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Antimicrobial screening of polyherbal formulations traditionally used against gastrointestinal diseases

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ABSTRACT

Emerging antibiotic resistance has become a cosmopolitan problem and evoking researchers to search for new antimicrobials from natural constituents. The present study was intended to test the antimicrobial potential of traditionally used unexplored polyherbal recipes for curing digestive ailments. A total of 25 plants species were combined in different ratios to form 14 polyherbal recipes. After collecting and grinding plant parts, methanolic extracts of 14 polyherbal recipes were prepared by the cold maceration process. Antibacterial and antifungal activity of the polyherbal extracts was checked by agar well diffusion method at a concentration of 50 mg/ ml while minimum inhibitory concentration (MIC) was determined by serial dilution method. Polyherbal recipes B and D showed significant inhibition zone each against *Vibrio cholerae* (25.63; $p < 0.001$). Recipe G (23.33; $p < 0.001$) showed better efficacy against *Escherichia coli*. Recipe E and G significantly inhibited *Proteus* species (28.33; 24.33; $p < 0.001$). Recipe B was highly effective against *Salmonella typhi*. Recipe C, A and F had significant antifungal affect and inhibited *Aspergillus nigar* (28.67; $p < 0.05$), *Aspergillus fumigatus* (27; $p < 0.01$) *Trichoderma* (30; $p < 0.001$), *Rhizopus* (19.67; $p < 0.01$), and *Fusarium graminearum* (28.67;$p < 0.001$). Polyherbal formulations A, B, D, K, and N were active with the lowest concentration. MIC ranges within 3.12–25 mg/ml while minimum bactericidal concentration (MBC) between 12.5 and 50 mg/ml. Polyherbal recipes' A, B, D, G, K and N have enhanced antimicrobial potential with better efficacy than tested antibiotics and should be evaluated for further scientific validation.

1. Introduction

Polyherbal therapy has been used in Ayurvedic, Chinese, and, Unani medicines, for thousands of years, yet scientific evidence of their therapeutic benefits is mostly lacking. In these systems, different chronic diseases are better managed by polyherbal formulations instead of monoherbal due to synergism and lesser side effects. The concept of polyherbal combination has been well established and achieved remarkable success in western medicine offering new hope to patients. In pharmaceutical industries, there are many research studies in which combination therapy of plants and antibiotics showed effective results for diabetes and cancer as compared to monotherapy (Patel and Saravolatz, 2006). A five-year literature review reported the in-vitro antimicrobial findings of synergy both within plant extracts and between plant extracts and antibiotics. Plant extracts and their combinations were more efficient than individual constituents (Abd El-Kalek and Mohamed, 2012; Mundy et al., 2016). In recent years, antimicrobial activities of mono herbal have been increasingly reported while there are very few studies on the biological activities of traditionally used polyherbal formulations.

Gastrointestinal diseases are common complaints caused by food poisoning and pathogenic microorganisms. Food poisoning and spoilage are mostly due to contamination with bacterial and

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fungal pathogens (Pandey and Singh, 2011; Solomakos et al., 2008). Some of the most common gastrointestinal problems in Pakistan are diarrhea, cholera, dyspepsia, stomachaches, cramps, vomiting, indigestion, colon cancer, and gastric ulcer. Every year approximately 4.0 million cases and 0.15 million deaths are reported worldwide due to gastrointestinal infections like diarrhea and cholera. Correspondingly in Pakistan, diarrhea deaths in children under 5 years remained at 0.1 million in 2015 (Ali et al., 2015). In recent years, antibiotic resistance is an emerging problem worldwide that raised the interest of the researcher to develop more potent antimicrobial agents to combat microbial resistance. Natural products remained a major source of new drugs that can offer a wide range of complex, pure secondary metabolites and structurally diverse compounds as potential antimicrobial agents (Mabona et al., 2013). These natural products regarded as nutritionally safe and easily degradable with no side effects due to antioxidant properties. An opportunity is to pharmacologically test the traditionally used polyherbal formulations being extensively used in the southern regions of Khyber Pakhtunkhwa to treat gastrointestinal tract infections. According to published literature there is scarce studies reported regarding pharmacology of traditionally used polyherbal formulation for gastrointestinal infections. There is need of time to evaluate polyherbal formulations using scientific methods such as clinical trials, possible bioactive constituents and mechanism of action for the future world. Hence, the present study has been designed with the objective to provide scientific background to the traditionally used polyherbal mixtures through *in-vitro* antimicrobial activities.

2. Materials and methods

2.1. Selection and preparation of polyherbal formulations

Polyherbal formulations used to treat digestive problems in rural and urban areas of district Dera Ismail Khan were selected (Mussarat et al., 2021). Plant parts of polyherbal recipes commonly used by the local people were collected and identified by Taxonomist at the Department of Botany, Kohat University of Science and Technology, Kohat. Collected plant parts were washed, cut into small pieces, shade dried, and crushed into powder form with the help of a grinder. Powder of individual plants was mixed according to the traditional description for a respective polyherbal mixture. A total of 25 plants species were combined in a different ratios, and 2–5 plants mixed to form these polyherbal formulations. *Foeniculum vulgare* was used in most of the polyherbal formulations (n = 7) followed by *Elettaria cardamomum* (n = 6) and *Cuminum cyminum* (n = 5). Fruit (n = 21) and seeds (n = 18) were the most used plants parts. Local names of all polyherbal recipes are given in the table and these are denoted by English alphabets (Table 1).

2.2. Preparation of polyherbal extract

A polyherbal mixture of 100 g of dried powder was soaked in 1000 ml methanol in 2000 ml flask and kept for seven days for allowing total extraction at room temperature by cold maceration process. After that, the soaked polyherbal mixture was filtered by Whattmann filter paper # 41. The filtrate was collected in a beaker and evaporates through a rotary evaporator. The semisolid extract was preserved for experimental purposes. For antimicrobial activity, crude extracts of polyherbal were dissolved in DMSO (50 mg/ml) to prepare a stock solution. In the present study 14 traditionally polyherbal formulations were tested for synergistic, antagonistic, and additive effects against selected pathogenic microbes.

2.3. Antibacterial activities of polyherbal mixtures

Antibacterial activities of 14 polyherbal crude extracts were checked at 50 mg/ml concentration against six bacterial strains *Vibrio cholerae, Shigella flexneri, Escherichia coli, Proteus mirabilis Proteus vulgaris, Pseudomonas aeruginosa,* and *Salmonella typhi* using the agar well diffusion method. All the equipment was autoclaved at 121 ℃ for 30 min. According to company instructions 11.4 g, Muller Hinton Agar was added in a 500 ml flask containing 300 ml distilled water. To dissolve all the ingredients, mixed and shake it well through an electric heater. After autoclaving, 20 ml of this media was poured aseptically into Petri plates and solidify for about 10 min. The bacterial strains were spread with sterile swabs on the nutrient agar. Wells was formed with a sterile cork borer. DMSO was used negative control while Meropenem standard disc (10 μg) as a positive control. To avoid any kind of contamination, all procedure was carried out in the laminar flow hood and then petri plates were incubated for 24 h at 37 °C in an incubator. Zones of inhibitions were measured in mm (Heatley, 1944; Kirby et al., 1956). All experiment was repeated three times and results recorded as mean values. The Minimum inhibitory concentration (MIC) was determined using the serial dilution method (NCCLS, 2000). The minimum bactericidal concentration (MBC) of the polyherbal extract was determined following the method of Spencer & Spencer (Spencer and de Spencer, 2004).

2.4. Antifungal activities

Antifungal activities of fourteen polyherbal crude extracts were checked at 50 mg/ml concentration against six fungal pathogens *Aspergillus nigar, Rhizopus, Fusarium oxysporum, Aspergillus fumigatus, Trichoderma,* and *Fusarium graminearum*. To prepare media for fungal activity, 6.5 g of SDA was taken in 100 ml of distilled water, mixed it well, autoclaved, and then cooled to 40 C°. About 20 ml of this media was poured aseptically into petri plates and solidified. A piece of 7 days old culture fungus with 4 mm diameter was placed on media and extract was poured in wells and labeled. Fluconazole was used as a standard for comparison of inhibition zone. All plates were incubated at 28 C° for 7 days. The experiment was repeated three times and the zone of inhibition was measured in mm.

2.5. Review on the antimicrobial analysis of individual plants

Selected fourteen polyherbal recipes were comprised of 25 individual medicinal plants with different ratios. Literature was searched about these individual plants online through different databases like Google Scholar, ISI Web of Knowledge, Science hub, Research gate, and Science Direct Navigator. A huge published data about the antimicrobial screening of methanolic extract of respective plant parts against selected bacterial and fungal strains were gathered. In this review table we focused on data of respective plant parts extracted with methanol solvent and whose extracts concentrations (mg/ml) were mentioned quantitatively i.e. milligrams of the extracts dissolved in milliliters. The concentration given in microgram was converted to the milligram. The review table is not only limited to antimicrobial activity, it contains phytocompounds isolated from methanolic extract of plant part which might be active constituent responsible for microbes inhibition (Table 2).

2.6. Data analysis

All results were arranged and analyzed using Microsoft 2007. The average zone of inhibition and Standard deviation were calculated. ANOVA was used to measure statistical significance (p-value) among polyherbal recipes producing inhibition zones for a single bacterial strain by Microsoft Excel.

Table 1
Polyherbal combinations used traditionally for gastrointestinal problems in Dera Ismail Khan.

Local name/ Abbreviation	Individual Plants in Polyherbal formulation	Local name	Habit	Part used	Disease name
(Phakki) A	*Withania coagulans* (Stocks) Dunal. Solanaceae	Paneer	Shrub	Fruit	Powder used for all digestive problems including diarrhea
	Foeniculum vulgare Mill. Apiaceae	Sounf	Herb	Seeds	
	Cuminum cyminum L. Apiaceae	Jeera	Herb	Seeds	
	Curcuma zedoaria Rosc. Zingiberaceae	Kachoor	Herb	Rhizome	
	Terminalia chebula Retz. Combretaceae	Hareer	Herb	Rhizome	
(Podeena qehwa) B	*Mentha piperita* L. Lamiaceae	Podeena	Herb	Leaves	Decoction (tea) used for nausea, vomiting and diarrhea
	Camellia sinensis L. Kuntze Theaceae	Sabz chaey	Herb	Leaves	
	Elettaria cardamomum (L.) Maton. Zingiberaceae	Sabz illaichi	Tree	Fruit	
(Savi chah) C	*Foeniculum vulgare* Mill. Apiaceae	Sounf	Herb	Seeds	Decoction used for gastric pain, mensis pain, stomach ache
	Withania coagulans (Stocks) Dunal. Solanaceae	Paneer	Shrub	Fruit	
	Camellia sinensis L. Kuntze Theaceae	Sabz chaey	Herb	Leaves	
	Elettaria cardamomum (L.) Maton. Zingiberaceae	Sabz illaichi	Tree	Fruit	
(Zeera sounf phakki) D	*Terminalia chebula* Retz. Combretaceae	Hareer	Herb	Rhizome	Intestinal problem
	Cuminum cyminum L. Apiaceae	Zeera	Herb	Seeds	
	Foeniculum vulgare Mill. Apiaceae	Sounf	Herb	Seeds	
(Hazma Phakki) E	*Withania coagulans* (Stocks) Dunal. Solanaceae	Paneer	Shrub	Fruit	Constipation, Obesity, indigestion, gastric pain
	Piper nigrum L. Piperaceae	Kali mirch	Shrub	Buds/ seeds	
	Trachyspermum ammi L. Apiaceae	Ajwain	Herb	Seeds	
	Cuminum cyminum L. Apiaceae	Zeera	Herb	Seeds	
	Foeniculum vulgare Mill. Apiaceae	Sounf	Herb	Seeds	
(Dawai dard) F	*Foeniculum vulgare* Mill. Apiaceae	Saunf	Herb	Fruit	Digestive problems like constipation, gastric pain, intestinal worms
	Cuminum cyminum L. Asteraceae	Sufaid Zeera	Herb	Fruit	
	Citrullus colocynthis (L.) Schrad. Cucurbitaceae	Kortuma	Herb	Fruit	
	Piper nigrum L. Piperaceae	Kali mirch	Herb	Fruit	
	Elettaria cardamomum (L.) Zingiberaceae	Choti ilaichee	Tree	Fruit	
	Plantago ovate Forssk. Plantaginaceae	Ispaghol	Herb	Fruit, husk	
	Ferula asafetida L. Apiaceae	Heeng	Tree	Fruit	
(Podina Sharbat) G	*Elettaria cardamomum* (L.) Zingiberaceae	Choti ilaichee	Tree	Fruit	Used for 24 hrs. vomiting and indigestion
	Syzygium aromaticum L. Myrtaceae	Lowng	Tree	Buds	
	Cinnamomum verum J. Presl Lauraceae	Dar cheeni	Tree	Bark	
	Mentha piperita L. Lamiaceae	Podina	Herb	Leaves	
	Rosa indica L. Rosaceae	Arq e Gulab	Shrub	Petal Extract	
(Safoof) H	*Mentha piperita* L. Lamiaceae	Podina	Herb	Leaves	Diarrhea, Dysentery
	Punica granatum L. Lythraceae	Anar sakh	Tree	Fruit cover	
(Adrak qehwa) I	*Camellia sinensis* L. Kuntze Theaceae	Sabz chaey	Herb	Leaves	Obesity/ Indigestion
	Citrus limon (L.) Osbeck. Rutaceae	Nimbo	Tree	Fruit	
	Zingiber officinale Roscoe. Zingiberaceae	Adrak/ Sund	Herb	Rhizome	
(Powder) J	*Syzygium cumini* (L.) Skeels Myrtaceae	Jaman	Tree	Seeds	Typhoid, diarrhea, and useful for diabetes patient
	Punicum granatum L. Lythraceae	Anar	Tree	Fruit cover	
(Keero ki dawa) K	*Cocos nucifera* L. Arecaceae	Nareal	Tree	Fruit	Intestinal worms
	Punicum granatum L. Lythraceae	Anar	Tree	Seeds	
(Arq) L	Cassia fistula L. Fabaceae	Gardnali	Tree	Seeds cover	Constipation
	Foeniculum vulgare Mill. Apiaceae	Sounf	Herb	Seeds	
(Chaata) M	Butea monosperma (Lam.) Kuntze Fabaceae	Chichra	Shrub	Seeds	Constipation and bronchial problems
	Achyranthes aspera L. Amaranthaceae	Puhutkanda	Herb	Seeds	
	Elettaria cardamomum (L.)Maton. Zingiberaceae	Sbz illachi	Herb	Fruit	
(Haiza recipe) N	*Ocimum basilicum* L. Lamiaceae	Niaz boo	Herb	Leaves/ seeds	Diarrhea, Cholera
	Mentha piperita L. Lamiaceae	Podina	Herb	Leaves	

Table 2
Review table of individual plants in polyherbal formulations against bacterial and fungal pathogens.

Plant name/ Part used	Pathogen	Concentration (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Compounds	References
A. aspera Seeds	*E. coli*	0.25–25	5–20	1.024	NA	Alkaloids, tannins, saponins, glycosides and flavonoids	(Abi Beaulah et al., 2011; BK et al., 2013; Kaveti et al., 2013; Srinivasulu et al., 2016; Talreja et al., 2017
	K. pneumoniae	0.25–1	11.2–20	1.024			
	S. flexneri	0.25–1	10.1–13.8	NA			
	P. vulgaris	0.25–1	8.8–25				
	P. aeruginosa	0.25–1	12–20				
	S. aureus	0.25–25	1–13.7				
	S. typhi	NA					
	P. mirabilis						
	V. cholerae						
	A. nigar						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
B. monosperma Seeds	*S. typhi*	50	7	NA	NA	Polyphenols, glycosides, quinines, anthracyanosides, flavonic glycosides, coumarins	(Maharjan et al., 2011)
	E. coli	NA					
	S. flexneri						
	P. mirabilis						
	P. vulgaris						
	V. cholerae						
	P. aeruginosa						
	K. pneumoniae						
	S. aureus						
	A. nigar						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
C. colocynthis Fruit	*A. nigar*	15–100	5–23	1–3.12	6.25	α-Dglucopyranoside, O-α-D-glucopyranosyl, phthalic acid and y-tocopherol. Alkaloid ,Tannins, Saponins , Flavonoids Unsaturated sterols and terpens, Sterol and steroid Terpenoids and Cardiac glycosides, Cucurbitacin	(Ali et al., 2013; Doss et al., 2011; Eidi et al., 2015; Gurudeeban et al., 2010; Hameed et al., 2020; Hany and Neelam, 2020; Hussain et al., 2011; Idan et al., 2015; Nora et al., 2015; Thangavel and Ramasamy, 2019)
	A. fumigatus	15–100	6–19	1.56	3.12		
	E. coli	40–60	3–16	0.5–13.9	NA		
	P. mirabilis	0.3	2–10	1			
	P. vulgaris	0.3	12.3	1			
	S. aureus	25–100	2.9–22	0.25–10.8			
	P. aeruginosa	25–100	4.9–19	0.5–13.9			
	K. pneumoniae	25–100	9.4–19	1–13.9			
	S. typhi	NA	10	1			
	F. oxysporum	25–100	10–15	NA			
	S. flexneri	NA	5				
	V. cholerae	NA					
	Rhizopus						
	Trichoderma						
	F. graminearum						
C. cyminum Seeds	*E. coli*	0.5–250	2–31	0.12–20	0.25–40	Gallic acid, tannin, Coumarin, terpenoids, 2-methyl-4isopropyliden-cyclopentan-1-al, 1-methyl-4-isopropyl-3-cyclohexen-1-ol, monoterpene, 4-isopropylcyclohex-1, 3-dien-1-yl) methanol, 4-isopropyl-1-cyclohexen-1-carbaldehyde, (3,4-dimethyl-2-oxo-cyclopenten-1-yl) p-cymene, 8a-methyl octahydro-2(1H)-naphthalenone, p-cymen-7-ol, o-cymen-5-ol, p-cymen-3-ol,6-allyl-4,5 dimethoxy-1,3 benzodioxole 3-isopropyl phenol,	(Akbar et al., 2019; Allaithy, 2017; Chaudhary et al., 2014; Dua et al., 2013b; Mostafa et al., 2018; Saee et al., 2016; Saeidi et al., 2015; Shahid et al., 2013; Sheikh et al., 2010; Shete and Chitanand, 2014; Singh and Tripathi, 2018; Srivastava et al., 2016)
	P. aeruginosa	2–33.33	10–25	0.25–6.25	0.5		
	S. typhi	0.5–250	8–35	40	60		
	K. pneumoniae	0.5–250	8–22	0.12–40	0.25–60		
	S. aureus	0.5–250	9–36	0.12–40	0.25–60		
	P. mirabilis	50–100	11.5–12	NA			
	V. cholerae	0.5	8–17				
	A. nigar	0.5	17				
	A. fumigatus	0.5	15				
	P. vulgaris	NA					
	S. flexneri						
	Rhizopus						
	F. oxysporum						
	Trichoderma						
	F. graminearum						

Table 2 (*continued*)

Plant name/ Part used	Pathogen	Concentration (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Compounds	References
C. fistula Seeds, seed cover	*E. coli* *S. typhi* *S. aureus* *S. flexneri* *P. mirabilis* *P. aeruginosa* *K. pneumoniae* *A. nigar* *F. oxysporum* *A. fumigatus* *Trichoderma* *P. vulgaris* *V. cholerae* *Rhizopus* *F. graminearum*	100 100 25–100 100 100 100 100 100 100 100 100 NA	4.83–16 4.15–25 5–18 9 6 5.73 5 5.8 5 6 5	50 3.12 12.5 NA	NA	2,4-Dihydroxy-2,5-dimethyl-39(2H)-furan-3-one, á-D-Glucopyranoside , O-á-D-glucopyranosyl-(1. fwdarw.3)-ß-D-fruc, d-Mannose, 5,7-Dodecadiyn -1,12- diol, 3- Tr ifluor oacetoxypentadecane, 3-Trifluoroacetoxypenta decane, Pterin-6-carboxylic acid, Imidazole-4-carboxylic acid ,2-fluoro-1-methoxymethyl-,ethyl ester, D-Carvone,	(Gupta et al., 2015; Kadhim et al., 2016; Subramanion et al., 2010)
C. limon Fruit juice/ extract	*E. coli* *S. aureus* *K. pneumoniae* *S. typhi* *P. aeruginosa* *A. nigar* *P. vulgaris* *S. flexneri* *V. cholerae* *P. mirabilis* *Rhizopus* *F. oxysporum* *A. fumigatus* *Trichoderma* *F. graminearum*	0.2–1 0.2 0.2 0.1–1 0.2 0.2 0.2 100-1000 ug/ disc NA	11–19 14–26 18–30 6–30 10–19 4–12 17–20 9-15	0.5–50 0.025–25 0.0035 0.025–12.5 0.0125 0.05 0.001–6.5 NA	0.1–1 0.05 0.05 0.05 0.05 0.1 NA	Alkaloids, flavonoids, phenols, quinines, terpenoids and carbohydrate, cyanogenetic, cardic and steroidal glycosides, tannins, saponins, and water-soluble vitamins	(Singh et al., 2020b) (Bhuiyan et al., 2019; Ekawati and Darmanto, 2019; Hindi and Chabuck, 2013; Kumari et al., 2014; Liya and Siddique, 2018; Oikeh et al., 2016; Okeke et al., 2015; Sah et al., 2011; Shakya et al., 2019)
C. nucifera Fruit	*E. coli* *S. aureus* *P. aeruginosa* *A. nigar* *V. cholerae* *S. flexneri* *P. mirabilis* *K. pneumoniae* *P. vulgaris* *S. typhi* *Rhizopus* *F. oxysporum* *A. fumigatus* *Trichoderma* *F. graminearum*	0.5–1.5 0.5–1.5 NA NA	0–10 8–15 16 12	NA	NA	Phenols, flavonoids, alkaloids, tannins and saponins	(Chakraborty and Mitra, 2008; Igwe and Ugwunnaji, 2016)
C. sinensis leaves	*P. aeruginosa* *E. coli* *S. flexneri* *S. aureus* *S. typhi* *V. cholerae* *K. pneumoniae* *P. mirabilis* *A. nigar* *F. oxysporum* *A. fumigatus* *P. vulgaris* *Rhizopus* *Trichoderma* *F. graminearum*	0.1–200 0.1–200 3–48 0.1–200 3–48 3–48 NA NA	10–19 3.6–32 2–26 0.8–20 2.3–28.4 1.6–30.1 22 25 5 6 30	5 3.25–100 2.5–12 0.1–0.39 12 6 0.512 NA	>5 >5 >5 1.56 60 NA	Phenol, flavonoids, catechin, alkaloids, tannins and alkaloid	(Agbom et al., 2020; Archana and Abraham, 2011; Bashir et al., 2014; Chakrabort and Chakrabort, 2010; Dzotam and Kuete, 2017; Farooqui et al., 2015; Fazal and Rauf, 2015; Latteef, 2016; Mehta et al., 2016; Rajeswari, 2015; Roy et al., 2018; Vasudeo and Sonika, 2009)
C. zedoaria Rhizome	*V. cholerae* *E. coli* *S. typhi* *P. aeruginosa* *K. pneumoniae* *S. aureus* *A. nigar* *A. fumigatus* *Trichoderma* *S. flexneri*	0.5 0.5–40 0.5 0.5–40 0.5 0.5–40 0.5–40 40 40 NA	0 9–18 16 5–13 0 16 11–14 12 5	NA	NA		(Das and Rahman, 2012; Shahriar, 2010) 26]

(*continued on next page*)

Table 2 (*continued*)

Plant name/ Part used	Pathogen	Concentration (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Compounds	References
	P. mirabilis						
	P. vulgaris						
	Rhizopus						
	F. oxysporum						
	F. graminearum						
C. zeylanicum Bark	*S. aureus*	10–150	11–14	25	NA	NA	(Aneja et al., 2009; Saliem and AbedSalih, 2018; Singh et al., 2020a; Vyas et al., 2015)
	E. coli	0.1–10	4–12	NA			
	P. aeruginosa	10–150	9–14				
	K. pneumoniae	1–2	9–10				
	A. nigar	10	13				
	V. cholerae	NA					
	S. flexneri						
	P. mirabilis						
	P. vulgaris						
	S. typhi						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
E. cardamomum Fruit/seeds	*S. aureus*	100	6–38	2–50	2.5–>50	terpenoids flavonoids and glycosides	(Al-Judaibi et al., 2014; Aneja and Joshi, 2009; Bano et al., 2016; Islam et al., 2010; Kaushik et al., 2010; Singh et al., 2008)
	S. typhi	100	6.5–22	25	50		
	P. aeruginosa	100	8.5–16	49	75		
	K. pneumoniae	100	14	50	50		
	E. coli	50	8.5-16.5	NA			
	V. cholerae	NA					
	S. flexneri						
	P. mirabilis						
	P. vulgaris						
	A. nigar						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
E. jambolana Seeds	*S. aureus*	0.050–1	6–25	0.125	0.125	NA	(Chandrasekaran and Venkatesalu, 2004; Mehreen et al., 2016; Ogato et al., 2015; Raju et al., 2011; Saha et al., 2013a)
	E. coli	0.050–1	4–15	0.250	0.5		
	S. typhi	0.050–1	5–18	0.125	0.5		
	P. aeruginosa	0.050–1	5–23	0.250	0.5		
	K. pneumoniae	0.050–1	4	0.25	0.5		
	A. nigar	0.050–1	6–11	0.0625	0.125		
	Rhizopus	0.050–1	10	0.0625	0.125		
	A. fumigatus	0.050–1	12	0.0625	0.250		
	S. flexneri	25–100	20–26	NA			
	V. cholerae	NA					
	P. mirabilis						
	P. vulgaris						
	F. oxysporum						
	Trichoderma						
	F. graminearum						
F. asafetida Root/ Gum resine	*S. aureus*	1–5	5–20	0.5	NA	Alakaloids, tannins, glycosoids, flavonoids, saponins	(Patil et al., 2015; Sharma et al., 2016; Shrivastava et al., 2012)
	E. coli	1–5	16–19	1			
	K. pneumoniae	2–4	14–17	1			
	A. nigar	2–4	14–17	1			
	P. aeruginosa	1–5	13.9	NA			
	S. flexneri	NA					
	V. cholerae						
	P. mirabilis						
	P. vulgaris						
	S. typhi						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						

Table 2 (*continued*)

Plant name/ Part used	Pathogen	Concentration (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Compounds	References
F. vulgare Seeds	*S. aureus*	0.05–0.4	3.33–20	0.0125–0.5	NA	terpenoids, tannins, steroids, alkaloids and glycosides, gallic acid, catechin, quercetin	(Agarwal et al., 2017; Al-Hadid, 2017; Al Akeel et al., 2014; Allaithy, 2017; Arman et al., 2019; Beyazen et al., 2017; Chang et al., 2016; Dua et al., 2013a; Jayalakshmi et al., 2011; Salami et al., 2016; Shahid et al., 2013)
	E. coli	0.4–100	2–19	0.015–0.25			
	S. typhi	1–15	11–25	0.015–0.5			
	P. aeruginosa	0.4–100	1.33,14–19	>33.33			
	V. cholerae	0.4	4.33	NA			
	P. mirabilis	50-100	10.5-16				
	P. vulgaris	0.0044	12				
	K. pneumoniae	10-15	12-15				
	A. nigar	NA	15.6				
	S. flexneri		NA				
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
M. piperita Leaves	*E. coli*			0.21	0.49		(Ali et al.; Elansary et al., 2020)
	P. aeruginosa			0.16	0.47		
	S. aureus			0.17	0.37		
	A. nigar		19				
	A. fumigatus		15				
	V. cholerae	NA					
	S. flexneri						
	S. typhi						
	K. pneumoniae						
	P. mirabilis						
	P. vulgaris						
	Rhizopus						
	F. oxysporum						
	Trichoderma						
	F. graminearum						
O. basilicum Seeds	*S. aureus*	25–100	5–11	0.125	NA	NA	(Adigüzel et al., 2005; Ahmad et al., 2016; Gajendiran et al., 2016; Kadhim et al., 2016; Saha et al., 2013b)
	E. coli	25–100	11–12	0.25			
	V. cholerae	300 μg/disc	10	NA			
	P. mirabilis	50–75	4–12				
	S. typhi	50–75	9–10				
	P. aeruginosa	75	16				
	K. pneumoniae	50–75	4–13				
	A. nigar	1–6	63–100%				
	Rhizopus	1–6	56–100%				
	A. fumigatus	1–6	58–100%				
	S. flexneri	NA					
	P. vulgaris						
	F. oxysporum						
	Trichoderma						
	F. graminearum						
P. granatum Fruit cover	*S. aureus*	4–100	7–29	0.1–50	>10–60	steroids, triterpenoides, alkaloids, flavonoids, saponins, tannins and carbohydrates, Anthraquinones	(Al-Zoreky, 2009; Ali, 2017; Dahham et al., 2010) (Abdollahzadeh et al., 2011; Kesur et al., 2016; Mathabe et al., 2006; Raju et al., 2011) (Abdu et al., 2020; Alemu et al., 2017; Barathikannan et al., 2016; Bereksi et al., 2018; Rajeswari, 2015; RASHID et al.; Sajjad et al., 2015; Salmen et al., 2016; Shafiq et al., 2013; Shahbazi, 2017; Ullah et al., 2012)
	E. coli	0.03–0.05	13–27	1–12.5	>10–32		
	S. typhi	14–24	1–13–37	25	>10–32		
	P. aeruginosa	1–100	8–26	12.5	NA		
	K. pneumoniae	0.125–1	7-18	2			
	P. mirabilis	0.03–0.05	12-20	NA			
	P. vulgaris	NA	6	12.5			
	S. flexneri		16-25	NA			
	A. nigar		7-23				
	F. oxysporum		13				
	V. cholerae						
	Rhizopus						
	A. fumigatus						
	Trichoderma						
	F. graminearum						

(*continued on next page*)

Table 2 (*continued*)

Plant name/ Part used	Pathogen	Concentration (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Compounds	References
P. nigrum Seeds, fruit	*E. coli*	1–2	4-11-11.8	NA			(Sharma et al., 2016) Antibacterial activity for fruit peel Methanol extract of Punica granatumLinn. (Punicaceae) Afzan Mahmad
	P. aeruginosa	1–200	10-16				
	K. pneumoniae	1–2	10-10.9				
	S. aureus	1–200	4-10.3-16				
	V. cholerae	NA					
	S. flexneri						
	P. mirabilis						
	P. vulgaris						
	S. typhi						
	A. nigar						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
P. ovate Fruit, husk	*E. coli*	50–400	7–10	NA		saponin, tannin, flavonoids, alkaloids, steroids	(Motamedi et al., 2010)
	P. mirabilis	50–400	0				
	P. vulgaris	5–50	8.5–12				
	S. typhi	50–400	0				
	P. aeruginosa	400	7				
	K. pneumoniae	400	7				
	S. aureus	5-400	9-18	20	>200		
	V. cholerae	NA					
	S. flexneri						
	A. nigar						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
R. indica Petal extract/ flower	*S. aureus*	200	17–22	4.5	NA	Phenolic compounds, flavonoids, tannins, alkaloids	(Mishra et al., 2011; Pathak et al., 2019; Rikhi et al., 2015; Safdar and Malik; Sowmya et al., 2017)
	E. coli	200	12–15	3.7			
	P. aeruginosa	200	18–21	4.5			
	S. typhi	20	6–10	NA			
	K. pneumoniae	NA	22				
	V. cholerae		10–17				
	S. flexneri	NA					
	P. mirabilis						
	P. vulgaris						
	A. nigar						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
S. aromaticum Buds	*V. cholerae*	NA	NA	0.025	NA	alkaloid, terpenoids, flavonoids, steroid, saponin Anthraquinones, and tannin, phenolic compounds	(Abd El Azim et al., 2014; Aneja and Joshi, 2010; Dua et al., 2014; Ghulam and Ahmad, 2014; Mehrotra and Srivastava, 2010; Okmen et al., 2018; Pandey and Singh, 2011; Prajapati et al., 2018; Sharma et al., 2016; Vizhi et al., 2016; Wankhede, 2015)
	E. coli	1–500	5–24	3.12–125	12.5–125		
	S. flexneri	25–500	7–19	6.25–125	50–125		
	P. vulgaris	250–500	7–11	31.25	62.5		
	S. typhi	25–500	10–16	3.9–6.25	7.8–25		
	P. aeruginosa	1–100	10–30	1.95–12.5	50		
	K. pneumoniae	25–500	7.5–15	6–7.8	15.6–25		
	S. aureus	1–350	5–28	0.98–3.25	25		
	A. nigar	NA	4-14	NA			
	F. oxysporum		9				
	Trichoderma		24				
	P. mirabilis	NA					
	A. fumigatus						
	Rhizopus						
	F. graminearum						

Table 2 (*continued*)

Plant name/ Part used	Pathogen	Concentration (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Compounds	References
T. chebula Rhizome	*V. cholerae*	10	15	0.25	1.5	Phenolic compounds, flavonoids, Alkaloid, Tannin, Steroid, Cardiac glycosides, terpenoids	(Bajpai et al., 2010; Baliah and Astalakshmi, 2014; Jayalakshmi et al., 2011; Monisha et al., 2013; Mostafa et al., 2011; Rai and Joshi, 2009; Sharma et al., 2012; Singh et al., 2012; Zearah, 2014)
	E. coli	10–500	14–30	50–100	NA		
	S. aureus	1–500	22–35	3.12–25			
	P. mirabilis	10	20.6	12.5			
	P. aeruginosa	125–500	18–30	12.5			
	F. oxysporum	1.5	23	0.5			
	S. flexneri	10	12	NA			
	S. typhi	10	16–25				
	K. pneumoniae	125–500	17–30				
	P. vulgaris	NA					
	A. fumigatus						
	A. nigar						
	Rhizopus						
	Trichoderma						
	F. graminearum						
T. ammi Seeds	*E. coli*	NA	9–20	1–12.5	25	NA	(BASHYAL and GUHA, 2018; Hassan et al., 2016; Sharma et al., 2018; Sharma and Shrivastava; Shokrian et al., 2016)
	S. typhi		9–12	1	25		
	S. aureus		9–20	1	50		
	P. aeruginosa	0.025	8–21	1	NA		
	A. nigar	NA	15	NA			
	S. flexneri	NA					
	P. vulgaris						
	P. mirabilis						
	V. cholerae						
	K. pneumoniae						
	Rhizopus						
	F. oxysporum						
	A. fumigatus						
	Trichoderma						
	F. graminearum						
W. coagulance Fruit	*E. coli*	15	10–21	NA		terpenoids, flavonoids and tannin	(Peerzade et al., 2018; Shahid et al., 2013; Sudhanshu et al., 2012)
	S. flexneri	50–250	8–13				
	P. vulgaris	50–250	0–16				
	S. typhi	1–250	7–16				
	P. aeruginosa	1–250	13–20				
	K. pneumoniae	1–250	10–22				
	S. aureus	1–250	11–19				
	A. nigar	50–250.	7–11				
	A. fumigatus	0.025	29				
	V. cholerae	NA					
	P. mirabilis						
	Rhizopus						
	F. oxysporum						
	Trichoderma						
	F. graminearum						
Z. officinale Rhizome	*E. coli*	0.025–80	2.9–22	3.5	40	alkaloid, phlobotannins, flavanoids, glycosides, saponins, tannin and terpenoids. zingiberene, β-bisabolene , α-farnesne, β-sesquiphellandrene ,α-curcumene and gingerol and shogaol	(Agrawal et al., 2018; Azadpour et al., 2016; BASHIR et al., 2015; Bhargava et al., 2012; El-Mesallamy et al., 2017; Hasan et al., 2012; Iotsor et al., 2019; Kaushik and Goyal, 2011; Njobdi et al., 2018; Riaz et al., 2015; Sunilson et al., 2009; Ushimaru et al., 2007; Yadufashije et al., 2020; Yassen and Ibrahim, 2016; Yusuf et al., 2018)
	S. aureus	0.025–50	3.75–31	0.052–1.75	0.1		
	P. aeruginosa	0.025–0.1	4–26	0.416–1.75	0.416		
	P. mirabilis	3.1–50	1.9–12	NA			
	S. typhi	0.025–0.1	6–21				
	S. flexneri	20	11				
	K. pneumoniae	0.025-50	4.93-29				
	A. nigar	3	11–25				
	F. oxysporum	3	29				
	V. cholerae	NA					
	P. vulgaris						
	Rhizopus						
	A. fumigatus						
	Trichoderma						
	F. graminearum						

3. Results

3.1. Antibacterial activities

On observation of antibacterial screening, all bacterial pathogens were sensitive towards tested polyherbal crude extracts; indicating the efficacy of these extracts, however, they vary in inhibition zone against the tested micro-organisms (Table 3). Polyherbal extract A, B and D produced the least number of colonies of bacterial strains *V. cholerae, E. coli,* and *S. typhi* on agar plate and statistically significant inhibition ($p < 0.01$). Polyherbal recipes B, and D showed a significant inhibition zone against *Vibrio cholerae* (25.63;$p < 0.001$).

Across all the polyherbal extracts, compared with antibiotic, polyherbal recipe E and G were very effective for four bacterial isolates *P. vulgaris* (28.33;$p < 0.001$), *P. mirabilis* (24.33; $p < 0.001$) *P. aeruginosa* (19.67 ± 0.5; $p < 0.0001$) and *S. flexneri* (13.67 ± 2.3). *S. typhi* was resilient towards recipe E with zero inhibition. Both the *P. mirabilis* and *S. flexneri* were also more sensitive to polyherbal extract K. Polyherbal recipe N showed potent activity against *E. coli* and *S. flexneri*. Among all the tested polyherbal extracts A, B, K, D, and N showed minimum inhibition and bactericidal effect at very low concentrations (3.12–6.25) (Table 4). Minimum inhibitory concentration ranges within (3.12–<6.25 mg/ml) while bactericidal concentration was (12.5–50 mg/ml).

3.2. Antifungal activities

Polyherbal crude extracts showed potential antifungal activity. Polyherbal extracts A, C, D, and F showed good inhibition than Fluconazole (Table 5). *A. nigar* and *A. fumigatus* tended to be more sensitive for polyherbal extract C with the least number of colonies on SDA plate and statistically significant inhibition (28.67; $p < 0.05$) and (27; $p < 0.01$), respectively.

Rhizopus (19.67; $p < 0.01$) and *Trichoderma* (30; $p < 0.001$) were more sensitive and produced the least number of colonies after treatment with polyherbal recipe A. Polyherbal recipe D and F showed higher significant inhibition against *F. oxysporum* (31; $p < 0.001$) and *F. graminearum* (28.67;$p < 0.001$), respectively as compared to standard antifungal (19.67 mm). *Trichoderma* was resistant to polyherbal recipe G, I, K, and L and showed no inhibition.

4. Discussion

Antimicrobial resistance is an alarming threat to human health. The rate of development of novel medicine is limited and slow. In the present study, the assessment of selective polyherbal combinations showed synergistic, antagonistic, and additive interactions. Polyherbal recipes A, B, D, E, N, K, and H were more potent and showed good antimicrobial effect. Synergism of polyherbal formulation provides a direction to develop effective antibiotics with

Table 3
Antibacterial activities (inhibition zone in mm) by polyherbal crude extract at the concentration of 50 mg/ml.

	V. cholerae	*E. coli*	*S. flexneri*	*P. mirabilis*	*P. vulgaris*	*S. typhi*	*P. aeruginosa*
A	24.33 ± 2.1	22.66 ± 2.3	12 ± 0	11.33 ± 1.1	20.67 ± 1.1	20.67 ± 1.1	13.33 ± 2.3
B	25.63 ± 3.5	19.33 ± 2	14 ± 3.6	15 ± 0	13 ± 1.7	24.67 ± 2.2	18 ± 2.6
C	13 ± 3.6	20.33 ± 0.5	12 ± 1	20.67 ± 1.1	20 ± 0	13.67 ± 1.5	14 ± 1.7
D	25.63 ± 0.5	18.67 ± 3.2	12 ± 1.7	21.33 ± 1.1	27.33 ± 1.5	14.33 ± 3.7	15 ± 4.3
E	16 ± 1.7	19.33 ± 1.1	13.67 ± 2.3	24.33 ± 1.1	28.33 ± 0.5	0	19.67 ± 0.5
F	12.67 ± 1.1	18.33 ± 0.5	10 ± 0	6.33 ± 5.5	15.67 ± 1.1	11.33 ± 1.1	18.67 ± 1.1
G	17 ± 3.5	23.33 ± 4.1	8 ± 1.7	24.33 ± 1.1	20.67 ± 1.1	14 ± 2	19.33 ± 4.0
H	25.33 ± 1	15.67 ± 4.0	4 ± 3.6	6.67 ± 5.7	25 ± 0	15.33 ± 3.5	17.67 ± 2.5
I	21 ± 1	22 ± 0	10.67 ± 1.1	15.33 ± 0.5	15.33 ± 0.5	11.67 ± 0.5	16.33 ± 1.5
J	24.67 ± 2.5	14.33 ± 1.1	11.67 ± 1.5	20 ± 0	15.33 ± 0.5	13 ± 2	18.33 ± 2
K	21 ± 1	18 ± 2	17 ± 1	24.33 ± 1.1	14.67 ± 0.5	12.33 ± 1.5	16 ± 2.8
L	16.33 ± 3.8	18.33 ± 0.5	13 ± 1.7	10.67 ± 1.1	15.33 ± 0.5	13.33 ± 1.5	16.67 ± 4.1
M	9.33 ± 2.5	15 ± 0	6.67 ± 5.7	14 ± 1.7	13 ± 1	10.67 ± 1.1	13.33 ± 1.5
N	15 ± 2	18.33 ± 1.1	11.67 ± 0.5	4 ± 3.6	19.67 ± 0.5	9.67 ± 0.5	16.67 ± 2.8
AB	22.67 ± 3	19.33 ± 1.5	21.33 ± 1.1	23.33 ± 1.1	20 ± 0	21.33 ± 1.1	23.67 ± 1.1
P value	*p < 0.001*	*p < 0.001*	*p < 0.001*	*p < 0.001*	*p < 0.001*	*p < 0.001*	*p < 0.0001*
DMSO	0	0	0	0	0	0	0

p value for ANNOVA, AB = Antibiotics.

Table 4
MIC and MBC of polyherbal extracts.

Recipe	*V. cholerae*		*E. coli*		*S. flexneri*		*P. mirabilis*		*P. vulgaris*		*S. typhi*		*P. aeruginosa*	
Crude extract (mg/ml)	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A	3.12	50	< 6.25	25	25	>50	6.25	>25	6.25	>25	6.25	>25	50	>100
B	12.5	>50	6.25	>25	6.25	>25	25	50	25	>50	6.25	>25	25	>50
C	< 6.25	25	< 6.25	50	25	>50	6.25	25	6.25	>25	50	100	50	>100
D	6.25	25	6.25	>50	25	>50	6.25	>25	6.25	>25	50	100	25	>50
E	6.25	>25	< 6.25	50	12.5	>50	6.25	>25	25	>50	–	–	25	>50
F	25	>50	25	100	50	100	50	100	50	100	50	>100	25	>50
G	6.25	50	< 6.25	25	50	100	6.25	>25	50	100	50	>100	25	>50
H	50	100	6.25	50	50	100	50	100	6.25	>25	50	100	50	100
I	< 6.25	25	< 6.25	25	25	>50	25	50	50	100	50	>100	50	100
J	< 6.25	50	< 12.5	25	25	>50	25	50	50	100	50	>100	50	100
K	< 6.25	50	< 6.25	25	25	>50	6.25	>25	50	100	50	>100	50	100
L	6.25	25	< 6.25	>50	12.5	>50	25	>50	50	100	50	>100	50	100
M	6.25	50	25	>50	50	100	25	>50	50	100	50	>100	50	>100
N	6.25	50	12.5	>25	12.5	>50	25	100	25	>50	50	>100	25	>50

Table 5
Antifungal activities (inhibition zone in mm) by crude extract at 50 mg/ml.

Polyherbal crude extract	*A. nigar*	*Rhizopus*	*F. oxysporum*	*A. fumigatus*	*Trichoderma*	*F. graminearum*
A	24.67 ± 1.5	19.67 ± 4	17 ± 1.7	5 ± 5	30 ± 0	20 ± 0
B	25.67 ± 6	0	17.67 ± 2.5	25.33 ± 5	29 ± 1	20 ± 0
C	28.67 ± 1.1	1.67 ± 2.8	25.33 ± 1.1	27 ± 7	18.67 ± 1.1	25 ± 0
D	25 ± 2	0	31 ± 1.7	14.33 ± 8.1	15.67 ± 1.1	25 ± 0
E	24 ± 3.6	11.67 ± 10.4	24 ± 1	3.33 ± 2.8	27 ± 1.7	20 ± 0
F	13.33 ± 12.5	13.33 ± 2.8	23.33 ± 1.5	19.33 ± 1.1	15 ± 0	28.67 ± 1.1
G	22 ± 6	6.67 ± 5.7	23.33 ± 3	16.67 ± 10	0	20 ± 0
H	25 ± 5	9.33 ± 1.1	24.33 ± 2	22.33 ± 2.5	19.33 ± 1.1	20 ± 0
I	24.33 ± 1.1	14 ± 5.5	25.33 ± 1.5	24.67 ± 4.5	0	25.67 ± 1.1
J	26.33 ± 2.8	12 ± 10.5	25.67 ± 1.1	15.67 ± 4.9	24.67 ± 0.5	25.33 ± 0.5
K	21.33 ± 1.1	16.67 ± 5.7	20 ± 3.4	10.33 ± 8.9	0	23.33 ± 1.5
L	22.33 ± 2.5	9.33 ± 1.1	19 ± 8.5	12.67 ± 11	0	20.33 ± 0.5
M	22.67 ± 6.8	10 ± 0	24.33 ± 0.5	15 ± 10	15.67 ± 1.1	20.33 ± 0.5
N	23.33 ± 2.8	16.33 ± 7.0	24 ± 2	17.67 ± 2.5	29.67 ± 0.5	19.67 ± 0.5
Fluconazole	19.33 ± 0.5	16.33 ± 2.3	19.67 ± 2.5	26.67 ± 2.8	21.33 ± 1.1	22.33 ± 2.08
P-value	*p < 0.5*	*p < 0.01*	*p < 0.001*	*p < 0.01*	*p < 0.001*	*p < 0.001*
DMSO	0	0	0	0	0	0

changing ratio in active constituents. A review reported the five-year literature regarding antimicrobial activities and plant synergy concluded that synergism both within plants extracts and between plants and antibiotics can enhance the antimicrobial effect (Mundy et al., 2016).

Polyherbal recipe B, the mixture of highly used three individual plants *Mentha piperita, Camellia sinensis,* and *Elettaria cardamomum,* was more effective at least concentration against common gastrointestinal pathogens.*piperita*. Capsules of *E. cardamomum* have been used since ancient times for treating various respiratory and digestive problems. In the traditional system of Chinese medicine, it was used to treat constipation, stomach ache, and dysentery in children. *M. piperita* and *E. cardamomum* individually have not been tested against theselected pathogens yet, however *C. sinensis* showed good effect with least concentration (Adwan et al., 2010). Adwan et al. (Adwan et al., 2010) studied the synergistic effects of plant combinations and found a decrease in MIC value against bacterial pathogens. Polyherbal formulation with *S. aromaticum*, *Zingiber officinalis*, and *T. ammi* used to cure digestive ailments. Presence of 1, 8-cineole, α-ter-pinyl acetate, α-terpineol and sabinene compounds in cardamom oil can serve as natural source of antimicrobial agent (Ashokkumar et al., 2020).

Least concentration of MIC and MBC were shown by mixture A, B, D, and N i.e (3.12 mg/ml) and (<25 mg/ml), respectively. Lower concentration of MIC may be due to damage of inner and outer membrane of the bacterial cell and releasing of all cell materials observed under the Transmission electron microscope. As synergistic effects of Amoxicillin with the combination of essential oil studied by El-Kalek and Mohamed (Abd El-Kalek and Mohamed, 2012). Recipe D was a mixture of three plants i.e rhizome of *Terminalia chebula* (Combretaceae), seeds of *Cuminum cyminum,* and *Foeniculum vulgare* (Apiaceae) with equal ratio showed higher significant inhibition against tested pathogens. Individually *F. vulgare* showed good inhibition with increasing concentration but its methanolic extract was not tested yet for selected fungal and *S. flexneri* as well there is a lack of information available about MBC values. Methanolic extract of *T. chebula* and *C. cyminum* also have increasing inhibition with increasing concentration but lack proof for *S. flexneri* and fungal isolates. Essential oil from seeds of *F. vulgare* has potential to inhibit and kill gram-positive and gram-negative as well fungal pathogens at very low concentrations. Anethole and fenchone, are considered as the main components of its oil (Al-Hadid, 2017). MIC of anethole and fenchone reported in literature against *Aspergillus species* were 1.8 and 5.3 ul/ml, respectively (Mimica-Dukić et al., 2003). The mechanism of action of essential oil might be acting on the membrane integrity and releasing all the cellular contents (Diao et al., 2014). Dua et al. (Dua et al., 2013a) found that the antibacterial effect of *F. vulgare* is due to the presence of a higher quantity of flavonoids like gallic acid, caffeic acid, ellagic acid, quercetin, and kaempferol in its methanolic extract. As in most of the polyherbal recipes used in the present study were consists of *F. vulgare* with other plants so, increased in inhibiton zone may be due to these diverse compounds.

Polyherbal recipe G was a mixture of *Elettaria cardamomum*, *Syzygium aromaticum, Cinnamomum zeylanicum*, *Mentha piperita*, and *Rosa indica* with a ratio of 1:2:2:3:3, respectively, and showed good inhibition zones against *E. coli*, and Proteus species. *C. zylinicum* was also a part of polyherbal formulation with *A. indica*, *C. longa*, *A. sativum*, *O. sanctum*, and *T. indica* studied by Bhinge et al. (Bhinge et al., 2017). This polyherbal recipe showed additive effects when mixed with synthetic base and exhibited maximum activity. Chandra et al. (Chandra et al., 2017) reported medicinal plants such as decoction of *Coriandrum sativum* leaves, *Cinnamomum* spp., *Syzygium aromaticum*, which eliminate or inhibit the growth of *E. coli*; the most common causal agent of urinary tract infections.

Recipe E was a combination of *Withania coagulans*, *Piper nigrum*, *Trachyspermum ammi*, *Cuminum cyminum*, *Foeniculum vulgare*. Two plants species *Cuminum cyminum*, *Foeniculum vulgare* were belonged to Apiaceae were part of many polyherbal mixtures and reported for digestive problems and have a good antibacterial effect. Literature showed that these plants have extra amounts of essential oil and flavonoids. Piperine extracted from *Piper nigrum* was studied for antibacterial activity and its synergistic effect with Ciprofloxacin at a very low concentration (20 µg/mL) against *Escherichia coli* and *Bacillus subtilis* (Maitra, 2017). Acetone and ethanol extract of polyherbal formulation of *Trachyspermum ammi*: *Cinnamonum zeylanicum*: *Syzygium aromaticum* with the ratio of 1:1:1, 1:2:1, and 1:1:2, respectively showed enhanced activity as compared to individual plants in literature against *E. coli* and *P. mirabilis.* Phytochemical analysis confirms the presence of different secondary metabolites responsible for activity (Reji and Rajasekaran, 2015). A polyherbal formulation Laxisen found significantly effective ($p < 0.003$) for acute and chronic constipation is common symptom of gastrointestinal infections and badly affecting the quality of life (Sheikh et al., 2014). Polyherbal recipes that have not shown good antimicrobial effect asrecipe E showed no activity against *S. typhi* may have an inadequate quantity of active constituents that can kill or inhibit the bacterial population. The effectiveness of polyherbal or combination of plant extract with antibiotic may be due to modification or blocking of resistance

mechanism so that bacterium becomes sensitive to these antibacterial extract in lower concentrations. Synergistic action of plant extract with antibiotics is a potential approach to overcome bacterial resistance (Stefanović, 2018).

Crude methanolic extract of polyherbal mixture A, C, D and F showed statistically significant antifungal effect against *A. nigar, A. fumigatus* and *F. graminearum* compared to Fluconazole (22.33 mm). These polyherbal mixtures share common plants with different ratios might be the reason for the change in the inhibition zone. It is noted that polyherbal mixture consist of plants from Apiaceae and Zingiberaceae showed maximum inhibition zone which may be due to essential oil present in the extracts. Polyherbal mixture comprised of three plants *Azadirachta indica*, *Cichorium intybus*, and *Trigonella foenum graecum*; demonstrated synergistic broad spectrum antimicrobial potential for pathogenic bacterial and fungal strains (minimum inhibition concentration: 5–7 mg/ml) (Yadav et al., 2019). In literature, there is a lack of studies reported for these individual plants against selected fungal pathogens. There is less data reported for MIC and MBC against bacterial and fungal strains, that need to be tested.

5. Conclusions

The present study confirms the efficacy of polyherbal formulations used traditionally to treat different gastrointestinal infections. All the 14 selected polyherbal crude extracts showed potential antimicrobial activity; however, they vary in inhibition effect against the micro-organisms tested. Polyherbal recipes A, B, D, G, K, N, and H and A, C, D, F showed higher significant inhibition against tested bacterial and fungal pathogens, respectively. MIC ranges within 3.12–25 mg/ml while MBC between 12.5 and 100 mg/ml.

Foeniculum vulgare (Apiaceae) is used in most of the polyherbal formulations. Polyherbal formulations consist of Apiaceae species show good inhibition may be due to the presence of essential oil in the extract. Comparison with review table individual plants of selected polyherbal mixtures are not studied for MIC and MBC. There are scarce studies reported for these plants extracts against *Fusarium* species, *Shigella,* and *Proteus* species.

Antimicrobial activity in combination gives a synergistic and boosted inhibition against pathogenic bacteria and fungi and thus leading towards developing more potent drugs. Traditionally used polyherbal formulations provide new hope to solving the microbial resistance issue. So, there is a need to further evaluate these polyherbal mixtures for clinical, and in vivio trials against respective diseases. The study will also provide the basis for the isolation of bioactive synergistic compounds and drug discovery after toxicity evaluation.

Polyherbal recipes A, B and N highly used recipes for curing diarrhea at local level and were more potent against pathogenic bacteria that are highly involved in diarrhea. So, these recipes should be recommended for in vivo antidiarrheal activity.

Ethical statement

After taking the oral consent from traditional healers the study was approved by the advanced board of research studies KUST.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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