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Research Article

Comparative Antibacterial Activity of *Syzygium aromaticum* and *Cinnamomum verum* Against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

The present study was conducted to comparatively evaluate the antibacterial activity of *Syzygium aromaticum* (clove) and *Cinnamomum verum* (cinnamon) against *Escherichia coli* and *Staphylococcus aureus*. Aqueous and ethanolic extracts of both medicinal plants were prepared and tested using disc diffusion assay and Minimum Inhibitory Concentration (MIC) analysis. The results demonstrated that both clove and cinnamon extracts exhibited antibacterial activity against the tested bacterial strains. However, clove extracts showed comparatively stronger antibacterial effects than cinnamon extracts. Among all the samples, the aqueous extract of clove at 10 mg/mL concentration produced the highest zone of inhibition against *E. coli*. The antibacterial activity of all extracts increased with increasing concentration, indicating concentration-dependent inhibition. MIC analysis further confirmed that clove extracts exhibited greater bacterial growth suppression compared to cinnamon extracts. Comparative analysis also revealed that E. coli was more susceptible to the plant extracts than *S. aureus*. Gas Liquid Chromatography (GLC) analysis confirmed the presence of eugenol as the major bioactive compound in clove and cinnamaldehyde in cinnamon, which are responsible for their antimicrobial properties. The study suggests that clove and cinnamon possess potential as natural antibacterial agents for pharmaceutical and food preservation applications.

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INTRODUCTION

Medicinal plants have been widely utilized in traditional healthcare systems because of their diverse pharmacological properties and rich phytochemical composition. Natural products derived from spices and herbs have gained considerable scientific attention due to their antimicrobial, antioxidant, anti-inflammatory, and therapeutic activities1. Among various medicinal spices, *Syzygium aromaticum* (clove) and *Cinnamomum verum* (cinnamon) are extensively used in traditional medicine, food preservation, and pharmaceutical preparations because of their biologically active constituents.

Syzygium aromaticum, commonly known as clove, belongs to the family Myrtaceae and is recognized for its high content of eugenol, a phenolic compound responsible for its characteristic aroma and antimicrobial activity. Eugenol has been reported to exhibit strong antibacterial properties through disruption of bacterial cell membranes, inhibition of enzyme activity, and interference with cellular metabolism (2). In addition to antibacterial activity, clove possesses antioxidant and preservative properties, making it an important medicinal spice in pharmaceutical and food industries (3).

Cinnamomum verum belonging to the family Lauraceae, is another important medicinal spice widely known for its therapeutic and preservative applications. Cinnamon contains cinnamaldehyde, flavonoids, tannins, and essential oils that contribute to its biological activities. Previous studies have demonstrated that cinnamaldehyde possesses significant antimicrobial properties by altering membrane permeability and inhibiting bacterial growth (4). Cinnamon extracts have also been reported to exhibit antioxidant and antibacterial effects against several bacterial species (5).

Comparative evaluation of medicinal plants is important for identifying more effective natural antibacterial agents and understanding variations in phytochemical activity. Although clove and cinnamon have individually been investigated for their antibacterial properties, limited studies have comparatively analyzed their aqueous and ethanolic extracts under identical laboratory conditions. Furthermore, solvent extraction plays a crucial role in determining the recovery of active phytochemicals, which may influence antibacterial efficacy (6).

Therefore, the present study was designed to comparatively evaluate the antibacterial activity of aqueous and ethanolic extracts of *Syzygium aromaticum* and *Cinnamomum verum* against Escherichia coli and Staphylococcus aureus using disc diffusion assay and Minimum Inhibitory Concentration (MIC) analysis. Hence the

main objectives of the study were as follows.

• Comparatively evaluate the antibacterial activity of aqueous and ethanolic extracts of *Syzygium aromaticum* and *Cinnamomum verum* against Escherichia coli and Staphylococcus aureus using disc diffusion assay and MIC analysis.

• Identify and compare the major bioactive phytochemical constituents responsible for antibacterial activity through Gas Liquid Chromatography (GLC) analysis.

MATERIALS AND METHODS

Collection and authentication of Plant Materials

Dried flower buds of *Syzygium aromaticum* and bark of *Cinnamomum verum* were procured from local spice markets of Brahmapur, Odisha. The plant materials were authenticated at Department of Botany, Khallikote Unitary University, Berhampur, Odisha. The plant materials were cleaned, shade dried, and ground into coarse powder using a mechanical grinder.

Preparation of Plant Extracts

Aqueous and ethanolic extracts of clove and cinnamon were prepared using solvent extraction method. About 10 g of coarse powdered plant materials were mixed with 100 mL of distilled water and ethanol separately. The mixtures were kept under continuous shaking for 72 hours at room temperature. The extracts were filtered using Whatman No.1 filter paper and concentrated using rotary evaporation. The concentrated extracts were stored at 4°C in a desiccator until further use.

Test microorganisms

The bacterial strains used in the present study were Escherichia coli (MTCC-443) and Staphylococcus aureus (MTCC-1430). Pure cultures were obtained from the Regional Institute of Biotechnology (RIB), Brahmapur, and sub-cultured onto solidified nutrient agar medium using a sterile inoculation loop and incubated at optimum temperature for growth. The cultures were sub-cultured every 2–3 days to maintain sterility and active growth.

Preparation of Culture Media

Nutrient agar and nutrient broth media were prepared and sterilized by autoclaving at 121°C for 15 minutes. The sterilized media were cooled and poured aseptically into sterile Petri plates for antibacterial assay.

Preparation of Bacterial Inoculum

Fresh bacterial inoculum was prepared by inoculating bacterial colonies into sterile nutrient broth followed by incubation at 37°C for 18–24 hours. The turbidity of bacterial suspension was adjusted to match McFarland standard for uniform inoculation.

Preparation of Antibiotic Discs

Sterile paper discs were prepared from thick chromatography paper and sterilized

using 70% ethanol followed by ultraviolet (UV) irradiation inside a laminar airflow chamber. Uniform circular discs were punched out aseptically using a sterile paper puncher. Crude extracts of clove and cinnamon were prepared at a stock concentration of 100 mg/mL. Working concentrations of 10 mg/mL and 5 mg/mL were prepared separately for both aqueous and ethanolic extracts using sterile distilled water and ethanol respectively. The sterile paper discs were immersed in the prepared extract solutions for approximately 15 minutes to ensure proper impregnation. After soaking, the discs were removed aseptically using sterile forceps and dried inside the laminar airflow chamber before use in antibacterial assay.

Antibacterial Activity by Disc Diffusion Method

The antibacterial activity of aqueous and ethanolic extracts of *Syzygium aromaticum* and *Cinnamomum verum* was evaluated using disc diffusion assay by pour plate technique. Sterile molten nutrient agar medium was inoculated with bacterial cultures and poured aseptically into sterile Petri plates. After solidification of the medium, sterile filter paper discs impregnated with plant extracts were carefully placed on the agar surface using sterile forceps. Standard antibiotic discs were used as positive control, while solvent-treated discs served as negative control. The plates were incubated at 37°C for 24 hours, and antibacterial activity was determined by measuring the diameter of zones of inhibition.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of plant extracts was determined using standard serial broth dilution method. The first test tube contained 10 mL sterile nutrient broth, while subsequent tubes contained 9 mL broth. Stock extract (100 mg/mL) was diluted to prepare 10 mg/mL working solution. Serial two-fold dilutions were carried out to obtain concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, and 0.312 mg/mL. Each tube was inoculated with standardized bacterial suspension and incubated at 37°C for 24 hours. Following incubation, bacterial growth was assessed by measuring optical density (OD) at 600 nm using a spectrophotometer. The MIC was considered as the lowest concentration showing no visible growth or significant reduction in turbidity compared to the control.

Gas Liquid Chromatography (GLC) Analysis

GLC analysis was performed to identify the major phytochemical constituents present in clove and cinnamon extracts. The chromatographic peaks obtained were analyzed to identify major bioactive compounds responsible for antibacterial

activity, including eugenol in clove and cinnamaldehyde in cinnamon.

RESULTS

Zone of Inhibition against E coli

The aqueous extract of *Syzygium aromaticum* at 10 mg/mL concentration exhibited the highest antibacterial activity against Escherichia coli with a zone of inhibition of 1.5 cm, whereas the 5 mg/mL concentration showed comparatively lower inhibitory activity. Ethanolic extract of clove also demonstrated significant antibacterial activity against E. coli at both concentrations. In comparison, aqueous and ethanolic extracts of *Cinnamomum verum* exhibited lower zones of inhibition against Escherichia coli than clove extracts. The standard antibiotic control, Vancomycin, showed the maximum antibacterial activity in cinnamon extract.

Zone of Inhibition against straphylococus aureus

Against Staphylococcus aureus, the aqueous extract of clove produced inhibition zones of 1.0 cm and 1.3 cm at 5 mg/mL and 10 mg/mL concentrations respectively. Ethanolic clove extract demonstrated inhibition zones of 0.9 cm and 1.1 cm at 5 mg/mL and 10 mg/mL concentrations respectively. The aqueous extract of cinnamon exhibited inhibition zones of 0.3 cm and 0.5 cm at 5 mg/mL and 10 mg/mL concentrations respectively against Staphylococcus aureus. Similarly, the ethanolic extract of cinnamon showed inhibition zones of 0.5 cm and 0.7 cm at 5 mg/mL and 10 mg/mL concentrations respectively. Norfloxacin exhibited the highest antibacterial activity against Staphylococcus aureus in cinnamon extract.

Table 1: Zone of Inhibition of aqueous extracts against *Escherichia coli*

Concentration	Clove extract (Aqueous)	Cinnamon extract (Aqueous)
5mg	10mm	4mm
10mg	15mm	5mm
Vancomycin	12mm	11mm

Table 2: Zone of Inhibition of ethanolic extracts against *Escherichia coli*

Concentration	Clove extract (Ethanol)	Cinnamon extract (Ethanol)
5mg	9mm	2mm
10mg	12mm	3mm
Vancomycin	12mm	11mm

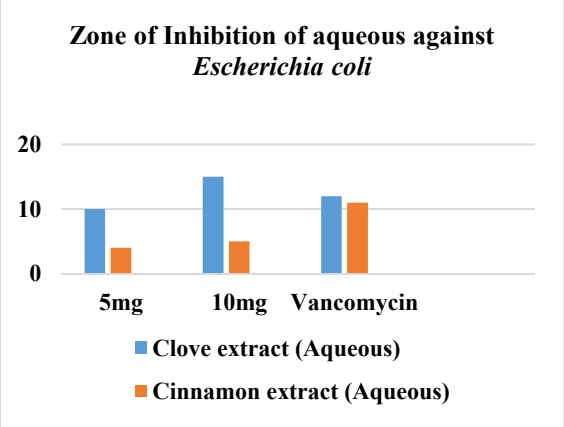


Figure 1: Zone of Inhibition of aqueous extracts against *Escherichia coli*

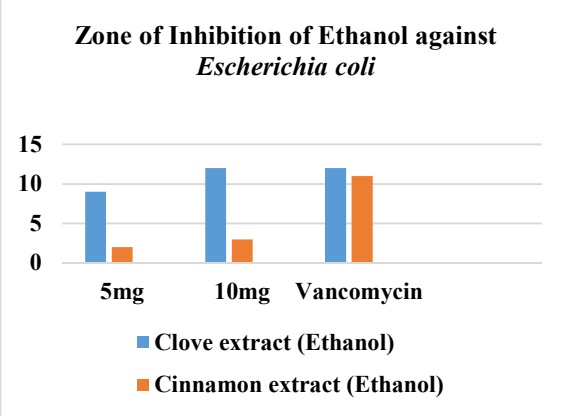


Figure 2: Zone of Inhibition of ethanolic extracts against *Escherichia coli*

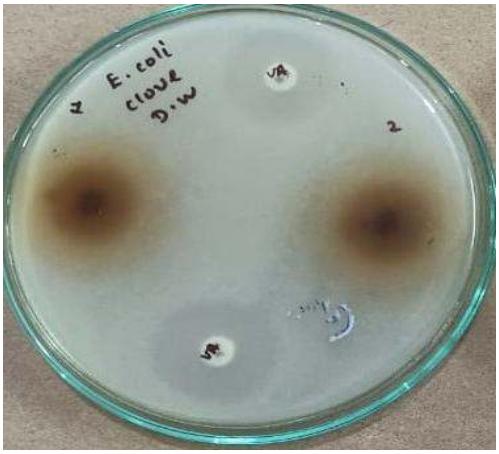


Figure 3: Zone of Inhibition of aqueous extract of clove against *Escherichia coli*

Figure 4: Zone of Inhibition of aqueous extract of cinnamon against *Escherichia coli*

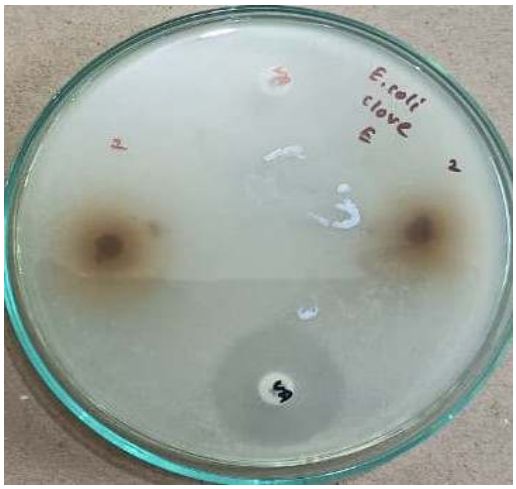


Figure 5: Zone of Inhibition of ethanolic extract of clove against *Escherichia coli*

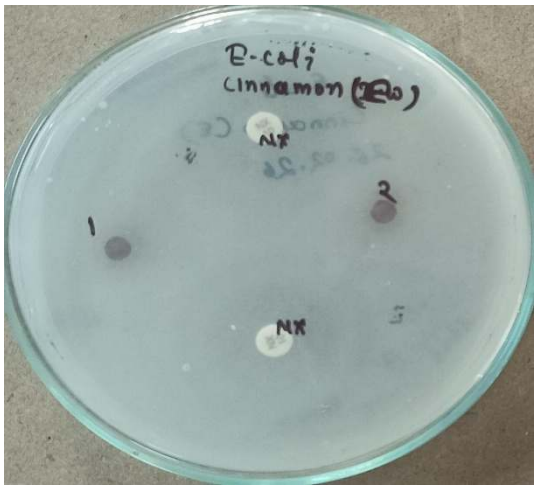


Figure 6: Zone of Inhibition of ethanolic extract of Cinnamon against *Escherichia coli*

Table 3: Zone of Inhibition of aqueous extracts against *Straphylococus aureus*

Concentration	Clove extract (Aqueous)	Cinnamon extract (Aqueous)
5mg	11mm	4mm
10mg	13mm	5mm
Norfloxacin	13mm	12mm

Table 4: Zone of Inhibition of ethanolic extracts against *Straphylococus aureus*

Concentration	Clove extract (Ethanol)	Cinnamon extract (Ethanol)
5mg	10mm	2mm
10mg	12mm	2mm
Norfloxacin	13mm	12mm

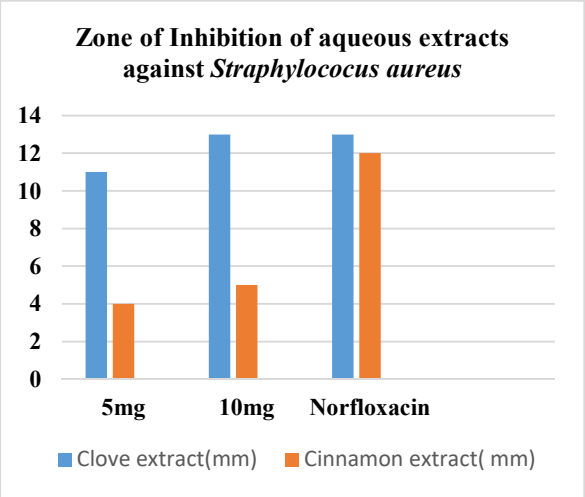


Figure 7: Zone of Inhibition of aqueous extracts against *Straphylococus aureus*

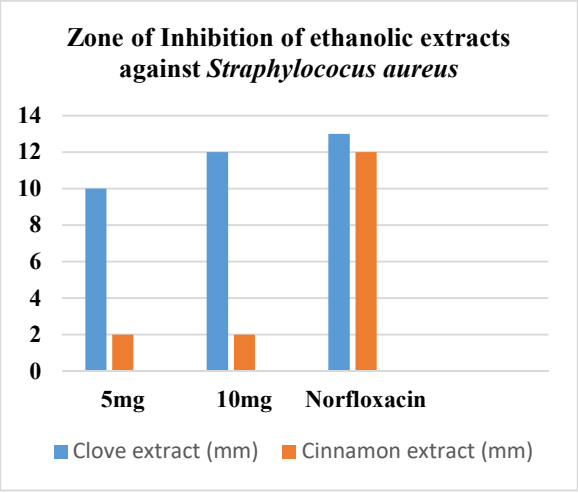


Figure 8: Zone of Inhibition of ethanolic extracts against *Straphylococus aureus*

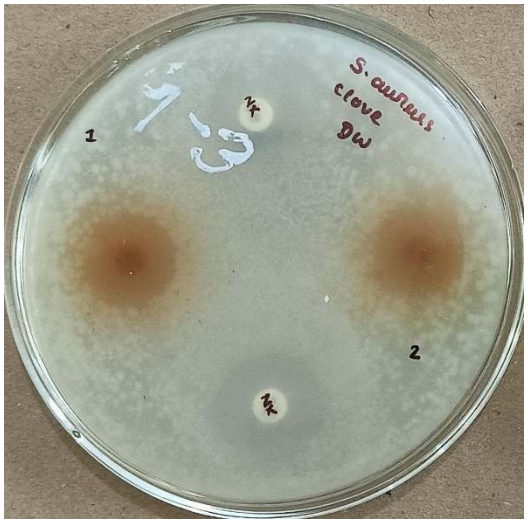


Figure 9: Zone of Inhibition of aqueous extract of clove against *Straphylococus aureus*

Figure 10: Zone of Inhibition of aqueous extract of cinnamon against *Straphylococus aureus*

Figure 11: Zone of Inhibition of ethanolic extract of clove against *Straphylococus aureus*

Figure 12: Zone of Inhibition of ethanolic extract of cinnamon against *Straphylococus aureus*

Comparative evaluation demonstrated that *Syzygium aromaticum* exhibited stronger antibacterial activity than *Cinnamomum verum* against both Escherichia coli and Staphylococcus aureus. Aqueous extracts of both medicinal plants showed comparatively greater antibacterial efficacy than ethanolic extracts, indicating better extraction of bioactive phytochemical constituents in distilled water. The antibacterial activity of all extracts increased with increasing concentration from 5 mg/mL to 10 mg/mL. Among the tested bacterial strains, Escherichia coli showed comparatively higher susceptibility to the plant extracts than Staphylococcus aureus.

Minimum Inhibitory Concentration The Minimum Inhibitory Concentration (MIC) analysis demonstrated that ethanolic extracts of both clove and cinnamon exhibited greater bacterial growth inhibition compared to aqueous extracts. Optical density (OD) values measured at 600 nm showed reduced bacterial growth with increasing extract concentration. Clove extracts exhibited lower optical density values than cinnamon extracts against both Escherichia coli and Staphylococcus aureus, indicating comparatively stronger antibacterial efficacy. The inhibitory effect was concentration dependent, with higher extract concentrations showing greater bacterial growth suppression.

Table:5 Minimum Inhibitory Concentration of aqueous extracts on *Escherichia coli*

Concentration	Clove extract	Cinnamon extract
10	0.76	0.69
5	0.64	0.81
2.5	0.78	0.67
1.25	0.76	0.68
0.62	0.55	0.67
0.31	0.36	0.71

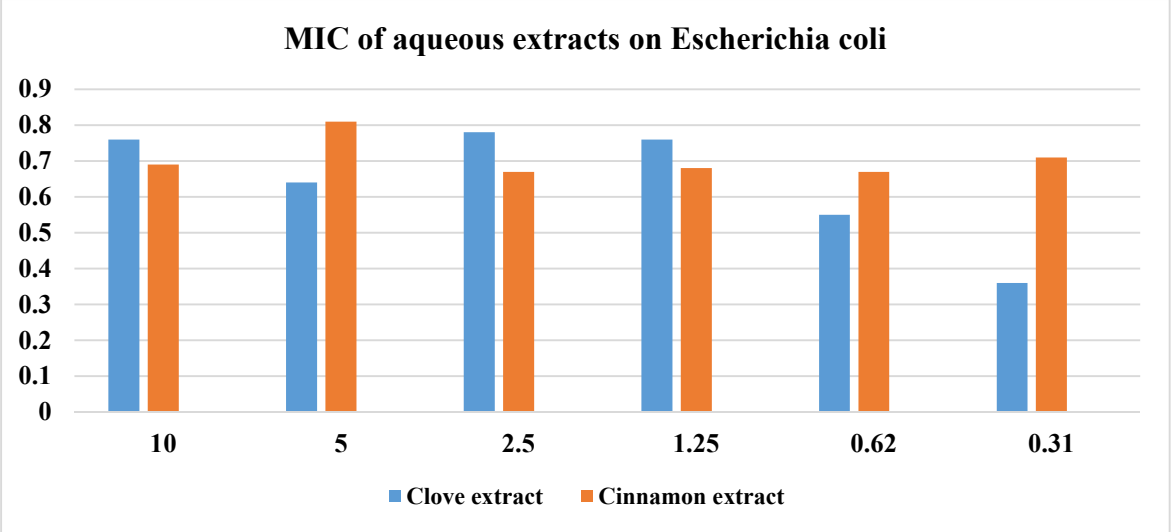


Figure 13: Minimum Inhibitory Concentration of aqueous extracts on *Escherichia coli*

Table:6 Minimum Inhibitory Concentration of ethanolic extracts on *Escherichia coli*

Concentration	Clove extract	Cinnamon extract
10	0.35	0.76
5	0.59	0.68
2.5	0.35	0.76
1.25	0.49	0.75
0.62	0.45	0.63
0.31	0.36	0.56

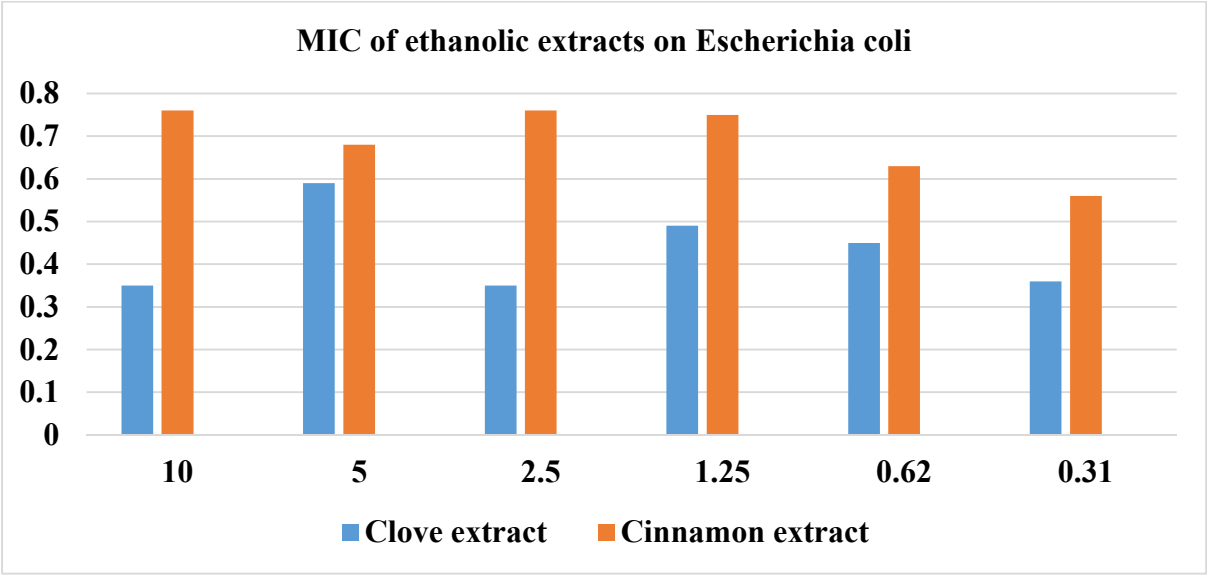


Figure 14: Minimum Inhibitory Concentration of ethanolic extracts on *Escherichia coli*

Table 7: Minimum Inhibitory Concentration of aqueous extracts on *Straphylococus aureus*

Concentration	Clove extract	Cinnamon extract
10	0.86	1.09
5	0.79	0.94
2.5	0.39	0.70
1.25	0.74	0.80
0.62	0.40	0.71
0.31	0.34	0.74

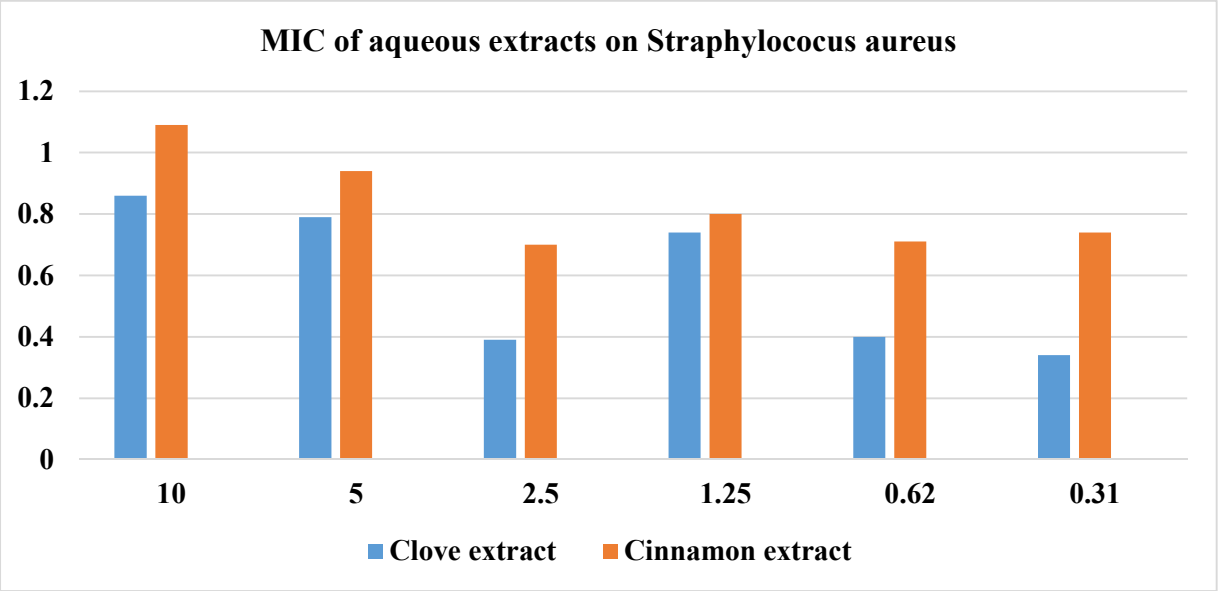


Figure 15: Minimum Inhibitory Concentration of aqueous extracts on *Straphylococus aureus*

Table 8: Minimum Inhibitory Concentration of ethanolic extracts on *Straphylococus aureus*

Concentration	Clove extract	Cinnamon extract
10	1.69	0.89
5	0.57	0.70
2.5	0.32	0.71
1.25	0.38	0.84
0.62	0.37	0.60
0.31	0.31	0.57

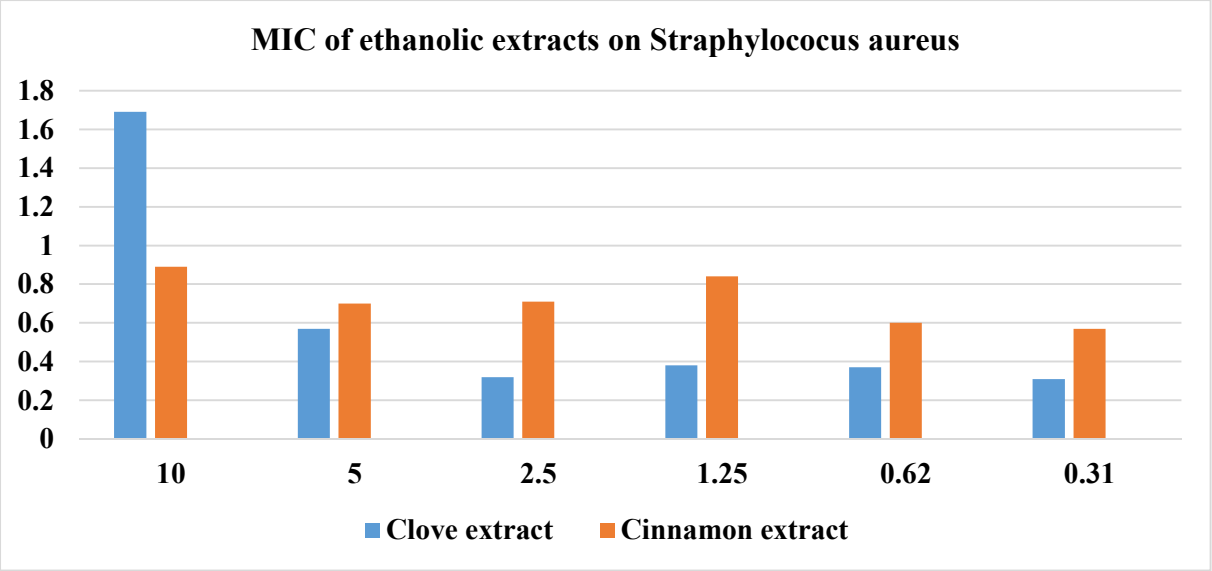
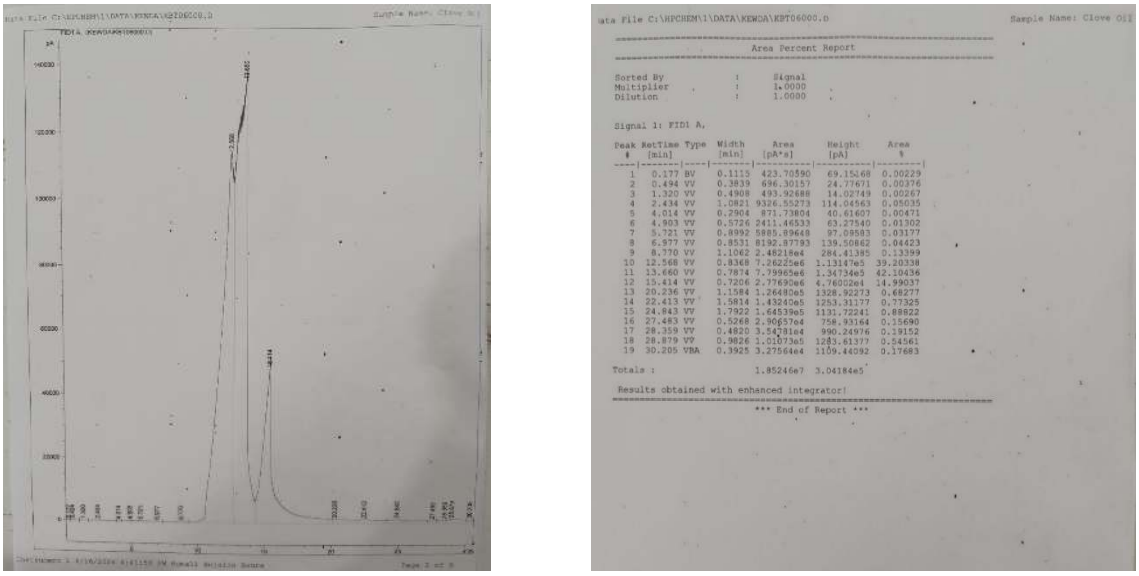


Figure 16: Minimum Inhibitory Concentration of ethanolic extracts on *Straphylococus aureus*

Gas Liquid Chromatography Analysis

Gas Liquid Chromatography (GLC) analysis confirmed the presence of major phytochemical constituents in both medicinal plants. Eugenol was identified as the predominant bioactive compound in clove extract, whereas cinnamaldehyde was identified as the major compound present in cinnamon extract. The chromatographic analysis suggested that the antibacterial activity observed in the present study may be associated with these bioactive phytochemical constituents. The higher antibacterial activity of clove extracts compared to cinnamon extracts may be attributed to the comparatively stronger antimicrobial action of eugenol.



Data File C:\HPCHEM\1\DATA\KEWDA\KBT06000.D Sample Name: Clove Oil

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

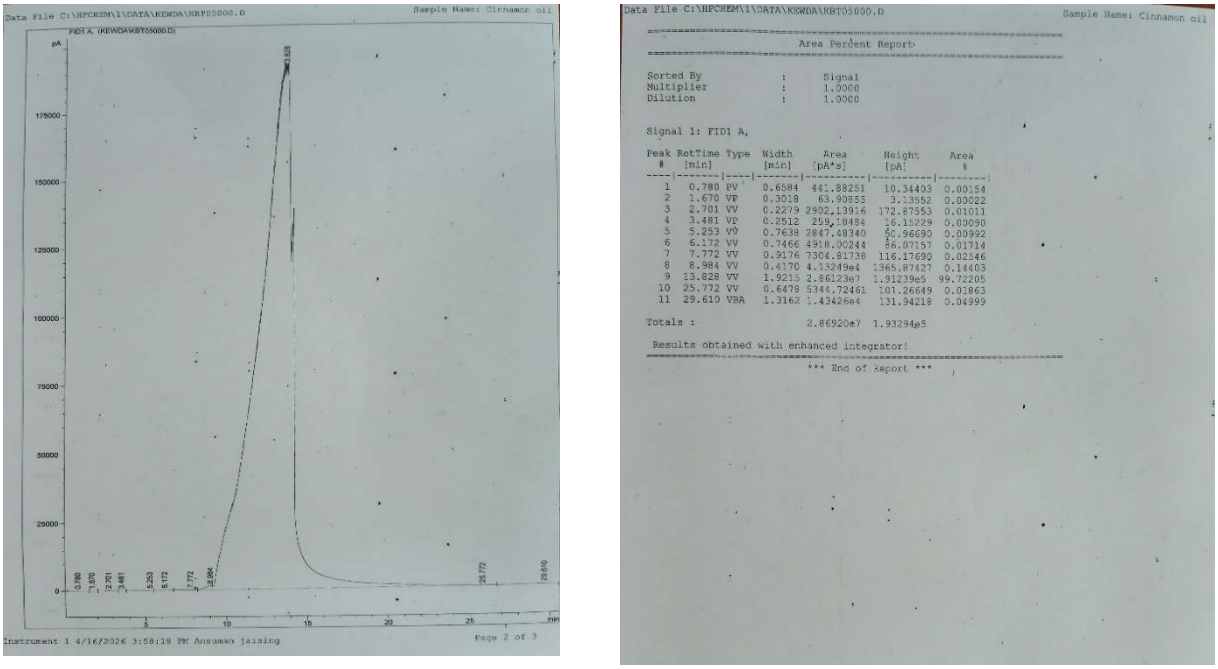
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	0.177	BV	0.1115	423.70590	69.15168	0.00229
2	0.494	VV	0.3839	696.30157	24.77671	0.00376
3	1.320	VV	0.4908	493.92688	14.02749	0.00267
4	2.434	VV	1.0821	9326.55273	114.04563	0.05035
5	4.014	VV	0.2904	871.73804	40.61607	0.00471
6	4.903	VV	0.5726	2411.46533	63.27540	0.01302
7	5.721	VV	0.8992	5885.89648	97.09583	0.03177
8	6.977	VV	0.8531	8192.87793	139.50862	0.04423
9	8.770	VV	1.1062	2.48218e4	284.41385	0.13399
10	12.568	VV	0.8368	7.26225e6	1.13147e5	39.20338
11	13.660	VV	0.7874	7.79965e6	1.34734e5	42.10436
12	15.414	VV	0.7206	2.77690e6	4.76002e4	14.99037
13	20.236	VV	1.1584	1.26480e5	1328.92273	0.68277
14	22.413	VV	1.5814	1.43240e5	1253.31177	0.77325
15	24.843	VV	1.7922	1.64539e5	1131.72241	0.88822
16	27.483	VV	0.5268	2.90657e4	758.93164	0.15690
17	28.359	VV	0.4820	3.54781e4	990.24976	0.19152
18	28.879	VV	0.9826	1.01073e5	1283.61377	0.54561
19	30.205	VBA	0.3925	3.27564e4	1109.44092	0.17683

Totals : 1.85246e7 3.04184e5

Results obtained with enhanced integrator!

*** End of Report ***

Figure 17: GLC Analysis of Clove extract



Data File C:\HPCHEM\1\DATA\KEWDA\KBT05000.D Sample Name: Cinnamon oil

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	0.780	PV	0.6584	441.88251	10.34403	0.00154
2	1.670	VB	0.3018	63.90855	3.13552	0.00022
3	2.701	VV	0.2279	2902.13916	172.87353	0.01011
4	3.481	VB	0.2512	259.18484	16.15229	0.00090
5	5.253	VV	0.7638	2847.48340	60.96690	0.00992
6	6.172	VV	0.7466	4918.00244	86.07157	0.01714
7	7.772	VV	0.9176	7304.81738	116.17690	0.02546
8	8.984	VV	0.4170	4.13249e4	1365.87427	0.14403
9	13.828	VV	1.9215	2.86123e7	1.91239e5	99.72205
10	25.772	VV	0.6479	5344.72461	101.26649	0.01863
11	29.610	VBA	1.3162	1.43426e4	131.94218	0.04999

Totals : 2.86920e7 1.93294e5

Results obtained with enhanced integrator!

*** End of Report ***

Figure 18: GLC Analysis of Cinnamon extract

DISCUSSION

Medicinal plants and aromatic spices have been widely investigated for their pharmacological and antimicrobial properties due to the presence of biologically active phytochemicals such as phenolics, flavonoids, tannins, and essential oils. Plant-derived antimicrobial compounds have gained considerable scientific interest because of their natural origin and broad-spectrum biological activities (1). Several studies have

evaluated the antibacterial properties of clove extracts and cinnamon extracts against both Gram-positive and Gram-negative bacteria.

Syzygium aromaticum is an important medicinal spice belonging to the family Myrtaceae and is widely recognized for its antimicrobial, antioxidant, anti-inflammatory, and preservative properties. The major bioactive compound present in clove is eugenol, which has been reported to possess strong antibacterial activity through disruption of bacterial cell membranes and inhibition of enzyme systems (3).

Clove essential oil has been reported to exhibit significant antibacterial activity against Staphylococcus aureus due to the presence of eugenol, which disrupts bacterial membrane integrity and interferes with cellular metabolism (2).

Several studies evaluating plant essential oils demonstrated that clove oil possesses strong inhibitory activity against both Escherichia coli and Staphylococcus aureus, suggesting its broad-spectrum antibacterial potential (6).

Eugenol derivatives isolated from clove have also been shown to possess antibacterial and antioxidant properties, indicating the therapeutic significance of phenolic compounds present in clove extracts (7).

Cinnamomum verum, belonging to the family Lauraceae, is another important medicinal spice extensively used in traditional medicine and food preservation. Cinnamon contains cinnamaldehyde as its principal bioactive compound along with flavonoids, tannins, and volatile oils (8).

Cinnamon oil and cinnamaldehyde have been reported to exhibit effective antibacterial activity against various pathogenic bacterial strains through disruption of membrane permeability and inhibition of essential metabolic pathways (4).

The antimicrobial mechanisms of cinnamaldehyde against foodborne and pathogenic bacteria have been extensively studied, and cinnamon-derived compounds have been suggested as potential natural antimicrobial agents for pharmaceutical and food preservation applications (9).

Studies comparing extraction solvents have demonstrated that ethanolic extracts of cinnamon exhibit stronger antibacterial activity than aqueous extracts due to improved extraction of phenolics and essential oils (5).

Essential oils containing phenolic compounds such as eugenol and cinnamaldehyde have been reported to alter bacterial membrane structure and cause leakage of intracellular components, leading to bacterial growth inhibition (10).

The biological activities of essential oils are strongly influenced by phytochemical composition and extraction methods, which significantly affect their antimicrobial efficacy against microorganisms (3).

Both clove and cinnamon essential oils have demonstrated inhibitory activity against antibiotic-resistant bacterial strains, including extended-spectrum β-lactamase-producing bacteria (11).

Although numerous studies have independently investigated the antibacterial properties of clove and cinnamon, limited comparative studies have simultaneously evaluated their aqueous and ethanolic extracts against both Gram-positive and Gram-negative bacteria under identical laboratory conditions. Therefore, comparative analysis of these medicinal plants remains important for identifying more effective natural antibacterial agents.

The present study confirmed that both *Syzygium aromaticum* and *Cinnamomum verum* possess significant antibacterial activity against Escherichia coli and Staphylococcus aureus. Comparative analysis showed that clove extracts exhibited stronger antibacterial activity than cinnamon extracts in both aqueous and ethanolic forms. The aqueous extract of clove showed the highest zone of inhibition, particularly against E. coli, indicating greater susceptibility of this bacterium to the plant extracts. The antibacterial activity of all extracts increased with increasing concentration, demonstrating concentration-dependent inhibition. MIC analysis further supported the antibacterial potential of the extracts, with clove showing better inhibitory effects compared to cinnamon. GLC analysis confirmed the presence of important bioactive compounds such as eugenol in clove and cinnamaldehyde in cinnamon, which are mainly responsible for their antimicrobial properties. Overall, the findings suggest that clove and cinnamon can serve as effective natural antibacterial agents and may have potential applications in pharmaceutical preparations, traditional medicine, and food preservation systems. Further research on purification of active compounds and detailed mechanism of action may help in developing plant-based antimicrobial drugs.

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