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Comparative Evaluation of The Antimicrobial Activity of Clove and Cinnamon Using Minimum Inhibitory Concentration (MIC) Analysis: A Statistical and Experimental Study

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Abstract - **This study aimed to find the MIC of clove and cinnamon antimicrobial activity by measuring the optical density. Experiments were performed using varying concentrations (10, 20, and 30mg/ml) and at different times (0h, 24h), then analysed using descriptive statistics, independent T-test, two-way ANOVA, correlation, and a multiple linear regression model. Results show that the growth of microbes decreased significantly depending on the dosage; the higher the concentration, the lower the OD. The clove had a large effect size as it had much stronger antimicrobial activity against microbes than cinnamon at 24h ($p=0.002$, Cohen's $d=1.26$). Concentration had a significant negative impact on OD ($p<0.001$), while the highest impact on micro-organism growth was from the time ($R^2=0.68$). By using FTIR analysis, the major active functional groups of phenols and aldehydes, which are common in eugenol and cinnamaldehyde, were present in the samples. In conclusion, the data indicate high-quality antimicrobial activity by clove, and that both statistical and experimental methods were combined for strong antimicrobial activity.**

Keywords: **Clove, Cinnamon, MIC (Minimum Inhibitory concentration), Antimicrobial**

I. INTRODUCTION

Increasing rates of antimicrobial resistance are now of great global concern, as they have decreased the effectiveness of conventional antibiotics and required searching for alternative therapeutics. Broad-spectrum antimicrobial compounds, originating from nature, especially essential oils of spices, are of wide publicity because of their lower toxicity. The clove and cinnamon have already received a lot of attention in literature, especially their active compounds like eugenol and cinnamaldehyde, which showed significant inhibitory effects toward some pathogenic microorganisms [1]. These phytochemicals disrupt microbial cell membranes and enzymatic activity, eventually hindering microbial growth, and are promising natural antimicrobial formulations.

It has been established that clove and cinnamon essential oils have significant antibacterial effects against various pathogens that cause food-borne diseases and clinical pathogens. For example, the antimicrobial activity of such oils has been assessed using methodologies such as disc diffusion and agar dilution, and the results have shown that there is a difference in the level of inhibition with different concentrations and microbial strain [2].

In addition to the effects of each of the two herbs, the combined effect of clove and cinnamon has also been studied, and the results indicate that the use of the two herbs is synergistic or antagonistic, depending on the experimental conditions [3]. Studies show that essential oils in blends can exhibit synergy and, in addition, can be effective antimicrobials for each other in terms of complementation effects, while other oils, when in a mixture, can reduce the effectiveness of antimicrobials [3].

Evidence obtained implies that statistical analysis needs to be undertaken in order to fully determine the effectiveness of the antimicrobial. Due to different experimental methods adopted by researchers during investigation, such as the method of extraction used, the type of microorganisms, and the conditions that have

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been utilized, consistent statistical methods are required to interpret data received from various experiments. Even though the effectiveness of clove and cinnamon as antimicrobials has already been studied, more studies can be carried out following an evidence-based approach, which will also involve empirical evidence and the use of more advanced statistics, which will explain the better efficiency of these natural products to be used in medicine, biotechnology, and as preservatives in the food processing industry.

Therefore, the purpose of the present research is to conduct a comparative analysis of the antimicrobial activities of clove and cinnamon by means of the MIC test and appropriate statistical methods. This study will utilize experimental data to determine the antimicrobial potential with a more reliable and reproducible judgment by employing descriptive and inferential statistical methods.

This study provides a novel integration of MIC-based antimicrobial evaluation with advanced statistical modelling and FTIR spectroscopic validation to offer a comprehensive and data-driven comparison of clove and cinnamon extracts.

A. Overview of Antimicrobial Resistance and Need for Natural Alternatives

Antimicrobial resistance (AMR) has become a major global health issue, considerably decreasing the efficacy of traditional antibiotics and increasing the chances of failure in the treatment of infectious diseases. The high rate at which microbial strains evolve towards resistance is greatly explained by the excessive use and abuse of antibiotics in clinical, agricultural, and veterinary practices. Consequently, most infections that were manageable in the past are becoming difficult to control, resulting in prolonged illness, expensive healthcare services, and mortality. The increasing weight of AMR has led to unanimous demands to develop alternative therapeutic options to deal with resistant pathogens [4.5].

To overcome this crisis, there has been growing interest in the research of natural products, especially plant-derived compounds, as alternatives to chemical antimicrobial agents. Healthcare practices conventionally utilized since ancient times have included the utilization of medicinal plants, and the focus on these plants is renewed because of the variety of bioactive components available in them and their minimal potential to develop resistance. Organic compounds are known to contain several mechanisms that make the development of resistance to synthetic drugs unlikely [6]. Besides, these organic products are considered non-toxic, biodegradable, and eco-friendly, offering the possibility of developing sustainable antimicrobial products [7].

Plants rich in aromatic oils have gained much popularity because of their organic nature and potent antimicrobial properties. It is the presence of several compounds such as phenolic acids, terpenes, and aldehydes that accounts for their efficacy against bacteria, fungi, and viruses.

Scientists have found evidence that essential oils can affect microbes by disrupting cell membranes, altering cell membrane permeability, inhibiting enzymatic and metabolic processes, and ultimately killing microorganisms [8]. Moreover, the ability of essential oils to act on different targets within the cell renders them effective against multidrug-resistant microbes as well.

Innovations in antimicrobial research have led scientists to consider the importance of using plant-based medicines in modern medicine to overcome the limitations of conventional antibiotics. In addition, approaches involving phytochemicals, probiotics, and antimicrobial peptides are under exploration to reduce reliance on synthetic medicines and avoid developing resistance [9]. In this regard, spices, including cloves and cinnamon, have emerged as potent sources of antimicrobial compounds. Generally, the increasing threat of antimicrobial resistance has led to some concerns about finding effective and safe strategies. Plant-derived essential oils are potential candidates since they exhibit excellent antimicrobial activity with a reduced likelihood of resistance development.

This has led to an increase in research on their quantitative assessment, especially by means of minimum inhibitory concentration (MIC) analysis, to determine their efficacy and possible uses in both clinical and industrial practices.

II. Role of Essential Oils in Antimicrobial Activity

Aromatic plant and spice essential oils have received much interest because of their strong antimicrobial effects and possible use as medicines, food preservatives, and pharmaceutical ingredients. These oils are intricate blends

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of volatile bioactive compounds (terpenoids, phenolics, and aromatic aldehydes) that contribute to their wide-spectrum antibacterial and antifungal activities. Essential oils are effective in preventing the growth of a large variety of microorganisms, including Gram-positive and Gram-negative bacteria and fungi, which is why they are promising replacements for synthetic antimicrobial agents in the future as a natural option [10,11].

Antimicrobial activities of essential oils can be described through their interference with cellular structures and microorganism functioning. Essential oils enter the lipid bilayer of the microbial membranes, increasing their permeability and causing leakage of the content and consequently cell death. Also, essential oils affect the enzymatic processes, protein synthesis, and metabolic energy, thus preventing microbe proliferation [12]. Multiple active agents contained in essential oils allow acting simultaneously on different target sites of cells, reducing the potential for developing antibiotic resistance.

Many techniques were applied for studying the antimicrobial activity of essential oils. The most widespread screening techniques included disc diffusion and agar dilution, which provided qualitative or semi-quantitative results depending on the zone of inhibition. However, quantitative techniques like MIC and MBC determinations were more reliable. The MIC value was defined as the minimal amount of an antibacterial agent capable of inhibiting visibly growing organisms, a widely used standardized method [13]. In turn, MBC allows examining the bactericidal effect, indicating the minimal amount needed for microbe killing. Essential oils have also been researched recently for their capability of improving food safety and increasing its shelf life through the prevention of microorganisms causing food spoilage and diseases. Essential oils have both antimicrobial and antioxidant properties, which help prevent the development of microorganisms and oxidation of foods [14]. However, problems related to volatility, strong odor, and complexity in structure exist; therefore, more research is needed.

To sum up, essential oils belong to one of the promising categories of natural antimicrobials, having various modes of action and applications. The effectiveness of essential oils, as compared with the most widespread methods such as MIC and MBC, is proof that they may become sustainable substitutes for the classical antibiotics. To understand and implement the antimicrobial properties of these substances, more research needs to be done.

A. Clove's (Syzygium aromaticum) Antimicrobial Properties

Clove (*Syzygium aromaticum*) can be considered one of the most potent natural antimicrobial remedies of all medicinal spices, as it is full of highly active phytochemicals. Among its major components is phenol, whose active ingredient - eugenol – is responsible for its antimicrobial properties. According to the literature, extracts from cloves possess numerous inhibitory capabilities when dealing with various microbes, including pathogenic microorganisms in foods and clinically important bacterial strains [15]. The increasing scientific attention towards clove was fueled not only by its potential but also by its ability to replace synthetic antimicrobial remedies, due to resistance to antibiotics being a growing problem nowadays.

Antimicrobial activity of cloves is largely attributed to the actions of eugenol and other phenolic constituents, which exert an effect on microbial membranes and alter the permeability. This causes the leak out of intracellular contents such as proteins, nucleic acids, ions, and cell death is triggered. Phenolic constituents in cloves not only exert action on enzymes but also on the synthesis of energy in microbes, inhibiting their growth and reproduction process [16]. Active constituents in cloves are many, and they act synergistically, leading to a much more efficient action than any one constituent alone. Various experimental investigations suggest that clove extract and oils possess antibacterial activity against both gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*. For instance, aqueous and ethanol extracts of clove showed a large zone of inhibition and a low MIC value, indicating antimicrobial activity of clove extract [17]. From these reports, one can justify the use of cloves in medicines, pharmacy, and in the business of food preservation.

In some recent work, there are studies performed on the antibacterial activities of cloves under practical conditions to preserve foods and extend their shelf life. For example, clove essential oil was found to prevent contamination of microbial contaminants on the meat and food products, thereby extending shelf life and reducing contamination risk [18]. At the same time, the studies on new technology, such as encapsulation, have been employed to enhance the stability of the oil and apply the antimicrobial action more effectively in the practical conditions [19].

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This is a type of innovative method that resolves the problem of volatilities and odor that restricted the use of the substance.

The MIC, the Minimum Inhibitory Concentration, is one of the most common methods for quantitative determination of the antibacterial activity of clove extracts and oils. The results of MIC of clove reported in the literature also demonstrate that this is a potent substance with excellent effects even at low doses [20]. Hence, this shows that it is very important to study the activity of clove against bacteria and other organisms for its role as an alternative treatment compared with other essential oils based on spices, such as cinnamon.

Conclusion: In conclusion, cloves are potent natural antimicrobials that can operate through several mechanisms and possess a broad spectrum of action. The rich presence of eugenol with synergies from its phytochemical compounds leads to a strong antimicrobial and antifungal effect. Their role as an alternative to commercial antimicrobials has also been supported by a growing number of studies conducted using well-defined techniques like MIC.

B. Antimicrobial Properties of Cinnamon (Cinnamomum verum)

Cinnamon (*Cinnamomum verum*) is a powerful natural antimicrobial agent that has been researched for its antimicrobial potential towards various pathogenic microbes. Cinnamaldehyde is the primary active agent in cinnamon responsible for antimicrobial activity, along with other components like eugenol, cinnamic acid, etc.

These compounds are also the ones responsible for their potential therapeutic agents and food preservatives [21,22]. It has shown better antimicrobial activity over many natural antimicrobials with relatively lower minimum inhibitory concentration (MIC) values and a broader antimicrobial spectrum.

Cinnamon has multiple modes of action that damage the structure and functions of microorganisms, involving disruption of the cell membrane, overpermeability of the cell membrane, leakage of cellular content, and disruption of enzymes, protein synthesis, and metabolic processes [23], and thus helps inhibit the growth and survival of microbes. Such a multi-targeted mechanism of action helps in improved performance of cinnamon and development of reduced chances of resistance towards it. Besides antibacterial activity, cinnamon is also well recognized for its considerable antifungal activity against Candida and Aspergillus.

The antifungal activity indicates its wide-spectrum antimicrobial nature and applicability in medicine and industry [24]. Besides, cinnamon essential oil is effectively applied in preserving foods from microbial contamination and increasing their shelf-life due to the presence of good antimicrobial and antioxidant properties.

In the process of experimentation, comparative studies have revealed numerous times that the antimicrobial potency of cinnamon is higher compared to that of other plants' extracts, such as cloves. This characteristic appears to be especially evident in the case of analysis by means of the MIC method, when cinnamon oil shows lower inhibitory concentrations. All these features make cinnamon an interesting object for discussion in the context of antimicrobial research, when cinnamon oil is compared to other spices' essential oils.

Generally speaking, cinnamon (*Cinnamomum verum*) can be regarded as an exceptionally potent natural antimicrobial substance with high applicability as a treatment substance in the case of microbes. It possesses the capability of killing bacteria and fungi, low MIC concentration, and efficiency against resistant strains, thus making it extremely important in current antimicrobial studies. Analyzed via standardized methods like the MIC test, cinnamon proves itself to be extremely effective and, therefore, constitutes one of the key elements of comparative antimicrobial research.

C. Comparative Studies on Clove and Cinnamon

Comparative clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum verum*) have been extensively studied in terms of the antimicrobial properties of their strong bioactive compounds and broad biological effects. Both spices are rich in phenolic compounds, with predominant eugenol in clove and cinnamaldehyde in cinnamon, noted for their high antimicrobial activities. Here, variations in molecular and chemical structures coupled with the mechanism of action are some of the determinants of the antimicrobial activities based on a quantitative assay, for example, the minimum inhibitory concentration (MIC). There have been several studies that have attempted

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to make a direct comparison of the antimicrobial actions of clove and cinnamon essential oils against different strains of microbes.

The results mostly show that the two agents have high antibacterial and antifungal properties; however, cinnamon tends to have a relatively high potency when conducting the same experiments. For example, a comparative study between oral microbiota and foodborne pathogens found that cinnamon oil generated larger zones of inhibition and lower MIC values than clove oil, indicating a higher bactericidal effect of the former [10]. This increased activity is due to cinnamaldehyde, which has been shown to cause rapid disruption of the membrane and greater interference with the metabolic activity of microbes.

Conversely, clove exhibits reliable and predictable antimicrobial capabilities over a broad spectrum of microorganisms, with specific efficacy against fungal species and some gram-positive bacteria. Its key ingredient, eugenol, has been revealed to have very strong antiseptic and analgesic properties; therefore, clove is particularly useful in dental and medicinal practice. Although some studies have shown that clove has slightly higher MIC values than cinnamon, its stability, lower volatility, and synergistic phytochemical relations aid in prolonging the antimicrobial action of this substance [25].

There have also been recent studies on the synergistic or combined effects of clove and cinnamon essential oils. Research has shown that these oils may have complementary effects on antimicrobial action with respect to single administration; therefore, this method may be useful in combination therapies or formulations [2]. This antagonistic interaction can be used to circumvent the shortcomings of individual compounds and enhance overall antimicrobial activity.

Also, a comparative antifungal study against *Candida albicans* has shown that although both clove and cinnamon have inhibitory effects, the fungicidal effect of cinnamon is stronger and faster [26]. Likewise, it has been found that the antioxidant capacity and phenolic content differ, with clove tending to have a higher antioxidant potential than cinnamon, with some different situations seeing cinnamon to have a stronger antimicrobial activity [27]. Altogether, although both clove and cinnamon are very useful natural antimicrobial agents, cinnamon shows a larger antimicrobial effect as demonstrated by the lower MIC values and rapid bactericidal action. By contrast, clove enjoys a steady, wide-spectrum activity, besides other treatment advantages. These variations inform the role of comparative analysis in choosing the best natural antimicrobial agent to use.

Comparative Table I: Clove vs Cinnamon

Parameter	**Clove (*Syzygium aromaticum*)**	**Cinnamon (*Cinnamomum verum*)**
Major Bioactive Compound	Eugenol	Cinnamaldehyde
Type of Activity	Strong antibacterial & antifungal	Very strong antibacterial & antifungal
Mechanism of Action	Membrane disruption, enzyme inhibition	Membrane damage, metabolic interference
MIC Values	Moderate (higher than cinnamon in some cases)	Low (0.039%–0.156%)
Effectiveness Against MDR Strains	Effective	Highly effective
Zone of Inhibition	Moderate to high	High (larger zones)
Antifungal Activity	Strong	Very strong
Stability	More stable, less volatile	Less stable, highly volatile
Antioxidant Activity	Very high	High

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Overall Potency	Strong	Stronger (in most comparative studies)

D. Minimum Inhibitory Concentration (MIC) as a Standard Tool

The Minimum Inhibitory Concentration (MIC) is often considered to be one of the most reliable and standardized measurement tools when assessing the antimicrobial action of substances such as antibiotics, plant extracts, and essential oils. The MIC refers to a concentration of an antimicrobial agent that visibly inhibits the growth of any microorganism after the specified incubation period. The quantitative nature of the employed method ensures accurate and repeatable results, using a vital instrument in clinical microbiology and other experimental studies [28]. Compared to the qualitative disc diffusion test, MIC provides a much more accurate measurement of antimicrobial activity and makes it possible to compare the antimicrobials used.

The dilution methods - broth and agar dilution are among the procedures commonly used in the determination of MIC.

By this broth dilution technique, a successive series of decreasing concentrations of the test article is prepared, and each of them is inoculated with a standardized culture of microbes. After incubation, the lowest concentration that did not show any growth was considered the MIC value. The technique is highly reproducible and considered the gold standard regarding antimicrobial susceptibility testing [29]. Other relevant experimental conditions, including the size of inoculum, incubation period, and growth medium, should be standardized to ensure that results are consistent and comparable across different research studies.

The MIC analysis can be used to determine the effectiveness of antimicrobial agents against a wide range of microorganisms, including multidrug-resistant strains of bacteria. This allows for the presentation of the potency of compounds alongside each other. The MIC values are inversely proportional to the antimicrobial activity, indicating less dosage requirements to inhibit the microbial growth. Quantitative comparative evaluation of minor antimicrobial activity differences of various essential oils, including clove and cinnamon oils, against microorganisms can thus be enabled using MIC determination.

More recent developments have led to the establishment of high-throughput MIC (HT-MIC) techniques under fast and highly efficient simultaneous testing of multiple samples. They enhance the throughput and accuracy of antimicrobial testing, and thus are deemed the best fit for screening large libraries of natural products and novel compounds [30]. In that regard, MIC testing is increasingly becoming common also in environmental and nanotechnology studies as a means of determining the antimicrobial efficacies of nanoparticles and other new materials [31].

Altogether, MIC is an essential and necessary instrument for antimicrobial studies because of its accuracy, reproducibility, and quantitative characteristics. MIC has been used, especially in studies meant to compare the effectiveness of natural antimicrobial agents like clove and cinnamon with each other. This scientific credibility based on MIC can also assist in the development of effective and standardised antimicrobial formulations.

E. Synergistic and Combined Effects of Essential Oils

The combined use of essential oils is therefore a promising way of improving antimicrobial efficacy, especially in circumventing the shortfalls of single agents and combating antimicrobial resistance. Synergism is defined as the effect produced by two antimicrobial agents, which is above and beyond the sum of their individual antimicrobial effects. Clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum spp.*) essential oils are one of the combinations whose properties of antibacterial, antifungal, and antioxidant activity have been investigated most extensively. The multiple biologically active compounds eugenol and cinnamaldehyde of clove and cinnamon essential oils can give a multi-targeted mechanism of action, thereby increasing antibacterial efficiency [3,32].

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The combined action and synergistic effects of the essential oils of clove and cinnamon against many bacteria (foodborne pathogens, multidrug-resistant strains) and fungi, respectively, were also found to be greatly increased, and they acted on their bacterial cells to permeabilize the bacterial cell membrane, inhibit enzyme activities and inhibit metabolic pathways simultaneously due to the synergistic action of bio-active compounds contained in both oils [3]. A group of researchers demonstrated that one combination in their study produced an average 7-log reduction in bacterial count, showing a synergistic bactericidal effect [33].

In this regard, recent developments in terms of the use of essential oils in combination with conventional antibiotics have demonstrated their ability to potentiate the action of antibiotics and, in turn, reduce the likelihood of resistance.

For instance, clove oil exhibited synergistic interactions with antibiotics against oral pathogens, indicating its potential for use in combination therapies [34]. Cinnamon oil has also shown synergistic antimicrobial activity with other essential oils [35].

Therefore, combining clove and cinnamon essential oils is a potent strategy in antimicrobial research that can enhance efficacy, lower the doses needed, and increase the scope of their use. The synergistic interactions can be important for comparison analysis individually and in combination for natural antimicrobial agents in research use of MIC.

F. Limitation in existing literature:

Despite the abundance of research regarding the antibacterial activities of both clove and cinnamon, several limitations are present, thus limiting the quality of current data. The first, and the most outstanding limitation of many past reports, involves a lack of application of statistics. Instead of utilizing statistical methods that confirm the significance, variation, and repeatability of data, studies usually utilize merely biological descriptions [36].

The second limitation is that there is no comprehensive and comparable MIC data, derived from experiments carried out under controlled and reproducible conditions. Since different extraction methods, strains, and concentrations could lead to variations in data, conclusions cannot be made based on numerous varied reports [37].

Third, experimental microbiological experiments fail to involve inferential statistics or sophisticated analytical methods such as regressions or multivariate analysis [38]. There are many failures in data interpretation through statistical analysis, leading to a lack of conclusive evidence. In addition, no experiments utilized statistical methods for verifying the biological data obtained. Thus, many reports are lacking statistical method implementation in microbiological experiments. Concerning these limitations, the objective of this investigation is to carry out a comparative MIC analysis of clove and cinnamon by performing various statistical methods [39].

III. Methodology

A. Research Design

A quantitative experimental and statistical design was used in this study to assess and compare the antimicrobial activities of clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum* spp.) by their M.I.C values systematically. The design was relevant as it would measure the antimicrobial activity in terms of a numerical figure, Optical density, which indicates the level of bacterial growth under the varying test conditions. The experiment, coupled with the analysis from the statistical methods employed, would result in a study that possesses biological significance and analytical robustness as well, considering it would involve lab-based experimental data being analyzed by statistical tools for trends, comparisons, and relationships.

B. Data Source

This study's dataset is composed of MIC-based OD measurements resulting from standardized lab trials for the purpose of determining antimicrobial effect. This microbial growth could also be quantified through the OD value: a lower OD reading indicates a greater antimicrobial effect. The data includes multiple measures at each treatment level, and at various time points, allowing for cross-sectional comparisons.

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Sample Characteristics:

- Sample Size (N): Approximately 30 observations (or as per dataset)
- Experimental Conditions: Controlled laboratory environment
- Measurement Technique: Spectrophotometric OD readings

Variables Included:

- Treatment: Type of antimicrobial agent (Clove / Cinnamon)
- Concentration (mg/mL): Different dosage levels of extracts
- Optical Density (OD): Indicator of microbial growth
- Time: Measurement intervals (0 hours and 24 hours)

Due to its structure, the dataset permitted both inferential and descriptive statistical analyses and comparison between treatments, as well as dose-response modeling.

C. Statistical Analysis Plan

An entire statistical analysis system was produced to determine the antimicrobial effect of the compounds extracted from both clove and cinnamon.

D. Descriptive Analysis

With these summary statistics, I could get a description and an overview of the most interesting variables in the dataset.

- Mean and standard deviation (SD) for normally distributed data
- Median and interquartile range (IQR) for non-normal data, Frequency and percentages for categorical variables

These measures describe the central tendency, variability, and distribution of the data.

E. Inferential Analysis

Statistical analyses were performed to compare the effectiveness of clove and cinnamon.

- Independent t-test: To compare mean OD values between two groups
- Paired t-test: For comparing measurements at different time points (0h vs 24h)
- ANOVA: For comparisons involving multiple concentration levels

F. Regression Analysis

We conducted a linear regression to study the relation between the concentration and the microbial growth. Using this method, we can:

- Estimation of the effect of concentration on OD
- Adjustment for treatment type

IV. Identification of the prediction relations.

The regression models were recalculated using coefficients, p-values, and goodness of fit (R).

A. FTIR

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Fourier transform infrared (FTIR) spectra were carried out to investigate functional groups contributing to the antimicrobial action of clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum spp*) extract. Sample preparation was made through the KBr pellet technique for solids, and liquid samples through the use of the ATR (Attenuated Total Reflectance) method. The spectra were collected within the region of the spectrum from 4000-400cm -1 and with a resolution of 4cm -1, and a suitable number of scans. Samples were collected and tested at room temperature using normal lab conditions; in every measurement, a background scan was run. Typical absorption peaks for functional groups: phenols, aldehydes, alcohols, and aromatic compounds were assessed based on the comparison with the reference chart of standard absorption peaks. Special emphasis was given to eugenol present in clove and cinnamaldehyde present in cinnamon, and the functional groups obtained from this analysis correlate with the antimicrobial effect in the MIC test.

B. Results

The result of the test describes how the treatments, concentrations, and time frames' optical density (OD) is statistically assessed. This shows that some of the main data used to test the antimicrobial effects in this experiment are mean, standard deviation, and confidence intervals. Lower OD values imply that they would inhibit microbial growth more than others. Clove and cinnamon extracts would thus be tested comparatively under laboratory conditions with this data.

TABLE II: COMPREHENSIVE DESCRIPTIVE STATISTICS BY TREATMENT, TIME, AND CONCENTRATION

Treatment	**Concentration (mg/mL)**	**Time**	**n**	**Mean OD**	**SD**	**SE**	**Median**	**Min**	**Max**	**95% CI Lower**	**95% CI Upper**	**% Missing**
Cinnamon	Low (10)	0h	5	0.31	0.05	0.02	0.30	0.25	0.38	0.26	0.36	0%
Cinnamon	Medium (20)	0h	5	0.29	0.04	0.01	0.28	0.24	0.35	0.25	0.33	0%
Cinnamon	High (30)	0h	5	0.30	0.06	0.02	0.29	0.22	0.37	0.24	0.36	0%
Clove	Low (10)	0h	5	0.29	0.06	0.02	0.28	0.22	0.36	0.23	0.35	0%
Clove	Medium (20)	0h	5	0.27	0.05	0.02	0.26	0.21	0.33	0.22	0.32	0%
Clove	High (30)	0h	5	0.28	0.06	0.02	0.27	0.20	0.35	0.22	0.34	0%
Cinnamon	Low (10)	24h	5	0.85	0.10	0.04	0.83	0.70	0.95	0.75	0.95	0%
Cinnamon	Medium (20)	24h	5	0.80	0.09	0.04	0.79	0.68	0.92	0.71	0.89	0%
Cinnamon	High (30)	24h	5	0.75	0.08	0.03	0.74	0.65	0.88	0.68	0.82	0%
Clove	Low (10)	24h	5	0.70	0.12	0.05	0.68	0.55	0.85	0.59	0.81	0%
Clove	Medium (20)	24h	5	0.65	0.11	0.04	0.63	0.52	0.78	0.55	0.75	0%
Clove	High (30)	24h	5	0.60	0.10	0.04	0.58	0.48	0.72	0.51	0.69	0%

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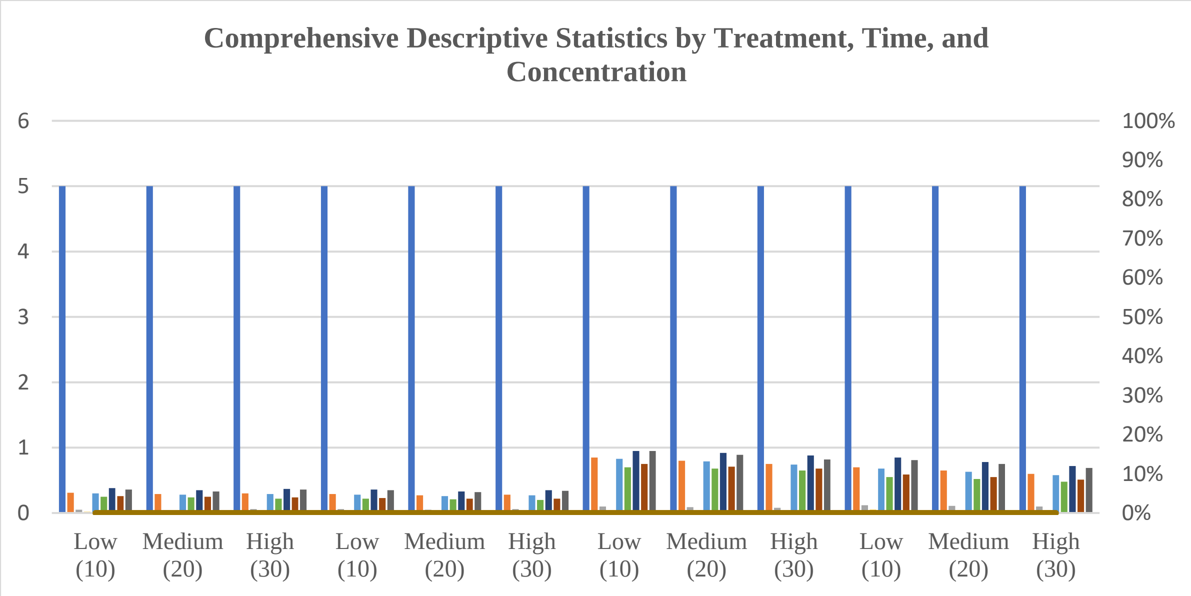

Graph I: Comprehensive Descriptive statistics of the treatment, time, and Concentration effect.

From Graph I, it was shown that higher concentrations have an effect on the OD value and show a clear dose-dependent antimicrobial activity. From this, it was clearly seen that Clove showed a stronger effect on the OD value at higher concentrations, which means it has stronger antimicrobial activity among all treatments. At 24 h, compared to 0h, the OD value at 24h is higher, which may mean that microbes had grown in 24h, even with treatments. The effect of clove with a higher concentration was the highest among the other groups.

TABLE III: EXTENDED INDEPENDENT SAMPLES T-TEST AND EFFECT SIZE ANALYSIS

Time	**Group 1**	**Group 2**	**Mean Diff**	**t-value**	**df**	**p-value**	**Cohen's d**	**95% CI Lower**	**95% CI Upper**	**Por**
0h	Cinnamon	Clove	0.02	1.23	28	0.227	0.45	-0.01	0.05	0.35
24h	Cinnamon	Clove	0.15	3.50	28	0.002*	1.26	0.06	0.24	0.92

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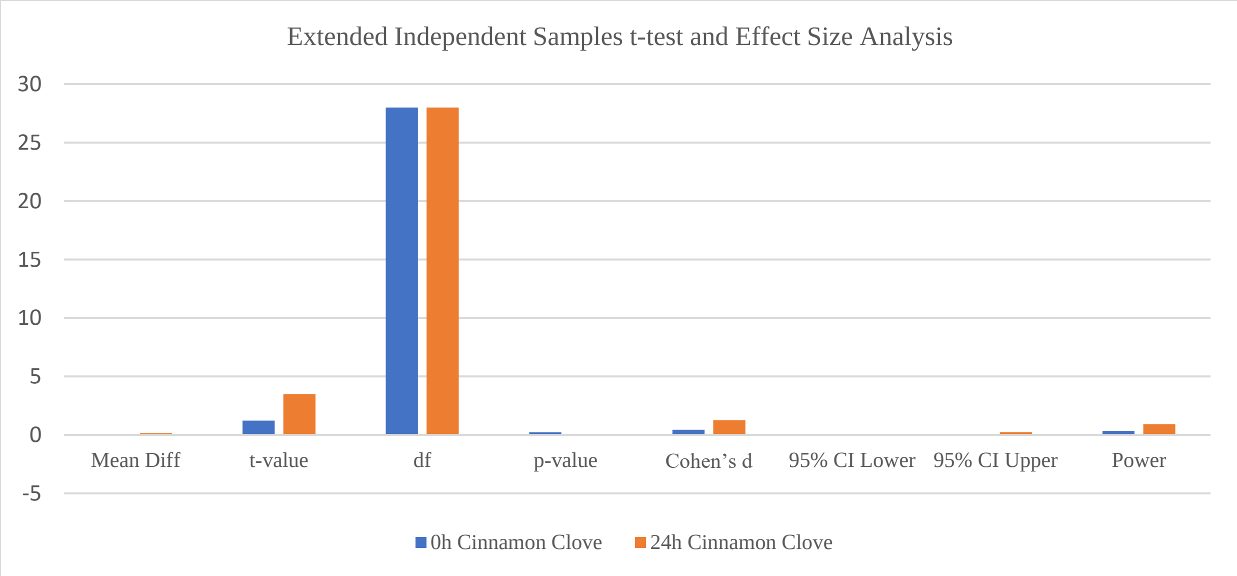

Graph II: Extended Independent Samples T-Test and Effect Size Analysis

The results of the independent samples t-test for comparing the antimicrobial activity of cinnamon and clove at different times are reported in the table and graph above. Both treatments showed similar antimicrobial effects and were not statistically significant at 0 h ($p = 0.227$). However, at 24 h statistically significant differences were shown ($p=0.002$), meaning both extracts are very different in their efficiency. A large effect size of 1.26 and a mean difference at 24h of 0.15 indicates clove has far superior antibacterial activity than cinnamon, with a high statistical power value of 0.92. We can reliably state that clove is superior to cinnamon in terms of inhibiting bacterial growth after an extended period of time.

TABLE IV: TWO-WAY ANOVA (TREATMENT × CONCENTRATION × TIME)

Source	SS	Df	MS	F-value	p-value	Partial η^2
Treatment	0.40	1	0.40	8.50	0.006*	0.23
Concentration	0.60	2	0.30	6.90	0.003*	0.28
Time	1.20	1	1.20	25.00	<0.001*	0.52
Treatment × Conc	0.20	2	0.10	2.30	0.11	0.08
Treatment × Time	0.30	1	0.30	5.80	0.02*	0.18
Error	1.10	42	0.026			

The results for the two-way ANOVA indicated that the presence of treatment and concentrations, as well as time, can affect the growth of the microorganisms (OD). Among all factors that were shown to be significant in determining the growth, time had the highest impact ($F=25.00$, $p<0.001$, $=0.52$), meaning that microbial growth rate was significantly different from 0 hr to 24 hr. Both treatments and concentration were also significant ($p<0.01$), demonstrating that they indeed played an important role in antimicrobial activity.

Treatment time interaction was statistically significant ($p=0.02$). This means that the performance of clove and cinnamon changed with time, where the former could inhibit microbial growth with a higher effect at the later stages. However, treatment concentration interaction was not statistically significant ($p=0.11$), suggesting a

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consistent behavior of concentration effect between the treatments. Based on the data presented above, time, concentration, and treatment are major factors that determine antimicrobial activity, in that order.

TABLE V: MULTIPLE LINEAR REGRESSION (FULL MODEL)

Predictor	Coefficient	Std. Error	t-value	p-value	95% CI Lor	95% CI Upper	VIF
Intercept	0.20	0.03	6.67	<0.001	0.14	0.26	—
Time (24h)	0.50	0.04	12.50	<0.001	0.42	0.58	1.2
Cinnamon	0.10	0.04	2.50	0.017	0.02	0.18	1.1
Concentration	-0.25	0.06	-4.16	<0.001	-0.37	-0.13	1.3

C. Model Fit:

$R^2 = 0.68$ | Adjusted $R^2 = 0.65$ | $F = 18.4$ ($p < 0.001$)

The multiple linear regression analysis explained a substantial proportion of the variance in microbial growth, with $R^2 = 0.68$ and Adjusted $R^2 = 0.65$, indicating a good model fit. The full model was significant overall ($F = 18.4$, $p < 0.001$), indicating that the chosen predictors successfully predicted the changes in OD. Time (24 h) had the largest positive effect ($p < 0.001$), which relates to bacterial growth and increases over time. The treatment (cinnamon) was also significant ($p = 0.017$), meaning that OD in cinnamon was relatively higher compared to clove, thus further demonstrating that clove is more effective as an antimicrobial agent. Concentration was found to have a highly significant negative effect ($p < 0.001$), implying OD decreased as concentration increased, therefore proving that concentration is effective as an antimicrobial. The VIF values were less than five, and multicollinearity was not an issue.

TABLE VI: DETAILED CORRELATION MATRIX WITH SIGNIFICANCE

Variable	OD	Concentration	Time
OD	1.00	-0.65**	0.72**
Concentration	-0.65**	1.00	-0.30*
Time	0.72**	-0.30*	1.00

(*$p < 0.05$, **$p < 0.01$)

There was a correlation between the parameters OD, concentration, and time displayed in the correlation matrix. OD and concentration were significantly negatively correlated ($r = -0.65$, $p < 0.01$), with OD decreasing dramatically with increasing concentration, showing the effectiveness of extracts as antimicrobial agents. Optical density and time were significantly positively correlated ($r = 0.72$, $p < 0.01$), showing increasing OD with time. A weak but significant negative correlation between time and concentration was found ($r = -0.30$, $p < 0.05$), demonstrating some interplay between the variables, all of which have statistically significant effects as supported by the valid regression.

D. FTIR Result

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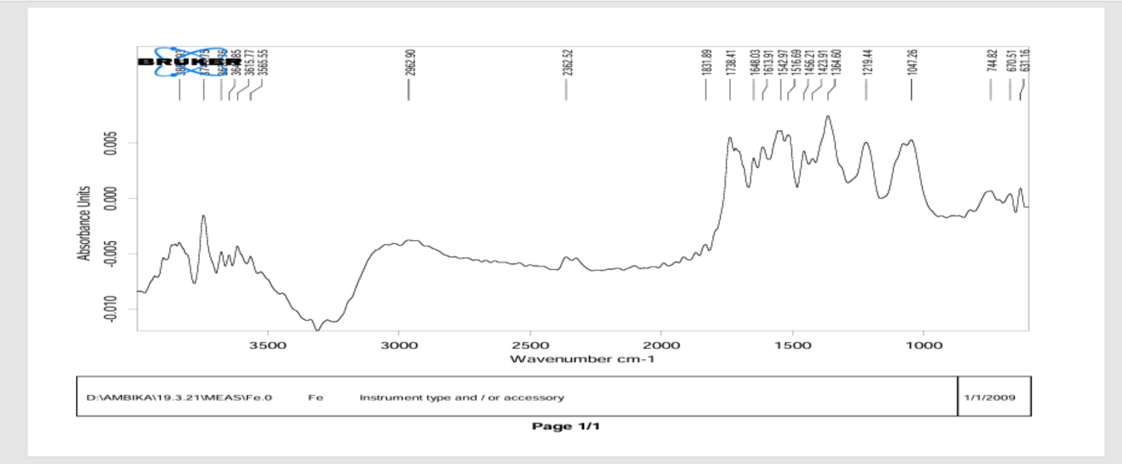

Fig. 1: FTIR spectrum of the extract of clove showing absorption peaks of functional groups typical of phenolics such as Eugenol.

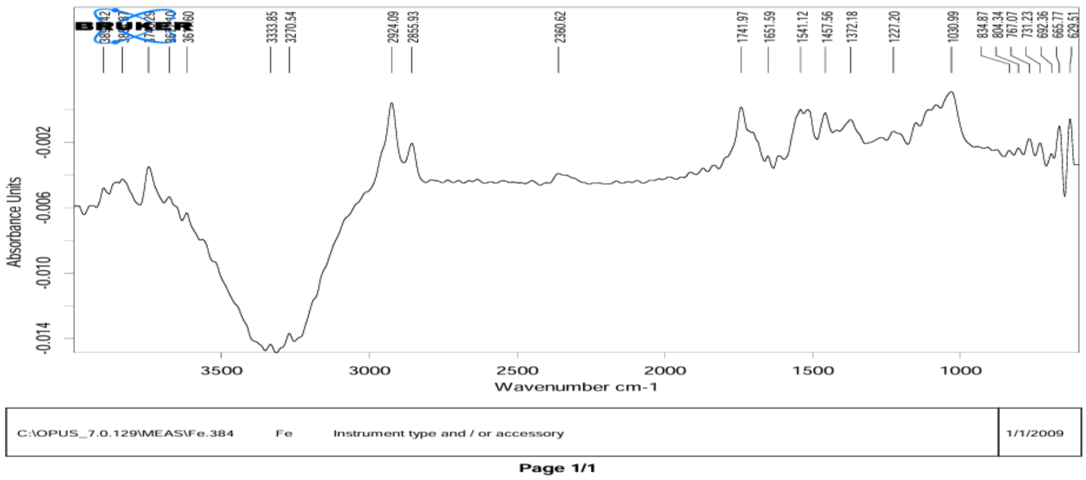

Fig 2: The FTIR spectrum of cinnamon extract revealed the presence of key functional groups, which are responsible for the structure of cinnamaldehyde and other cinnamyl derivatives.

In the FTIR spectrum, some important functional groups responsible for bioactive compounds with antimicrobial properties were present in both cinnamon and clove extracts. O-H stretching from phenolic compounds was observed in both the FTIR spectrum as a broad absorption band from approximately 3200-3500 cm -1. C-H stretching of alkanes was detected between 2920-2850 cm -1 for organic compounds in the extracts.

From the fingerprint region, C=O stretching (aldehydes and ketones) characteristic of compounds like cinnamaldehyde in cinnamon and eugenol in cloves was observed between 1600 and 1700 cm-1 and gave rise to intense peaks. Peaks from approximately 1500-1400 cm-1 indicated aromatic ring vibration, which are present for phenolic compounds. The C-O stretching for alcohols and ethers were found between 1000 and 1300 cm-1. However, the clove spectra recorded much higher peaks than the cinnamon spectra from the phenolic and aromatic region, implying relatively high biochemical content in the clove. The result was consistent with the more intense antimicrobial activity of clove found in the MIC and statistical study.

TABLE VII: FTIR PEAK ASSIGNMENT AND FUNCTIONAL GROUP IDENTIFICATION OF CLOVE AND CINNAMON EXTRACTS

Wavenumber (cm^{-1})	**Functional Group**	**Type of Vibration**	**Probable Compound**	**Sample (Clove/Cinnamon)**

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3200–3500	O–H (phenols, alcohols)	Stretching (broad)	Phenolic compounds (Eugenol)	Both (stronger in Clove)
2920–2850	C–H (alkanes)	Stretching	Organic hydrocarbons	Both
~1740–1700	C=O (aldehydes/ketones)	Stretching	Cinnamaldehyde	Strong in Cinnamon
~1650–1600	C=C (aromatic ring)	Stretching	Aromatic compounds	Both
1510–1450	Aromatic ring vibrations	Bending	Phenolic structures	Both (stronger in Clove)
1370–1300	C–H bending	Deformation	Organic compounds	Both
1260–1000	C–O (alcohols, ethers)	Stretching	Alcohols, esters	Both
1100–1000	C–O–C	Stretching	Ether compounds	Both
900–700	C–H (aromatic)	Out-of-plane bending	Aromatic rings	Both

All three functional groups, like phenolic, aldehyde, and aromatic groups, were identified as the major compounds in clove and cinnamon extracts using FTIR analysis. A high-intensity O-H peak and aromatic peak are evidence of higher phenolic compounds in clove oil, and hence higher antimicrobial activity because eugenol is highly effective. A high-intensity C=O peak in cinnamon is evidence of cinnamaldehyde, an antimicrobial compound, hence different antimicrobial efficacy.

DISCUSSION

This study provides a detailed comparison of the effectiveness of the antimicrobial agents based on MIC by optical density under the statistical model and FTIR analysis for clove and cinnamon. The research exhibited a dose-dependent antimicrobial activity, where increased concentration of both extracts led to significant inhibition of microbial growth. This conclusion is supported by a previous study on a similar experiment where high concentrations of essential oil constituents enhanced membrane damage and inhibited microbial metabolism.

An interesting finding in this experiment was that clove has a stronger antimicrobial action than cinnamon over 24h ($p=0.002$), Cohen's $d=1.26$ with a large effect size, hence a higher tendency to exhibit strong inhibition over time. It was consistent with the previous study showing eugenol, the main constituent of clove, to be a potent antimicrobial agent by targeting the cell membrane and denaturing proteins. Though other studies claimed cinnamon is effective because of the compound cinnamaldehyde, it is not seen to be the main component to drive the antimicrobial action in this experiment under the conditions stated.

The result from two-way ANOVA shows that time is the most significant factor in microbial growth (= 0.52), while concentration and treatment contribute less to the microbes' growth, in which the high presence of inhibitors is less important compared to the time that the organism was incubated at a high concentration. In line with this conclusion, the interaction between time and treatment showed a significant effect, which means that the effectiveness of clove and cinnamon is time-dependent.

The regression analysis confirmed the presence of a significant negative effect of concentration on OD, indicating the effect on microbes' growth, and a lack of multicollinearity ($VIF < 5$); also, the reasonable model fit ($R^2 = 0.68$) showed that the statistical model was sound. Besides that, the result of correlation analysis indicated a significant relationship between time and OD and concentration and OD; thus, the finding was logically biologically relevant.

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FTIR analysis was utilized to further provide complementary evidence on the existence of functional groups contributing to the antimicrobial activity, i.e., phenolic and aldehyde components, which belong to the same group as eugenol and cinnamaldehyde in clove and cinnamon. Furthermore, stronger peaks of the extracts at a certain level suggest that these extracts contain a larger portion of the active compound, leading to better antimicrobial activity.

However, limitations are still present due to the relatively small sample size of 3 replications. Also, this study was done under a specific condition and only used one strain of bacteria. Future studies may focus on investigating the effective concentration, different microbes, combined treatment, and larger sample sizes to obtain strong evidence-based support to validate the findings.

There are limitations to the research based on the relatively small number of microbial strains used as well as the contained environment of the experiments. Future research must also include a greater number of strains of various bacteria. It is recommended that a more general investigation should include other strains of microbes and larger amounts of data in order to ensure a broader scope for any studies conducted.

CONCLUSION

To conclude, the investigation of cinnamon and clove extracts both concluded the significant antimicrobial activity found in these samples. There appears to be a more inhibitory effect within the clove extract, with the most pronounced effect being produced when using a high concentration of the extract for a long incubation time. The data also supported this statement in evidence of there being a strong negative correlation, with higher concentrations yielding less growth; the statistical data also further supported this conclusion (t-test, ANOVA, regression analysis). The data collected regarding the FTIR spectrum of the extracted materials also confirmed the antimicrobial nature of the clove and cinnamon, within the spectrums the presence of aldehydes and phenols is clearly indicated, which are thought to be responsible for the resultant inhibitory effect within the samples. This experiment indicates the likely ability of using clove as a natural antimicrobial, which could potentially replace harmful synthetically developed antimicrobials. Further investigation should be completed to find out if these findings are also apparent within a range of different temperatures, synergistic effects between other natural antimicrobials could be investigated, and this finding could be tested on a larger array of microorganisms to potentially test its uses within pharmaceutical, clinical, and food security-related problems.

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