Antimicrobial screening of polyherbal formulations traditionally used against gastrointestinal diseases

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Original article

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...
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 crashed 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 to 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 Whatmann 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*, *Pseudomonas aeruginosa*, and *Salmonella typhi* using the agar well diffusion method. All the equipment was autoclaved at 121 °C 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 inhibition 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 niger*, *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 phytochemicals 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**/ | **Individual Plants in Polyherbal formulation** | **Habit** | **Part used** | **Disease name** |
| Abbreviation 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 | |
|                | Terminalia chebula Retz. Combretaceae | Kachoor | Herb | Rhi zone | |
| **(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 | |
|                | Cuminum cyminum L. Apiaceae | Zeera | Herb | Seeds | |
| **(Zeera sounf phakki) D** | Terminalia chebula Retz. Combretaceae | Hareer | Herb | Rhi zone | Intestinal problem |
|                | Withania coagulans (Stocks) Dunal. Solanaceae | Sounf | Herb | Seeds | |
| **(Hazna Phakki) E** | Piper nigrum L. Piperaceae | Kali mirch | Shrub | Buds/ | |
|                | Elettaria cardamomum (L.) Zingiberaceae | Choti ilai chee | Tree | Fruit | |
|                | Plantago ovate Forsk. Plantaginaceae | Isphaghol | Herb | Fruit, husk | |
| **(Dawai dard) F** | Foeniculum vulgare Mill. Apiaceae | Saunf | Herb | Fruit | Digestive problems like constipation, gastric pain, intestinal worms |
|                | Withania coagulans (Stocks) Dunal. Solanaceae | Sounf | Herb | Seeds | |
|                | Piper nigrum L. Piperaceae | Kali mirch | Herb | Fruit | |
|                | Elettaria cardamomum (L.) Zingiberaceae | Choti ilai chee | Tree | Fruit | |
| **(Podina Sharbat) G** | Syzygium aromaticum L. Myrtaceae | Lowng | Tree | Buds | |
|                | Cinnamomum verum J. Presl Lauraceae | Dar cheeni | Tree | Bark | |
|                | Mentha piperita L. Lamiaceae | Podina | Herb | Leaves | |
|                | Ruscus aculeatus 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 | Herb | Fruit | |
|                | Zingiber officinalis Ros. Zingiberaceae | Adrak/sund | Herb | Rhi zome | |
| **(Powder) J** | Syzygium cumini (L.) Skeels Myrtaceae | Jaman | Tree | Seeds | Typhoid, diarrhe a, and useful for diabetes patient |
|                | Punica granatum L. Lythraceae | Anar | Tree | Fruit cover | |
| **(Keero ki dawa) K** | Coccos nucfera L. Areaceae | Nareal | Tree | Fruit | Intestinal worms |
|                | Punica granatum L. Lythraceae | Anar | Tree | Seeds | |
| **(Arq) L** | Cassia fistula L. Fabaceae | Gardnali | Tree | Seeds cover | Constipation |
| **(Chaata) M** | Foeniculum vulgare Mill. Apiaceae | Sounf | Herb | Seeds | Constipation and bronchial problems |
|                | Butea monosperma (Lam.) Kuntze Fabaceae | Chichra | Shrub | Seeds | |
|                | Achyranthes aspera L. Amaranthaceae | Puhutkanda | Herb | Seeds | |
|                | Elettaria cardamomum (L.)Maton. Zingiberaceae | Shz illaichi | Herb | Fruit | |
| **(Haiza recipe) N** | Ocimum basilicum L. Lamiaceae | Niaz boi | Herb | Leaves/ seeds | Diarrhea, Cholera |
|                | Mentha piperita L. Lamiaceae | Podina | Herb | Leaves | |

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### 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 |
|                       | K. pneumoniae     | 0.25–1                | 11.2–20              | 1.024       | NA          |                      |
|                       | S. flexneri       | 0.25–1                | 10.1–13.8            |             |             |                      |
|                       | P. vulgaris       | 0.25–1                | 8.8–25               |             |             |                      |
|                       | P. aeruginosa     | 0.25–1                | 12–20                |             |             |                      |
|                       | S. aureus         | 0.25–25               | 1–13.7               |             |             |                      |
|                       | 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 |
|                       | E. coli           |                       |                      |             |             | (Maharjan et al., 2011) |
|                       | S. flexneri       |                       |                      |             |             |                      |
|                       | P. mirabilis      |                       |                      |             |             |                      |
|                       | P. vulgaris       |                       |                      |             |             |                      |
|                       | V. cholerae       |                       |                      |             |             |                      |
|                       | F. oxysporum      |                       |                      |             |             |                      |
|                       | A. fumigatus      |                       |                      |             |             |                      |
|                       | Trichoderma       |                       |                      |             |             |                      |
|                       | F. graminearum    |                       |                      |             |             |                      |
| **C. colocynthis Fruit** | A. nigar       | 15–100                | 5–23                 | 1–3.12      |             | 6.25                  |
|                       | A. fumigatus      | 15–100                | 6–19                 |             |             | 3.12                  |
|                       | E. coli           | 40–60                 | 3–16                 | 0.5–13.9    |             |                      |
|                       | P. mirabilis      | 0.3                   | 2–10                 | 1           | NA          |                      |
|                       | 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                    | NA          |             |                      |
|                       | V. cholerae       | NA                    |                      |             |             |                      |
|                       | F. oxysporum      | NA                    |                      |             |             |                      |
|                       | A. fumigatus      |                       |                      |             |             |                      |
|                       | Trichoderma       |                       |                      |             |             |                      |
|                       | F. graminearum    |                       |                      |             |             |                      |
| **C. cyminum Seeds**  | E. coli           | 0.5–250               | 2–31                 | 0.12–20     |             | 0.25–40              |
|                       | P. aeruginosa     | 2–33.33               | 10–25                | 0.25–6.25   |             | 0.25–60              |
|                       | S. typhi          | 0.5–250               | 8–35                 | 40          |             | 0.5–60               |
|                       | 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       | NA                    |                      |             |             |                      |
|                       | Rhizopus          |                       |                      |             |             |                      |
|                       | F. oxysporum      |                       |                      |             |             |                      |
|                       | Trichoderma       |                       |                      |             |             |                      |
|                       | F. graminearum    |                       |                      |             |             |                      |
| 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       | 100                   | 4.83–16              | 50          | NA          | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, á-D-Glucopyranoside, O-á-D-glucopyranosyl-(1,6-fwardw.3)-ß-D-fruc, d-Mannose, 5,7-Dodecadiyn-1,12-diol, 3-Trifluoroacetoxypentadecane, 3-Trifluoroacetoxypentadecane, 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) |
|                      | S. typhi       | 100                   | 4.15–25              | 3.12        | NA          |                                                                          |                                                                           |
|                      | S. aureus      | 25–100                | 5–18                 | 12.5        | NA          |                                                                          |                                                                           |
|                      | P. mirabilis   | 100                   | 5                    | NA          | NA          |                                                                          |                                                                           |
|                      | P. aeruginosa  | 100                   | 5.73                 | 12.5        | NA          |                                                                          |                                                                           |
|                      | K. pneumoniae  | 100                   | 5                    | NA          | NA          |                                                                          |                                                                           |
|                      | A. niger       | 100                   | 5.8                  | 12.5        | NA          |                                                                          |                                                                           |
|                      | F. oxysporum   | 100                   | 5                    | NA          | NA          |                                                                          |                                                                           |
|                      | A. fumigatus   | 100                   | 6                    | NA          | NA          |                                                                          |                                                                           |
|                      | Trichoderma    | 100                   | 5                    | NA          | NA          |                                                                          |                                                                           |
|                      | P. vulgaris    | NA                    |                      | NA          | NA          |                                                                          |                                                                           |
|                      | V. cholerae    | NA                    |                      | NA          | NA          |                                                                          |                                                                           |
| C. limon Fruit juice/ extract | E. coli       | 0.2–1                 | 11–19                | 0.5–50      | 0.1–1       | Alkaloids, flavonoids, phenols, quinines, terpenoids and carbohydrate, cyanogenetic, cardiac and steroidal glycosides, tannins, saponins, and water-soluble vitamins | (Singh et al., 2020b) |
|                      | S. aureus      | 0.2                   | 14–26                | 0.025–25    | 0.05        |                                                                          |                                                                           |
|                      | K. pneumoniae  | 0.2                   | 18–30                | 0.0035      | 0.05        |                                                                          |                                                                           |
|                      | S. typhi       | 0.1–1                 | 6–30                 | 0.025–12.5  | 0.05        |                                                                          |                                                                           |
|                      | P. aeruginosa  | 0.2                   | 10–19                | 0.0125      | 0.05        |                                                                          |                                                                           |
|                      | A. niger       | 0.2                   | 4–12                 | 0.05        | 0.1         |                                                                          |                                                                           |
|                      | P. vulgaris    | 0.2                   | 17–20                | 0.001–6.5   | NA          |                                                                          |                                                                           |
|                      | S. flexneri    | 100-1000 ug/disc      | 9–15                 | NA          | NA          |                                                                          |                                                                           |
|                      | V. cholerae    | NA                    |                      | NA          | NA          |                                                                          |                                                                           |
|                      | P. mirabilis   | RHizopus              |                      | NA          | NA          |                                                                          |                                                                           |
|                      | F. oxysporum   | A. fumigatus          | Trichoderma          | F. graminearum |            |                                                                          |                                                                           |
| C. nuicifera Fruit | E. coli       | 0.5–1.5               | 0–10                 | NA          | NA          | Phenols, flavonoids, alkaloids, tannins and saponins                  | (Chakraborty and Mitra, 2008; Igwe and Ugwumaja, 2016) |
|                      | S. aureus      | 0.5–1.5               | 8–15                 | NA          | NA          |                                                                          |                                                                           |
|                      | P. aeruginosa  | NA                    | 16                   | NA          | NA          |                                                                          |                                                                           |
|                      | A. niger       |                      | 12                   | NA          | NA          |                                                                          |                                                                           |
|                      | V. cholerae    |                      |                      | NA          | NA          |                                                                          |                                                                           |
|                      | S. flexneri    |                      |                      | NA          | NA          |                                                                          |                                                                           |
|                      | P. mirabilis   |                      |                      | NA          | NA          |                                                                          |                                                                           |
|                      | K. pneumoniae  |                      |                      | NA          | NA          |                                                                          |                                                                           |
|                      | P. vulgaris    |                      |                      | NA          | NA          |                                                                          |                                                                           |
|                      | F. oxysporum   | A. fumigatus          | Trichoderma          | F. graminearum |            |                                                                          |                                                                           |
| C. sinensis leaves | P. aeruginosa  | 0.1–200               | 10–19                | 5           | >5          | 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) |
|                      | E. coli       | 0.1–200               | 3.6–32               | 3.25–100    | >5          |                                                                          |                                                                           |
|                      | S. flexneri    | 3–48                  | 2–26                 | 2.5–12      | >5          |                                                                          |                                                                           |
|                      | S. typhi       | 3–48                  | 2.3–28.4             | 12          | 60          |                                                                          |                                                                           |
|                      | V. cholerae    | 3–48                  | 1.6–30.1             | 6           | NA          |                                                                          |                                                                           |
|                      | K. pneumoniae  | NA                    | 22                   | 0.512       | NA          |                                                                          |                                                                           |
|                      | P. mirabilis   | NA                    | 25                   | NA          | NA          |                                                                          |                                                                           |
|                      | A. niger       | NA                    | 5                    | NA          | NA          |                                                                          |                                                                           |
|                      | F. oxysporum   | A. fumigatus          | Trichoderma          | F. graminearum |            |                                                                          |                                                                           |
|                      | P. vulgaris    | NA                    |                      | NA          | NA          |                                                                          |                                                                           |
|                      | RHizopus       | Trichoderma           |                      | F. graminearum |            |                                                                          |                                                                           |
| C. zedoaria Rhizome | V. cholerae   | 0.5                   | 0                    | NA          | NA          |                                                                          | (Das and Rahman, 2012; Shahrir, 2010) |
|                      | E. coli       | 0.5–40                | 9–18                 | NA          | NA          |                                                                          |                                                                           |
|                      | S. typhi       | 0.5                   | 16                   | NA          | NA          |                                                                          |                                                                           |
|                      | P. aeruginosa  | 0.5–40                | 5–13                 | NA          | NA          |                                                                          |                                                                           |
|                      | K. pneumoniae  | 0.5                   | 0                    | NA          | NA          |                                                                          |                                                                           |
|                      | S. aureus      | 0.5–40                | 16                   | NA          | NA          |                                                                          |                                                                           |
|                      | A. niger       | 0.5–40                | 11–14                | NA          | NA          |                                                                          |                                                                           |
|                      | A. fumigatus   | 40                    | 12                   | NA          | NA          |                                                                          |                                                                           |
|                      | Trichoderma    | 40                    | 5                    | NA          | NA          |                                                                          |                                                                           |
|                      | S. flexneri    | NA                    |                      | NA          | NA          |                                                                          |                                                                           | (continued on next page)
| 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                    | 11–14                 | NA                   |             |             |                                     |                                                                          |
| P. aeruginosa       | 10–150                    | 9–14                  | NA                   |             |             |                                     |                                                                          |
| K. pneumoniae       | 1–2                       | 9–10                  | NA                   |             |             |                                     |                                                                          |
| A. niger            | 10                        | 13                    |                      |             |             |                                     |                                                                          |
| V. cholerae         |                           |                       |                      |             |             |                                     |                                                                          |
| 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          | 75          |                                     |                                                                          |
| P. aeruginosa       | 100                       | 8.5–16                | 49                   | 50          | 50          |                                     |                                                                          |
| K. pneumoniae       | 100                       | 14                    | 50                   |             |             |                                     |                                                                          |
| E. coli             | 50                        | 8.5–16.5              | NA                   |             |             |                                     |                                                                          |
| V. cholerae         |                           |                       |                      |             |             |                                     |                                                                          |
| S. flexneri         |                           |                       |                      |             |             |                                     |                                                                          |
| P. mirabilis        |                           |                       |                      |             |             |                                     |                                                                          |
| P. vulgaris         |                           |                       |                      |             |             |                                     |                                                                          |
| A. niger            |                           |                       |                      |             |             |                                     |                                                                          |
| 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. niger            | 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         |                           |                       |                      |             |             |                                     |                                                                          |
| P. mirabilis        |                           |                       |                      |             |             |                                     |                                                                          |
| P. vulgaris         |                           |                       |                      |             |             |                                     |                                                                          |
| S. typhi            |                           |                       |                      |             |             |                                     |                                                                          |
| Rhizopus            |                           |                       |                      |             |             |                                     |                                                                          |
| F. oxysporum        |                           |                       |                      |             |             |                                     |                                                                          |
| A. fumigatus        |                           |                       |                      |             |             |                                     |                                                                          |
| Trichoderma         |                           |                       |                      |             |             |                                     |                                                                          |
| F. graminearum      |                           |                       |                      |             |             |                                     |                                                                          |
| F. asafetida Root/Gum resine | S. aureus | 1–5                   | 5–20                 | 0.5         | NA          | Alkaloids, 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. niger            | 1–5                      | 14–17                 | 1                    |             |             |                                     |                                                                          |
| P. aeruginosa       | 1–5                      | 13.9                  | NA                   |             |             |                                     |                                                                          |
| S. flexneri         |                           |                       |                      |             |             |                                     |                                                                          |
| V. cholerae         |                           |                       |                      |             |             |                                     |                                                                          |
| P. mirabilis        |                           |                       |                      |             |             |                                     |                                                                          |
| P. vulgaris         |                           |                       |                      |             |             |                                     |                                                                          |
| S. typhi            |                           |                       |                      |             |             |                                     |                                                                          |
| Rhizopus            |                           |                       |                      |             |             |                                     |                                                                          |
| F. oxysporum        |                           |                       |                      |             |             |                                     |                                                                          |
| A. fumigatus        |                           |                       |                      |             |             |                                     |                                                                          |
| Trichoderma         |                           |                       |                      |             |             |                                     |                                                                          |
| F. graminearum      |                           |                       |                      |             |             |                                     |                                                                          |
| 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; Aliasothy, 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  | 0.015–0.5   | >33.33    |            |
|                      | S. typhi | 1–15                  | 11–25                |             |             |           |            |
|                      | P. aeruginosa | 0.4–100          | 1.33–14–19          |             |             |           |            |
|                      | 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. nigra  | NA                   | 15.6               | NA          |             |           |            |
|                      | S. flexneri | Rhizopus          |                     |             |             |           |            |
|                      | R. oxysporum | F. graminearum |                     |             |             |           |            |
|                      | 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. nigra  | 19                   |                     |             |             |           |            |
|                      | A. fumigatus | 15                |                     |             |             |           |            |
|                      | V. cholerae | NA                 |                     |             |             |           |            |
|                      | S. flexneri |                     |                     |             |             |           |            |
|                      | S. typhi  |                     |                     |             |             |           |            |
|                      | P. aeruginosa |                     |                     |             |             |           |            |
|                      | P. vulgaris |                     |                     |             |             |           |            |
|                      | K. pneumoniae |                     |                     |             |             |           |            |
|                      | A. nigra  | 1–6                  |                     |             |             |           |            |
|                      | R. oxysporum |                     |                     |             |             |           |            |
|                      | A. fumigatus |                     |                     |             |             |           |            |
|                      | S. flexneri |                     |                     |             |             |           |            |
|                      | P. vulgaris |                     |                     |             |             |           |            |
|                      | 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        | NA          | NA        |            |
|                      | 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                | 63–100%     |             |           |            |
|                      | A. nigra  | 1–6                  | 56–100%             |             |             |           |            |
|                      | R. oxysporum | 1–6               | 58–100%             |             |             |           |            |
|                      | A. fumigatus | 1–6              |                     |             |             |           |            |
|                      | S. flexneri |                     |                     |             |             |           |            |
|                      | P. vulgaris |                     |                     |             |             |           |            |
|                      | F. oxysporum |                     |                     |             |             |           |            |
|                      | Trichoderma |                     |                     |             |             |           |            |
|                      | F. graminearum |                     |                     |             |             |           |            |
| P. granatum Fruit cover | S. aureus | 4–100               | 7–29                | 0.1–50      | >10–60      | steroids, triterpenoids, alkaloids, flavonoids, saponins, tannins and carbohydrates, Anthraquinones | (Al-Zoreky, 2009; Ali, 2017; Daflham et al., 2010) (Abdollahzadeh et al., 2011; Keser 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; Shabazi; 2017; Ullah et al., 2012) |
|                      | E. coli  | 0.03–0.05            | 13–27               | 1–12.5      | >10–32      | NA        |            |
|                      | S. typhi | 14–24                | 1–13–37             | 25          | >10–32      | NA        |            |
|                      | P. aeruginosa | 1–100             | 8–26                | 12.5        | >10–32      | NA        |            |
|                      | K. pneumoniae | 0.125–1         | 7–18                | 2           | >10–32      | NA        |            |
|                      | P. mirabilis | 0.03–0.05        | 12–20               | NA          | >10–32      | NA        |            |
|                      | P. vulgaris | NA                 | 6                   | NA          | >10–32      | NA        |            |
|                      | S. flexneri | 16–25             | 12.5                |             | NA          |           |            |
|                      | A. nigra  | 7–23                | 13                  |             |             |           |            |
|                      | F. oxysporum | V. cholerae       |                     |             |             |           |            |
|                      | R. oxysporum |                     |                     |             |             |           |            |
|                      | A. fumigatus | Trichoderma      |                     |             |             |           |            |
|                      | F. graminearum |                     |                     |             |             |           |            |

(continued on next page)
| 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) |
| | *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* | 1–200 | 10-11.8 | | | | |
| | *S. typhi* | 1–200 | 10-11.8 | | | | |
| | *A. niger* | 1–200 | 10-10.9 | | | | |
| | *Rhizopus* | 1–200 | 10-10.9 | | | | |
| | *F. oxysporum* | 1–200 | 10-10.9 | | | | |
| | *A. fumigatus* | 1–200 | 10-10.9 | | | | |
| | *Trichoderma* | 1–200 | 10-10.9 | | | | |
| | *F. graminearum* | 1–200 | 10-10.9 | | | | |
| | *S. Mussarat, M. Adnan, S. Begum et al. Saudi Journal of Biological Sciences 28 (2021) 6829–6843* | | | | | | |
| *P. ovata* 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 | 20 | 9-18 | >200 | | |
| | *V. cholerae* | NA | | | | | |
| | *S. flexneri* | | | | | | |
| | *A. niger* | | | | | | |
| | *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* | NA | 10–17 | | | | |
| | *S. flexneri* | | | | | | |
| | *P. mirabilis* | | | | | | |
| | *P. vulgaris* | | | | | | |
| | *A. niger* | | | | | | |
| | *Rhizopus* | | | | | | |
| | *F. oxysporum* | | | | | | |
| | *A. fumigatus* | | | | | | |
| | *Trichoderma* | | | | | | |
| | *F. graminearum* | | | | | | |
| | *S. aromaticum* Buds | *V. cholerae* | NA | NA | 0.025 | NA | alkaid, terpenoids, flavonoids, steroid, saponin, Anthraquinones, and tannin, phenolic compounds | (Abd El Azim et al., 2014; Aneja and Joshi, 2010; Dua et al., 2014; Ghalam and Ahmad, 2014; Mehrotra and Srivastava, 2010; Okinen 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 | 10–10 | 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. niger* | NA | 4–14 | NA | | | |
| | *F. oxysporum* | NA | 9 | | | | |
| | *Trichoderma* | NA | 24 | | | | |
| | *P. mirabilis* | NA | | | | | |
| | *A. fumigatus* | NA | | | | | |
| | *Rhizopus* | NA | | | | | |
| | *F. graminearum* | NA | | | | | |
| 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 (Bagpai 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               | 25          |             |                     |
|                     | *K. pneumoniae* | 125–500              | 17–30               | 25          |             |                     |
|                     | *P. vulgaris* | 1.5                  | 23                  | NA          |             |                     |
|                     | *A. fumigatus* | NA                  | 15                  | NA          |             |                     |
|                     | *A. nigar*  | NA                    | 15                  | NA          |             |                     |
|                     | *Rhizopus*  | NA                    | 15                  | NA          |             |                     |
|                     | *F. graminearum* | NA               | 15                  | NA          |             |                     |
| **T. ammi Seeds**    | *E. coli*  | 10                    | 10–21               | 1–12.5      | 25          | NA (BASHYAL and GUHA, 2018; Hassan et al., 2016; Sharma et al., 2018; Sharma and Shrivastava; Shokrani et al., 2016) |
|                     | *S. aureus* | 10                    | 10–21               | 1–12.5      | 25          |                     |
|                     | *P. aeruginosa* | 0.025               | 8–21                | 1           | 50          |                     |
|                     | *A. nigar*  | NA                    | 15                  | NA          |             |                     |
|                     | *S. flexneri* | NA                  | 15                  | NA          |             |                     |
|                     | *P. vulgaris* | NA                  | 15                  | NA          |             |                     |
|                     | *P. mirabilis* | NA               | 15                  | NA          |             |                     |
|                     | *V. cholerae* | NA                  | 15                  | NA          |             |                     |
|                     | *K. pneumoniae* | NA               | 15                  | NA          |             |                     |
|                     | *Rhizopus*  | NA                    | 15                  | NA          |             |                     |
|                     | *F. graminearum* | NA               | 15                  | NA          |             |                     |
| **W. coagulans 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                | NA          |             |                     |
|                     | *P. vulgaris* | 50–250               | 0–16                | NA          |             |                     |
|                     | *S. typhi*  | 1–250                 | 7–16                | NA          |             |                     |
|                     | *P. aeruginosa* | 1–250               | 13–20               | NA          |             |                     |
|                     | *K. pneumoniae* | 1–250              | 10–22               | NA          |             |                     |
|                     | *S. aureus* | 1–250                 | 11–19               | NA          |             |                     |
|                     | *A. nigar*  | 50–250.0             | 7–11                | NA          |             |                     |
|                     | *A. fumigatus* | 0.025               | 29                  | NA          |             |                     |
|                     | *V. cholerae* | NA                  | 29                  | NA          |             |                     |
|                     | *P. mirabilis* | NA               | 29                  | NA          |             |                     |
|                     | *Rhizopus*  | NA                    | 29                  | NA          |             |                     |
|                     | *F. graminearum* | NA               | 29                  | NA          |             |                     |
| **Z. officinales Rhizome** | *E. coli*  | 0.025–80             | 2.9–22              | 3.5         | 40          | alkaid, phlobotannins, flavonoids, glycosides, sapoxins, 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; Kaustik and Goyal, 2011; Njobdi et al., 2018; Riaz et al., 2015; Sunilson et al., 2009; Ushimaru et al., 2007; Yadufashije et al., 2020; 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                | NA          |             |                     |
|                     | *S. flexneri* | 0.025–50.0         | 11                  | NA          |             |                     |
|                     | *K. pneumoniae* | 0.025–50.0         | 11–25               | NA          |             |                     |
|                     | *A. nigar*  | 3                     | 11–25               | NA          |             |                     |
|                     | *F. oxysporum* | 3                   | 11–25               | NA          |             |                     |
|                     | *V. cholerae* | NA                  | 11–25               | NA          |             |                     |
|                     | *P. vulgaris* | NA                  | 11–25               | NA          |             |                     |
|                     | *Rhizopus*  | NA                    | 11–25               | NA          |             |                     |
|                     | *A. fumigatus* | NA                 | 11–25               | NA          |             |                     |
|                     | *Trichoderma* | NA                 | 11–25               | NA          |             |                     |
|                     | *F. graminearum* | NA             | 11–25               | NA          |             |                     |
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).

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 |
| B          | 25.63 ± 3.5 | 19.33 ± 2   | 14 ± 3.6     | 15 ± 0      | 20.67 ± 1.1 | 13.33 ± 2.3  |
| C          | 13 ± 3.6    | 20.33 ± 0.5 | 12 ± 1       | 20.67 ± 1.1 | 21.33 ± 1.1 | 27.33 ± 1.5  |
| D          | 25.63 ± 0.5 | 18.67 ± 3.2 | 12 ± 1.7     | 21.33 ± 1.1 | 23.67 ± 1.1 | 19.67 ± 0.5  |
| E          | 16 ± 1.7    | 19.33 ± 1.1 | 13.67 ± 2.3  | 24.33 ± 1.1 | 38.33 ± 0.5 | 0             |
| F          | 12.67 ± 1.1 | 18.33 ± 0.5 | 10 ± 0       | 6.33 ± 5.5  | 15.67 ± 1.1 | 11.33 ± 1.1  |
| G          | 17 ± 3.5    | 23.33 ± 4.1 | 8 ± 1.7      | 24.33 ± 1.1 | 20.67 ± 1.1 | 14 ± 2        |
| H          | 25.33 ± 1   | 15.67 ± 4.0 | 4 ± 3.6      | 6.67 ± 5.7  | 25 ± 0     | 15.33 ± 3.5  |
| I          | 21 ± 1      | 22 ± 0      | 10.67 ± 1.1  | 15.33 ± 0.5 | 15.33 ± 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       |
| K          | 21 ± 1      | 18 ± 2      | 17 ± 1       | 24.33 ± 1.1 | 14.67 ± 0.5 | 12.33 ± 1.5  |
| L          | 16.33 ± 3.8 | 18.33 ± 0.5 | 13 ± 1.7     | 16.07 ± 1.1 | 15.33 ± 0.5 | 16.33 ± 1.5  |
| M          | 9.33 ± 2.5  | 15 ± 0      | 6.67 ± 5.7   | 14 ± 1.7    | 13 ± 1    | 16.67 ± 1.1  |
| N          | 15 ± 2      | 18.33 ± 1.1 | 11.67 ± 0.5  | 3 ± 6       | 17.67 ± 0.5 | 16.67 ± 2.8  |
| AB         | 22.67 ± 3.3 | 19.33 ± 1.5 | 21.33 ± 1.1  | 23.33 ± 1.1 | 20 ± 0    | 23.67 ± 1.1  |
| P value    | p < 0.001   | p < 0.001   | p < 0.001    | p < 0.001   | p < 0.001 | p < 0.001    |
| DMSO       | 0           | 0           | 0            | 0           | 0        | 0            |

p value for ANNOVA, AB = Antibiotics.

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

| Polyherbal crude extract | A. niger | Rhizopus | F. oxysporum | A. fumigatus | Trichoderma | F. graminearum |
|--------------------------|---------|---------|-------------|-------------|-------------|--------------|
| A                        | 24.67 ± 1.5 | 19.67 ± 4 | 17 ± 1.5 | 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 | 2 | 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                        | 23.33 ± 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 ± 3.3 | 1.1 | 14.55 | 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 | 25.63 ± 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 | 19.85 | 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

| 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-value | p < 0.5 | p < 0.01 | p < 0.001 | p < 0.001 | p < 0.001 | p < 0.001 |
|---------|---------|----------|----------|----------|----------|----------|

DMSO

| DMSO | 0 | 0 | 0 | 0 | 0 | 0 |

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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 the selected 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. aroumaticum, Zingiber officinalis, and T. ammi used to cure digestive ailments. Presence of 1, 8-cineole, α-terpinyl acetate, α-terpineol and sabine 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, and D respectively (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 μl/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 inhibition zone may be due to these diverse compounds.

Polyherbal recipe G was a mixture of Elettaria cardamomum, Syzygium aromaticum, Cinnaomonum 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. zylincum 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, Cinnaomonum 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, Trachyspernum 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 (Maira, 2017). Acetone and ethanol extract of polyherbal formulation of Trachyspernum ammi: Cinnaomonum 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 (Stefanovic, 2018).

Crude methanolic extract of polyherbal mixture A, C, D and F showed statistically significant antifungal effect against A. niger, 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, 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 vivo 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.

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