



In vitro antibacterial activity of *Hibiscus rosa sinensis*, *Chrysanthemum indicum*, and *Calendula officinalis* flower extracts against Gram negative and Gram positive food poisoning bacteria

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Abstract

Safely management of food spoilage and foodborne illness is primarily achieved by applying chemical additives that have adverse effects along with health risk, increment chemical in food, and reduced bacterial susceptibility to antimicrobials. In the present study, antimicrobial efficacy of extracts from 3 different flowers (*Hibiscus rosa sinensis*, *Chrysanthemum indicum*, and *Calendula officinalis*) was examined towards seven food poisoning bacterial strains, four gram-positive strains (*Staphylococcus aureus* MTCC 87, *Bacillus cereus* MTCC 430, *Clostridium perfringens* MTCC 450, *Listeria monocytogenes* MTCC 657), and three gram-negative strains (*Escherichia coli* MTCC 43, *Salmonella typhi* MTCC 1264 and *Pseudomonas aeruginosa* MTCC424) using well diffusion assay. Aqueous extracts from all three of the flowers were similarly efficient with variable antimicrobial efficiency against the examined bacterial strains, while ethanol and methanol extracts from *C. officinalis* were highly efficient against all tested pathogenic bacteria. Ethanolic extract of *C. indicum* was the most efficient flower extract after *C. officinalis* against *C. perfringens*, *L. monocytogenes*, and *S. typhi*. *H. rosa sinensis* ethanol extract exhibited bactericidal action against *S. aureus*, *B. cereus*, and *P. aeruginosa*. For most extracts, the minimum inhibitory concentration (MIC) ranged from 3.75 to 7.5% and minimum bactericidal concentration (MBC) of 1.87–3.75% except for *C. perfringens*, and *L. monocytogenes* those were less sensitive with MIC 20%, and MBC 20%. Such flower extracts, which are potentially efficient, would be utilized to manage foodborne illness and protect food items from spoilage and minimize safety hazards generated due to chemically preservatives.

Keywords Food poisoning · Flower extracts · *Hibiscus rosa sinensis* · *Chrysanthemum indicum* · *Calendula officinalis*

Introduction

Food poisoning is identified in underdeveloped and developing countries as one of the severe causes of foodborne diseases and mortality. In specific, Gram-negative bacteria, including *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa*, are mostly associated with bacteria-based food poisoning cases (Mostafa et al. 2018; Maema et al. 2020; Arullappan et al. 2009). Certain Gram-positive bacteria, particularly *Staphylococcus aureus* and *Bacillus cereus*, were also described as the causative agent of

gastrointestinal illnesses or food contamination (Mostafa et al. 2018). The conventional use of chemical additives implies preventing food spoilage and survival of causative organisms or products. Despite their established effectiveness in eliminating and managing food poisoning, their regular uses often led to the deposition of chemical substances in the food and food chain, development of microbial tolerance to the chemicals applied, and adverse human health consequences (Karwa and Rai 2012). Due to this issue, efforts have focused on developing potentially efficient, safer, and more natural food preservatives. In these contexts, bioactive compounds could be used as antimicrobials to preserve food from spoilage, causing organisms and foodborne pathogens (Chen and Xie 2019).

Plants are used globally to prevent and cure various diseases and the continuous development of new drugs. It has been reported that about 20,000 plant varieties are used as conventional pharmaceutical products, and many of these

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bioactive molecules are utilized to formulate new drugs globally (Mostafa et al. 2018; Maema et al. 2020). Plants have been traditionally used to prevent and cure various ailments, including gastroenteritis, oral diseases, cold, fever, contraception, and fertility control worldwide. Contaminated food is typically designated as the critical source of several infections in human beings. The existence and activities of microorganisms in food may impair them and deteriorate nutritional quality.

Furthermore, the occurrence of a range of drug resistance in pathogenic microorganisms has become a significant concern for both human and veterinary therapeutic sectors. Therefore, it is required to continuously discover and identify novel antimicrobial substances to reduce microbial antibiotic tolerance. Plant essential oils and extracts provide substantial scope for naturopathic products to prevent and care for human and animal infections (Maema et al. 2020). These plant-derived molecules contain anti-microbial agents, anti-cancer, antioxidants, and free radicals. Many scholars have become quite concerned about utilizing plant antimicrobial molecules with drug-resistant microbes for human welfare. Massive numbers of plant species were identified as essential resources of natural antibacterial agents as a replacement of antibiotics that could effectively manage drug-resistant bacterial infections (Maema et al. 2020).

According to the World Health Organisation (WHO), plant-derived products may become the safest way to get a spectrum of pharmacological drugs. The antimicrobial potential of plant-derived extracts is governed by various factors, including the plant species, method of cultivation, chemical properties, the procedure of extraction applied, and the solvent type used (Vijayakumar et al. 2018). The mode of action of bioactive molecules on bacterial cells is complicated. This bioactive molecule can trigger cell wall breakdown, cytoplasmic membrane instability, inactivation of cellular enzymes, and, in addition, may also restrict replication and transcription process by interacting with DNA or RNA (Maema et al. 2020).

Medical professionals have long recognized the medicinal powers of flowers over many decades (Vijayakumar et al. 2018). Some of the critical benefits of flowers and plants are that these have entirely natural therapeutic qualities, even without the harmful adverse effects of synthetic medicines and drugs. Plant-based medicines are much cheaper than medical products or branded medicines. The Hibiscus plant is commonly cultivated in tropical areas like the Caribbean, Australia, Brazil, Central America, India, Africa, the United States, and the Philippines (Arullappan et al. 2009). Hibiscus flowers, rhizome, and foliage are utilized in traditional tribal medicine in many countries and communities. Hibiscus plant extract is often reported in India to reduce high

blood pressure and to boost the liver's functioning (Vijayakumar et al. 2018). Hibiscus powders are also utilized as nutritional supplements and nutraceuticals worldwide. Dried calyces infusion from Hibiscus flower used to prepare tea (hibiscus tea) that holds antimicrobial properties to secure from urinary tract (UTIs) diseases (Ruban and Gajalakshmi 2012).

Chrysanthemum is a widely recognized herbal tea in Thailand and China made from *Chrysanthemum indicum* (Nepali et al. 2018). The entire plant has therapeutic effects; however, the popular component is the flower utilized in chrysanthemum tea. *Chrysanthemum indicum* is also used in conventional medicine formulations to cure various illnesses like influenza, colitis, stomatitis, diarrhea, fatigue, soreness, vertigo, pertussis, and hypertensive symptoms (Nepali et al. 2018). *Chrysanthemum indicum* active compounds are glycosides, adenine, and flavonoids. Earlier studies (Mostafa et al. 2018; Nepali et al. 2018) have shown that *Chrysanthemum indicum* can function as an antibiotic against several microorganisms.

Calendula officinalis is an annual Asteraceae family plant that flowers from May to October. Its flowers have been reported to use in preparations of various medicines. The plant is chemically abundant in sesquiterpenes, phenolic acids, flavonoid glycosides, triterpene saponins, triterpene alcohol, flavonoids, carotenoids, xanthophylls, tocopherol, and calenduline (Tresch et al. 2019). The *Calendula officinalis* concentrate was commonly known as a superficial anti-inflammatory drug. In vivo experiments utilizing *Calendula officinalis* oral rinses have shown this herb's potential in minimizing gingival bleeding (Khairnar et al. 2013). A previous study indicates the existence of the antimicrobial action of *Calendula officinalis* against periodontal-pathogenic bacteria (Khairnar et al. 2013). *Camellia sinensis* (L.) Kuntze leaves used in green tea, which has been used in Japan and China for centuries and are widely cultivated in these countries. Animal experimentation has proven that *Calendula sinensis* derivatives are antibacterial to *Streptococcus mutans* (Anushree et al. 2015). Furthermore, experiments with *Calendula sinensis* have shown good antimicrobial activity against periodontal pathogenic agents like *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (Anushree et al. 2015).

The objective of the present study was to evaluate the antimicrobial effect of three medically essential flowers (*Hibiscus rosa sinensis*, *Chrysanthemum indicum*, and *Calendula officinalis*) against food poisoning Gram-positive (*Staphylococcus aureus* MTCC 87, *Bacillus cereus* MTCC 430, *Clostridium perfringens* MTCC 450, *Listeria monocytogenes* MTCC 657) and Gram-negative (*Escherichia coli* MTCC 43, *Salmonella typhi* MTCC 1264 and *Pseudomonas aeruginosa* MTCC424) bacterial spp.

Materials and methods

Plant material (flower) collection and identification

In this study, flowers from 3 plant species (*Hibiscus rosa sinensis*, *Chrysanthemum indicum*, and *Calendula officinalis*) were obtained from the regional market of Panchkula, Haryana, India and then further identified in Herbarium (Botanical Herbarium) Himachal Pradesh University, Shimla, Himachal Pradesh. The flowers were rinsed and cleaned with sterilized distilled water to remove almost all unwanted contaminants and air-dried under shade for three days at room temperature (25 °C with a relative humidity of 41.0% ± 0.4%) (Sharma and Karnwal 2018; Fernandes et al. 2018). The dried portion was pulverized in a mortar and pestle to get fine powder of flowers of each plant species and further sieved to get uniformed particle size powder. The dried powder was kept in airtight aseptic glass containers till further research.

Test bacterial strains collection

Each flower extract's antibacterial efficacy was determined using seven bacterial species that cause food poisoning diseases (Kappeli et al. 2019). Four strains of Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*) and three strains of Gram-negative (*Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) bacteria were collected from IMTECH, Chandigarh, India MTCC culture collection.

Inoculums preparation

Bacterial Inoculums were standardized to 0.5 McFarland (1.5×10^8 CFU/mL) to provide a uniform bacterial density during experiments. The test bacterium was inoculated into 5.0 ml of the nutrient broth and cultivated at 32 ± 2 °C for 24 h. Further, 0.2 ml of 24 h grown bacterial cultures were inoculated to 20 ml of sterile nutrient broth and incubated for 5–6 h to get bacterial density to 10^8 CFU/ml (equivalent to 0.5 McFarland). To avoid any changes in the inoculum concentration, plates with nutrient agar were inoculated after 15 min of standardization (Al-Qurainy et al. 2013; Dike-Ndudim et al. 2016).

Antimicrobial assay

Antimicrobial susceptibility assay with antibiotic discs

To confirm the resistance of test bacterial spp. towards commercially available antibiotic discs (Hi-media, India), the

Kirby-Bauer technique (disc diffusion method) was applied (Sharma and Karnwal 2018). In compliance with the CLSI (Clinical Laboratory Standards Institute) recommendations (Humphries et al. 2018), different antibiotic discs (Himedia, India) were placed on the surface of the priory-inoculated Muller-Hinton agar plates with test organism and incubated at 32 ± 2 °C for 24 h (Fayemi et al. 2017). After 24 h of incubation, plates were observed for any bacterial growth within the inhibition zone. Three sets of discs were used, i.e., specific for Gram-negative (Gn), Gram-positive (Gp), and standard for both types of Gram organisms. For Gram-negative, specifically, the following antibiotic discs were used: Azithromycin (AZM), Aztreonam (AT), Carbenicillin (CB), Cefamandole (FAM), Cefoperazone (CPZ), Cefprozil (CPR), and Enoxacin (EN). For Gram-positive specifically, the following antibiotic discs were used: Ampicillin (AMP), Cefazolin (CZ), Erythromycin (E), clindamycin (DA), and Oxacillin (OX). Few antibiotic discs were commonly applied for both type of organism includes Amikacin (AK), Amoxycylav (AMC), Azlocillin (AZ), Cefaclor (CF), Cefdinir (CDR), Cefmetazole (CMZ), Cefoxitin (CX), Cefpodoxime (CPD), Chloramphenicol (C), Cinoxacin (CIN), and Ertapenem (ETP).

Floral extract preparation

Aqueous extract

The methodology for aqueous extract preparation was used as described by Rahman et al. (2009). A measured amount (50 g) of air-dried powder of each flower was extracted with 200 ml of sterilized distilled water by shaking and percolating for seven days, subsequently purified and dehydrated under vacuum (Fernandes et al. 2018). 200 mg of crude extract was immersed in 0.4 ml of dimethyl sulfoxide (DMSO) to yield 500 mg/ml of concentrate (Humphries et al. 2018). The dry aqueous extract was stored at -4 °C for antibacterial assay.

Organic solvent extraction

Ethanol and methanol extract: 50 g of dried flower powder was processed using ethanol (95%) and methanol (80% v/v) with 48 h stirring at room temperature. After 48 h, the mixture was filtered with double sheets of muslin cloth and further centrifuged for 10 min at 4000 g and then filtered with Whatman filter paper No. 41 for clear filtrate (Khairnar et al. 2013). The filtrates were dissipated and dried at 40 ± 2 °C under low pressure using a rotatory vacuum evaporator. The extracted amount was weighed and collected in small airtight bottles in the refrigerator -4 °C.

The extract percentages were estimated using the following equation for both types of extractions: Extract yield (%) = (FR × 100)/FS

(where FR; weight of extracted residues of the flower after solvent removal and FS; weight of flower sample).

Antimicrobial susceptibility assay of the floral extract

Antimicrobial Susceptibility assay of the floral extract was executed using the agar diffusion assay as outlined by Sharma and Karnwal (2018). About 100 µl (10⁸ CFU/ml) of freshly grown test bacterial culture was spread on Muller Hilton agar plates with the non-toxic swab. Four wells of 6 mm diameter were punched in the agar media with a sterile cork-borer. The known amount (50 mg) of the dried extract was dissolved in 2.5 ml of respective solvent and sterilized with a bacterial filter (pore size 0.22 µm). 100 µl of the prepared mixture were transferred aseptically with a micropipette in each well. Dimethyl sulfoxide (DMSO) was used as a negative control. The inoculated plates were permitted to stay for one hour to enable the extract to pre-diffuse into the growth medium and incubated aerobically at 32 ± 2 °C for 48 h. The development of inhibition zones was assessed by Vernier-Calliper, reported, and perceived as an indicator of antibacterial activity.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination of floral extract

The micro-dilution method was used to assess the MIC of flower extracts. Extracts were diluted two-fold using Mueller–Hinton Broth for bacterial growth at a concentration of 15%, 7.5%, 3.75%, and 1.87% v/v. The amount of 20 µl (1 × 10⁸ CFU/ml) of bacterial suspension was poured in each tested concentration and allowed to incubate for 24 h at 32 ± 2 °C. The MIC value was determined by looking for the lowest concentration of the extract that would fully inhibit the bacterial growth (Maema et al. 2020). Minimum bactericidal concentration (MBC) has been calculated from the MIC range (Sharma and Karnwal 2018). The Mueller–Hinton agar plates were subcultured and incubated for 24 h at a

temperature of 32 ± 2 °C. Petri dishes were examined, and the smallest concentration correlated with flower extract that developed no bacterial growth in the medium was recognized as MBC.

Results and discussion

Resistance to antibiotics in both emerging and industrialized countries is an issue that continues to challenge the healthcare services in most of the world. The evolution and spreading of multidrug-resistant bacteria have considerably challenged the latest antibacterial therapy (Ma et al. 2018). It has prompted the lookout for an alternative source of antimicrobials, including plants because they contain a wide range of bioactive molecules with proven medicinal potential. This study was performed to evaluate the antimicrobial efficacy of floral extracts against foodborne pathogens.

The antibiotic profile of each tested bacterium was determined using specified antibiotic discs. The Kirby-Bauer technique (disc diffusion method) was applied for this analysis. Seven bacterial spp. was tested with different, commercially available antibiotic discs (Karwa and Rai 2012). Three sets of the disc were used as per the type of bacterial pathogen. Gram-negative (GN) bacterial strains tested specifically for Azithromycin (AZM), Aztreonam (AT), Carbenicillin (CB), Cefamandole (FAM), Cefoperazone (CPZ), Cefprozil (CPR), and Enoxacin (EN). Most of the Gram-positive bacteria are entirely covered with a thick peptidoglycan cell wall. This structure provides limited tolerance to small molecules' diffusion like antimicrobials (Karwa and Rai 2012). However, in Gram-negative bacteria, the cell wall covers itself with a second membrane called LPS, which serves as an efficient shield against antimicrobials. Previous findings (Al-Qurainy et al. 2013; Duraipandiyam and Ignacimuthu 2009; Fazly Bazzaz et al. 2016) reported strong tolerance of *P. aeruginosa* to Aztreonam & ceftazidime and confirmed that *P. aeruginosa* strain was susceptible to polymyxin B, levofloxacin, and ciprofloxacin. Researchers (Al-Qurainy et al. 2013; Yakha et al. 2015) also identified the high tolerance potential of *P. aeruginosa* for ceftazidime and polymyxin B antibiotics. In the present study, GN isolates *Escherichia coli* was resistant to 5, *Salmonella typhi* was resistant to 3, and *Pseudomonas aeruginosa* was resistant

Table 1 Antibiotic susceptibility results of tested Gram-negative bacteria

Gram type	Bacterial sample	Antibiotic disc						
		AZM	AT	CB	FAM	CPZ	CPR	EN
Gram negative	<i>Escherichia coli</i> MTCC 43	R	R	S	R	R	I	S
	<i>Salmonella typhi</i> MTCC 1264	S	R	I	S	R	S	S
	<i>Pseudomonas aeruginosa</i> MTCC 424	S	S	I	R	I	S	R

S sensitive, R resistant, I intermediate

to 4 of 7 antibiotics used. Antibiotic AZM, and CPR are highly effective for GN bacterial *Salmonella typhi* and *Pseudomonas aeruginosa*. However, both pathogens were resistant to CB antibiotic but this antibiotic shown bactericidal activity for highly resistant *Escherichia coli* (Table 1). Yakha et al. (2015); Kosari et al. (2020); Saha et al. (2017) were reported similar inferences that imipenem, amikacin, and gentamycin were most effective against *E. coli* isolates however has shown resistances to cefuroxime, ciprofloxacin, and ofloxacin antibiotics.

Similarly, Gram-positive (GP) strains were tested for antibiotics Ampicillin (AMP), Cefazolin (CZ), Erythromycin (E), clindamycin (DA), and Oxacillin (OX). GP isolate *Staphylococcus aureus* was resistant to 4, *Bacillus cereus* was resistant to 3, *Clostridium perfringens*, and *Listeria monocytogenes* were resistant to 2 antibiotics of the total five antibiotics used (Table 2). Our research coincides with Tekwu et al. (2012) observations, who reported that significant percentages of *S. aureus* are susceptible to CZ and E antibiotics. Yakha et al. (2015) documented strong resistance of *S. aureus* to penicillin and oxacillin. An Antibio-gram of antibiotics used, especially for GP bacteria, showed that antibiotics E and CZ are highly effective for tested GP bacteria. All four GP bacteria showed complete resistance

against OX antibiotics, whereas only three bacteria instead of *Listeria monocytogenes* showed resistance to AMP antibiotic discs (Table 2).

Separately, a total of 11 broad-spectrum antibiotic discs were used for both types of bacterial pathogen to evaluate their efficiency to work against these bacterial spp. as results shown in Table 3. During the study, it was observed that GN bacterial *Escherichia coli* were highly resistant for 81% and GP bacteria *Clostridium perfringens* for 72% tested antibiotics, as shown in Table 3. Out of 11 tested broad-spectrum antibiotics, CIN and ETP have shown more than 57% of bactericidal potential for 4 out of seven bacterial strains. Antibiotic AK, CF, and CDR showed the next highest bactericidal activity (42%) against 3 out of seven bacterial strains. *Escherichia coli* mostly sensitive to CB, EN, AZ, and C antibiotics. *Escherichia coli* shown intermediate resistance to AT, CPR, AMC, and CF antibiotic discs. *Salmonella typhi* mostly sensitive to AZM, FAM, CPR, EN, CF, CDR, CMZ, CPD, and CIN antibiotics.

Bacterial strain *Staphylococcus aureus* was shown no growth against CZ, AK, AMC, CX, CIN, AND ETP antibiotic discs, as shown in Tables 2 and 3. The details of individual antibiotic-resistant profiles of individual bacteria are presented (Tables 1, 2, 3). Thus, all isolated bacterial

Table 2 Antibiotic susceptibility results of tested Gram-positive bacteria

Gram type	Bacterial sample	Antibiotic disc				
		AMP	CZ	E	DA	OX
Gram positive	<i>Staphylococcus aureus</i> MTCC 87	R	S	I	R	R
	<i>Bacillus cereus</i> MTCC 430	R	I	S	S	I
	<i>Clostridium perfringens</i> MTCC 450	I	S	S	S	I
	<i>Listeria monocytogenes</i> MTCC 657	S	S	S	R	R

S sensitive, R resistant, I intermediate

Table 3 Antibiotic susceptibility results of tested Gram-positive and Gram-negative bacteria against 11 common antibiotics

Gram type	Bacterial sample	Antibiotic disc										
		AK	AMC	AZ	CF	CDR	CMZ	CX	CPD	C	CIN	ETP
Gram positive	<i>S. aureus</i> MTCC 87	S	S	I	R	R	I	S	I	R	S	S
	<i>B. cereus</i> MTCC 430	R	I	S	S	R	I	R	R	I	S	R
	<i>C. perfringens</i> MTCC 450	R	R	R	I	S	S	I	S	I	R	R
	<i>L. monocytogenes</i> MTCC 657	S	I	R	S	S	I	R	I	R	I	S
Gram negative	<i>E. coli</i> MTCC 43	R	I	S	I	R	R	R	R	S	R	R
	<i>S. typhi</i> MTCC 1264	R	R	R	S	S	S	R	S	I	S	S
	<i>P. aeruginosa</i> MTCC 424	S	R	R	I	R	R	R	I	S	S	S

S sensitive, R resistant, I intermediate

strains were MDR. Statistics of the WHO (2014) revealed that around 40 percent of human infections are resistant to several antibiotics. The growing pattern in antibiotic resistance may be attributed to frequent, excessive misuse of antibiotics and longer hospitalization. Antibiotic susceptible microorganisms have limited pathogenicity as they are controlled in vivo using antimicrobials (Yakha et al. 2015). The host protection mechanism often manages pathogens at a specific pathogen density while the antimicrobials work as a limiting factor. As known, antimicrobial-producing microbes harbor genes resistant to antibiotics in extrachromosomal elements and bacterial chromosomes for protection (Ghosh et al. 2008).

Consequently, the susceptible bacterial community collects genes through transformation or conjugation to become resistant species for antimicrobials. In addition, in the vicinity of an antimicrobial as a stress factor, bacteria with simple genomes induced by inherent (mutations) or acquired genetic modifications (conjugations and transformation) changes and become resistant to antimicrobial agents (Maema et al. 2020; Yakha et al. 2015). It was reported that the tolerance of bacteria to antibiotics was more vital in places where different classes of antibiotics are used in routine, like hospitals (Yakha et al. 2015; Al-Qurainy et al. 2019). Indeed, the horizontal gene transfer between two individuals occurs quicker than mutational modifications, this process commonly described as 'the evolution of quantum leaps.' Progressively, the usage of several antimicrobials of higher generations to manage bacterial infections has led to multiple drug resistances (Kosari et al. 2020).

Extraction yields and antibacterial potential of the flowers extract

Plant are a natural source of bioactive molecules used to discover novel drugs against pathogenic microbes. Segregated bioactive molecules are used to synthesize bioactive products and as a lead component for synthesis of drugs at laboratory level (Tekwu et al. 2012). Phyto-chemical extraction assay from plant materials is an important stage for optimizing the desired molecule concentration and functions. The choice of an effective extraction solvent and process is also critical for optimizing and standardization of plant products used to separate appropriate soluble components from the non-targeted portion of extract using the suitable solvent (Fazly Bazzaz et al. 2016; Moryl et al. 2015). In the present study, using the cold percolation method, the highest yield was obtained from *Chrysanthemum indicum* extract of 9.3% by using an aqueous solvent and followed by 9.1% with ethanol while the least yield was of *Calendula officinalis* 4.8% with methanol solvent. Solvent-wise, the aqueous extract of *Chrysanthemum indicum* given maximum yield compared to the other two flowers (Table 4).

Table 4 Yield % of different flower extract used in the study

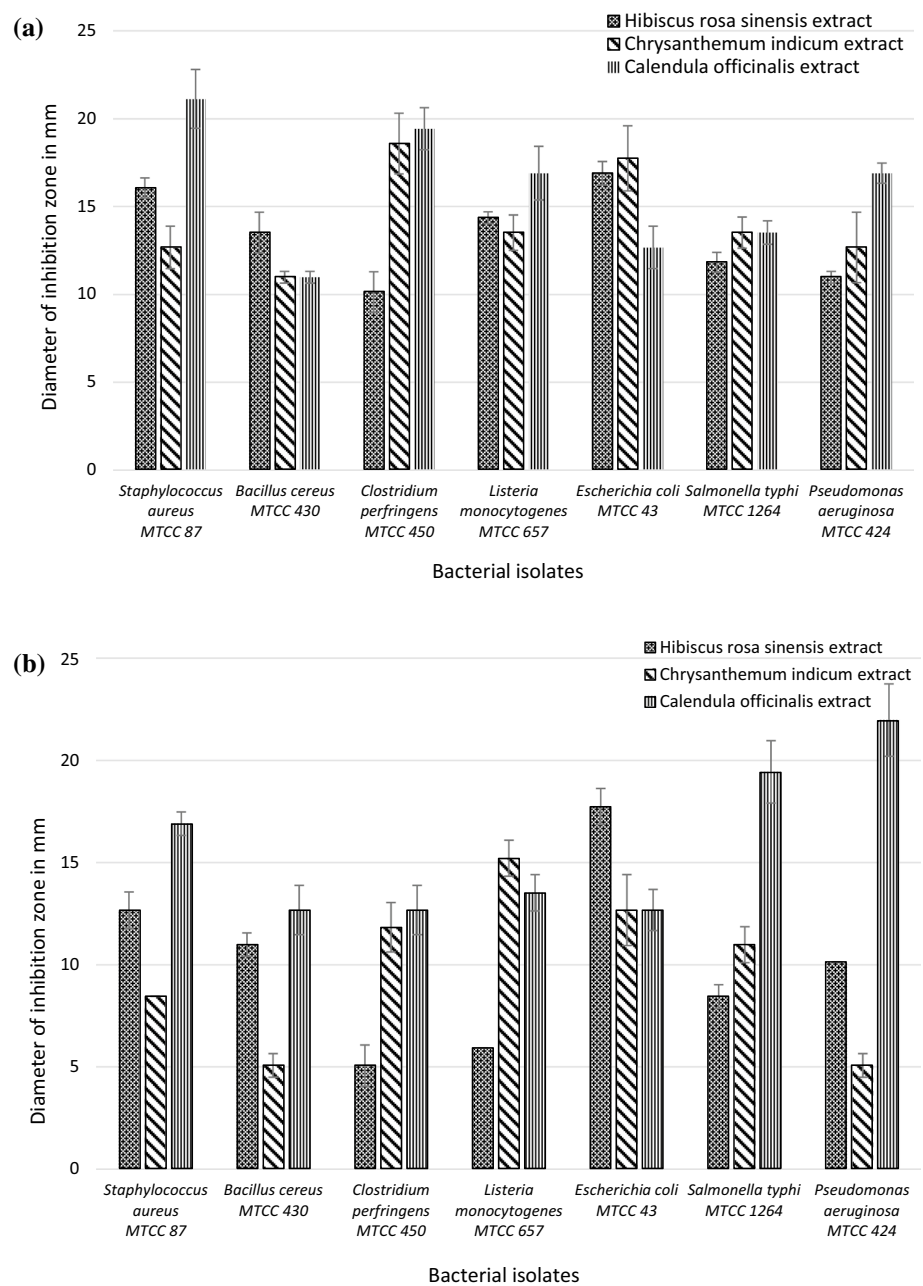
Plant species	Extract yield (%) with solvent used		
	Aq	Et	Me
<i>Hibiscus rosa sinensis</i>	8.7	7.8	6.9
<i>Chrysanthemum indicum</i>	9.3	9.1	5.2
<i>Calendula officinalis</i>	7.3	6.4	4.8

Similar results were observed with ethanol-based extract for *Chrysanthemum indicum*. Whereas, maximum yield was obtained from *Hibiscus rosa sinensis* extract of 6.9% by using Methanol solvent followed by *Chrysanthemum indicum* and *Calendula officinalis*. This result indicates that the type of solvents being used could significantly influence the recovered amounts of crude concentrate obtained from different flowers. Our results were in agreement with earlier research (Do et al. 2014) conducted with *Limnophila aromatica* and *Phoenix dactylifera* L., where variation in yield occurs due to the solubility difference of biomolecules in different solvents. These results indicated that aqueous, absolute methanol or mixture of water and methanol, ethanol, was the preferred solvent for extracting large amounts of extractable solids from plant materials (Pires et al. 2018). The difference in yield percentage can also be clarified by the varying polarities of molecules that appeared quite readily soluble in specific solvents.

In this present work, aqueous, ethanol, and methanol extracts of flowers of all three tested flowers were evaluated for their antibacterial property against seven food poisoning causing bacterial spp.

Evaluation of the antimicrobial activity of three different extracts (aqueous, ethanol, and methanol) for all three tested plant species was determined initially by the disc diffusion method against test bacterial pathogens (Fig. 1). The study showed that all flower extracts used in the study exhibited varying antimicrobial activity against all microorganisms tested. It was observed that methanolic extract of *Calendula officinalis* was the most effective among the three flower extracts tested for *Staphylococcus aureus* and *Pseudomonas aeruginosa* with 25 mm zone of inhibition (Table 5). Our findings are in line with the results, Cwikla et al. (2010), who documented assertive antimicrobial behavior of petroleum ether, chloroform, ethanol, and water extracts of *C. officinalis* against *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *C. albicans*, and *A. niger*. In another study, Brito-Junior et al. (2012) reported significant inhibition potential of *C. officinalis* extracts against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* sp., coagulase-positive *Staphylococcus* sp., as well as coagulase-negative *Staphylococcus* sp., *C. albicans*, and *Candida parapsilosis*. Various studies (Pires et al. 2018; Kuok et al. 2017; Ramesh et al. 2015)

Fig. 1 Antimicrobial activity (in mm) of different flower extracts against tested bacterial isolates **a** aqueous extract; **b** ethanolic extract; **c** methanolic extract

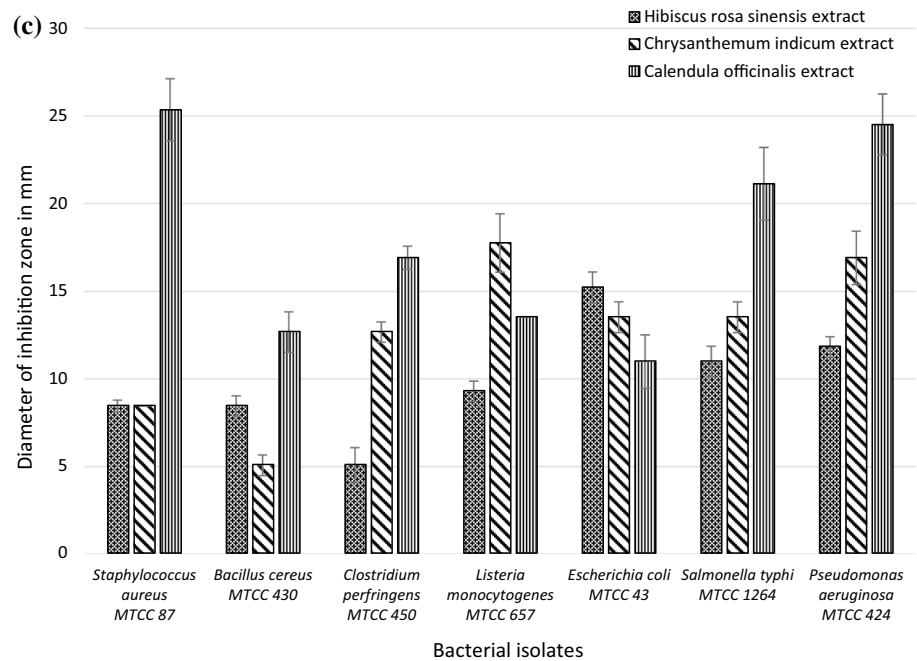


indicated a significant degree of antimicrobial behavior of *C. officinalis* extracts against various bacterial and fungal pathogens. It has been documented that *C. sinensis* antimicrobial effect was due to its vital molecule, catechin, which directly acts on the cell membrane of pathogenic bacteria and causes non-recoverable damage and bacterial death (Pires et al. 2018). *C. officinalis* also showed strong topical anti-inflammatory activity due to saponins and flavonoids in contrast to the antimicrobial function. Zitterl-Eglseer et al. (1997) reported that *C. officinalis* derivatives had identical anti-inflammatory efficacy to prostaglandin blockers.

All flower extracts showed a zone of inhibition (ZOI) against all Gram-negative and Gram-positive bacteria

tested, as presented in Table 5. It was observed that aqueous extract of all tested plant species showed better antibacterial activity against all tested bacterial pathogens compared to methanolic and ethanolic extracts, as shown in Table 5 and Fig. 1a. *Calendula officinalis* was effective against Gram-positive (*Staphylococcus aureus*, and *Clostridium perfringens*) and Gram-negative (*Salmonella typhi*, and *Pseudomonas aeruginosa*) bacteria. The extract from *Hibiscus rosa sinensis* showed better ZOI against *Bacillus cereus* as well as *Escherichia coli*. Bacterial growth inhibition by the floral extracts could be explained by the presence of certain effective substances in flower concentrates (Arullappan et al. 2009). Such

Fig. 1 (continued)



bioactive components could inhibit bacterial growth alone or in combination with other bioactive molecules available in extracts. Crude plant concentrates contain various natural biomolecules, including flavonoids, tannins, alkaloids, triterpenoids, which have been reported to possess antibacterial properties (Kilic et al. 2019). Floral extracts are rich in phenolic and tannins that are having very strong antimicrobial attributes. This has been reported (Turker and Usta 2008) that the metabolites are crucial for medicinal plants to achieve therapeutic functionality against infection. Notably, flavonoids are recognized as being an efficient antimicrobial tool for a vast array of microorganisms. The antimicrobial behavior can be attributed to their ability to interact with additional cellular and soluble proteins and bacterial cell-wall. Growth of *Listeria monocytogenes* was maximally inhibited by using *Chrysanthemum indicum* extract in increasing order from aqueous > Ethanolic > Methanolic extract (Table 5). Of all seven species, *Staphylococcus aureus* and *Bacillus cereus* were a more susceptible strain to most of the flower extracts. Furthermore, several people (Al-Qurainy et al. 2013; Saha et al. 2017; AftabUddin et al. 2017) who manage different skin disorders and other ailments with plant extract use the water-based extract of plants. This was accepted with the work done by Dike-Ndudim et al. (2016) and Muhuha et al. (2018), who observed that the *M. oleifera* leaf aqueous extract has effective antibacterial efficacy for both Gram-negative and Gram-positive bacterial species.

MIC and MBC of flower extracts

Furthermore, two-fold dilution approaches were employed to determine the minimum inhibitory concentration (MIC) for the experimental flower extracts capable of generating an inhibition zone in the course of the screening procedure. MIC was performed for only those organisms that showed a zone of inhibition and were sensitive to the flower extracts in the previous antimicrobial assay using the agar well diffusion method.

The aqueous flower extract of *Hibiscus rosa sinensis* had the lowest MIC value, 3.75% for *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Escherichia coli*, with the lowest MBC value 1.87% mg/ml for *Staphylococcus aureus*, *Salmonella typhi*, and 3.75% for *Pseudomonas aeruginosa*, and *Escherichia coli* (Table 6). However, ethanolic flower extract of *Hibiscus rosa sinensis* had the lowest MIC value, 3.75% for *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli* with the lowest MBC value 1.87% mg/ml for *Staphylococcus aureus*, *Bacillus cereus*, and 3.75% for *Escherichia coli*. The lowest MIC and MBC values for methanolic extract were recorded with 3.75% and 1.87% for only one bacterial species, *Escherichia coli*, all other six bacterial shown higher MIC and MBC value for *Hibiscus rosa sinensis* methanolic extract as represented in Table 6.

Similarly, the other two flower extracts were also tested for their MIC and MBC value against seven bacterial pathogens. Aqueous extract of *Chrysanthemum indicum* had the

Table 5 Antibacterial activity (zone of inhibition) generated by different flower extract against bacteria

Gram type	Bacterial isolates	Diameter of inhibition zone in mm								
		<i>H. rosa sinensis</i> extract			<i>C. indicum</i> extract			<i>C. officinalis</i> extract		
		Aq	Et	Me	Aq	Et	Me	Aq	Et	Me
Gram-positive	<i>S. aureus</i> MTCC 87	16±0.58 ^a	13±0.88	8±0.3	13±1.2	8±0.0	8±0.01	21±1.66	17±0.57	25±1.76
	<i>B. cereus</i> MTCC 430	14±1.15	11±0.58	8±0.60	11±0.33	5±0.56	5±0.58	11±0.33	13±1.20	13±1.15
	<i>C. perfirngens</i> MTCC 450	10±1.02	5±1.00	5±1.01	19±1.73	12±1.20	13±0.58	19±1.20	13±1.18	17±0.67
Gram-negative	<i>L. monocytogenes</i> MTCC 657	14±0.33	6±0.0	9±0.60	14±1.00	15±0.88	18±1.67	17±1.52	14±0.86	14±0.0
	<i>E. coli</i> MTCC 43	17±0.67	18±0.85	15±0.90	18±1.86	13±1.73	14±0.82	13±1.20	13±1.00	11±1.52
	<i>S. typhi</i> MTCC 1264	12±0.58	8±0.55	11±0.89	14±0.88	11±0.88	14±0.85	14±0.67	19±1.55	21±2.08
	<i>P. aeruginosa</i> MTCC 424	11±0.33	10±0.0	12±0.60	13±2.00	5±0.57	17±1.53	17±0.57	22±1.76	25±1.73

^aValues are mean ± SE

Table 6 MIC and MBC values of *Hibiscus rosa sinensis* extract against bacterial pathogens

Gram type	Bacterial isolates	Different concentrations (%) of <i>Hibiscus rosa sinensis</i> extract against food poisoning causing bacterial isolates with reference to MIC and MBC																	
		Aqueous						Ethanol 95%						Methanol 80%					
		15	7.50	3.75	1.87	MIC	MBC	15	7.50	3.75	1.87	MIC	MBC	15	7.50	3.75	1.87	MIC	MBC
Gram-positive	<i>S. aureus</i> MTCC 87	+	+	+	-	3.75	1.87	+	+	+	-	3.75	1.87	+	+	+	-	7.5	3.75
	<i>B. cereus</i> MTCC 430	+	+	-	-	7.5	7.5	+	+	+	-	3.75	1.87	+	+	+	-	7.5	3.75
	<i>C. perfirngens</i> MTCC 450	+	+	-	-	7.5	7.5	-	-	-	-	NA	NA	+	-	-	-	15	7.5
Gram-negative	<i>L. monocytogenes</i> MTCC 657	+	+	-	-	7.5	3.75	-	-	-	-	NA	NA	+	-	-	-	15	7.5
	<i>E. coli</i> MTCC 43	+	+	+	-	3.75	3.75	+	+	+	-	3.75	3.75	+	+	+	-	3.75	1.87
	<i>S. typhi</i> MTCC 1264	+	+	+	-	3.75	1.87	+	+	+	-	15	7.5	+	+	-	-	7.5	7.5
	<i>P. aeruginosa</i> MTCC 424	+	+	+	-	3.75	3.75	+	+	+	-	7.5	7.5	+	+	+	-	7.5	7.5

Table 7 MIC and MBC values of *Chrysanthemum indicum* extract against bacterial pathogens

Gram type	Bacterial isolates	Different concentrations (%) of <i>Chrysanthemum indicum</i> extract against food poisoning causing bacterial isolates with reference to MIC and MBC																	
		Aqueous					Ethanol 95%					Methanol 80%							
		15	7.50	3.75	1.87	MIC	MBC	15	7.50	3.75	1.87	MIC	MBC	15	7.50	3.75	1.87	MIC	MBC
Gram-positive	<i>S. aureus</i> MTCC 87	+	+	-	-	7.5	3.75	+	-	-	-	15	15	+	+	-	-	7.5	7.5
		+	+	-	-	7.5	3.75	+	-	-	-	15	7.5	+	-	-	-	15	7.5
	<i>C. perfringens</i> MTCC 450	+	+	+	-	3.75	1.87	+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75
		+	+	+	-	3.75	1.87	+	+	-	-	7.5	3.75	+	+	+	-	3.75	3.75
	Gram-negative	<i>E. coli</i> MTCC 43	+	+	+	-	3.75	3.75	+	+	-	-	7.5	7.5	+	+	+	-	3.75
+			+	-	-	7.5	3.75	+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75
<i>S. typhi</i> MTCC 1264		+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75
		+	+	-	-	7.5	3.75	+	+	-	-	15	7.5	+	+	+	-	3.75	1.87
<i>P. aeruginosa</i> MTCC 424		+	+	-	-	7.5	3.75	+	-	-	-	15	7.5	+	+	+	-	3.75	1.87

lowest possible MIC (3.75%) and MBC (1.87%) value for three bacterial pathogens, namely, *Clostridium perfringens*, *Listeria monocytogenes*, and *Escherichia coli*, as shown in Table 7. The ethanolic extract of *Chrysanthemum indicum* was not too effective as aqueous and methanolic extracts. Ethanolic extract recorded with the lowest MIC (7.5%) and MBC (3.75%). MIC and MBC value with *Calendula officinalis* aqueous extracts shown minimum MIC (3.75%) for *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa*. In contrast, minimum MBC (1.87%) was recorded for *Staphylococcus aureus*, *Clostridium perfringens*, and *Listeria monocytogenes* (Table 8). Ethanolic extract of *Calendula officinalis* shown minimum MIC and MBC for only one bacterial pathogen, namely, *Pseudomonas aeruginosa* with 3.75% and 1.87%, respectively. The methanolic extract of *Calendula* flowers also showed good MIC and MBC for all tested bacterial pathogens, as shown in Table 8. A lower MIC/MBC value signifies that a minimum amount of flower extract is used, whereas a higher value signifies the use of comparatively more amount of plant extract to control any bacterium (Sharma and Karnwal 2018). These outcomes are also in accordance with Arullappan et al. (2009), Vijayakumar et al. (2018), Ruban and Gajalakshmi (2012) results. In several studies (Nepali et al. 2018; Rios-Chavez et al. 2019; Fayemi et al. 2017), variations in MIC results of *Hibiscus* extract were reported; those could be due to the method of extraction used, the bio-active molecules available in extract, and the bacterial strains used for the study. Also, variability in MIC of various plant extracts can often arise through differences in their phytochemical compounds and the volatile nature of molecules available in extract (Rios-Chavez et al. 2019). In our study, mostly screened extracts of (3.75% v/v) content were effective in controlling the growth of bacterial pathogens. Abudunia et al. (2017) mentioned that for *Salmonella blockley*, *Salmonella aequatoria*, *Salmonella braenderup*, *E. coli* enteropathogens, *E. coli*, *E. coli* ATCC, and *E. coli* MDR, the MIC values of methanol extracts of *C. arvensis* flowers were 12.5–25 µg/mL. However, the hexanolic extract MIC values ranged from 6.25 to 12.5 µg/mL and were bacteriostatic for all tested bacteria, although methanolic and aqueous extracts were bactericidal. Fayemi et al. (2017) confirmed that the development of *Listeria monocytogenes* in the beef burger was inhibited by 2 percent of methanol extract from *C. citrinus* extract.

Cock (2012) mentioned that the methanol extract of *C. citrinus* leaf had been ineffective in preventing the growth of *K. pneumonia* and *E. coli*, whereas the *C. citrinus* flower extract had the antibacterial effect for *K. pneumoniae* only. Few investigators (Nepali et al. 2018; Fayemi et al. 2017; Cock 2012) studied the effectiveness, as antimicrobial agents, of the plant extracts and their potent substances to regulate pathogenic and spoilage-causing bacteria in food.

Table 8 MIC and MBC values of *Calendula officinalis* extract against bacterial pathogens

Gram type	Bacterial isolates	Different concentrations of <i>Calendula officinalis</i> extract against food poisoning causing bacterial isolates with reference to MIC and MBC																	
		Aqueous					Ethanol 95%					Methanol 80%							
		15	7.50	3.75	1.87	MIC	MBC	15	7.50	3.75	1.87	MIC	MBC	15	7.50	3.75	1.87	MIC	MBC
Gram-positive	<i>S. aureus</i> MTCC 87	+	+	+	-	3.75	1.87	+	+	-	-	7.5	3.75	+	+	-	-	3.75	1.87
		+	+	-	-	7.5	3.75	+	+	-	-	7.5	7.5	+	+	-	-	7.5	3.75
	<i>B. cereus</i> MTCC 430	+	+	+	-	3.75	1.87	+	+	-	-	7.5	3.75	+	+	-	-	7.5	7.5
		+	+	+	-	3.75	1.87	+	+	-	-	7.5	3.75	+	+	-	-	7.5	7.5
Gram-negative	<i>L. monocytogenes</i> MTCC 657	+	+	+	-	3.75	1.87	+	+	-	-	7.5	7.5	+	+	-	-	7.5	7.5
		+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75	+	+	-	-	7.5	7.5
Gram-negative	<i>E. coli</i> MTCC 43	+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75	+	+	-	-	7.5	7.5
		+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75	+	+	-	-	7.5	7.5
	<i>S. typhi</i> MTCC 1264	+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75	+	+	-	-	7.5	3.75
+		+	+	-	3.75	1.87	+	+	-	-	3.75	1.87	+	+	-	-	3.75	1.87	

Some studies also indicated that the antimicrobial compounds of plant-derived extracts react with the enzymes and proteins of the microbial cell membrane inducing cell death or inhibiting the enzymes required for the biosynthesis of amino acids. Other investigators referred the inhibitory actions of plant concentrates to their hydrophobic nature, allowing extracts to interact with cellular membrane proteins and mitochondria and disrupt or modify their structures (Tresch et al. 2019; Khairnar et al. 2013; Sharma and Karnwal 2018). The findings of this study suggested that plant (flower) extracts, which are potentially efficient, may be considered natural preservatives to manage food poisoning diseases and protect food by minimizing hazardous chemical preservatives.

Conclusion

Foodborne illness and spoilage were mostly associated with the growth of many pathogenic bacterial strains in food. Mitigating food spoilage in the food processing industries is entirely dependent on the usage of chemical preservatives. The harmful impact of such synthetic chemicals on human health raises the need to find potentially efficient, healthy, and natural food preservatives. The gradual emergence of microbes' resistance to chemical therapeutic agents (mainly antibiotics) has also made it essential to realize suitable alternative and efficient therapeutic agents like herbs. This investigation had the possible value of developing herbal remedies as antimicrobial agents against susceptible bacterial spp. that promoted the potential application of plant (flower) derivatives to prevent food spoilage and foodborne illness. All such active compounds would provide valuable information to identify novel bioactive molecules with better efficiency and more resistance (MDR) to susceptible microorganisms concerned for food-based disease than compared to antibiotics available in the market.

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