pm 1.5$	---	---	---
18	**Terbinafine (+)**	---	---	---	---	$31.7^{A} \pm 0.21$	---
19	**Fluconazol (+)**	---	---	---	---	$31^{A} \pm 0.10$	---
20	**Ascorbic acid (+)**	---	---	---	---	---	$84.86^{A} \pm 0.31$

Samples-a = Fractions from crude methanolic extract, **samples-b** = Fractions from crude cell mass, **mm** = millimeter. **ZOI** = Zone of inhibition in millimeter, n=15, *Means sharing same alphabets are statistically non-significant (P>0.05).

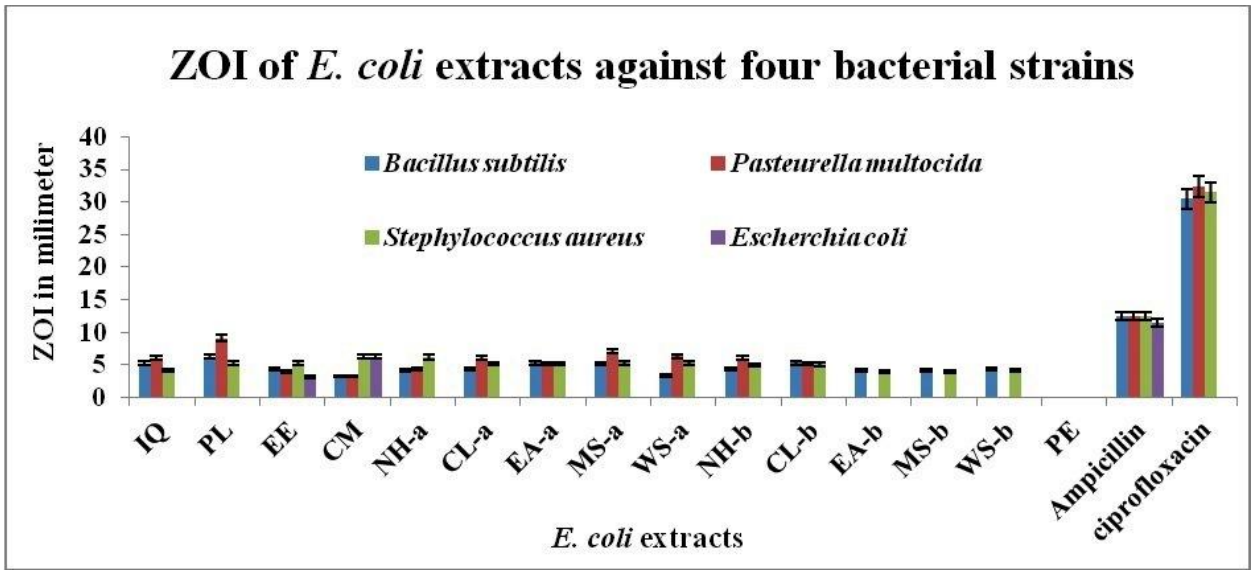

Fig 1. Graphical representation of antibacterial activity of *E. coli* extracts against four strains with Ampicilin and Ciprofloxacin as positive controls. Each bar represents data from at least three independent experiments with error bars showing standard deviation. Maximum antibacterial activity was shown by polar lipids.

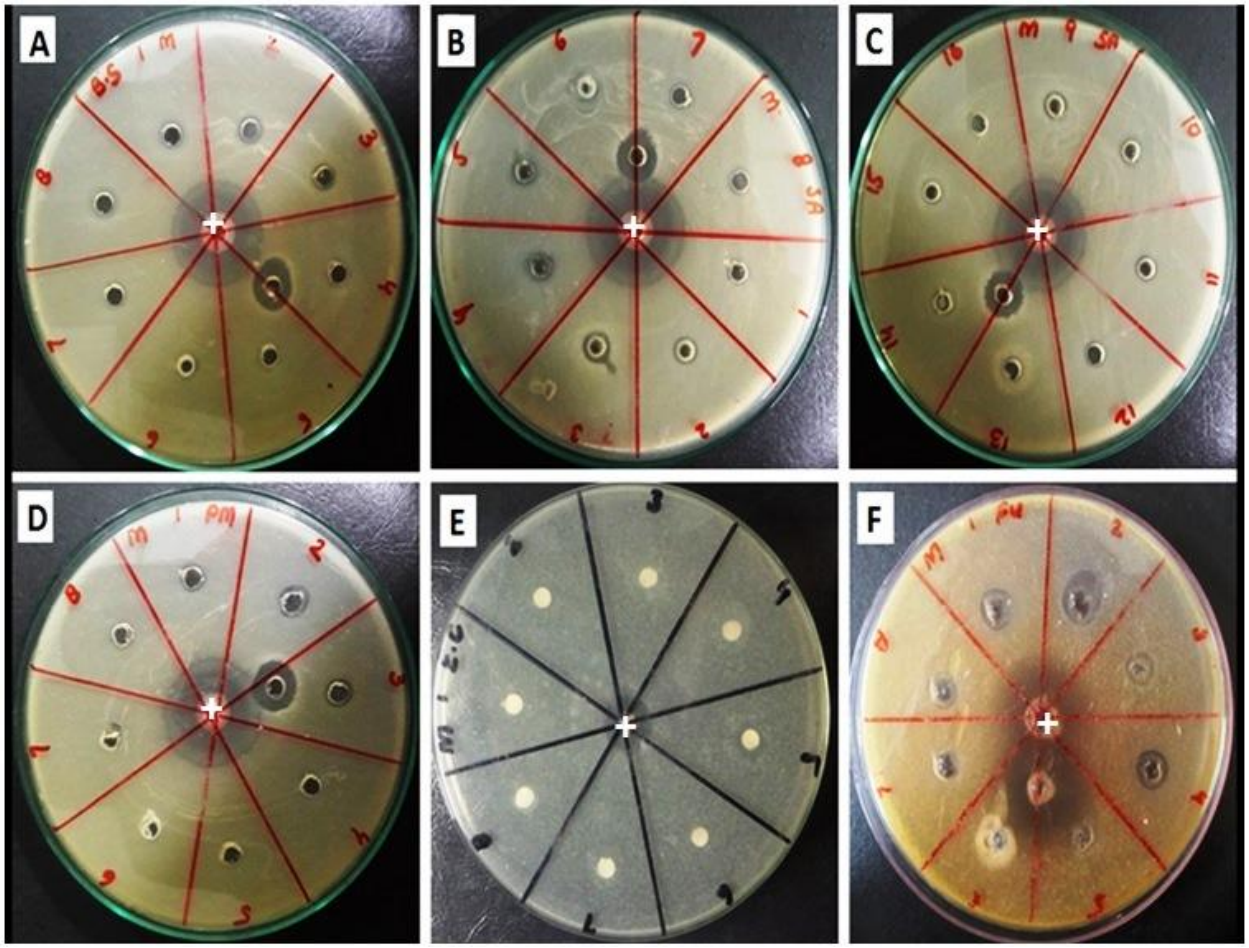

Fig 2. Antibacterial activity of different *E. coli* (ATCC 35218) extracts against *Bacillus subtilis* (A), *Staphylococcus aureus* (B, C), *Pasteurella multocida*(D), Local *Escherichia coli* (E) and antifungal activity against *Fusarium solani* (F).

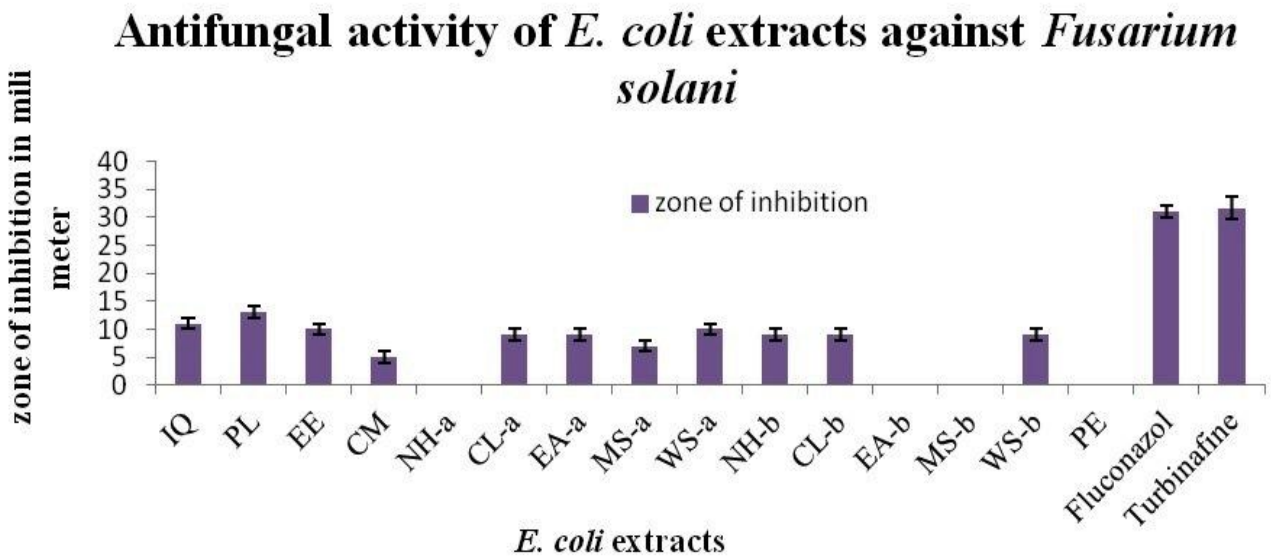

Fig 3. Antifungal activity of *E. coli* extracts against *Fusarium solani.* Each bar represents data from at least three independent experiments with error bars showing standard deviation. Maximum antifungal activity was shown by polar lipids and minimum activity was shown by crude methanolic extracts.

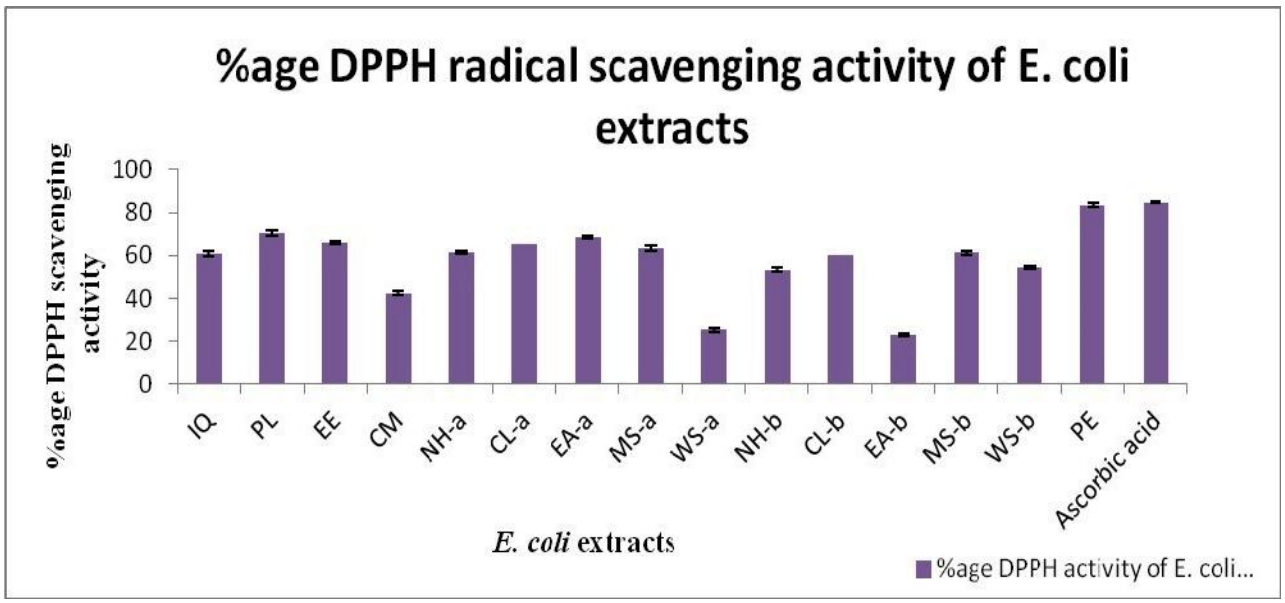

Fig 4.Percentage antioxidant activity of *E. coli* extracts. Antioxidant activity of *E. coli* extracts and ascorbic acid as a standard. Each bar represents data from at least three independent experiments with error bars showing standard deviation. Protein extracts showed maximum antioxidant activity and EA-b showed minimum activity. Other extracts also showed significant DPPH free radical scavenging activity.

Conclusion: On the basis of these studies it is suggested that *E. coli* extracts like CL, ME and crude protein, EA, PL can be used as antimicrobial and antioxidant agents respectively. Still there is a need of extensive studies in the field of microbes based drugs designing. *In vivo* studies may be helpful in determining the real potential usefulness of these *E. coli* extracts for the treatment of infectious diseases. In future each of these extracts can further be analyzed for the presence of active components that are responsible for their respective bioactive potential.

Acknowledgments: We are very grateful for the co-operation and facilities of chemicals and equipments received from Dr. Muhammad Shahid of PMBL (Protein Molecular Biology Laboratory) and MBL (Molecular Biology Laboratory), Department of Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan.

REFERENCES

Balachandrana, C., V. Duraipandiyana, Y. Arund, B. Sangeethae, N. Emib, N.A. Al-Dhabic, S. Ignacimuthua, Y. Inagumab, A. Okamotob, and P.T. Perumald (2016). Isolation and characterization of 2-hydroxy-9,10-anthraquinone from *Streptomyces olivochromogenes* (ERINLG-261) with antimicrobial and antiproliferative properties. Rev. Bras. Farmacogn. 26: 285–295.

Barros, L., R. C. Calhelha, J.A. Vaz, I. C. F.R. Ferreira, P. Baptista, and L. M. Estevinho (2007). Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. Eur. Food Res. Technol. 225: 151–156.

Beauchamp, C., and I. Fridovich (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287.

Bhagavathy, S., and P. Sumathiand I.J.S. Bell (2011). Green algae *Chlorococcumhumicola*-a new source of bioactive compounds with antimicrobial activity. Asian Pac. J. Trop. Biomed. 1: 1-7.

Bizuye, A., F. Moges, and B. Andualem (2013). Isolation and screening of antibiotics producing *actinomycetes* from soils in Gondar town, North West Ethopia. Asian Pac. J. Trop. Dis. 3: 375-381.

Borquaye, L.S., D. Godfred, O. Edward, and A. Emmanuel (2015). Antimicrobial and antioxidant properties of the crude peptide extracts of *Galatea paradoxa* and *Patella rustica*. Springer Plus. 4: 500.

Bowman, J.P., (2007). Bioactive compound synthetic capacity and ecological significance of marine bacterial genus *Pseudoalteromonas*. Mar. Drugs. 5: 220-241.

Bradford, M.M. (1976). A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Brand, W.W., M.E. Cuvelier, and C. Berset (1999). Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss. Technol. 28: 25-30.

Candan, F., M. Unlu, B. Tepe, D. Daferera, M. Polissiou, A. Sökmen, and H.A. Akpulat (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* sub sp. *Millefolium* A fan. (Asteraceae). J. Ethnopharmacol. 87: 215–220.

Ciencewicki, J., S. Trivedi, and S.R. Kleeberger (2008). Oxidants and the pathogenesis of lung diseases. J. Allergy Clin. Immunol. 122: 456-468.

Dorosz, J., Y. Gofman, S. Kolusheva, D. Otzen, N. Ben-Tal, N.C. Nielsen, and R. Jelinek (2010). Membrane interactions of Novicidin, a novel antimicrobial peptide: phosphatidylglycerol promotes bilayer insertion. J. Phys. Chem. B. 114: 11053-11060.

Emran, B.T., M.A. Rahman, M.M.N. Uddin, M.M. Rahman, M.Z. Uddin, R. Dash, and C. Layzu (2015). Effects of organic extracts and their different fractions of five Bangladeshi plants on *in vitro* thrombolysis. J. Altern. Complement Med. 15: 2-8.

Etherton, P.M.K., M. Lefevre, G.R. Beecher, M.D. Gross, C.L. Keen, and T.D. Etherton (2004). Bioactive compounds in nutrition and health research methodologies for establishing biological functions: The antioxidant and anti-inflammatory effects of flavonoids on Atherosclerosis. Annu. Rev. Nutr. 24: 511-538.

Nighat, F and Mushtaq, Z (2019). *In vitro* antimicrobial and antioxidant activities of organic and aqueous extracts of *Bacillus clausii* KP10**.** J. Chem. Soc. Pakistan 41 (1): 161-168.

Fenical, W. and P.R. Jensen (1993). Marine microorganisms: A new biomedical resource. Pharm. Bio. Natural Prod. 1: 419-459.

Ferber, D., (2010). From Pigs to People: The emergence of a new superbug. Science. 329(5995): 1010-1011.

Frank, D.N., A.L.S. Amand, R.A. Feldman, E.C. Boedeker, N. Harpaz, and N.R. Pace (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. USA. 104: 13780–13785.

Gao, M., H. Liu, M. Yang, J. Hu, and B. Shao (2004). Indirect identification of isoprenoid quinones in *Escherichia coli* by LC-MS with atmospheric pressure chemical ionization in negative mode. J. Basic Microbiol. 44: 424–429.

Guo, B., Y. Wang, X. Sun and K. Tang (2008). Bioactive natural products from endophytes: A review. Appl. Biochem. Microbiol. 44: 136–142.

Hogan, S., L. Zhang, J. Li, H. Wang and K. Zhou (2009). Development of antioxidant rich peptides from milk protein by microbial proteases and analysis of their effects on lipid peroxidation in cooked beef. Food Chem. 117: 438–443.

Inouye, M., S. Halegoua, and J. Beckwith (2008). Secretion and membrane localization of proteins in *Escherichia Coli*. Crit. Rev. Biochem. 7: 339-371.

Islam, M.A., Z.A. Mahmud, S.M.A. Rahman, M. Monirujjaman and S.K. Saha (2013). Evaluation of thrombolytic activity and brine shrimp lethality bioassay of methanol extract of stems

of *Tinosporacrispa*. Int. J. Pharm. Sci. Res. 4: 1148-1153.

Jamil, A., M. Shahid, K.M. Masud-ul-Haq and M. Ashraf (2007). Screening of some medicinal plants for isolation of antifungal proteins and peptides. Pakistan J. Bot. 39: 211-221.

Li, Y., F.G. Naghdi, S. Garg, T.C. Adarme-Vega, K.J. Thurecht, W.A. Ghafor, S. Tannock, and P.M. Schenk (2014). A comparative study: the impact of different lipid extraction methods on current microalgal lipid research. Microbial. Cell Fact.13: 2-9.

Minnikin, D.E., A.G. Donnell, M. Goodfellow, G. Alderson, M. Athalye, A. Schaal and J.H. Parlett (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J. Microbiol Meth. 2: 233-241.

Müller, A., D. Behsnilian, E. Walz, V. Gräf, L. Hogekamp and R. Greiner (2016). Effect of culture medium on the extracellular synthesis of silver nanoparticles using *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas jessinii*. Biocatal. Agric. Biotechnol. 6: 107–115.

Nimse, S.B. and D. Palb (2015). Free radicals, natural antioxidants, and their reaction mechanisms. RSC. Adv. 5: 27986–28006.

Omonhinmin, A.C., I.P. Dike and S.O. Rotimi (2015). Phytochemical, cytotoxicity and antioxidant activities of five anti-malaria plants. Res. J. Med. Plant. DOI:10.3923: 1-9.

Omura, S., H. Ikeda, J. Ishikawa, A. Hanamoto, C. Takahashi, M. Shinose, Y. Takahashi, H. Horikawa, H. Nakazawa, T. Osonoe, H. Kikuchi, T. Shibai, Y. Sakaki and M. Hattori (2001). Genome sequence of an industrial microorganism *Streptomyces avermitilis*: Deducing the ability of producing secondary metabolites. Proc. Natl. Acad. Sci. USA. 98: 12215–12220.

Peiru, S., H.G. Menzella, E. Rodrguez, J. Carney and H. Gramajo (2004). Production of the potent antibacterial polyketide Erythromycin C in *Escherichia coli.* Appl. Environ. Microbiol. 71: 2539-2547.

Roopa, G., M.C. Madhusudhan, K. Triveni, N.E. Mokaya, H.S. Prakash, and N. Geetha (2015). Evaluation of antioxidant properties of *Salacia macrosperma* leaf extracts. IJRSSET. 2: 58-63.

Rosenfeld, Y., and Y. Shai (2006). Lipopolysaccharide (Endotoxin)-host defense antibacterial peptides interactions: Role in bacterial resistance and prevention of sepsis. Biochim. Biophys. Acta. 1758(9): 1513–1522.

Saha, S., F. Samadi, J.N. Jaiswal and U. Ghoshal (2010). Antimicrobial activity of different endodontic sealers: an *in vitro* evaluation. J. Indian Soc. Pedod. Prev. Dent. 28: 251-257.

Serban, E.S., M. Ionescu, D. Matinca, C.S. Maier, and M.T. Bojiţa (2011). Screening of the antibacterial and antifungal activity of eight volatile essential oils. Farmacia. 59: 440-446.

Tepea, B., E. Donmeza, M. Unlub, F. Candanc, D. Dafererad, G. Vardar-Unlub, M. Polissioud, and A. Sokmena (2004). Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chem. 84: 519–525.

Villoslada, F.L., M. Olivares, S. Sierra, J.M. Rodrıguez, J. Boza, and J. Xaus (2007). Beneficial effects of probiotic bacteria isolated from breast milk. Br. J. Nutr. 98: 96–100.

Yadav, V., J. Gupta, R. Mandhan, A.K. Chhillar, R. Dabur, D.D. Singh and G.L. Sharma (2005). Investigations on anti-Aspergillus properties of bacterial products. Lett. Appl. Microbiol. 41: 309-14.

Yasuhiro, K., A. Yuzuru, and N. Shoshichi (1967). Composition and turnover of the phospholipids in *Escherichia coli.* Biochim. Biophys. Acta. 144: 382-390.

Zaidan, M.R.S., A.N. Rain, A.R. Badrul, A. Adlin, A. Norazah, and I. Zakiah (2005). *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop. Biomed. 22: 165–170.

Zhang, L., J. Li and K. Zhou (2010). Chelating and radical scavenging activities of soy protein hydrolysates prepared from microbial proteases and their effect on meat lipid peroxidation. Bioresour. Technol. 101: 2084–2089.