



Antimicrobial and Antioxidant Efficacy of Garlic Essential Oil, Fenugreek Essential Oil and their Blends for Potential Applications in Food Industry

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ABSTRACT

This study evaluates the antimicrobial and antioxidant properties of two essential oils i.e. garlic essential oil (GEO) and fenugreek essential oil (FEO), along with their 1:1 blend (BEO), under *in vitro* conditions. Antibacterial effectiveness was measured using agar well diffusion and minimum inhibitory concentration (MIC) methods against four bacterial strains viz. *Staphylococcus aureus* (MTCC 96), *Bacillus cereus* (MTCC 1272), *Escherichia coli* (MTCC 723), and *Salmonella enterica serovar Typhi* (MTCC 733). Antioxidant activity was assessed by determining radical scavenging capacity through ABTS and DPPH assays. GEO demonstrated strong antibacterial activity, with inhibition zones between 12 mm and 27 mm and MIC values ranging from 8500 to 16000 ppm, showing the highest effectiveness against *B. cereus*. FEO exhibited selective antibacterial properties, notably inhibiting gram-negative bacteria like *E. coli* and *S. typhi* but was ineffective against *B. cereus*. BEO displayed improved antibacterial activity, likely due to synergistic interactions between GEO and FEO. While both essential oils demonstrated good antioxidant activity individually, their blend showed enhanced antioxidant capacity, evidenced by DPPH inhibition of 71.77% and ABTS inhibition of 62.44% at the highest concentration tested. In conclusion, the combination of these essential oils exhibits significant antimicrobial and antioxidant properties, indicating potential as a natural preservative in the food industry.

HIGHLIGHTS

- Garlic essential oil possess strong antibacterial activity, however, fenugreek essential oil has lower to moderate activity against tested organisms.
- On blending, the antimicrobial and antioxidant activity enhanced significantly.
- Blended essential oils offer promising potential as natural food preservatives due to synergistic effects.

Keywords: Garlic essential oil, Fenugreek essential oil, blends, DPPH, ABTS, antimicrobial, MIC

Growing concerns about health risks associated with synthetic preservatives have triggered a global shift toward safer and more natural food preservation alternatives. Common artificial preservatives like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ), which are frequently used in food systems, are associated with harmful effects, including DNA damage and carcinogenesis (Dhankhar *et al.*, 2021). This has spurred interest in natural compounds that can maintain food safety without sacrificing quality. Among them, plant-derived essential oils (EOs) are emerging as strong

candidates due to their abundance of bioactive compounds such as terpenes, phenolics and flavonoids. These are valued for their potent antimicrobial, antioxidant and anti-inflammatory properties (Tariq *et al.*, 2019). Essential oils exhibit broad-spectrum antibacterial activity, with their effectiveness often influenced by their chemical composition and other factors like time of harvesting of

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plant etc. Gram-positive bacteria, such as *Staphylococcus aureus* and *Listeria monocytogenes*, along with Gram-negative strains like *Escherichia coli* and *Salmonella typhimurium*, exhibit varying sensitivities to essential oils (Amor *et al.*, 2019; Punya *et al.*, 2019). This characteristic is especially important in meat preservation, where microbial spoilage poses a significant risk. Meat has expanded its role as a vital component in the human food basket and consumer's preference for ready to eat foods including meat products has increased multifold in last few years (Mehta *et al.*, 2013; Mehta *et al.*, 2015). However, the growing concerns regarding the adverse health impact of synthetic preservatives has steered the research towards natural alternatives and essential oils are being projected a most favoured choice. Garlic essential oil (GEO), high in allicin and sulfur-containing compounds, is noted for its antimicrobial and antioxidant properties (Bhatwalkar *et al.*, 2021). Likewise, fenugreek essential oil (FEO), which contains linoleic acid and other bioactive substances, shows similar functional benefits (Munshi *et al.*, 2020; Raza *et al.*, 2022). Utilizing these oils singly has sometimes proven less effective due to varied sensitivity for foodborne pathogens but combining various essential oils may help address these limitations by boosting their efficacy through synergistic effects (Goswami *et al.*, 2022). This study aimed to investigate the antimicrobial and antioxidant properties of garlic and fenugreek essential oils, both individually and in combination, to assess their potential as natural preservatives in meat model system for ensuring food safety, substituting synthetic additives.

MATERIALS AND METHODS

Garlic and Fenugreek essential oil: Source and composition

Garlic essential oil (GEO) was sourced from Moksha Lifestyle Products in New Delhi, India. It was a pale to transparent yellow and had a distinct pungent aroma. The specific gravity ranged from 0.910 to 0.980 at 20°C, while the refractive index ranged between 1.559 and 1.579 at the same temperature. Fenugreek essential oil (FEO) was acquired from Young Chemist Pvt. Ltd. in Gujarat, India. According to the manufacturer's certificate, this oil varied from pale to deep yellow and had a thick consistency. Its specific gravity was 0.900 to 0.960, with a relative density of 0.924 and a refractive index of 1.474 at 20°C.

Bacterial strains, antimicrobial and antioxidant activity estimation

Four pure freeze-dried cultures of Gram-positive bacteria (*Bacillus cereus* MTCC 1272 and *Staphylococcus aureus* MTCC 96) and Gram-negative bacteria (*Escherichia coli* MTCC 723 and *Salmonella Typhi* MTCC 733) were procured from IMTECH, Chandigarh and were revived and maintained at -80°C through routine passaging. The antimicrobial activity of both essential oils and their blends (1:1) was estimated through agar-well diffusion assay as per method followed by Kalembe and Kunicka (2003), with slight modifications. Minimum inhibition concentration (MIC) values were estimated against the tested strains using the method as described by Gulluce *et al.* (2003). The antioxidant potential of GEO, FEO and their blends was evaluated using DPPH and ABTS radical scavenging assays as per methods described by Blois (1958), Yang *et al.* (2010) and Kumar *et al.* (2017).

Statistical analysis

Statistical analysis of data was performed using SPSS-24.0 (SPSS Inc., Chicago, IL USA). All the experiments were repeated thrice (n=3) for each parameter. The statistical significance was determined at 5 % level (P<0.05) using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Antimicrobial activity of Garlic and Fenugreek essential oil and their blends

Antimicrobial activity was assessed using the disc diffusion method against four foodborne pathogens and the results for the antimicrobial potential of garlic oil, fenugreek oil and their blends (1:1) are presented in Figs. 1 and 2. The *in vitro* antimicrobial activity of garlic essential oil exhibited a significant degree of inhibition against all evaluated strains, with zone sizes ranging from 12 mm to 27 mm. Maximum growth inhibition, indicated by the larger diameter, was observed against *Bacillus cereus*, followed by *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. For fenugreek essential oil, maximum growth inhibition as evident by larger diameter was observed against *Escherichia coli* followed by *Staphylococcus aureus* and *Salmonella typhi*; however

for *Bacillus cereus* no inhibition zone was observed. Both essential oils were blended in 1:1 ratio and the results revealed that blends exhibited significant zones of inhibition for all four bacteria tested in present study. It could be due to the fact that mixture of two or more essential oils can increase the diversity of components and result in multiple sites of action. The greatest antibacterial activity of the blended oil was observed against *E. coli* followed by *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhi*. The synergistic effect of essential oils arises from the complementary actions of their chemical compounds, the potential to enhance each other's bioavailability and their ability to target distinct mechanisms of action (Ju *et al.*, 2022). The results showed a strong correlation between zone of inhibition diameter and MIC values.

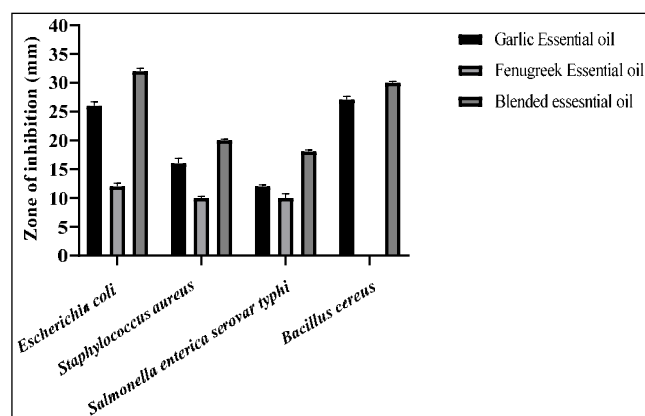


Fig. 1: Zone of inhibition assay (mm) of Garlic and Fenugreek essential oil and their blends (Mean $\pm$ S.E.); n=3

MIC represents the minimal concentration that prevents visible growth of microorganisms. For garlic essential oil it ranged from 8500 ppm to 16000 ppm, indicating effective antibacterial action against both Gram-positive and Gram-negative bacteria. The antimicrobial effect of GEO is attributed to sulphur-containing compounds, particularly allicin, which disrupts lipid biosynthesis and RNA synthesis in microbial cells (Jepson *et al.*, 2016; Yasin *et al.*, 2022). Similar findings were reported by Yasin *et al.* (2022) and Somrani *et al.* (2020), who observed GEO's effectiveness against a wide range of pathogens, including *Listeria monocytogenes*. Manjhi *et al.* (2024) also demonstrated strong antibacterial activity of diallyl sulfide, a major component of GEO, against *B. cereus*, with MIC value of 54 mM. Variations in ZoI and

MIC values between studies may result from differences in essential oil composition, influenced by environmental conditions, harvest time and geographic origin (Kanth *et al.*, 2018; Kumar *et al.*, 2017). A higher concentration of fenugreek essential oil was required for inhibition of tested organisms. A study by Alwan *et al.* (2017) evaluated the antibacterial potential of methanolic seed extract of fenugreek (*T. foenum-graecum*) and reported significant inhibitory activity, against *Escherichia coli* and *Staphylococcus aureus*. Sharma *et al.* (2020) reported that fenugreek essential oils showed 80–100% inhibition against *S. aureus* and 0–20% against *E. coli* at a minimum concentration of 3.125%. For BEO, MIC values ranged from 4000 to 8000 ppm.

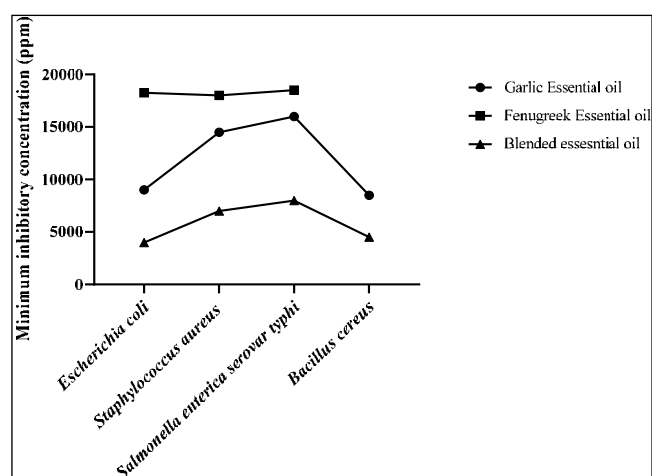


Fig. 2: Minimum Inhibitory concentration (ppm) of Garlic and Fenugreek essential oil and their blends (Mean values); n=3

Individual essential oils, when combined, can exhibit various effects, including indifferent, additive, synergistic or antagonistic outcomes (Burt *et al.*, 2004). Pei *et al.* (2009) found that the combination of certain essential oils exhibited synergistic effects, with the most significant interactions observed between eugenol and cinnamaldehyde, as well as between eugenol and thymol. The minimum inhibitory concentrations (MICs) for eugenol, cinnamaldehyde, thymol and carvacrol were 1600, 400, 400, and 400 mg/L against *E. coli*, respectively. When combined, the MICs of these components decreased to 400, 100, 100, and 100 mg/L, indicating enhanced antimicrobial activity. Similar results regarding the synergistic effect of blended essential oils were evaluated by Gadisa and Usman (2021). They reported significant inhibition zones and low MIC/MBC

values, particularly for blended oils of *Rumex abyssinicus* and *Discopodium penninervium*. In favor of the current findings, Wasim-Akram *et al.* (2024) also reported that the combined outcome of essential oils (*Trachyspermum ammi* and *Anethum sowa*) in the ratio of 1:1 exhibited excellent inhibition against six pathogenic bacterial and fungal strains outperforming individual essential oils. At the highest concentration of 100 µg/mL, the zone of inhibition for *Staphylococcus aureus*, *Bacillus cereus* and *E. coli* was 36.13 mm, 37.13 mm, and 25.9 mm, respectively.

Antioxidant activity of Garlic and Fenugreek essential oil and their blends

Antioxidants are crucial for neutralizing free radicals and preventing oxidative damage in biological systems (Engwa *et al.*, 2022). The antioxidant capacity of garlic essential oil (GEO), fenugreek essential oil (FEO) and their blends (BEO) was assessed through DPPH and ABTS radical scavenging assays, covering a concentration range from 100 to 20,000 ppm (Fig. 3 and 4). DPPH, a stable free radical, shifts from violet to yellow when it interacts with antioxidants that provide hydrogen atoms (Gulcin and Alwasel, 2023). A dose-dependent increase in the radical scavenging activity of GEO, FEO, and BEO was noted. The antioxidant potential of GEO is mainly attributed to its sulfur-containing compounds such as allicin, diallyl disulfide and diallyl trisulfide, alongside selenium and amino acids like cysteine and methionine (Dorrigiv *et al.*, 2020). These bioactive compounds not only neutralize free radicals but also enhance the activity of natural antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase. Importantly, allicin interacts with the free thiol groups of enzymes and captures hydroxyl radicals, further contributing to garlic's potent antioxidant properties (Ashraf *et al.*, 2019). Chung *et al.* (2011) reported an increase in antioxidant activity of garlic essential oil with increasing concentration, confirming a dose-dependent response. For FEO, the percent inhibition ranged from 8.73% to 43% in DPPH assay. This activity is attributed to the presence of antioxidant compounds like palmitic acid and phytol (Mehmood *et al.*, 2017). Akbari *et al.* (2019) reported a similar trend, with FEO showing an IC₅₀ of 172.6 ± 3.1 µg/mL. For GEO, % Radical inhibition in ABTS assay varied from 6.68% to 40.27% and the highest inhibition was observed at the 20,000 ppm

concentration. FEO demonstrated stronger scavenging activity against ABTS radicals than DPPH, likely due to the greater sensitivity and faster kinetics of the ABTS method. Akbari *et al.* (2019) also reported higher ABTS activity for fenugreek oil (IC₅₀ = 161.3 ± 2.21 µg/mL) compared to DPPH.

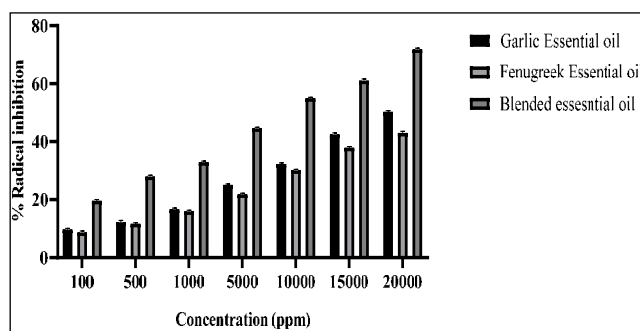


Fig. 3: DPPH Radical scavenging activity (%) of Garlic and Fenugreek essential oil and their blends (Mean ± S.E.); n=3

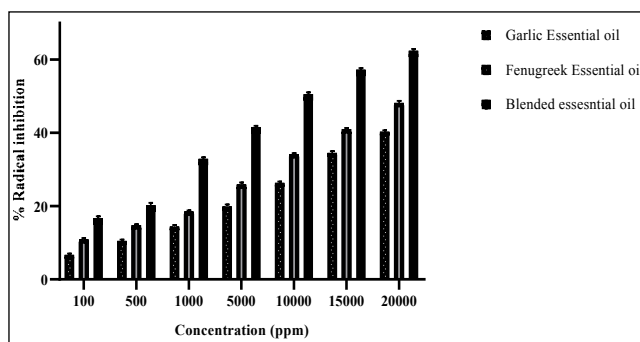


Fig. 4: ABTS Radical scavenging activity (%) of Garlic and Fenugreek essential oil and their blends (Mean ± S.E.); n=3

Consistent with individual oils, the antioxidant activity of a 1:1 blend of garlic and fenugreek essential oils showed a concentration-dependent increase. However, BEO demonstrated superior % radical inhibition compared to individual oils, as indicated by DPPH and ABTS assays. Supporting this, Wasim-Akram *et al.* (2024) found that a blend of *Trachyspermum ammi* and *Anethum sowa* essential oils exhibited enhanced antioxidant activity, reaching up to 84.02% inhibition in the DPPH assay, with IC₅₀ values of 4.69 µg/mL (DPPH), 18.37 µg/mL (ABTS), 16.46 µg/mL (superoxide) and 15.58 µg/mL (H₂O₂ scavenging). Similarly, Bag and Chattopadhyay (2015) noted synergistic antioxidant effects in specific

combinations of herbs and spices, particularly coriander and cumin seed oils, highlighting that certain pairings can boost proton-donating capacity.

CONCLUSION

The present study highlighted the significant antimicrobial and antioxidant potential of garlic and fenugreek essential oils, both individually and in combination. Garlic essential oil demonstrated strong antibacterial activity, particularly against *Bacillus cereus*, whereas fenugreek essential oil was more effective against Gram-negative bacteria, such as *Escherichia coli* and *Salmonella typhi*. Antioxidant assays confirmed the concentration-dependent radical scavenging activity of both oils, with fenugreek showing relatively higher efficacy in ABTS assays. Notably, the blended essential oil exhibited a synergistic effect, resulting in enhanced antibacterial efficacy and superior antioxidant capacity compared to the individual oils. These findings suggest that the combined use of garlic and fenugreek essential oils could serve as a potent natural alternative to synthetic preservatives in food preservation, contributing to improved food safety and shelf-life. Further research is recommended to explore their practical applications in meat and other perishable food products.

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