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IN VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SELECTED INDIAN SPICES AND HERBS

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ABSTRACT

This study investigates the antioxidant and antimicrobial properties of ethanolic extracts and essential oils derived from eight commonly used Indian spices and herbs, including ajwain, fennel, bay leaves, coriander, dill (leaves and seeds), kalonji, and scallions. Phytochemical extraction was performed using Soxhlet and Clevenger methods, and antioxidant activity was assessed via DPPH radical scavenging assay. Antimicrobial efficacy was evaluated using disc diffusion, vapor phase, and microdilution methods against selected bacterial and fungal strains. Results revealed that ethanolic extracts generally exhibited stronger antioxidant activities than their essential oil counterparts, with ajwain and coriander showing the highest radical scavenging potential. Conversely, essential oils demonstrated broader antimicrobial activity, particularly those from fennel and ajwain, which inhibited both bacterial and fungal growth effectively. The vapor phase assay showed heightened sensitivity in fungal strains to essential oils, while MIC values indicated that ethanolic extracts were more efficient at lower concentrations. Ajwain emerged as the most potent spice in both antioxidant and antimicrobial assays, attributed to compounds such as thymol and carvacrol. The findings highlight the potential of these spices and herbs as natural preservatives and therapeutic agents, supporting their integration in food safety, cosmetic formulations, and alternative medicine.

KEYWORDS: Antioxidant activity, antimicrobial activity, Spices, Herbs, ethanolic extract, essential oil.

INTRODUCTION

Natural antimicrobial agents, derived from plant, animal, or microbial sources, are increasingly favored in food preservation for their safety, sustainability, and compatibility with biodegradable materials. Unlike synthetic agents, natural antimicrobials are generally recognized as safe (GRAS), making them more acceptable to both regulatory bodies and consumers.[1,2] Spices and herbs have long been used in traditional medicine and culinary applications due to their flavor-enhancing properties and health benefits. Essential oils and plant extracts, derived from herbs and spices such as oregano, thyme, and cinnamon, are rich in phenolic compounds and terpenes, which exhibit strong antimicrobial properties. These natural compounds disrupt microbial cell membranes, inhibit enzymatic activity, and interfere with energy production pathways. Essential oils are particularly effective against a broad spectrum of pathogens, including *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella spp.*[3,4,5]

The growing demand for clean-label products and eco-friendly solutions is driving the shift toward natural alternatives, supported by advancements in extraction,

encapsulation, and controlled-release technologies. Synthetic antimicrobial agents, especially those derived from non-renewable resources, have significant environmental drawbacks. They are often non-biodegradable, contributing to pollution and ecological imbalances. For example, the persistence of synthetic preservatives in wastewater has been linked to environmental toxicity.[6] Natural agents, on the other hand, are biodegradable and derived from renewable sources, making them more environmentally friendly. Essential oils and plant extracts decompose naturally, reducing their ecological footprint. However, large-scale extraction of natural compounds may raise sustainability concerns, such as overharvesting of plant resources.[7,8] This study focuses on comparing the antioxidant and antimicrobial efficacy of ethanolic extracts and essential oils derived from 8 commonly used Indian spices and herbs.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation of Plant Materials

A total of six spices, including fennel seeds **(***Foeniculum vulgare*), ajwain seeds (*Trachyspermum ammi*), bay leaves (*Laurus nobilis*), dill seeds (*Anethum graveolens*), coriander seeds (*Coriandrum sativum*), and kalonji (*Nigella sativa*), and two herbs: dill leaves (*Anethum graveolens*) and spring onion leaves (*Allium fistulosum*) were selected due to their potential antimicrobial properties. The samples were procured from local markets, cleaned and dried under controlled conditions. **Spices** were dried at 40°C in a hot-air oven for 48 hours to achieve uniform moisture reduction. **Herbs** were dried at a slightly lower temperature (35°C) to preserve volatile compounds and chlorophyll content. Once dried, the samples were ground into a fine powder using a mixer grinder. The powdered plant material was sieved through a 40-mesh sieve to ensure uniform particle size and were subsequently stored in sterile, airtight containers to prevent exposure to moisture or environmental contaminants.

2.2 Preparation of Ethanolic Extracts and Essential Oil Extraction

Phytochemicals were extracted using Soxhlet's distillation method. 100 g of dried powdered samples were extracted using 200 ml of ethanol in Soxhlet apparatus for 48 hours. The crude extracts were concentrated by rotary vacuum flash evaporation, weighed and stored at refrigerated condition. For extraction of essential oils, 500 grams of dried samples in powdered form were subjected to hydrodistillation in a Clevenger apparatus for 4-5 hours. The essential oil was collected, dried under anhydrous sodium sulphate and stored at 4^0C.[9]

2.3 Antioxidant Activity by DPPH assay

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was determined according to Baliyan et al, 2022.[10] To determine the antioxidant activity of the extract, DPPH solution (0.5 mM) was prepared in methanol. Standard calibration curve was plotted using gallic acid (100 µg/ml) as standard antioxidant. Ethanolic extracts and essential oils of the spices were diluted with methanol. For the assay, 100µl of either of the standard gallic acid solution, ethanolic extracts or essential oils at different concentrations was added in the wells of the microtiter plate. 100µl of DPPH solution was then added to each well and the contents were mixed thoroughly. A control sample containing methanol and DPPH was set up for comparison. The microtiter plate was then incubated at room temperature for 30 minutes in dark condition as DPPH is sensitive to light and undergoes degradation. After incubation, the absorbance of the samples was measured at 517 nm using a microplate reader. The antioxidant activity was calculated by determining the percentage of DPPH radical scavenging, using the formula:

DPPH Scavenging Activity(%)=$[A_{control}-A_{sample})/A_{control}]\times100$

where $A_{control}$ is the absorbance of the control sample, and A_{sample} is the absorbance of the sample.

2.4 Determination of Antimicrobial Activity

To determine the antibacterial activity and antifungal activity of the spices and herbs, the standard cultures were obtained from NCIM, Pune. The chosen test cultures included gram-negative bacteria (*Escherichia coli* NCIM 5346), *Salmonella typhimurium* NCIM 2501), gram positive bacteria (*Staphylococcus aureus* NCIM 5720, *Bacillus cereus* NCIM 2217), yeast (*Candida albicans* NCIM 3665) and molds (*Aspergillus niger* NCIM 1269, *Penicillium expansum* NCIM 1349)

2.4.1 Disc Diffusion assay

Disc diffusion method was used to determine the antimicrobial activity of the ethanolic extracts against the standard test organisms as described by Becerril et al, 2007.[3] Extracts were diluted using 10% Dimethyl sulfoxide. Sterile filter paper discs, each with a standard diameter of 6 mm, were impregnated with 20 microlitres of the prepared extract solutions. The discs were aseptically placed onto the surface of Mueller Hinton agar plates. pre-inoculated with the standard test cultures. The agar plates were incubated at 37°C for 24 hours. The zones of inhibition around the discs were measured in millimeters to determine the antimicrobial activity. Tests were performed in duplicates.

2.4.2 Vapour Phase assay

The antimicrobial activity of essential oils was evaluated using a vapour phase diffusion assay as described by Balaguer et al, 2013.[11] The test cultures were inoculated on Mueller and Hinton agar medium. Sterile filter paper disks impregnated with essential oils were placed on the lid of the Petri dish. The Petri dishes were then sealed with sterile adhesive tape and incubated at 37°C for 24 hours. For fungal cultures, the plates were incubated at room temperature for 3-4 days. Presence or absence of

growth on the plate was recorded for the inoculated organisms based on its sensitivity to the essential oils in the test compounds.

2.4.3 MIC determination by broth microdilution

The Minimum Inhibitory Concentration (MIC) of the extracts was determined using the microdilution broth susceptibility assay in a 96- well microtitre plate.[12] 200 µL of Mueller and Hinton broth was added to each well. The extracts were prepared at different concentrations by diluting them with DMSO or distilled water. Specifically, the extract volumes used were 0 µL, 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL across different wells, with corresponding adjustments to the diluent volume to ensure a total volume of 250 µL in each well. 10 µL of the microbial culture was inoculated in each well after the dilution of the extract. Appropriate controls were also set up for the experimental design. The plates were then sealed with parafilm and incubated at 37°C for 24 hours. After incubation, 10 µl of 0.02% resazurin solution, prepared by dissolving 2 mg of resazurin in 10 ml of sterile PBS (pH 7.4), was added to each well. The resazurin acted as a redox indicator, changing color in the presence of living microorganisms. Wells with microbial growth showed a pink color, indicating active metabolism, while those without growth remained blue, indicating the inhibition of microbial activity. The MIC was defined as the lowest concentration of the extract or essential oil that resulted in no visible growth after 24 h of incubation at 37°C.

3. RESULTS AND DISCUSSION

3.1 *Determination of Antioxidant Activity*

Antioxidant activity of ethanolic extracts and essential oils were determined using DPPH assay and Gallic acid as standard. Figure 1 represents the antioxidant activity of ethanolic extracts and essential oils used in this study.

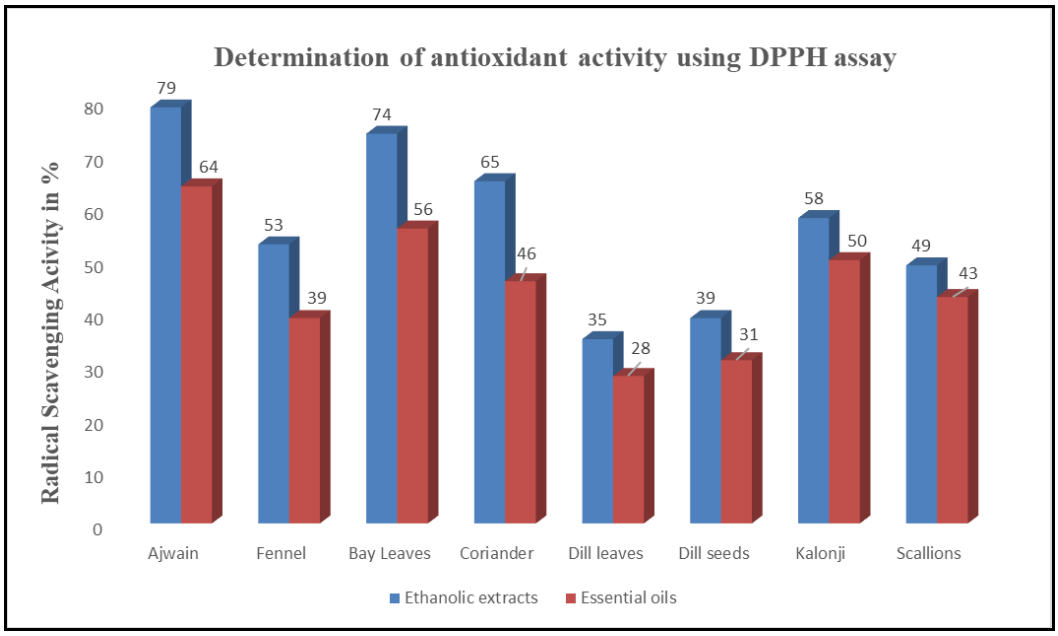

Figure 1: Radical Scavenging Activity of the extracts.

Ajwain demonstrates the highest antioxidant potential, with 79% radical scavenging activity for ethanolic extracts and 64% for essential oils. Similarly, Bay Leaves and Coriander also show significant antioxidant activity, with ethanolic extracts reaching 74% and 65%, respectively, while their essential oils exhibit 56% and 46% activity. Fennel, although moderately effective, displays a lower radical scavenging activity of 53% for ethanolic extracts and 39% for essential oils. On the other hand, Dill leaves and Dill seeds exhibit the lowest antioxidant activity, with ethanolic extracts reaching 35% and 39%, while their essential oils show even lower values of 28% and 31%, respectively. Kalonji and Scallions fall in the mid-range of antioxidant activity, with Kalonji showing 58% for ethanolic extracts and 50% for essential oils, while Scallions demonstrate 49% and 43%, respectively.

The results demonstrated that ethanolic extracts exhibited higher antioxidant activity than essential oils, suggesting that phenolic and flavonoid compounds present in the ethanolic extracts play a crucial role in their radical scavenging ability. Similar studies have reported a strong correlation between phenolic content and antioxidant activity, emphasizing the role of flavonoids and phenolic acids in neutralizing free radicals.[13,14] Further research indicates that spices and herbs with high phenolic content exhibit superior antioxidant properties, making them suitable candidates for natural preservatives and medicinal formulations.[15] Factors such as extraction method, solvent type, and processing conditions influence the antioxidant activity of spices and herbs. Ethanol and methanol extract often show higher DPPH radical scavenging activity compared to aqueous extracts due to better solubility of polyphenols in organic solvents.[16] Furthermore,

cultivation, storage conditions and thermal processing may alter the antioxidant potency of these natural products.[17,18]

3.2 *Determination of Antimicrobial Activity*

3.2.1 Disc Diffusion Assay

The inhibitory effects of the ethanolic extracts of the spices and herbs were tested against the standard test organisms and the bacterial and fungal isolates using disc diffusion method.

Table 1 represents the diameter of the inhibition zones for each extract against the standard test organisms. No inhibition was observed for the control alcohol and DMSO discs. The results indicate that the ethanolic extracts of ajwain, bay leaves and coriander seeds are most effective in inhibiting the growth of both bacteria and fungi as evident from the mean diameter of the inhibition zones. Fennel ethanolic extracts were found to be least inhibitory. The ethanolic extracts of dill seeds, dill leaves, kalonji and scallion were found to have moderate inhibitory activity against the tested organisms. The bacterial strains *E.coli, S.typhi.* are seen to exhibit resistance to most of the ethanolic extracts whereas *S.aureus* and *B.cereus* are sensitive to most of extracts. Yeasts are more sensitive than molds. The standard strain *P.expansum* and *A.niger* show resistance to all the extracts.

Table 1: Antimicrobial activity of the ethanolic extracts expressed as diameter of inhibition zones in mm.

	Diameter of Inhibition Zones in mm							
Test organism	**Ajwain**	**Fennel**	**Bay Leaves**	**Coriander seed**	**Dill leaves**	**Dill seeds**	**Kalonji**	**Scallions**
E.coli NCIM5346	17 ± 2	-	12 ± 1	19 ± 1	-	-	-	-
S.typhi NCIM 2501	10 ± 1	-	10 ± 1	6 ± 2	-	-	-	-
S.aureus NCIM 5720	38 ± 1	-	15 ± 1	26 ± 1	-	8 ± 2	14 ± 1	10 ± 2
B.cereus NCIM 2217	33 ± 2	-	8 ± 1	18 ± 2	8 ± 1	6 ± 1	6 ± 2	10 ± 1
Candida albicans NCIM 3665	28 ± 1	-	12 ± 2	11 ± 1	-	-	-	10 ± 1
Penicillium expansum NCIM 1348	-	-	-	-	-	-	-	-
Aspergillus niger NCIM1269	12± 1	-	-	-	-	-	-	-

The inhibitory potential of ajwain, bay leaves and coriander ethanolic extracts were analyzed statistically using unpaired t-test as mean diameter of the inhibition zones of these extracts demonstrated maximum inhibition for most of the test organisms including bacteria and fungi. However, when used alone, these extracts do not have a significant effect on bacteria and fungi ($p>0.05$). The combination of ajwain and bay leaves ethanolic extract or ajwain with coriander ethanolic extract will have a significant effect against the bacteria and fungi ($p<0.05$). A marginally significant effect between the combination of bay leaves and coriander leaves is expected ($p=0.05$).

Among the tested extracts, ajwain exhibited the highest antimicrobial activity, with inhibitory effect against both bacteria and fungi. This could be attributed to the presence of bioactive compounds such as thymol and carvacrol, which have well-documented antibacterial properties.[19,20] Similarly, coriander seeds showed strong antimicrobial effects, likely due to the presence of essential oil linalool, a potent antimicrobial agent.[21,22]

Fennel, Dill seeds and Bay Leaves also demonstrated moderate antibacterial activity, particularly against Gram-negative bacteria, suggesting the presence of secondary metabolites that interfere with bacterial cell wall synthesis or metabolic pathways.[23, 24, 25] In contrast, Scallions exhibited relatively lower antibacterial potential, indicating that their bioactive components might be more effective in different concentrations or extraction solvents.[26] The antifungal effects may be attributed to the presence of phenolic compounds and essential oils, which are known to disrupt fungal cell membranes and inhibit spore germination.[22,27]

3.2.2 Vapour phase assay

The vapor phase assay was performed to evaluate the antimicrobial activity of volatile compounds in essential oils. Table 2 summarizes the antimicrobial activity of essential oils of the spices and herbs against the various test organisms.

The results of the vapour phase assay indicate that organisms show variable response to essential oils of different spices and herbs. Fennel essential oil is found to be the most effective antimicrobial oil as it shows broad inhibition of bacterial and fungal spp. Ajwain, coriander seed, dill seed and kalonji essential oils have a varying effect on organisms as they show a mixture of sensitivity and resistance response from organisms. Bay leaves, Dill leaves and Scallion essential oils are relatively ineffective as most of the tested organisms exhibit resistance to these oils.

Amongst the bacterial organisms tested, *E.coli, S.aureus* and *B.cereus* are sensitive to most of the essential oils tested, whereas *S.typhi* exhibit resistance to almost all the oils tested. Fungal species are seen to be more sensitive to the essential oils used than bacterial species, showing

mixed response to the oils. *P.expansum* and *C.albicans* show very high sensitivity to most of these essential oils.

Table 2: Antimicrobial activity of essential oils.

Test organism	Ajwain	Fennel	Bay Leaves	Coriander seeds	Dill leaves	Dill seeds	Kalonji	Scallions
E.coli NCIM5346	S	R	R	S	R	R	R	R
S.typhi NCIM 2501	R	R	R	R	R	R	R	R
S.aureus NCIM 5720	S	S	S	S	R	R	S	R
B.cereus NCIM 2217	S	R	S	S	S	R	R	R
C.albicans NCIM 3665	S	S	S	S	R	S	R	R
P. expansum NCIM 1348	R	S	S	S	S	S	S	S
A. niger NCIM 1269	S	R	S	S	R	S	R	R

Key: S- Sensitive to essential oil, R- Resistant to essential oil

Essential oils of dill, coriander, ajwain, and cinnamon have been consistently reported to have strong antibacterial and antifungal properties.[25, 28,29] In a study carried out by Delaquis et al in 2002,[25] using dill, celery, eucalyptus, and coriander; celery oil exhibited the strongest antibacterial efficacy. It has been demonstrated that the antibacterial activity of fennel essential oils exhibits high degree of variation when tested against *Aspergillus flavus, Candida albicans, Bacillus cereus,* and *Staphylococcus aureus*.[30] Numerous investigations on essential oil blends have shown that different mixtures of essential oils have synergistic antibacterial effects.[31,32]

Certain components of essential oils have been shown to disrupt the lipids in cell membranes, resulting in intracellular material leakage and, eventually, cell lysis.[31,33] Some essential oils contain the phenolic compounds carvacrol, thymol, anethole, eugenol, linalool, pinene as main constituents with membrane disrupting ability. Monoterpenes and phenolics in essential oils are also antifungal in nature as they interfere with the germination of spores and affect the mycotoxin production of molds.[5,34]

3.2.3: MIC determination using broth microdilution

Tables 3 and 4 represent the MIC values of ethanolic extracts and essential oils of spices and herbs against the test microorganisms. Both ethanolic extracts and essential oils from different spices and herbs are found to be efficient against a variety of bacteria and fungi. The results suggest that ethanolic extracts are more efficient than essential oils at preventing the growth of most of the test organisms. The ethanolic extracts exhibit greater efficacy at lower concentrations, with ajwain, bay leaves and fennel being the most potent extracts which suppress growth for numerous species, including *S. aureus, S. typhi, and E. coli,* at 20 µL (Figure 2.26). Coriander extract is also found to be effective particularly against *E. coli, S. typhi, and S. aureus* at lower concentrations. However, essential oils are less effective than their corresponding ethanolic extracts, requiring larger concentrations (30–50 µL) to have comparable antibacterial properties. The dill leaves and dill seeds need larger amounts to exhibit any inhibitory action; these extracts are consistently efficient against a variety of bacteria. Kalonji and Scallions are reported to be the least effective in both forms. *B. cereus* also have been shown to exhibit sensitivity to both the ethanolic extracts and essential oils of various spices and herbs. *S. typhi* show resistance to both the ethanolic extracts and essential oils. Amongst fungi, *A. niger* and *P. expansum* have consistently been reported as resistant to both the ethanolic extracts and essential oils.

Table 3: MIC of ethanolic extracts against the test microorganisms in µl.

Test organism	Ajwain	Fennel	Bay Leaves	Coriander	Dill leaves	Dill seeds	Kalonji	Scallions
E.coli NCIM5346	S	20	S	20	R	30	R	R
S.typhi NCIM 2501	S	30	S	20	R	20	R	R
S.aureus NCIM 5720	S	20	S	S	R	R	R	R
B.cereus NCIM 2217	S	20	S	S	R	20	R	R
C.albicans NCIM 3665	S	20	S	S	R	20	R	R
P. expansum NCIM 1348	S	20	20	30	R	20	R	R
A. niger NCIM1269	S	20	S	10	R	20	R	R

Key: S- Sensitive- No growth in the lowest concentration added (10 microlitre),
R- Resistant- Growth upto 50 microlitres of extract

Table 4: MIC of essential oils against the test microorganisms in µl.

Test organism	Ajwain	Fennel	Bay Leaves	Coriander	Dill leaves	Dill seeds	Kalonji	Scallions
E.coli NCIM5346	S	R	S	S	50	R	R	R
S.typhi NCIM 2501	S	R	S	S	50	R	R	R
S.aureus NCIM 5720	S	R	S	S	50	R	R	R
B.cereus NCIM 2217	S	R	S	S	50	R	R	R
C.albicans NCIM 3665	S	R	S	S	S	S	R	R
P. expansum NCIM 1348	S	R	S	S	50	S	R	R
A. niger NCIM1269	S	R	S	S	50	S	R	R

Key: S- Sensitive- No growth in the lowest concentration added (10 microlitre),
R- Resistant- Growth upto 50 microlitres of extract

The results align with previous findings that essential oils and ethanolic extracts from a variety of herbs and spices have strong antimicrobial activity against a broad spectrum of fungi and bacteria.[12,35] Roby et al, 2013[30] showed that the essential oil and the organic solvent extracts of fennel and chamomile were effective against *Bacillus* and *Aspergillus spp.* Moreover, Kedia et al, 2015[28] reported that ajwain essential oil exhibited a strong antimicrobial activity due to the presence of a large amount of cymene, thymol, and terpines known to be effective on most of pathogenic strains. MICs for effective extracts ranged from 4.2 µl/ml to 5 mg/mL, depending on the microorganism and extract.[27,36] Some extracts, such as those from *Laurus nobilis*, exhibited higher antifungal activity than standard antifungal agents.[27] Generally, essential oils demonstrated greater antimicrobial potency compared to ethanolic extracts.[36] However, methanolic and ethanolic extracts of oregano displayed better antioxidant and free radical scavenging properties, respectively.[37] Clove and thyme ethanolic extract also demonstrated strong antimicrobial activity against various microorganisms, with clove extract showing greater potency.[38,39] These findings suggest that both essential oils and ethanolic extracts possess valuable antimicrobial properties, with varying efficacy depending on the plant source, extraction methods and target microorganisms.

4. CONCLUSION

This study highlights the significant antioxidant and antimicrobial activities of both ethanolic extracts and essential oils from selected spices and herbs. Spices such as Ajwain and Coriander seeds exhibited strong radical scavenging and antimicrobial activity, likely due to their high content of bioactive volatile compounds like thymol and thymoquinone. While ethanolic extracts offer robust antioxidant potential, essential oils exhibit stronger antimicrobial efficacy. These findings highlight the importance of spices and herbs as sources of natural antioxidants and antimicrobial agents with potential health benefits and applications in the food, cosmetic, and therapeutic industries as natural preservatives and additives. These natural substances hold promise as bioactive agents in food preservation and pharmaceutical formulations, contributing to improved shelf-life and safety of products.

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