

In Vitro Screening of *Ferula Galbaniflua* and *Ocimum Canum* Essential Oils Against of Food-Borne Bacteria

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Abstract: The ever-emerging resistant microorganisms towards multiple antibiotics and many side effects induced by prevalent antibiotics encouraged the exploration of the antimicrobial agents derived from medicinal plants. This study appraises the in vitro antibacterial activity of essential oils extracted from *Ferula galbaniflua* Boiss. (Apiaceae) and *Ocimum canum* Sims (Lamiaceae) against two common food-borne *Bacillus cereus* and *Escherichia coli*. Essential oils were extracted via hydro-distillation using Clevenger's apparatus and tested using disc diffusion, and agar microdilution method according to CLSI guidelines. *F. galbaniflua* oil displayed a zone of inhibition (ZOI) of 32.50 ± 8.42 mm against *B. cereus* and 14.39 ± 2.24 mm against *E. coli*. *O. canum* oil induced inhibition of 15.56 ± 2.74 mm against *B. cereus* and 35.00 ± 5.83 mm against *E. coli* in terms of ZOI. MIC and MBC values further confirmed the efficacy of *F. galbaniflua* against *B. cereus* (MIC: 0.5 mg ml⁻¹, MBC: 1.0 mg ml⁻¹) and *O. canum* against *E. coli* (MIC: 0.25 mg ml⁻¹, MBC: 0.5 mg ml⁻¹). The values are comparable to gentamicin and kanamycin as reference antibiotics in specific cases as *F. galbaniflua* against *B. cereus*, while *O. canum* against *E. coli*. These findings suggest that these essential oils could serve as natural alternatives to combat food-borne infections, highlighting their potential in food preservation and therapeutic applications. Further studies on their chemical constituents and in vivo efficacy are recommended.

Keywords: Essential oils, Antibacterial activity, *Ferula galbaniflua*, *Ocimum canum*, *Bacillus cereus*, *Escherichia coli*, Food-borne pathogens

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Introduction

The medicinal value of aromatic plant products has been recognized since ancient times, forming the cornerstone of traditional healing systems worldwide. According to the World Health Organization (WHO), approximately 75-80% of the global population, particularly in developing countries, relies on herbs and traditional medicines for primary healthcare needs [1,2]. In India, a biodiversity hotspot, over 2,000 medicinal and essential oil-bearing plants have been documented, yet only a fraction has been

thoroughly investigated for their pharmacological properties [3]. This untapped reservoir of natural compounds offers promising alternatives to synthetic pharmaceuticals, especially in the face of rising antibiotic resistance.

Bacillus cereus is a Gram-positive, spore-forming rod responsible for two types of food poisoning: emetic (vomiting) and diarrheal syndromes, often associated with rice and starchy foods. Symptoms appear slowly up to 16 hours post-ingestion, including nausea, vomiting, diarrhoea, and abdominal cramps [4,5,6]. *Escherichia coli*, a Gram-negative rod, causes food-borne infections through contaminated meat, water, or vegetables, leading to diarrhoea, abdominal cramps, haemolytic uremic syndrome, and urinary tract infections [7,8]. Food-borne illnesses, caused by bacteria like; *Bacillus cereus* and *Escherichia coli* affect millions annually, leading to symptoms ranging from mild diarrhoea and urinary tract infections to severe septicaemia [5,8]. Both pathogens exhibit increasing antibiotic resistance, necessitating natural alternatives. Natural plant products possess several advantages over conventional antibiotics, including affordability, widespread availability, minimal side effects, and reduced likelihood of inducing resistance. As bacterial pathogens evolve resistance to multiple drugs, plant-derived antimicrobials provide a sustainable solution by targeting diverse mechanisms, such as cell membrane disruption and enzyme inhibition [9]. With antibiotic resistance complicating treatment, exploring essential oils from traditional plants is crucial.

Ferula galbaniflua Boiss., commonly known as galbanum, belongs to the Apiaceae family and is native to regions including Iran and India. Its oleo-resin, known as 'Gandha-biroza' in local markets, is rich in monoterpenes like β -pinene, α -pinene, δ -3-carene, α -thujene, and myrcene, which contribute to its anti-inflammatory and antimicrobial properties. Traditionally, it has been used as a stimulant, carminative, expectorant, antispasmodic, diuretic, and tonic in Indian Complementary and Alternative Medicine systems. It is recommended for treating chronic bronchitis, asthma, and parasitic skin diseases [10,11].

Ocimum canum Sims, from the Lamiaceae family, is widely distributed in tropical regions, including India. Its essential oil contains major components such as methylchavicol, linalool, cineole, cymene, carvacrol, and camphor, known for their antibacterial efficacy, particularly against Gram-negative bacteria. Traditionally, its usage includes in treatment of respiratory ailments, indigestion, gastrointestinal upset, diarrhoea, and parasitic infections. It is also used as insect repellent [12,13,14].

These both plants yield essential oils in substantial quantities, making them viable for antimicrobial studies. This investigation aims to assess the antibacterial efficacy of *Ferula galbaniflua* and *Ocimum canum* essential oils against *Bacillus cereus* and *Escherichia coli* using standard *in vitro* methods and compare their activity to conventional antibiotics. The study seeks to contribute to the development of natural preservatives for food safety and therapeutic agents against these food borne strains.

Materials and Methods

Plant Material Collection and Essential Oil Extraction

Leaves of *Ocimum canum* were collected from the local areas of Khagaria district, Bihar, India, during the summer season when volatile oil content is optimal. Oleo-resin of *Ferula galbaniflua*, commercially known as 'Gandha-biroza', was procured from a reputable local market in the same region. Plant materials were authenticated based on botanical characteristics as described in standard floras. Essential oils were extracted using hydro-distillation in a Clevenger's apparatus for 4-6 hours. The obtained oils were dried over anhydrous sodium sulphate to remove moisture and stored in airtight, screw-capped vials in a cool, dark, dry place to prevent degradation of volatile compounds.

Test Pathogens and Culture Preparation

Strains of *Bacillus cereus* and *Escherichia coli* were isolated from contaminated food samples following Indian Standards IS:5887 (Part 1:2005 for *E. coli* and Part 6:2012 for *B. cereus*) [15,16]. Isolates were purified and maintained on master slants of Trypticase Soy Agar (TSA) for *B. cereus* and modified Nutrient Agar (NA) for *E. coli*, with sub-culturing every two months to ensure viability for working

cultures. For experiments unless otherwise stated, overnight cultures in Nutrient Broth adjusted to 0.5 McFarland standard, corresponding to approximately 1 to 2×10^8 cfu ml⁻¹ viability were used.

Antibacterial Assays

Disc Diffusion Method

Antibacterial activity was determined using the disc diffusion method as per CLSI guidelines [17] with some modifications. Solidified Mueller-Hinton Agar (MHA) plates (containing 0.5% Tween 80 v/v as surfactant) were spread with 100 µl of standardized bacterial suspension. Two sterile filter paper discs (6 mm diameter) having 5 µl of essential oil were placed on each plate. Reference antibiotics gentamicin (10 µg/disc) and kanamycin (30 µg/disc) were used as positive controls, while Tween 80 soaked discs served as negative controls. Plates were incubated at 37°C for 18-20 hours, and zones of inhibition (ZOI) were measured in millimetres. Experiments were performed in triplicate with four readings each (n=12), and mean ± standard deviation (SD) was calculated.

Minimum Inhibitory Concentration (MIC)

MIC was assessed using the agar microdilution method [18] with some modifications. Serial two-fold dilutions of essential oils (0.25-3.2% w/v consequentially converted in to weight by volume) were prepared in MHA (containing 0.5% Tween 80 v/v as surfactant) and incorporated into Petri plates. Bacterial inoculant (10 µl 0.5 McFarland standard suspension/spot) were spotted at the three pre-marked places on each plate after solidification and incubated at 37°C for 18-20 hours. The lowest concentration showing no visible growth was recorded as MIC. Antibiotics were tested similarly at concentrations suggested by CLSI [18].

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was established by sub-culturing swab from the MIC points exhibiting no growth onto fresh MHA plates without tween 80. This procedure is repeated from all the spots exhibiting negative visible growth that means the MIC concentration onwards up to the highest point of concentration to rule out any false positive or negative phenomenon. All the plates were incubated for 18-20 hours at 37°C, the minimum concentration resulting in no colony development (99.9% kill) was identified as the Minimum Bactericidal Concentration [19].

Statistical Analysis

All values were expressed as the mean of all readings with standard deviation wherever required.

Results

The results section of the study provides empirical data on the antibacterial activity of the essential oils from *Ferula galbaniflua* and *Ocimum canum* against the food-borne pathogens *Bacillus cereus* and *Escherichia coli*. The findings are based on bacterial identification, zone of inhibition (ZOI) measurements from disc diffusion assays, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values.

Bacterial Identification

Table 1 summarizes the morphological, biochemical, fermentation, and cultural characteristics confirming the identity of the test pathogens. The test pathogens were confirmed through a combination of morphological, biochemical, fermentation, and cultural characteristics, aligning with standard protocols for food-borne isolates (e.g., Indian Standards IS:5887). These traits match literature descriptions, confirming the isolates as typical food-borne strains of *B. cereus* as aerobic, Gram-positive rods having mannitol fermenting capability and characteristic pink colony on MYP Agar, while *E. coli* exhibits aerobic Gram-negative rod appearance having lactose fermenting ability with marked yellow colonies on Tergitol 7 Agar. This ensured the strains were authentic representations of common contaminants in food.

Table 1: Morphological, Biochemical and Cultural Characteristics of Bacteria

Characteristic	<i>Bacillus cereus</i>	<i>Escherichia coli</i>
Morphology	Aerobic, Gram-positive, motile large rod in chain	Aerobic, Gram-negative, motile rod
Biochemical	Indole –, MR –, VP +, Catalase +, Acid production +	Indole +, MR +, VP –, H ₂ S –, Urease –
Fermentation	Mannitol with ppt	Lactose with gas
Cultural	Pink colony with surrounding ppt on MYP Agar	Pink colony on McConkey Agar, Yellow colony on Tergitol 7 Agar

Zone of Inhibition

Table 2 presents the mean ZOI values for the essential oils and reference antibiotics. As clear from the experiments, *F. galbaniflua* oil produced a significantly larger ZOI against *B. cereus* (32.50±8.42) compared to both antibiotics, suggesting superior efficacy against Gram-positive bacteria. Conversely, *O. canum* oil showed the largest ZOI against *E. coli* (35.00±5.83), outperforming the antibiotics in this case. High standard deviation values were also observed in case of effective oils against these bacteria. Negative controls (Tween 80) showed no inhibition, confirming the activity was oil-specific.

It is also evident from the table that both oils exhibited the limited activity against the test pathogens. *F. galbaniflua* failed to control *E. coli* in disc diffusion with poor ZOI of 14.39±2.24, while *O. canum* was found ineffective against *B. cereus* in terms of ZOI (15.56±2.74).

Table 2: Zone of Inhibition Values of Oils and Antibiotics against Test Pathogens

Oil/Antibiotics	<i>Bacillus cereus</i> (mm ± SD*)	<i>Escherichia coli</i> (mm ± SD*)
<i>Ferula galbaniflua</i>	32.50 ± 8.42	14.39 ± 2.24
<i>Ocimum canum</i>	15.56 ± 2.74	35.00 ± 5.83
Gentamicin	22.92 ± 1.68	25.16 ± 1.44
Kanamycin	24.39 ± 2.66	28.94 ± 3.12

*Mean value of diameter of inhibition (n = 12) with SD

MIC and MBC Value

The MIC and MBC values of oils and antibiotics are represented in a bar graph (Figure 1). These values align with documented ranges for essential oils targeting analogous diseases. *F. galbaniflua* exhibited the lowest MIC/MBC against *B. cereus* (MIC: 0.5 mg ml⁻¹, MBC: 1.0 mg ml⁻¹), whilst *O. canum* shown the highest potency against *E. coli* (MIC: 0.25 mg ml⁻¹, MBC: 0.5 mg ml⁻¹). It is evident from the above-mentioned value; the oils signify the bactericidal activity as the MBC/MIC ratio is usually equalled twice the MIC value. Compared to reference antibiotics namely; gentamicin and kanamycin, the oils showed comparable or superior activity in specific cases as *F. galbaniflua* against *B. cereus* and *O. canum* against *E. coli*.

Graph 1 also exhibited the inefficacy of *F. galbaniflua* against *E. coli* (MIC 16 mg ml⁻¹ & MBC 32 mg ml⁻¹) and *O. canum* against *B. cereus* (MIC 4 mg ml⁻¹ & MBC 16 mg ml⁻¹) respectively; indicating the narrow spectrum of both the oils in terms of antibacterial property.

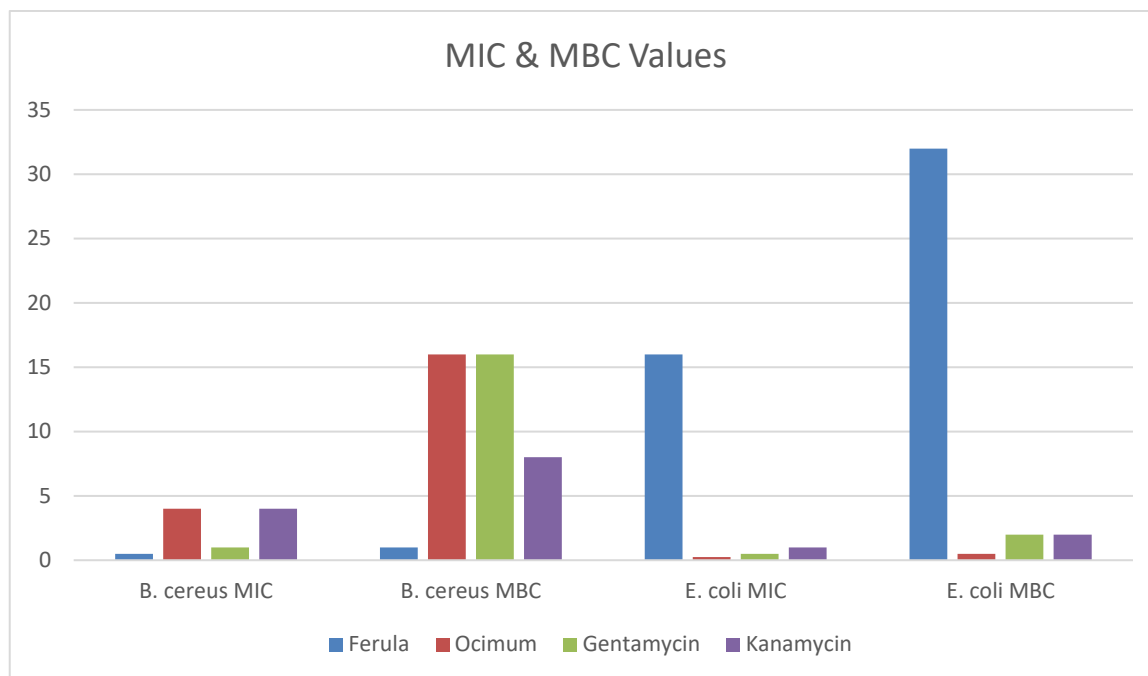


Figure 1: MIC and MBC Values of Essential Oil and Antibiotics against Test Pathogens

Discussion

The results align with prior research as galbanum have shown strong activity against Gram-positive bacteria like MRSA which in pattern of the present study as the oil exhibits good efficacy against Gram positive bacteria [10]. *Ocimum* oils have known broad range antibacterial efficacy. Usually, *O. canum* oil was found effective against many Gram-positive as well as Gram-negative pathogens [12,13,20] but present study shows the efficacy of oil against Gram-negative pathogen like *E. coli* only. The variation might involve the season or environment induced variability in chemical composition [13]. Interestingly, *O. canum* oil was reported to control range of Gram-positive (species of *Staphylococcus*, *Enterococcus*, *Listeria*, *Streptococcus*) and Gram-negative bacteria including *E. coli* but oil cannot control *B. cereus* as like present study [20].

The study highlights a clear specificity: *Ferula galbaniflua* oil was highly effective against Gram-positive *B. cereus* (large ZOI of 32.50 mm, low MIC of 0.5 mg/mL), likely due to its high monoterpene content (β -pinene, α -pinene, δ -3-carene, α -thujene, and myrcene). These compounds are known to disrupt cell membranes, leading to leakage, permeability, and cell death [10,21]. In contrast, *Ocimum canum* oil better performed activity against Gram-negative *E. coli* (ZOI 35.00 mm, MIC 0.25 mg/mL) might be attributed to their major components like as methylchavicol, linalool, carvacrol, and camphor, which can penetrate the outer lipopolysaccharide membrane of Gram-negative bacteria. These findings also clearly authenticate the present and earlier reports in which essential oils have high antimicrobial activity in synergism among components (likewise; terpenes and phenols) as these enhances overall potency against bacteria [21].

Variability in ZOI (high SD) is common in potent essential oils studied (SD value of 8.42 for *F. galbaniflua* vs. *B. cereus*, while SD value of 5.83 for *O. canum* against *E. coli*) may indicate the complex chemical composition and environmental factors induced variation among diffusion rates, volatility, and hydrophobicity. Usually, habitat, harvesting season, and age brought disparity in raw material collection and extraction process may develop batch differences affecting oil composition [13,22]. Use of surfactants were reported to overcome these procedural inconsistencies but they cannot rule out the unpredictability. These induced factors may be rule out through harmonised screening protocols for the standardization of screening breakpoints worldwide.

The above finding delves into new perspectives as *B. cereus* common in starchy foods (e.g., rice) and *E. coli* in contaminated milk, meat and vegetables, these oils could serve as natural preservatives in food packaging or processing to extend shelf life and minimize contamination. Their efficacy against resistant strains addresses global challenges, as per WHO reports on antibiotic resistance. As natural, affordable alternatives with minimal side effects, they meet consumer demands for "clean-label" products. Integrating them could reduce reliance on synthetic antibiotics, supporting sustainable solutions in food safety and therapeutics.

Conclusion

This study confirms the potent antibacterial activity of *F. galbaniflua* and *O. canum* essential oils against food-borne pathogens *B. cereus* and *E. coli*, with specificities aligning with their chemical profiles. These natural agents offer promising, side-effect-free alternatives to antibiotics, supporting their integration into food safety protocols and therapeutic strategies to mitigate antibiotic resistance. By bridging traditional knowledge with modern science, this research underscores the value of India's botanical heritage in addressing global health challenges.

The *in vitro* focus of study limits extrapolation to *in vivo* scenarios, where bioavailability and host factors may alter efficacy. Lack of GC-MS profiling for the specific oil batches used hinders precise attribution to compounds. Variability in natural products (due to geography, season) calls for standardization. Future work should include: chemical analysis via GC-MS, synergy tests with antibiotics, *in vivo* animal models, toxicity assessments, and clinical trials for therapeutic applications. Exploring formulations for food preservation could further validate practical use.

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Conflict of Interest

There is no conflict of interest.

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