Chapter 03

Medicinal Herbs Extracts as an Alternative Therapeutics Approach against Multi-Drug Resistant Diarrheagenic *Escherichia coli*

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ABSTRACT

Diarrhea is one of the most critical health issues responsible for the high rate of morbidity and mortality in the world. It is major health problem especially in developing countries. Different pathogenic bacteria and viruses cause diarrhea. Among the pathogenic bacteria, *Escherichia coli* (*E. coli)* is the major etiological agent of diarrhea by producing Shiga toxin that may damage the intestine. The diarrhea-genic *E. coli* is treated through the use of antimicrobial agents. The excessive and nonspecific use of antibiotics has resulted the emergence of multi-drug resistant strains of *E. coli,* which creates a serious threat to public health. This chapter provides brief overview of isolation and molecular identification of various multidrug resistive strains of *Escherichia coli* from diarrheal patients of different ecological zone of district Swat, Khyber-Pakhtunkhwa, Pakistan and to evaluate antimicrobial potential of medicinal herbs especially *Limonium cabulicum* against diarrhea-genic *E. coli*. The samples were collected from equal number of male and female diarrheal patients under the age group of five years. For antibiogram studies, ten different antibiotics from all of the four generations were tested following the guidelines of Centers for Disease Control and Prevention (CDC). The study also evaluated local medicinal plant i.e., *Limonium cabulicum* for their antibiotic potential against selective multidrug resistant *E. coli* strains. Sequencing of 16s rRNA and phylogenetic analysis for *E. coli* isolates were also performed in the current study. The data revealed high frequency of *E. coli* isolates were recorded in male children than females. The susceptibility pattern of isolates showed the existence of multi-drugs resistant strain of *E. coli*. Nine out of ten selected antibiotics were non-effective against most strain of *E. coli*. The only effective antibiotic was Fosfomycin. The results of study revealed that *Limonium cabulicum* roots and leaves extracts in ethanol and aqueous solvents had remarkable activities against the tested strains. Sequencing of the 16s rRNA and phylogenetic analysis confirmed the existence of *E. coli* strains in diarrheal patients.

KEYWORDS
Antibiogram; Diarrhea, Diarrhea-genic *E. coli; Escherichia coli;* Medicinal Plants, Multi-Drug resistant; *Limonium cabulicum;* Pathogenic Bacteria

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INTRODUCTION

Diarrhea is the release of three or more liquid stools in 24 hours with abnormal fluids contents or irregular increases in daily stool fluidity than normal (Haricharan, 2010). This disease is common for all age groups but severe in infants and children below the age group of 5 years. The prevalence of diarrhea revealed a major world health problem and responsible for the second-highest rate of mortality and morbidity, especially in under developing countries. The severity of this disease is found in the infant's age group under 5 years and reported as the fourth highest burden of child mortality across the world (Black et al., 2012).

Different pathogenic bacteria including *Salmonella enteritis, Shigella, Campylobacter, Staphylococcus aureus,*

Clostridioides difficile and *Yersinia* spp. can cause diarrhea. However, *E. coli* is the major causative agent of diarrhea. The pathogenic strains of *E. coli* causing diarrhea include STEC serotypes O157: H7, O103: H2, O118:H6, O26:H16, O26:H11, O111:H8 and O121:H19. The severity of the disease's symptoms varies depending on the toxins produced by *E. coli* in the intestine and may include vomiting, cramping in the muscles and abdomen, and diarrhea (Klein et al., 2002). Symptoms and transmission sources of pathogenic strains of *E. coli* are diverse. For example, the significant reservoir of *E. coli* (O157: H7) is cattle. However, sometimes other mammals including cats, dogs, rabbits, horses, pigs and insects (flies) also involve in the transmission of *E. coli*. Furthermore, contaminated food, vegetable and fruits irrigated by contaminated water are also the possible reservoir for the transmission of *E. coli* (Ferens and Hovde, 2011) (Fig. 1). The transmission of *E. coli* can be prevented by using proper cooked food, adopting the required safety barriers such as masks, gloves, awareness of the cooking policies, applying different scientific methods in sterilization pasteurization, canning, maintaining the water quality and quantity in foods, avoid raw and unpasteurized milk and juices (Taylor et al., 1995; Nicholls et al., 2002). Furthermore, the use of antiseptics for cleaning households, and following the safety precautions measure while pitting and contact with farm animals are also the possible measures for preventing the transmission of *E. coli* (Maunsell and Bolton, 2004).

Fig. 1: Different therapeutic approaches against the major causes of diarrhea.

The specific identification of *E. coli* is also a key for the proper management and control of *E. coli* infection. Different assays were used for the identification of *E. coli*. Biochemical test and genotypic techniques are commonly used for the identification purpose. Among the genotypic techniques 16S rRNA gene sequencing, is most accurate method for identification and characterization of the clinical isolates of *E. coli* (Rousselier et al., 2001; Deshmukh and Roy, 2021)

Materials and Methods

The current study was conducted at the Center for Biotechnology and Microbiology (CBandM) at the University of Swat Khyber Pakhtunkhwa in Pakistan. About 153 miles from Pakistan's capital city of Islamabad, the Swat Valley is situated on the Swat River at the coordinates 35°12′N 72°29′E. As seen in Fig. 2, four Tehsil Headquarter Hospitals (THQs) Madyan, Khawazakhela, Matta, and Kabal, three BHUs, (basic health units) Fatehpur, Islampur, Manglawar. and eight private hospitals in District swat were chosen for sample collection.

For the study, different samples were taken from diarrheic patients, predominantly from THQs and BHUs. The sampling procedure entails the collection of faeces (watery) from diarrheal patients of both genders and of all ages using sterile surgical swabs with labels indicating the patient's age, gender, and location. Using the standards procedure, a non-selective Nutrient ager (NA) media was created for the growth of bacteria from an inherently obtained sample. According to established protocols, MacConkey ager and Sorbitol MacConkey ager (SMAC) were utilized for identification of pathogenic strain O157:H7 *E. coli.*

Following the development of various colonies, the colonies were identified based on their architecture and colors (colony morphology). Following morphological identification, pure cultures were subjected to biochemical confirmation tests for authentic identification. Gram staining revealed that the samples were gram negative, while catalase and oxidase tests revealed that they were catalase positive and oxidase negative. To ascertain whether the microorganisms could produce -galactosidase enzymes that hydrolyzed O-Nitro phenyl—D-Galactopyranoside, the O-Nitro phenyl—D-

Galactopyranoside (ONGP) test was carried out (ONPG). Using the disc approach, the medium was infected with a significant amount of pure culture inoculum and incubated for four hours at 37 ºC. Yellow tint of the disc after 4 hours of incubation indicated ONGP positive for the chosen bacterium sample. The lysine decarboxylase test was used to identify isolated microorganisms that use lysine as a source of carbon and are referred as lysine decarboxylase positive. A further 24 hours were allocated to the medium for incubation, during which it was noted that the application of lysine caused the hue to return to purple from yellow. Another biochemical test to identify bacteria's capacity to convert tryptophan into indole was conducted, which was a sign that the strain can break down tryptophan and create indole.

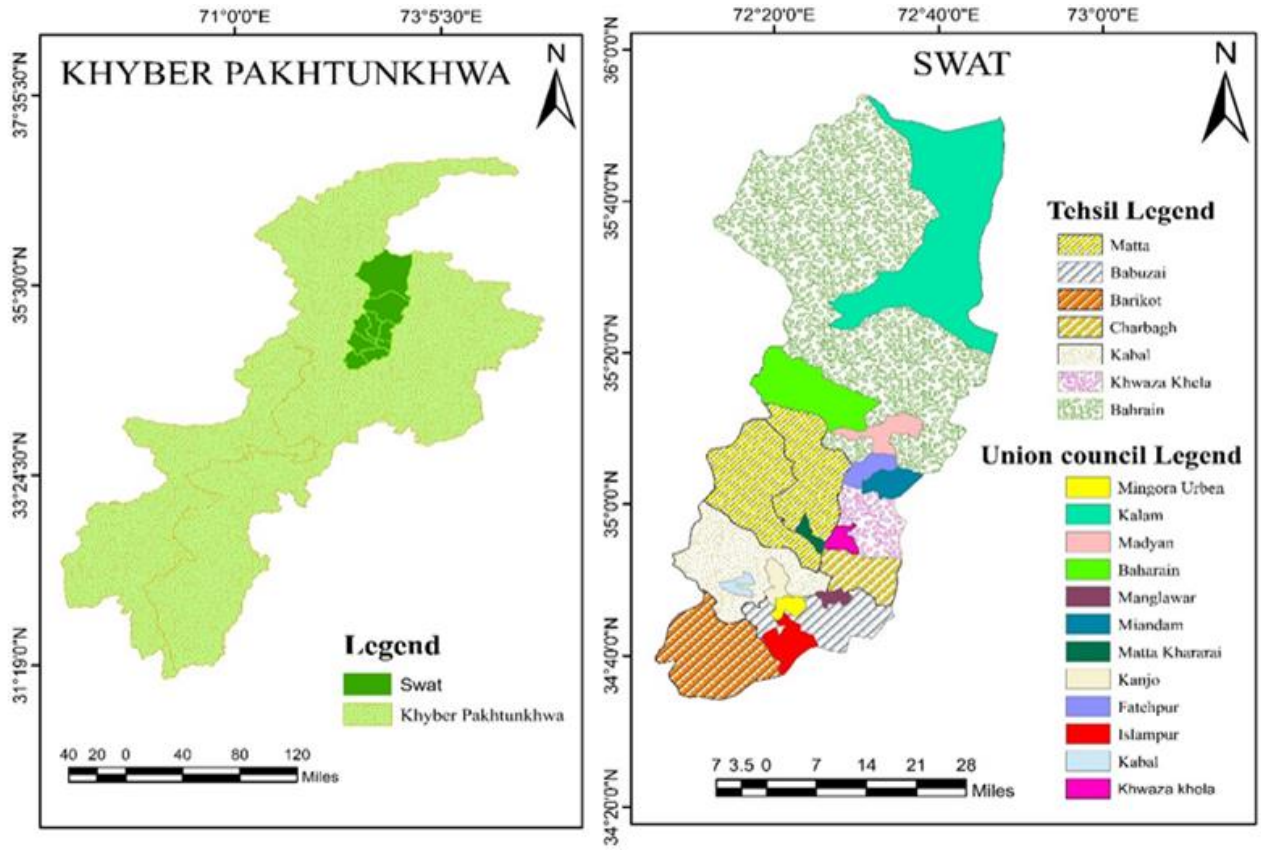

Fig. 2: Map of study area.

For identification purposes, genotypic approaches were also used in addition to other biochemical test procedures. The findings of BLAST in the NCBI database and sequencing the sample's 16s rRNA confirmed the presence of *E. coli* in the samples. Our query sequence from the isolate, according to the phylogenetic analysis, was also shown to be more closely related to *E. coli* strains from Pakistan, India, and Mexico.

By using the Kirby and Bayer-proposed disc diffusion approach, antibiogram patterns was carried out. Ten commonly used antibiotics were utilized in total. Muller Hilton (MH) agar was used to assess the antibiotic sensitivity of the chosen *E. coli* strains, following the manufacturer's recommendations. Premade antibiotic discs were utilized on the plates. Well diffusion method was employed to evaluate the antimicrobial sensitivity of medicinal plants against the diarrheal pathogenic *E. coli*. following National Committee for Clinical Laboratory Standards (NCCLS) protocols.

The Challenges of Antimicrobial Resistance Associated with Diarrhea Worldwide

Antimicrobial agents are used for the control of pathogenic strains of *E. coli*. Without any doubt antibiotics have revolutionized the treatment of bacterial infections of both humans and animals to a great extent. But the prevalence of antibiotic resistance to specific types of bacteria has alarmingly increased in recent years. Among those bacteria that pose the greatest threat to human health because of its growing resistance to antibiotics are the members of the *Enterobacteriaceae* family, particularly *E. coli*. The increase in antimicrobial resistance is a challenge in medical field, and the advance antimicrobial chemotherapy shows less effectiveness against the prevention of highly pathogenic strains of *E. coli* (Table 1). According to Centers for Disease Control and Prevention (CDC) O157: H7 is the most common *E. coli* strain isolated as a pathogen in the United States (Gonzalez-Escalona and Kase, 2019). The study conducted by Mora et al. (2005) reported that the STEC strains of *E. coli* includes O157: H7, O4:H4, O8:H21, O20:H19, O26:H11, O111:H, O118:H16, O128:H and O171:H25 shows highly resistance to more than five antibiotics. The increase in the prevalence and development of multi drug resistance is happening due to the misuse of antibiotics which can pose a great deal of threat to the normal

intestinal microbiota and therefore there is an urgent need to develop alternative drugs to treat resistant bacteria (Kaper and O'Brien, 1998; Rhee et al., 2009; Ifeanyi et al., 2015; Mutters et al., 2018; Gharbi et al., 2019)

In developing countries, the situation is getting worsens, as reported by national surveillance data from Mexico, China and Turkey, where *E. coli*-resistant strains has been shown to have a prevalence of > 40% to cephalosporin, quinolones and trimethoprim/sulfamethoxazole (TSX), drugs widely used around the world to empirically treat bacterial infections (Hamad, 2010; Shao et al., 2017). The study conducted on specimens from diarrheal patients in Bangladesh and screened for ETEC between 2005 and 2009 (Begum et al., 2016). The isolates were checked for antibiotic sensitivity and the resistance profile of ETEC isolates was observed as follows: ciprofloxacin 27%, ampicillin 66%, ceftriaxone 13%, streptomycin 48%, azithromycin 27%, doxycycline 44%, erythromycin 96%, nalidixic acid 83% cotrimaxazole 46%, tetracycline 42% and norfloxacin 27%, Furthermore, the antibiotic resistance in ETEC against ciprofloxacin is increased from 13% to 34% in duration of 4 years from 2005-2009.

The study conducted by Hamid et al. (2012) reported that Multi drug-resistant E coli has become a major public health concern in Sudan and many countries, causing failure in treatment with consequent huge health burden. They identified a total of 232 *E. coli* isolates from clinical specimens and tested their antimicrobial susceptibility. Out of the 232 *E. coli* isolates, 214 isolates were characterized as MDR *E. coli* (92.2%). The findings of the study revealed that the antibiotic resistance was recorded as, ciprofloxacin 58.4%, Ofloxacin 55.1%, amoxicillin-clavulanate 50.4%, cefuroxime 92.5%, amoxicillin 97.7%, trimethoprim-sulfamethoxazole 88.3%, amoxicillin 97.7%, gentamicin 35% nalidixic acid 72%, tetracycline 77.1%, ceftriaxone 64%, nitrofurantoin and ceftazidime each, 22.4%, tobramycin and chloramphenicol 18.2% each and amikacin 1.9%. The study reported the total of 53.3% isolates multi-drug-resistant (MDR) diarrheagenic *E. coli* (DEC) has rapidly spread worldwide and represents the most serious threat to the management of diarrhea in developing countries. During the period from March 2011 to January 2012, a total of 450 stool samples of diarrheal children with age of 0– 60 months were studied in Tabriz Iran, to detect enterohemorrhagic *E. coli* (EHEC) enterotoxigenic *E. coli* (ETEC) and tested for antimicrobial susceptibility. Diarrheagenic *E. coli* exhibited high-level resistance to aztreonam (80.7%), amoxicillin (74.4%), and tetracycline (69.3%). The finding of the study reported 86.4% of *E. coli* isolates as MDR (Memariani et al., 2015)

Antibiotics Key Codes

Ampicillin -AMP, Tetracycline- TET, Nalidixic acid-NA/NAL, Cephalosporins-CEP, Imipenem-IPM, Trimethoprim-TMP, Sulfa-Methoxazole-CMX, Chloramphenicol-C/CHL/VCL, Gentamicin-GEN, Streptomycin-STM, Cotrimoxazole-CMX, Azithromycin-AZI, Ciprofloxacin-CPFX/CIP, Teicoplanin-TEC, Quinolones- QNS, Vancomycin- VAN, Aminoglycosides-AMG, Sulfamethoxazole(TS)-SMZ, Amoxicillin-clavulanic acid-AMC, Cefotaxime-CTX, Cefuroxime- CXM, Kanamycin-KAN, Erythromycin-ERY, Spectinomycin-SPT/ SPTCM, Ceftriaxone-CRO.

Medicinal Plants– an Alternative Approach for the Control of Diarrhea-genic *E. coli*

Medicinal plants are getting high consideration among the scientific community due to their long historical background of being sources of natural bioactive compounds for the treatment of different infection caused by pathogens (Seyyed Nejad et al., 2010; Nabavi et al., 2015). The composition of medicinal plants is highly diverse and their extracts have antimicrobial potential against both Gram-positive and Gram-negative bacteria (Mustafa et al., 2019; Fernandes et al., 2022). The medicinal plants contain phenolic, terpenoids, alkaloids, Sulphur and nitrogen-containing compounds which exhibit antibacterial potential, by targeting the different component of microbial cell and generating the reactive oxygen species in the culture (Berni et al., 2018; Manzar Alam et al., 2022). Plant extracts and their compounds have been reported for Immune stimulatory effects (Amirghofran, Hashemzadeh, Javidnia, Golmoghaddam, and Esmaeilbeig, 2011). The extracts of *Tinospora crispa* stimulate innate immune responses in Wistar Kyoto rats (Bukhari et al., 2015). Similarly, the inhibition of arachidonic acid metabolism and cytokine production by eucalyptol has also documented in previous study. The others researchers also reported the anti-inflammatory potential of the bioactive compounds and plant extracts in the *in vivo* and *in vitro* studies (Gurgel et al., 2009; Ferreira et al., 2016). Furthermore, the antibiotic resistance modifying potential in plants extracts also gaining research interest in scientific community. Some plant extracts have shown to increase the antimicrobial potency of antibiotic against multidrug resistant bacterial species and suggested as antibiotic adjuvants (Vaverková et al., 2013; Kuete et al., 2015).

Medicinal plant extract has shown a remarkable antimicrobial potential against Gram-negative bacteria with least side effects (Fig 3) and have long been used for the treatment of gastrointestinal tract infections especially diarrhea. Previous published data revealed the antimicrobial potential of the different medicinal plants against the causative agent of diarrhea especially *E. coli*. For example, the ethanol extracts of *Acacia nilotica*, *Syzygium aromaticum* and *Cinnamomum zeylanicum* inhibited the growth of *E. coli* (Khan et al., 2009a; Khan et al., 2009b). Similarly, the ethanol and methanol extracts of *Oxalis corniculata*, *Punica granatum* and *Syzygium aromaticum* also displayed the antimicrobial potential against pathogenic strains of *E. coli* (Mostafa et al., 2018; Dahal et al., 2019). The extracts of other medicinal plants including *Gaultheria procumbens* leaf, ethenolic solvent of *Aloysia citrodora*, *Hibiscus sabdariffa*, *Phlomis brachyodon*, *Urtica pilulifera*, *Anchusa azurea*, *Pallenis spinose*, *Cirsium englerianum*, *Eucalyptus depauperata*, *Lippia adoensis*, *Discopodium penninervium* , *Rumex abyssinicus*, *Cyperus pustulatus*, *Cassia fi stula*, *Holarrhena antidysenterica*, *Terminalia alata*, *Terminalia arjuna*, *Paederia foetida*, *Zanthoxylum alatum*, *Ocimum sanctum*,

Verbascum Thapsus and *Bryophyllum* were also effective against pathogenic strain of *E. coli* (O157:H7), reported in the previous studies (Odugbemi et al., 2007; Dahiya and Purkayastha, 2012; Rawat et al., 2015; Ahmad et al., 2021; Tufa et al., 2021). In the present study *Limonium cabulicum* (Local name *Ghawakhai*) was found for the first time to have a strong antibacterial potential against multidrug resistance (MDR) strains of *E. coli*, producing maximum zone of inhibition of (19 mm).

Results

The antibiotics sensitivity test revealed that the diarrheagenic *E. coli* isolates was resistant to Cefoperazone/ Sulbactum (SCF), Norfloxacin (NOR), Ciprofloxacin (CIP), Septran/ Co-Trimexazole (SXT), Chloramphenicol (C), Cefixime (CFM), Trimethoprim (W), Pipemidic acid (PIP) and Nalidixic acid (NA). Furthermore, the findings of the current study showed that Fosfomycin (FOS) is a drug of choice for the control of *E. coli* as shown in the Table 2.

Table 1: Resistance of *E. coli* to antibiotics reported in different countries.

Country	Antibiotics and percentage of resistant *E. Coli* isolates										References
Iran	AMP 19.80%	TET 75.50%	NA/NAL 75.50%	IPM 100%	TMP 24%	CMX 24%	GEN 84.60%	CPFX/CIP 91.40%			(Farshad et al., 2012)
Qatar	AMP 70%	TET 35%	CEP 35%	TMP 30%	CMX 30%						(Yassine et al., 2020)
Korea	AMP 76% 10.40%	TET 66% 15.60%	NA/NAL 9.40%	STM 12.40%							(Kim et al., 2014) (Ryu et al., 2012)
Peru south America	AMP 85%	TET 65%	NA/NAL 28%	C/CHL/VCL 65%	CMX 79%						(Ochoa et al., 2009)
Vietnam	AMP 77.20%	C/CHL/ VCL 29.60%	CPFX/CI P 88.30%	CTX 19.10%	CXM 29.10%						(Weintraub et al., 2005)
China/ Shaanxi china	AMP 75.60%	TET 73.10%	CPFX/CI P 46%	CTX 46%	KAN 65.20%	ERY 65.20%	SPT/ SPTCM 50%				(Baloch et al., 2017; bdelgader et al., 2018)
Ghana	AMP 68%	TET 93.55%	TMP 58.60%	C/CHL/VCL 93.55%	STM 54%	AZI 70.97%	CPFX/CIP 61.29%	TEC 96.77%	(AMC), 70.90%	CRO 58.60%	(Huda et al., 2020)
United States/ Canada	AMP 13.7	CPFX/ CIP 19.30%	(AMC), 13.7								(Okeke et al., 2005)
Bangladesh	TET 89.44%	AZI 100%	ERY 88.89%								(Sobur et al., 2019)
Maxico	AMP 44%	TET 37%	CTX 44%								(Canizalez-Roman et al., 2019)
Japan	AMP 24%	TET 49%	NA/NAL 28%	CEP 18%	TMP 20%	TS 20%					(Nishikawa et al., 2017)
South africa	TET 91%	AZI 92%	TS 90%								(Ateba and Bezuidenhout, 2008)
Salvador, Brazil	AMP 13.30%	TET 13.30%	TMP 13.30%	C/CHL/VCL 13.30%	TS 13.30%						(Lima et al., 2017)
Egypt	AMP 18.90%	TET 27.50%	NA/NAL 1.80%	TMP 11.30%	CMX 11.30%	C/CHL/VC L 2.30%	STM 18.50%	CPFX/CIP 1.40%	CTX 4.50%	KAN 4.10%	(Yamasaki et al., 2018)
Ethiopia	AMP 83%	AMC 75.79%									(Tuem et al., 2018)
Portugal	TET 50%	QNS 50%	VAN 50%	AMG 50%	TS 50%						(Igrejas et al., 2016; Amaro et al., 2021)
England	AMP 5.80%	TET 2.80%	TMP 5.80%	C/CHL/VCL 2.10%	STM 5.80%	AZI 0.20%	CPFX/CIP 2.60%	TEC 5.80%	QNS 5.80%	VAN 2.80%	(Do Nascimento et al., 2017; Jenkins et al., 2020)

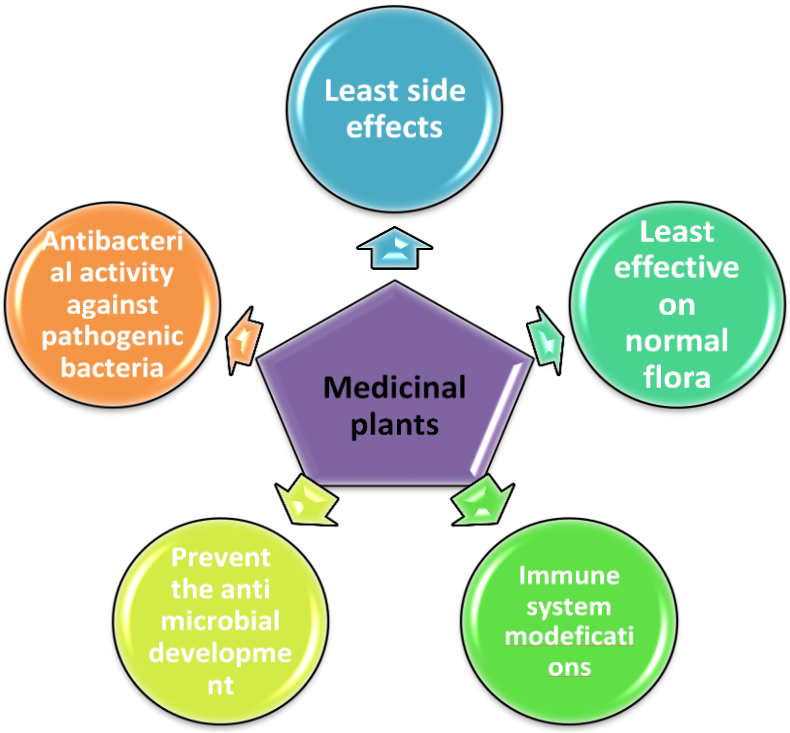

Fig. 3: Importance of medicinal plants as alternative therapeutics

Table 4.1: Antibiotic sensitivity test against pathogenic *E. coli* O157:H7 serotype.

ANTIBIOTICS NAMES	ABBREVIATIONS	DISK CONTENT	ANTIBIOTIC GENERATION	STANDARD DIAMETER OF INHIBITION ZONE (mm)			Average Zone Obtained In (mm)	RESULT
				R	I	S		
Fosfomycin	FOS	50 µg	3rd	≤12	12-16	≥16	27.5	Sensitive
Norfloxacin	NOR	10 µg	2nd	≤13	13-16	≥17	10.25	Resistant
Cefoperazone/Sulbactum	SCF	105 µg	3rd	≤15	16-20	≥21	12.75	Resistant
Ciprofloxacin	CIP	5 µg	2nd	≤15	16-20	≥21	11	Resistant
Septran/Co-Trimexazole	SXT	25 µg	2nd	≤12	12-16	≥16	00	Resistant
Chloramphenicol	C	30 µg	3rd	≤13	13-18	≥18	00	Resistant
Cefixime	CFM	5 µg	3rd	≤13	13-19	≥19	00	Resistant
Trimethoprim	W	1.25 µg	3rd	≤12	12-16	≥16	00	Resistant
Pipemidic acid	PIP	20 µg	2nd	≤13	13-18	≥18	00	Resistant
Nalidixic acid	NA*	10 µg	1st	≤13	14-18	≥19	00	Resistant

The findings of the current study revealed that the extracts of leaves and roots were active against the *E. coli* isolates. Among the tested extracts, the water extract of leaves showed a maximum zone of inhibition (ZI) of 15mm at the concentration of 1000 µg/ml. However, the antimicrobial activity of the plant extracts showed an increase when the concentration of the extract was increased. The water extract at 3000 µg/ml showed a higher ZI of 19mm. Similarly, at the same concentration, the ethanol solvent of the extract also produced a zone of inhibition of 15mm (ZI) against the tested isolates of *E. coli*. The current study also reported moderate anti-*E. coli* activity in n-hexane and ethyl acetate extract of leaves at the concentration of 3000 µg/ml. (Table 3). On the other hand, n-hexane extract did not show any activity at a concentration of 1000 µg/ml.

Among the root extracts of the plant, ethanol solvent extracted samples revealed a higher ZI with 19mm, 18mm, and 15mm at 3000, 2000, and 1000 µg/ml respectively. The extract of the water showed a uniform ZI with 15mm at each tested concentration. Although, the ethyl acetate solvent did not show any activity at both 2000 and 1000 µg/ml, except for a marginal 2mm ZI at 3000µg/ml (Table 3). Similarly, n-hexane extracts of root at 3000 and 2000 µg/ml showed 2mm of ZI, while no antibacterial activity was noted at 1000 µg/ml.

Overall, the average higher ZI was reported for ethanol solvent with 17mm ZI, followed by aqueous solvent with 15mm ZI, respectively. The current study revealed that ethanol and aqueous solvents are the optimum solutions for pant material extraction.

Phylogenetic analysis of sequenced *E. coli* isolates revealed that the bacterial strain isolated from diarrheal patients predominantly occupies the basal nodes. This analysis indicates that the selected strain is most closely related to *Escherichia coli* strain O55:H7 (accession number CP038295), followed by *Escherichia coli* strain LD39-1 (accession number

CP047658). The least similarity was observed with *Escherichia coli* O55:H7 strain DEC5D (accession number CP038386), as depicted in the cladogram (Fig. 4).

Table 3: Average zone of inhibition of *E. coli* isolates produced by root and leaves extracts of *L. cabulicum* at 1000, 2000 and 3000µg/ml

Plants	Parts used	Extract	Concentrations of extracts and Zone of Inhibition		
			1000 µg/ml	2000 µg/ml	3000 µg/ml
L. cabulicum		Ethanol	15±1.0	18±1.1	19±1.4
		Ethyl acetate	0±0.	02±0.9	02±0.5
	Root	N-hexane	0±0	0±0	02±0.7
		Water	15±1.1	15±1.0	15±1.1
		Ethanol	12±0.9	14.4±1.2	15.3±0.7
L. cabulicum	Leaves	Ethyl acetate	4±0.9	7.2±0.9	9.1±1.1
		N-hexane	0±0.7	6.3±0.8	7.4±1.0
		Water	15±1.1	18±0.9	19±1.1

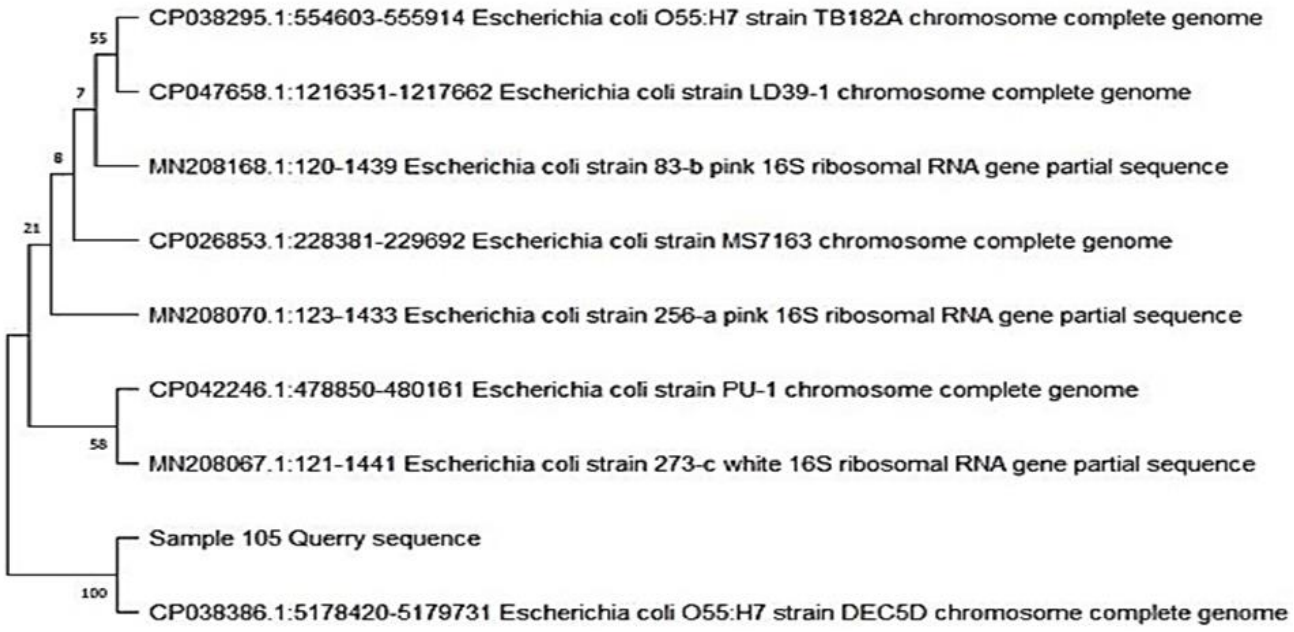

Fig. 4: Cladogram of the phylogenetic analysis

DISCUSSION

Diarrhea is one of the major intestinal diseases responsible for the second-highest rate of mortality and morbidity, across the world, widely in poor countries and also in industrialized and developing countries. Globally diarrhea affects people of all ages; however, children at an early age are more affected than others (Claeson et al., 2004). In 2015, among the under-age-five, ten highest mortality countries, Pakistan was ranked with the third highest neonates' mortalities rate caused by diarrhea (ADIELE, 2019; Rabbani and Qayyum, 2017). Among the most important various etiological agents, the strains of *E. coli* are the one responsible for chronic diarrheal disease (Mobley et al., 2004). These strains of diarrhoea-genic *E. coli* have acquired resistance through horizontal gene transfer and become multi-drug resistant (Gomes et al., 2016). The scientific community in the world is making huge efforts to combat the rising antimicrobial resistance. Traditionally medicinal plants have been utilized for the treatment of various diseases, including severe infectious diseases (Sheetal Verma and Singh, 2008). Therefore, keeping that in mind, the study was designed to evaluate the medicinal plant extracts against the MDR diarrheagenic *E. coli* as an alternative approach to the currently resistant antibiotics.

Fosfomycin was the only antibiotic found to be effective against the pathogen in the current study when isolated pathogenic *E. coli* O157:H7 was tested with 10 different antibiotics. Norfloxacin, cefoperazone/sulbactum, and ciprofloxacin, co-trimaxazole, chloramphenicol, cefixime, trimethoprim, pipemidic acid, and nalidixic acid were all found to be ineffective. The current result is consistent with a study by Pascual et al. (2006). The current study backs up previous findings that Fosfomycin has excellent anti-*E. coli* activity (Vila et al., 2001).

In the current research, the plant *Limonium cabulicum* local name *Ghawakai*, was selected because the other species of the family have been previously studied for their bioactivities. The *L. cabulicum* belongs to the family of *Plumbaginaceae*, which have a rich source of the bioactive compound containing species (Tripathi et al., 2012). However,

the current study on the antimicrobial activity of *L. cabulicum* spp. in different extraction solvents against the MDR diarrheagenic *E. coli* is novel and up to date has not been reported elsewhere.

Four different solvents (Ethanol, Ethyl acetate, N-hexane and Water) were used to optimize the extraction from root and leaves of *L. cabulicum*. The extracts of both root and leaves were screened in three different concentrations against the MDR strains of diarrheagenic *E. coli* (Table 3). The findings of the study revealed the effect of extraction solvent on antibacterial potential of root and leaves against diarrheagenic *E. coli*. In the extraction, solvents play a key role in the recovery of phyto active metabolites from plant material (Mutalib et al., 2013). Previous studies have also reported the effectiveness of extraction solvents on the bioactivities of plant (Sahle and Okbatinsae, 2017; Dixon et al., 2019; Habtom and Gebrehiwot, 2019). Overall, water extract of *L. cabulicum* leaves showed the highest zone of inhibition of 19mm (ZI) at 3000 µg/ml, followed by the ethanolic extract with an average of 17mm zone of inhibition. A similar study conducted by Uma et al. (2009) reported the phytochemical antibacterial activity of *Ficus bengalensis* against entero-toxigenic *E. coli* with 16mm and 12mm ZI at 4ml/disk with methanol and aqueous extract respectively. This study showed that even 1000 µg/ml extract of *L. cabulicum* is effective against the diarrheagenic *E. coli*. Similarly, the root extracted samples in ethanol solvent produced a zone of inhibition of 19mm ZI and 18mm at 2000 and 3000 µg/ml, respectively. The results of this study are further supported by Darwish and Aburjai (2010), who have reported more than a dozen medicinal plants' activity against the MDR strains of *E. coli*. The less or no activity of hexane extracts may be due to the least recovery of bioactive compounds from root and leaves of *L. cabulicum* in hexane solvent system. Our findings are aligned with the finding of Aibinu et al. (2007), where 7mm to 28mm zones of inhibition have been reported against MDR *E. coli*. Ethanol and aqueous extracts have the good antibacterial efficacy against both gram-negative and gram-positive bacteria and the anti-microbial potential of the medicinal plants depends on the nature of the solvents for the crude extraction (Rasool Hassan, 2012).

Phylogenetic analysis revealed that the isolated bacterial specie was most closely related to *Escherichia coli* strain O55:H7 (CP038295) and *Escherichia coli* strain LD39-1 (CP047658), with the least similarity to *Escherichia coli* O55:H7 strain DEC5D (CP038386) (Kyle et al., 2012). Notably, the O55:H7 *E. coli* strain has been reported to share significant similarities with the pathogenic O157:H7 strain (Kyle et al., 2012). Our study confirms the isolation of the O55 strain, which is considered the progenitor strain of *E. coli* O157:H7 and is recognized as a foodborne pathogen (Griffin and Tauxe, 1991; Weinroth and Bono, 2022). Furthermore, studies have highlighted the importance of monitoring and controlling the spread of *E. coli* O55:H7, as it can lead to severe foodborne illnesses (Nadon et al., 2017; Hemrajani et al., 2020).

Conclusion

The study concluded that, the diarrheagenic *E. coli* isolates were shown to be resistant to the following antibiotics: Cefoperazone/Sulbactum (SCF), Norfloxacin (NOR), Ciprofloxacin (CIP), Septran/Co-Trimexazole (SXT), Chloramphenicol (C), Cefixime (CFM), Trimethoprim (W), Pipemidic acid (PIP), and Nalidixic acid (NA). Additionally, the results of the present investigation demonstrated that the medicine fosfomycin (FOS) is a viable option for the management of *E. coli*. The results of the study conclude antibacterial potential of the root and leaves of *L. cabulicum* against diarrheagenic *E. coli*. The difference in antibacterial potential among the different extracts revealed that extraction solvent affects the recovery of bioactive compounds from plant material. Ethanol extract from root and water extract from the leaves exhibited strong anti- *E. coli* potential than the other tested extracts. The results of the study also concluded that the root and leaves of *L. cabulicum* might contain bioactive compounds having strong antibacterial potential against MDR *E. coli,* and recommended for the isolation and recovery of these compounds for the development of natural therapies in the treatment of highly resistant pathogens.

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