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Antibacterial activity of Sofoof polyherbal formulation against some gastrointestinal pathogens

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ABSTRACT

Introduction: The people of Forumad, a village in Semnan Province, Iran, have long used a polyherbal formulation called *Sofoof* to prevent and treat gastrointestinal disorders. This formulation comprises 11 medicinal plants: *Carum carvi*, *Cuminum cyminum*, *Pimpinella anisum*, *Anethum graveolens*, *Achillea millefolium*, *Glycyrrhiza glabra*, *Artemisia absinthium*, *Terminalia chebula*, *Peganum harmala*, *Ferula communis*, and *Trachyspermum ammi*. This study aimed to investigate the antibacterial activity of the *Sofoof* polyherbal formulation and its individual plant constituents against six species of gastrointestinal pathogenic bacteria. **Methods:** Extraction was performed using an infusion method with a solvent system of ethanol, 1-propanol, and water (1:1:1, *v*/*v*/*v*). Antibacterial activity was assessed using the agar well-diffusion assay, and the macrodilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). **Results:** Extracts of *T. chebula*, *P. harmala*, and *C. cyminum* showed significant antibacterial activity. At a concentration of 31.25 mg/mL, the inhibition zone diameters of *T. chebula* extract against *Escherichia coli*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Bacillus cereus* were 13, 10, 13, 12, 29, and 24.5 mm, respectively. The MICs for *T. chebula* against these bacteria ranged from 0.98 mg/mL (*Bacillus cereus*) to 31.25 mg/mL (*Shigella dysenteriae*). Notably, the extract of the *Sofoof* polyherbal formulation (comprising 11 plants) showed lower antibacterial activity than the individual extracts of *T. chebula* and *P. harmala*. **Conclusion:** This study validates the traditional use of the *Sofoof* polyherbal formulation and identifies *T. chebula*, *P. harmala*, and *C. cyminum* as its most active antibacterial constituents. Gram-positive bacteria demonstrated greater susceptibility to the extracts than Gram-negative bacteria. The lower activity of the *Sofoof* polyherbal formulation compared to certain individual plant extracts suggests possible antagonistic interactions, warranting further studies to elucidate constituent interactions within polyherbal formulations.

INTRODUCTION

In recent years, the use of medicinal plants from their native habitats has gained significant attention in medical science. These plants are considered environmentally sustainable and synthesize bioactive secondary metabolites, often in response to environmental stress, which contribute to their effectiveness in disease prevention and treatment.

The people of Forumad, a region in Semnan Province, Iran, have long used a polyherbal formulation called *Sofoof* (a traditional Persian term meaning 'powder') to prevent and treat gastrointestinal disorders. According to local traditional knowledge, this remedy has been passed down through generations and is used by local residents to prevent and treat many gastrointestinal disorders such as diarrhea, constipation, gastroesophageal reflux disease (GERD), bloating, abdominal pain, cramps, indigestion, nausea, and occasionally menstrual cramps and postpartum gastrointestinal complaints. Based on ethnographic observations, this polyherbal formulation is often suspended in water without heating to relieve

postpartum gastrointestinal discomfort. The resulting suspension is traditionally administered to infants, although safety data for this practice are lacking. The World Health Organization (WHO) estimates that approximately 80% of people in certain Asian and African countries rely on herbal remedies for at least part of their primary health care needs. Furthermore, WHO reports that about 21,000 plant species have potential medicinal applications [1].

The pharmaceutical industry has used natural products, particularly medicinal plants, as sources of therapeutic compounds for decades. Natural medicines are considered foundational to traditional medicine and, in certain situations, may represent the sole therapeutic option available [2]. For example, medicinal plants are widely used in the treatment of infectious diseases [3] and gastrointestinal disorders [4].

Antibiotic resistance has emerged as a global health issue since the late 20th century, with resistance rates of certain microorganisms to antibiotics exceeding 90% in some clinical settings. This challenge has heightened interest among researchers in developing safer and more potent antimicrobial agents to combat microbial resistance. Natural products represent a significant source of novel therapeutic agents, as they contain structurally diverse secondary metabolites with potential antimicrobial properties. Furthermore, antimicrobial compounds derived from plants exert antibacterial effects through mechanisms distinct from those of conventional antibiotics, which may be clinically important in the treatment of infections caused by antibiotic-resistant bacterial strains [5, 6]. Although natural products may offer favorable biodegradability and distinct pharmacological profiles, their safety must be evaluated individually.

Polyherbal formulations have been used for thousands of years in Ayurvedic and traditional Chinese medicine, as well as in ancient Greek medicine. Ayurveda, for instance, is an ancient traditional medical system originating in India that utilizes herbal formulations, dietary regimens, and detoxification therapies to restore physiological and psychological equilibrium. However, scientific evidence supporting their therapeutic benefits remains limited. Nevertheless, in these systems, various chronic diseases are believed to be more effectively managed with polyherbal formulations rather than monoherbal preparations, potentially due to synergistic effects and reduced adverse reactions. Several studies in pharmaceutical research have shown that, when compared to monotherapy, combination therapy involving herbal formulations and conventional drugs is effective in managing conditions such as diabetes and cancer [7, 8]. The biological activities of traditionally used polyherbal formulations have been the subject of relatively few studies, whereas the antimicrobial properties of individual herbs have received increasing attention in recent years.

Pathogenic microorganisms, including those causing food poisoning, are the main causes of gastrointestinal disorders. Among these, bacterial contamination is the primary cause of food poisoning and foodborne gastrointestinal infections. Diarrheal diseases caused an estimated 1.17–1.34 million deaths globally in 2021, with children under five years of age accounting for 30–40% of these fatalities [9]. Additionally, cholera-related deaths were estimated at 117,167 in 2019 [10]. These statistics underscore the urgent need for effective antimicrobial agents, including plant-based alternatives, to combat gastrointestinal pathogens.

The market for herbal supplements and traditional medicine systems is substantial and expanding rapidly worldwide, reflecting increased interest in natural therapeutics, including antimicrobial agents. The global herbal supplements market was valued at approximately $48 billion in 2023 and is projected to exceed $88 billion by 2032 [11]. Despite this growing interest, few studies have investigated the antimicrobial pharmacology of polyherbal formulations traditionally used for gastrointestinal infections, highlighting the need for research in this area.

The plants that comprise the *Sofoof* polyherbal formulation are collected from the mountainous and lowland areas of the Forumad region. After grinding, the plant materials are mixed in specific proportions (as detailed in Table 1) and consumed without further processing. The most important plants of this polyherbal formulation include *Carum carvi*, *Cuminum cyminum*, *Pimpinella anisum*, *Anethum graveolens*, *Achillea millefolium*, *Glycyrrhiza glabra*, *Artemisia absinthium*, *Terminalia chebula*, *Peganum harmala*, *Ferula communis*, and *Trachyspermum ammi*.

The aim of this study was to evaluate the antibacterial activity of the *Sofoof* polyherbal formulation and each of its individual plant constituents against six gastrointestinal pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Bacillus cereus*, *Enterococcus faecalis*, and *Salmonella enterica* serovar Typhimurium (hereafter *Salmonella typhimurium*).

MATERIAL AND METHODS

Study area and plant collection site. Plant materials were collected from the Forumad village, Meyami Village, Semnan Province, Iran (36°30′46″ N, 56°45′08″ E), which is characterized by a semi-arid climate.

Plant identification and collection. Field operations were carried out to identify the natural habitats of the target plant species. After collection, the plant specimens were authenticated at the Herbarium of Islamic Azad University, Gorgan Branch. The relevant plant parts (as specified in Table 1) were stored under controlled conditions (dark, dry environment at ambient temperature) and dried over 1 to 2 weeks. Plant materials

were spread in ventilated, shaded areas to avoid direct sunlight, which can degrade thermolabile compounds such as essential oils; this approach helped preserve color and volatile components. The dried plant materials were then ground using a mechanical grinder, and the resulting powder was used for extraction. In addition to preparing extracts from each of the plants that constitute the *Sofoof* polyherbal formulation, the complete *Sofoof* polyherbal formulation was also prepared by combining the plants in the proportions specified in Table 1.

Table 1. Constituent plants of the *Sofoof* polyherbal formulation, plant parts used, and their proportions in the formulation

Plant species	Part used	Proportion (% of total dry weight)
Carum carvi	Seed	10%
Cuminum cyminum	Seed	10%
Pimpinella anisum	Seed	10%
Anethum graveolens	Seed	10%
Achillea millefolium	Flower	15%
Glycyrrhiza glabra	Root	10%
Artemisia absinthium	Leaves and flowers	10%
Terminalia chebula	Fruit	5%
Peganum harmala	Seed	5%
Ferula communis	Leaves and stems	5%
Trachyspermum ammi	Seed	10%

Preparation of herbal extracts. Extracts were prepared using an infusion method with a solvent system of 96% ethanol, 1-propanol, and deionized water (1:1:1, *v/v/v*) [12]. Briefly, 10 g of powdered plant material was placed in muslin cloth bags and infused separately in 100 mL of the solvent mixture for 1 h at 70°C. This process was repeated once to ensure maximum extraction efficiency. After extraction, the solvent was removed using a rotary evaporator under reduced pressure. The concentrated extracts were stored at 4°C until testing. For the antibacterial assays, a stock concentration of 1000 mg/mL of each extract was prepared in dimethyl sulfoxide (DMSO), and serial two-fold dilutions were prepared in Nutrient Broth from this stock solution.

Bacterial strains. The standard bacterial strains were obtained in lyophilized form from the Iranian Research Organization for Science and Technology (IROST). These strains included three Gram-negative bacteria: *S. Typhimurium* (Persian Type Culture Collection [PTCC] 1709), *S. dysenteriae* (PTCC 1188), and *E. coli* (PTCC 1338), as well as three Gram-positive bacteria: *S. aureus* (PTCC 1112), *B. cereus* (PTCC 1154), and *E. faecalis* (PTCC 1778). The bacterial strains were revived in brain heart infusion (BHI) broth (Merck) at 37°C for 24 h and were verified using routine microbiological laboratory tests (including selective media culture, Gram staining, catalase and oxidase tests, and biochemical tests). Subsequently, multiple isolated colonies of each bacterial strain from the 24 h culture were inoculated into Nutrient Broth (Merck) and incubated at 37°C for 1 to 2 h until reaching a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) [13-16].

Agar well-diffusion antimicrobial assay. Using a sterile swab, the 0.5 McFarland suspension of each bacterial strain was uniformly spread onto Mueller-Hinton Agar (MHA) (Merck, Germany), and then 6-mm-diameter wells were punched into the inoculated agar plates using a cork borer. Each well was filled with prepared plant extracts ranging from 0.98 to 250 mg/mL, and the plates were then incubated for 24–48 h at 37°C. DMSO (at the highest concentration used in test wells) served as a negative control.

Following incubation, inhibition zone diameters were measured in mm [13-16]. An inhibition zone diameter of <7 mm was considered indicative of resistance, 7–9 mm as intermediate (relatively resistant), 10–12 mm as intermediate (relatively susceptible), and >12 mm as susceptible [17].

Determination of MIC and MBC. To determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of plant extracts, the method described by CLSI (2018) was used with minor modifications. For MIC determination, the macrodilution method with visual turbidity assessment was employed. Serial dilutions of each extract were prepared in Nutrient Broth, and each tube was inoculated with the 0.5 McFarland bacterial suspension to achieve a final concentration of 5×10^5 CFU/mL. Growth control tubes (bacterial suspension without extracts) and negative control tubes (extracts in Nutrient Broth without bacterial suspension) were included.

Visible turbidity was assessed following incubation at 37°C for 24 h. The MIC was defined as the lowest concentration at which no visible turbidity was detected. For MBC determination, tubes showing no turbidity (at the MIC and higher concentrations) were subcultured on MHA plates, and after 24 h of incubation at 37°C, the lowest concentration at which no colonies were observed, was recorded as the MBC [13, 15, 16].

Statistical analysis. Inhibition zone diameters and MIC/MBC values were analyzed using SAS software (Version 9.4 SAS Institute Inc., Cary, NC, USA) with a completely randomized design. All data were expressed as mean ± standard deviation (SD) (n = 3 replicates per treatment). Data were assessed for normality and homogeneity of variance prior to analysis. Mean comparisons were performed using Duncan's multiple

range test, with $P \leq 0.05$ considered statistically significant.

RESULTS

Agar well-diffusion antimicrobial assay results. The results demonstrated that the *Sofoof* polyherbal formulation and several of its constituent plant components exhibited significant antibacterial effects against the six tested bacterial species. Statistical analysis revealed significant differences among the antibacterial activities of the 11 individual plant extracts and the *Sofoof* polyherbal formulation against these bacteria ($P \leq 0.05$).

Among the components of the *Sofoof* polyherbal formulation, the extracts of *Terminalia chebula*, *Peganum harmala*, and *Cuminum cyminum* showed significant antibacterial activity. The mean inhibition zone diameters for the tested bacteria at various concentrations of these plant extracts are presented in Tables 2-4.

The mean inhibition zone diameters for *E. coli*, *S. typhimurium*, *E. faecalis*, *S. dysenteriae*, *S. aureus*, and *B. cereus* at a concentration of 31.25 mg/mL of *T. chebula* extract were 13, 10, 13, 12, 29, and 24.5 mm, respectively. Even at a concentration of 15.62 mg/mL, the extract exhibited antibacterial activity against all tested bacteria, although activity was weak against *S. dysenteriae*. The Gram-positive bacteria including *S. aureus* and *B. cereus* were the most susceptible, whereas the Gram-negative bacteria including *S. typhimurium* and *S. dysenteriae* were the most resistant to this extract (Table 2).

Table 2. Mean inhibition zone diameters of *Terminalia chebula* extract against tested bacteria (mm)

Plant species	Concentration (mg/mL)	Bacteria					
		E. coli	*S. typhimurium*	*E. faecalis*	*S. dysenteriae*	*S. aureus*	*B. cereus*
Terminalia chebula	250.00	26.5 ± 1.5	21.0 ± 0.5	22.5 ± 1.5	20.0 ± 2.0	31.0 ± 2.0	30.0 ± 0.5
	125.00	22.0 ± 1.0	17.5 ± 0.5	20.0 ± 1.0	20.0 ± 1.0	30.0 ± 1.5	28.5 ± 1.5
	62.50	16.0 ± 1.0	13.5 ± 1.0	19.0 ± 1.0	18.0 ± 1.5	24.5 ± 1.5	27.0 ± 1.0
	31.25	13.0 ± 1.0	10.0 ± 1.0	13.0 ± 0.5	12.0 ± 2.0	29.0 ± 1.0	24.5 ± 1.0
	15.62	11.5 ± 1.5	10.0 ± 2.0	12.0 ± 2.0	8.0 ± 1.0	22.5 ± 1.5	22.0 ± 1.0
	7.81	9.0 ± 1.0	—	—	—	16.0 ± 1.0	15.0 ± 1.0
	3.90	—	—	—	—	9.0 ± 1.0	13.0 ± 2.0
	1.95	—	—	—	—	—	10.0 ± 1.0
	0.97	—	—	—	—	—	—

Values are expressed as mean ± SD (n = 3). —, No inhibition zone detected.

The inhibition zone diameters for *E. coli*, *S. typhimurium*, *E. faecalis*, *S. dysenteriae*, *S. aureus*, and *B. cereus* at a concentration of 31.25 mg/mL of *Peganum harmala* extract were 12, 15, 15, 14, 14, and 16 mm, respectively. The *P. harmala* extract, at a concentration of 15.62 mg/mL, showed antibacterial activity against all tested bacteria. Based on the overall mean values across all concentrations, the most susceptible bacteria to *P. harmala* extract were *B. cereus* and *S. typhimurium*. Additionally, *S. aureus* was the most resistant bacterium to this extract, while *E. faecalis* showed intermediate susceptibility (Table 3).

Table 3. Mean inhibition zone diameters of *Peganum harmala* extract against tested bacteria (mm)

Plant species	Concentration (mg/mL)	Bacteria					
		E. coli	*S. typhimurium*	*E. faecalis*	*S. dysenteriae*	*S. aureus*	*B. cereus*
Peganum harmala	250	24.0 ± 2.0	27.0 ± 1.0	15.0 ± 0.5	21.0 ± 1.0	20.5 ± 1.5	21.0 ± 1.0
	125	21.0 ± 2.0	24.0 ± 1.0	14.0 ± 2.0	22.0 ± 1.0	19.0 ± 1.0	21.5 ± 1.5
	62.5	18.0 ± 1.5	18.0 ± 3.0	14.0 ± 2.0	18.0 ± 2.0	16.0 ± 2.0	20.0 ± 2.0
	31.25	12.0 ± 1.0	15.0 ± 1.5	15.0 ± 2.0	14.0 ± 1.5	14.0 ± 1.0	16.0 ± 2.0
	15.62	9.0 ± 1.0	14.0 ± 1.0	10.0 ± 1.5	10.0 ± 0.5	7.0 ± 0.5	12.0 ± 2.0
	7.81	7.0 ± 0.5	8.0 ± 1.0	8.0 ± 1.0	9.0 ± 1.5	—	8.0 ± 0.5

Values are expressed as mean ± SD (n = 3). —, No inhibition zone detected.

The extract of *Cuminum cyminum* at a concentration of 31.25 mg/mL showed antibacterial activity against all tested bacteria. The mean inhibition zone diameters for *E. coli*, *S. typhimurium*, *E. faecalis*, *S. dysenteriae*, *S. aureus*, and *B. cereus* at a concentration of 31.25 mg/mL of *C. cyminum* extract were 9, 13, 10, 9, 15, and 16 mm, respectively. *B. cereus* (Gram-positive) was the most susceptible to the *C. cyminum* extract, while *S. dysenteriae* (Gram-negative) was the most resistant bacteria to this extract (Table 4).

Notably, the extract of the *Sofoof* polyherbal formulation showed lower antibacterial activity than the extracts of *T. chebula*, *P. harmala*, and *C. cyminum*. The Sofoof polyherbal formulation at a concentration of 62.5 mg/mL showed antibacterial activity against all tested bacteria. The mean inhibition zone diameters for *E. coli*, *S. typhimurium*, *E. faecalis*, *S. dysenteriae*, *S. aureus*, and *B. cereus* at a concentration of 62.5 mg/mL of the *Sofoof* polyherbal formulation extract were 12.5, 13, 14, 13, 10, and 13 mm, respectively (Table 5). Based on overall mean values across all concentrations, *E. faecalis*

and *B. cereus* (Gram-positive bacteria) were the most susceptible, whereas *E. coli* and *S. dysenteriae* (Gram-negative bacteria) were the most resistant to the *Sofoof* polyherbal formulation extract.

Table 4. Mean inhibition zone diameters of *Cuminum cyminum* extract against tested bacteria (mm)

Plant species	Concentration (mg/mL)	Bacteria					
		E. coli	*S. typhimurium*	*E. faecalis*	*S. dysenteriae*	*S. aureus*	*B. cereus*
Cuminum cyminum	250	14.0 ± 2.0	22.0 ± 1.5	17.0 ± 1.0	14.0 ± 2.0	18.5 ± 1.5	21.5 ± 1.5
	125	13.5 ± 0.5	20.0 ± 1.0	15.0 ± 1.5	10.5 ± 1.5	17.0 ± 2.0	19.5 ± 1.5
	62.5	13.0 ± 2.0	18.5 ± 1.0	12.0 ± 2.0	11.0 ± 1.0	14.0 ± 3.0	18.0 ± 0.5
	31.25	9.0 ± 0.5	13.0 ± 1.0	10.0 ± 2.0	9.0 ± 1.0	15.0 ± 2.0	16.0 ± 1.0
	15.62	—	8.0 ± 1.0	9.0 ± 1.0	8.0 ± 1.0	9.0 ± 1.0	12.0 ± 1.0
	7.81	—	—	—	—	—	8.0 ± 1.0

Values are expressed as mean ± SD (n = 3). —, No inhibition zone detected.

Table 5. Mean inhibition zone diameters of *Sofoof* polyherbal formulation extract against tested bacteria (mm)

Extract source	Concentration (mg/mL)	Bacteria					
		E. coli	*S. typhimurium*	*E. faecalis*	*S. dysenteriae*	*S. aureus*	*B. cereus*
***Sofoof* polyherbal formulation**	250	15.5 ± 1.0	18.0 ± 1.0	17.0 ± 2.0	13.5 ± 0.5	21.0 ± 0.5	23.5 ± 1.5
	125	15.0 ± 1.0	17.0 ± 0.5	16.0 ± 1.0	13.0 ± 1.0	12.5 ± 1.0	15.0 ± 3.0
	62.5	12.5 ± 0.5	13.0 ± 2.0	14.0 ± 0.5	13.0 ± 1.0	10.0 ± 1.0	13.0 ± 2.0
	31.25	—	9.0 ± 0.5	11.0 ± 0.5	8.0 ± 0.5	8.0 ± 1.0	7.0 ± 0.5
	15.62	—	—	—	—	—	—

Values are expressed as mean ± SD (n = 3). —, No inhibition zone detected.

Table 6. MIC and MBC values of plant extracts (mg/mL)

Plant species	Parameter	Bacteria					
		E. coli	*S. typhimurium*	*E. faecalis*	*S. dysenteriae*	*S. aureus*	*B. cereus*
Terminalia chebula	MIC	15.62	15.62	15.62	31.25	1.95	3.90
	MBC	15.62	15.62	15.62	31.25	3.90	7.81
Peganum harmala	MIC	7.81	15.62	7.81	7.81	15.62	7.81
	MBC	15.62	15.62	15.62	15.62	15.62	15.62
Achillea millefolium	MIC	62.5	62.5	62.5	62.5	62.5	62.5
	MBC	125	62.5	125	62.5	125	62.5
Sofoof polyherbal	MIC	62.5	31.25	15.62	62.5	62.5	31.25
	MBC	62.5	31.25	31.25	62.5	62.5	31.25
Cuminum cyminum	MIC	15.62	31.25	15.62	15.62	15.62	7.81
	MBC	31.25	31.25	31.25	31.25	15.62	7.81
Glycyrrhiza glabra	MIC	62.5	62.5	31.25	62.5	31.25	15.62
	MBC	62.5	62.5	125	125	62.5	31.25
Pimpinella anisum	MIC	62.5	62.5	250	250	62.5	62.5
	MBC	125	250	500	500	62.5	62.5
Trachyspermum ammi	MIC	250	250	62.5	62.5	62.5	31.25
	MBC	500	500	125	500	125	125
Ferula communis	MIC	62.5	62.5	125	62.5	62.5	31.25
	MBC	250	125	250	500	125	125
Carum carvi	MIC	125	250	62.5	62.5	31.25	31.25
	MBC	125	250	125	125	125	125
Artemisia absinthium	MIC	62.5	62.5	125	62.5	31.25	31.25
	MBC	125	250	125	250	31.25	31.25
Anethum graveolens	MIC	62.5	62.5	62.5	62.5	62.5	31.25
	MBC	125	125	125	125	125	125

Values are expressed in mg/mL. MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

The *Sofoof* polyherbal formulation exhibited detectable antibacterial activity against all tested strains. Overall, assessment of antibacterial activity across all plant extracts indicated that Gram-positive bacteria such as *S. aureus* and *B. cereus* were more susceptible than Gram-negative bacteria, specifically *E. coli*, *S. dysenteriae*, and *S. typhimurium*.

MIC and MBC determination results. The results align with those of the agar well-diffusion assay, indicating greater antibacterial activity of *T. chebula* and *P. harmala* compared to others. The MICs of *T. chebula* extract against *E. coli*, *S. typhimurium*, *E. faecalis*, *S. dysenteriae*, *S. aureus*, and *B. cereus* were 15.62, 15.62, 15.62, 31.25, 1.95, and 3.90 mg/mL, respectively, and for *P. harmala* extract, 7.81, 15.62, 7.81, 7.81, 15.62, and 7.81 mg/mL, respectively. Similarly, the *Sofoof* polyherbal formulation, as observed in the agar well-diffusion assay, showed lower antibacterial activity than these two plant extracts. The MICs of the *Sofoof* polyherbal formulation against these bacteria were 62.5, 31.25, 15.62, 62.5, 62.5, and 31.25 mg/mL, respectively (Table 6).

Consistent with the results of the agar well-diffusion assay, greater susceptibility of Gram-positive bacteria

was observed. The MIC and MBC of *T. chebula* extract against *B. cereus* were 3.90 and 7.81 mg/mL, respectively, indicating the high susceptibility of *B. cereus* as a Gram-positive bacterium, compared to other bacteria studied, to the *T. chebula* extract. Furthermore, the MIC and MBC of *T. chebula* extract against *S. dysenteriae* were both 31.25 mg/mL, indicating that this Gram-negative bacterium is the most resistant to this extract (Table 6).

DISCUSSION

Traditional polyherbal formulations derived from native plants have been used for controlling pathogenic bacteria and treating diseases; this provided the rationale for investigating the antibacterial activity of the *Sofoof* polyherbal formulation in this study. The findings indicated that the *Sofoof* polyherbal formulation and several of its constituent plant components exhibited significant antibacterial activity against the tested bacteria.

Similarly, Mussarat *et al*. (2021) investigated the antimicrobial potential of polyherbal formulations prepared from 25 traditional plant species in Pakistan against selected gastrointestinal pathogens. The authors combined these 25 plant species in different proportions and prepared 14 different polyherbal formulations, and evaluated the antibacterial and antifungal activity at a concentration of 50 mg/mL using methanolic extracts of these formulations with the agar well-diffusion assay. Additionally, they determined the MIC and MBC. In that study, the antimicrobial activity of these 14 traditional polyherbal formulations was confirmed. Among them, the polyherbal formulation consisting of *Terminalia chebula*, *Cuminum cyminum*, *Withania coagulans*, *Foeniculum vulgare*, and *Curcuma zedoaria* (locally known as 'Phakki'); the formulation consisting of *Mentha piperita*, *Camellia sinensis*, and *Elettaria cardamomum* (locally known as 'Podeena qehwa'); and the formulation consisting of *Ocimum basilicum* and *Mentha piperita* (locally known as 'Haiza recipe') exhibited significant activity against diarrhea-causing microorganisms. The MICs of these traditional polyherbal formulations were reported to be in the range of 3.12–25 mg/mL, and their MBCs were 12.5–100 mg/mL [7]. These findings align with our results, where polyherbal formulations showed activity but individual plant extracts such as *T. chebula* were more potent.

In this study, greater susceptibility was noted in Gram-positive bacteria such as *B. cereus* and *S. aureus*, whereas Gram-negative bacteria such as *E. coli*, *S. dysenteriae*, and *S. typhimurium* showed greater resistance to plant extracts. The composition and structure of the bacterial cell envelope (including the cell wall) are related to susceptibility and resistance to plant extracts. Gram-positive bacteria have a thick multilayered peptidoglycan wall, whereas Gram-negative bacteria have a thinner peptidoglycan layer plus an outer membrane. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, absent in Gram-positive bacteria, which instead contain teichoic and lipoteichoic acids. This outer membrane serves as a barrier, reducing the penetration of antimicrobial compounds, including plant extracts, essential oils, and antibiotics, compared to the more permeable cell wall of Gram-positive bacteria. Additionally, various degradative enzymes and compounds in the periplasmic space (located between the inner and outer membranes of Gram-negative bacteria) can degrade or inactivate the active compounds in the extracts [18-20].

As noted above, among the plant extracts from the *Sofoof* polyherbal formulation, the antibacterial activity of *Terminalia chebula* and *Cuminum cyminum* was notably high, even exceeding that of the *Sofoof* polyherbal formulation itself. In the study by Mussarat *et al*. (2021), a polyherbal formulation locally known as 'Phakki' containing *T. chebula* and *C. cyminum* was reported to exhibit strong antibacterial activity [7]. Similarly, this polyherbal formulation is used in Pakistan as a powdered preparation for gastrointestinal disorders, including diarrhea.

Among the components of the *Sofoof* polyherbal formulation, *T. chebula* extract exhibited the highest antibacterial activity, surpassing both the other plant extracts and the complete formulation, as evidenced by the inhibition zone diameters and MIC values presented above. The antibacterial activity of *T. chebula* has been documented in previous studies [21-26].

Terminalia chebula exhibits a diverse phytochemical profile, characterized by numerous bioactive compounds that contribute to its medicinal properties. The main constituents identified primarily in the fruit of *T. chebula* include phenolics (including tannins and flavonoids), alkaloids, saponins, and glycosides, which confer its antioxidant, antibacterial, and anti-inflammatory activities [21, 26, 27].

The fruit is rich in phenolics, with at least 28 phenolic acids and 60 tannins among the 149 chemical compounds identified. *T. chebula* fruits contain particularly high concentrations of tannins, ranging from approximately 30–40% in mature fruits, although total tannin content varies significantly among genotypes. These tannins are primarily hydrolyzable. Flavonoids are present in multiple plant parts, including fruits and leaves, contributing to its antioxidant activity. Six flavonoids have been identified in the fruit, with notable concentrations detected in methanolic extracts [27, 28].

Alkaloids are detected in fruit extracts, contributing to its pharmacological potential. Saponins and glycosides are present in various extracts of the fruit and other plant parts, with the latter contributing to the plant's bioactivity. *T. chebula* fruit also contains cardiac glycosides, which have medicinal applications in treating heart failure and cardiac arrhythmias. Anthraquinones

are present as well, contributing to its biological activity. The fruit contains steroids and approximately 20 triterpenoids, contributing to its complex phytochemical profile [27–29]. Additionally, the fruit contains fatty acids (including palmitic, oleic, and linoleic acids), carbohydrates, proteins, and minerals (including iron, zinc, manganese, copper, calcium, magnesium, and potassium). Phytochemical content varies significantly among genotypes due to environmental and genetic factors, highlighting the need for standardization in medicinal use [27-29].

Khan *et al*. (2022) reported a higher abundance of phenolic compounds relative to flavonoids in *Terminalia chebula* extract and, consistent with the present study, demonstrated antibacterial activity of *T. chebula* extract against *E. coli*, *Bacillus* sp., and *Staphylococcus* sp. [30]. The MICs of this ethanolic extract against *E. coli*, *Bacillus* sp., and *Staphylococcus* sp. were reported to be 10, 2.5, and 5 mg/mL, respectively, while in this study, the values were 15.62, 1.95, and 3.90 mg/mL for *E. coli*, *S. aureus*, and *B. cereus*, respectively. These differences may be attributed to environmental and geographical factors, as well as to abiotic stresses at the site of plant collection, which significantly affect the production of secondary metabolites responsible for antibacterial activity. Other influential factors include differences in extraction methods and solvent types, which may also contribute to these variations [27-29].

Like *T. chebula* extract, the extract of *Peganum harmala* also exhibited significant antibacterial activity, even exceeding that of the *Sofoof* polyherbal formulation. Our data indicated that the mean inhibition zone diameters for *E. coli*, *S. typhimurium*, *E. faecalis*, *S. dysenteriae*, *S. aureus*, and *B. cereus* at a concentration of 31.25 mg/mL of *P. harmala* extract were 12, 15, 15, 14, 14, and 16 mm, respectively, and the MICs for *P. harmala* extract were 7.81, 15.62, 7.81, 7.81, 15.62, and 7.81 mg/mL, respectively. Abdulridha *et al*. (2019) reported that alkaloids were the most abundant compounds identified, followed by tannins, saponins, glycosides, terpenoids, steroids, and anthraquinones in the ethanolic extract of *Peganum harmala*. However, flavonoids, coumarin, and resin were not detected. Antibacterial activity of the ethanolic extract of *P. harmala* was reported against isolates of *Staphylococcus*, *Streptococcus*, *E. coli*, and *Acinetobacter*. However, two Gram-negative bacteria, *Aeromonas* and *Klebsiella*, were resistant to the extract. Alkaloids were found in high concentrations, and the authors attributed the antibacterial activity to this high alkaloid content [31].

Nenaah (2010) also reported that the antibacterial activity of ethanolic extract of *Peganum harmala* could be related to the presence of alkaloids such as harmine and harmaline [32]. Similarly, Wang *et al*. (2022) reported harmine and harmaline alkaloids as the most abundant compounds in *Peganum harmala*, and demonstrated that the antimicrobial activity of *P. harmala* extract against 12 bacterial and fungal strains, including *E. coli* and *S. aureus*, was attributable to these compounds. The authors suggested that, given the antimicrobial properties of these alkaloids, they could serve as leads for developing novel antimicrobial agents [33].

The extract of *Cuminum cyminum* at a concentration of 31.25 mg/mL showed antibacterial activity against all tested bacteria, with greater activity observed against Gram-positive bacteria. At this concentration, mean inhibition zone diameters ranged from 9–16 mm, with the highest values for *S. aureus* (15 mm) and *B. cereus* (16 mm). Other studies have also documented the antibacterial properties of *C. cyminum* [34-38]. Several bioactive compounds have been identified in *Cuminum cyminum* extract, including gallic acid, tannins, coumarins, terpenoids, monoterpenes, and cuminaldehyde. The antimicrobial activity of the *C. cyminum* extract may be attributable to the presence and concentration of these compounds [7, 35].

Similarly, Johri (2011) showed the antibacterial effect of cumin aqueous extract against Gram-positive and Gram-negative bacteria and attributed it to compounds such as carvone, limonene, linalool, and carvacrol present in *Cuminum cyminum* [39].

The use of polyherbal formulations in clinical treatments is not limited to the present study, and has been documented in numerous studies. Similar to the findings of the present study, where the polyherbal formulation showed lower antibacterial activity compared to some individual extracts, other studies have also reported synergistic, additive, and even antagonistic effects. In the study of Mussarat *et al*. (2021), the evaluation of selected polyherbal formulations showed synergistic, antagonistic, and additive interactions [7]. Mundy *et al*. (2016) conducted a review of five years of studies on antimicrobial activities and plant synergy and concluded that synergy, both in herbal extracts and between plants and antibiotics, can enhance the antimicrobial effect [40].

Yadav *et al.* (2019) evaluated the antimicrobial activity of *Azadirachta indica*, *Cichorium intybus*, and *Trigonella foenum-graecum* against bacterial and fungal pathogens responsible for vaginal infections using the agar well-diffusion method and demonstrated the antimicrobial efficacy of this polyherbal formulation [41]. Bhinge *et al.* (2017) examined the antimicrobial activity of two polyherbal formulations containing *Azadirachta indica*, *Curcuma longa*, *Allium sativum*, *Ocimum sanctum*, *Cinnamomum zeylanicum*, and *Tamarindus indica* against *S. aureus*, *E. coli*, *Bacillus subtilis*, and *Aspergillus niger* using the agar well-diffusion method and reported greater antimicrobial activity of polyherbal formulations compared to individual plant extracts [42].

Another example is a multi-herbal formula that is significantly effective for both acute and chronic constipation, which is a common symptom of gastrointestinal infections and has a serious negative impact on one's quality of life [43]. Adwan *et al*. (2010) also reported the synergistic effect of polyherbal formulations and the reduction of MIC values against bacterial pathogens [44]. However, polyherbal formulations do not always result in positive effects against microorganisms. For example, one of the Mussarat *et al*. (2021) polyherbal formulations consisting of *Withania coagulans*, *Piper nigrum*, *Trachyspermum ammi*, *Cuminum cyminum*, and *Foeniculum vulgare* (locally known as Hazma Phakki) failed to inhibit the growth of *Salmonella typhi* [7]. This lack of efficacy in some polyherbal formulations could be due to insufficient concentrations of active compounds to inhibit bacterial growth or exert bactericidal effects. Conversely, modification or inhibition of bacterial resistance mechanisms may enhance susceptibility to antibacterial extracts at lower concentrations, potentially explaining the effectiveness observed when polyherbal formulations or herbal extracts are combined with antibiotics. Indeed, combining herbal extracts with antibiotics has been proposed as a promising strategy to combat bacterial resistance [45].

Currently, there is growing interest within the global health community in developing therapeutics derived from natural sources, including plant-based antimicrobial agents. Therefore, conducting basic and applied phytochemical research to identify, extract, and characterize bioactive compounds from native medicinal plants with traditional therapeutic uses is essential. Such efforts should aim to optimize extraction methods and maximize the yield of bioactive secondary metabolites, ultimately facilitating the development of effective and safer plants-derived therapeutics. The findings of the present study showed both consistencies and discrepancies with previously published studies, as discussed above. These differences can be attributed to numerous factors, including environmental variables such as the time and place of plant collection, climate, and geographical conditions, as well as methodological variables such as solvent type and extraction method.

In conclusion, among the plants that make up the *Sofoof* polyherbal formulation, extracts of *Terminalia chebula*, *Peganum harmala*, and *Cuminum cyminum* showed greater antibacterial activity than other extracts and even the Sofoof polyherbal formulation itself. Additionally, Gram-positive bacteria demonstrated greater susceptibility to the plant extracts than did Gram-negative bacteria. Polyherbal formulations may represent promising candidates for future research aimed at addressing antibiotic resistance, and the use of this polyherbal formulation and its individual components, especially *Terminalia chebula*, *Peganum harmala*, and *Cuminum cyminum*, may hold therapeutic potential for the treatment of gastrointestinal diseases. Furthermore, this study provides a foundation for further investigation, including identification of bioactive compounds, *in vivo* experiments using animal models, and evaluation of plant fractions against a broader range of bacterial species. Isolation and characterization of active compounds may ultimately contribute to the development of novel plant-derived antimicrobial agents. However, this study is limited to *in vitro* testing against six bacterial strains and does not include *in vivo* evaluations or phytochemical fractionation, which should be addressed in future research. Furthermore, the study utilized a single solvent system for extraction; alternative extraction methods could yield extracts with different phytochemical profiles and potentially different biological activities. Additionally, phytochemical characterization of the extracts would aid in identifying the bioactive compounds responsible for the observed antibacterial activity. The lack of cytotoxicity testing, antibiotic controls, and synergy and antagonism assays is also a limitation of this study.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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AI DISCLOSURE

The authors did not use artificial intelligence (AI) to write this manuscript.

AUTHOR CONTRIBUTIONS

This research is derived from the thesis work of Mohammad Javad Nosrati, who performed the experiments under the supervision of Dr. Hadi Koohsari and supervised the statistical analysis and manuscript preparation. Dr. Morteza Noryan was the advisor for this thesis. MJN: Data curation; Funding acquisition; Investigation. HK: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing – original draft;

Writing – review and editing. MN: Software; Writing – review and editing.

DATA AVAILABILITY

The raw data (including zone diameters and MIC replicates) that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study involved *in vitro* experiments with standard strains exclusively and did not involve any research on animals or human subjects. Therefore, formal ethical approval from an institutional review board (IRB) or ethics committee was not required for this specific study. The original thesis from which this manuscript is derived included a statement confirming adherence to ethical guidelines for research not involving human or animal subjects. Furthermore, as a procedural requirement for thesis registration and archiving in Iran, an ethics compliance report pertaining to the original thesis has been submitted to IranDoc (in Persian)**,** as documented in the supplementary materials.

REFERENCES

1. Oyebode O, Kandala NB, Chilton PJ, Lilford RJ. Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan. 2016; 31 (8): 984-91.

2. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001; 109 (Suppl 1): 69-75.

3. Ngezahayo J, Havyarimana F, Hari L, Stévigny C, Duez P. Medicinal plants used by Burundian traditional healers for the treatment of microbial diseases. J Ethnopharmacol. 2015; 173: 338-51.

4. Khayyal MT, el-Ghazaly MA, Kenawy SA, Seif-el-Nasr M, Mahran LG, Kafafi YA, et al. Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination. Arzneimittelforschung. 2001; 51 (7): 545-53.

5. Mabona U, Viljoen A, Shikanga E, Marston A, Van Vuuren S. Antimicrobial activity of southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound. J Ethnopharmacol. 2013; 148 (1): 45-55.

6. Neu HC. The crisis in antibiotic resistance. Science. 1992; 257 (5073): 1064-73.

7. Mussarat S, Adnan M, Begum S, Rehman SU, Hashem A, Abd Allah EF. Antimicrobial screening of polyherbal formulations traditionally used against gastrointestinal diseases. Saudi J Biol Sci. 2021; 28 (12): 6829-43.

8. Patel SM, Saravolatz LD. Monotherapy versus combination therapy. Med Clin North Am. 2006; 90 (6): 1183-95.

9. Zhang Y, Zhang Y, Chen Z, Jia Z, Yu Y, Wang J, et al. Global burden, subtype, risk factors and etiological analysis of enteric infections from 1990-2021: population based study. Front Cell Infect Microbiol. 2025; 15: 1527765.

10. Ilic I, Ilic M. Global patterns of trends in cholera mortality. Trop Med Infect Dis. 2023; 8 (3): 169.

11. Market Research Future. Global herbal supplements market overview 2024 [Internet]. Pune (India): Market Research Future; 2024 [cited 2024 Jul 30]. Available from: https://www.marketresearchfuture.com/reports/herbal-supplements-market-3231

12. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015; 4 (3): 1000196.

13. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

14. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar well diffusion method. Acta Biol Med Exp. 1990; 5 (1): 113-5.

15. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: a review. J Pharm Anal. 2016; 6 (2): 71-9.

16. Hossain TJ. Methods for screening and evaluation of antimicrobial activity: a review of protocols, advantages, and limitations. Eur J Microbiol Immunol. 2024; 14 (2): 97-115.

17. Nostro A, Germano MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 2000; 30 (5): 379-84.

18. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015; 13 (1): 42-51.

19. Semeniuc CA, Pop CR, Rotar AM. Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal. 2017; 25 (2): 403-8.

20. Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harb Perspect Biol. 2010; 2 (5): a000414.

21. Baliah NT, Astalakshmi A. Phytochemical analysis and antibacterial activity of extracts from *Terminalia chebula Retz*. Int J Curr Microbiol Appl Sci. 2014; 3 (3): 992-9.

22. Mostafa M, Rahman M, Karim MM. Antimicrobial activity of *Terminalia chebula*. Int J Med Arom Plants. 2011; 1 (2): 175-9.

23. Aneja KR, Joshi R. Evaluation of antimicrobial properties of fruit extracts of *Terminalia chebula* against dental caries pathogens. Jundishapur J Microbiol. 2009; 2 (3): 105-11.

24. Zearah SA. Antifungal and antibacterial activity of flavonoid extract from *Terminalia chebula Retz*. fruits. J Basrah Res (Sci.). 2014; 40 (1): 122-31.

25. Sharma C, Aneja KR, Kasera R, Aneja A. Antimicrobial potential of *Terminalia chebula* Retz. fruit extracts against ear pathogens. World J Otorhinolaryngol. 2012; 2 (2): 8-13.

26. Singh D, Singh D, Choi SM, Zo SM, Ki SB, Han SS. Therapeutical effect of extracts of *Terminalia chebula* in inhibiting human pathogens and free radicals. Int J Biosci Biochem Bioinforma. 2012; 2 (3): 164-7.

27. Sultan MT, Anwar MJ, Imran M, Khalil I, Saeed F, Neelum S, et al. Phytochemical profile and pro-healthy properties of *Terminalia chebula*: a comprehensive review. Int J Food Prop. 2023; 26 (1): 526-51.

28. Wang C, Zhang H, Wang X, Wang X, Li X, Li C, et al. Comprehensive review on fruit of *Terminalia chebula*: traditional uses, phytochemistry, pharmacology, toxicity, and pharmacokinetics. Molecules. 2024; 29 (23): 5547.

29. Jamil A, Pasha I, Kamran Sharif M, Israr B. Functional characterization and phytochemical profiling of *Terminalia chebula* fruit. J Popul Ther Clin Pharmacol. 2023; 30 (18): 2440-9.

30. Khan I, Ullah Z, Shad AA, Fahim M, Öztürk M. *In vitro* antioxidant, anticholinesterase inhibitory, and antimicrobial activity studies of *Terminalia chebula* (Retz) and *Terminalia arjuna* (Roxb). S Afr J Bot. 2022; 146: 395-400.

31. Abdulridha MM, Abdulhussein HS, Alyaseen FF, Hassan BA. Phytochemical and antibacterial activity of the *Peganum harmala* seeds and its alkaloids. Plant Arch. 2019; 19 (1): 1439-44.

32. Nenaah GE. Antibacterial and antifungal activities of (beta)-carboline alkaloids of *Peganum harmala (L)* seeds and their combination effects. Fitoterapia. 2010; 81 (7): 779-82.

33. Wang N, An J, Zhang Z, Liu Y, Fang J, Yang Z. The Antimicrobial activity and characterization of bioactive compounds in *Peganum harmala L.* based on HPLC and HS-SPME-GC-MS. Front Microbiol. 2022; 13: 916371.

34. Akbar A, Ali I, Ullah S, Ullah N, Khan S, Rahman ZU, et al. Functional, antioxidant, antimicrobial potential and food safety applications of *curcuma longa* and *cuminum cyminum*. Pak J Bot. 2019; 51 (3): 1129-35.

35. Chaudhary N, Sultana S, Ali M. Chemical composition and antimicrobial activity of volatile oil of the seeds of *Cuminum cyminum L.* World J Pharm Pharm Sci. 2014; 3 (1): 1428-41.

36. Sheikh MI, Islam S, Rahman A, Rahman M, Rahman M. Control of some human pathogenic bacteria by seed extracts of cumin (*Cuminum cyminum L.*). Agric Conspec Sci. 2010; 75 (1): 39-44.

37. Saee Y, Dadashi M, Eslami G, Goudarzi H, Taheri S, Fallah F. Evaluation of antimicrobial activity of *Cuminum cyminum* essential oil and extract against bacterial strains isolated from patients with symptomatic urinary tract infection. Nov Biomed. 2016; 4 (4): 147-52.

38. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN, Bakri M. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J Biol Sci. 2018; 25 (2): 361-6.

39. Johri RK. *Cuminum cyminum* and *Carum carvi*: an update. Pharmacogn Rev. 2011; 5 (9): 63-72.

40. Mundy L, Pendry B, Rahman M. Antimicrobial resistance and synergy in herbal medicine. J Herb Med. 2016; 6 (2): 53-8.

41. Yadav SK, Jain GK, Mazumder A, Khar RK. Antimicrobial activity of a novel polyherbal combination for the treatment of vaginal infection. J Adv Pharm Technol Res. 2019; 10 (4): 190-4.

42. Bhinge SD, Bhutkar MA, Randive DS, Wadkar GH, Todkar SS, Kakade PM, et al. Formulation development and evaluation of antimicrobial polyherbal gel. Ann Pharm Fr. 2017; 75 (5): 349-58.

43. Sheikh ZA, Khan AA, Nawaz A, Zahoor A, Siddiqui ZA, Khan SS, et al. Development and clinical evaluation of polyherbal laxative laxisen. J Pharm Pharm Sci. 2014; 2 (2): 63-70.

44. Adwan G, Abu-Shanab B, Adwan K. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug–resistant *Pseudomonas aeruginosa* strains. Asian Pac J Trop Med. 2010; 3 (4): 266-9.

45. Stefanović OD. Synergistic activity of antibiotics and bioactive plant extracts: a study against Gram-positive and Gram-negative bacteria [Internet]. Rijeka (Croatia): InTech; 2018 [cited 2024 Jul 30]. Available from: https://doi.org/10.5772/intechopen.72026.

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