Extraction and Analysis of Eugenol from Clove

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ABSTRACT

Eugenol is a bioactive phenolic compound with antimicrobial, antioxidant and anaesthetic properties, but its extraction from locally available cloves and its characterization are still insufficiently reported. This study aimed to extract eugenol from clove (Syzygium aromaticum) buds, determine the percentage yield, characterize the extract and evaluate its antibacterial activity. Dried clove buds purchased from a local market in Birnin Kebbi were ground and extracted with 90% ethanol in a Soxhlet apparatus, and the solvent was removed with a rotary evaporator. The extract was characterized using colour tests (Marquis, Mecke and FeCl3 reagents), Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC-MS), and its antibacterial activity against Staphylococcus aureus and Escherichia coli was assessed at 30, 60 and 90 mg/cm3 using the agar well diffusion method. The extraction gave a percentage yield of 77.6% (crude extract). The Marquis, Mecke and FeCl3 reagents produced red, green-to-black and green colours, respectively, and the FTIR spectrum showed bands characteristic of the O-H, C-H, aromatic C=C and C-O groups of eugenol. GC-MS revealed a complex mixture in which eugenol was not the dominant peak, so further purification and confirmation against an authentic standard are required. The extract inhibited both bacteria, with zones of inhibition of up to 25 mm for S. aureus and 28 mm for E. coli at 90 mg/cm3. These findings support the potential of clove-derived eugenol as a natural antimicrobial agent for pharmaceutical and biomedical applications.

Keywords: Cloves, Eugenol, Antioxidant, GC-MS and Pharmacological Properties

INTRODUCTION

The medicinal benefits of cloves have been recognized for thousands of years (Bhowmik et al., 2012). The main constituent, eugenol, has been used as a topical anesthetic, adjunct component in dental applications, and recently studied for numerous other medicinal qualities (Zari et al., 2021, Mohtashami et al., 2024). Traditionally, eugenol is isolated in the essential oil of cloves which is produced via steam distillation of the dried flowering bud of the tree Syzygium aromaticum L., which is in the evergreen family (Elisha et al., 2022). The analytical profile of the essential oil reveals a secondary constituent of similar chemical structure with purported medical benefits, acetyl eugenol, also known as eugenol acetate, along with numerous other minor components such as terpenes, which are common to many essential oils derived from plants (Fitzgerald and Booker 2020).

Clove (Syzygium aromaticum) a highly aromatic spice has been cultivated and used by humans for centuries. Cloves were among the most sought-after spices during the spice trade era, driving significant global exploration and trade routes during the 15th and 16th centuries (Ravindran, 2023). Clove trees thrive in tropical climates, requiring abundant rainfall and well-drained soils. Today, cloves are commercially grown in countries such as Indonesia, Madagascar, Tanzania and Sri Lanka. Clove buds, the immature flower buds harvested for their flavour and medicinal properties marketed as spices or used for essential oil extraction (Mini et al., 2023).

Clove oil, extracted from clove buds, has been used for treating a variety of ailments such as toothaches, digestive disorders, and respiratory issues. The presence of eugenol in clove oil gives it strong analgesic and antiseptic properties, making it useful for treating minor infections and pain relief (Tungare et al., 2024).

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Eugenol has been approved to encompass numerous beneficial aspects against a capacious spectrum of life-threatening indispositions including oxidative stress, inflammation, hyperglycemia, elevated cholesterol level, neural disorders and cancer (Singh, H., & Pathak, A). In addition, eugenol has also shown strong potential as an antimicrobial agent against wide ranges of pathogenic and spoilage causing microorganisms (Hu et al., 2018). Predominantly, the principle mechanistic approaches associated with the therapeutic potential of eugenol include its free radical scavenging activity, hindrance of reactive oxygen species' generation, preventing the production of reactive nitrogen species, enhancement of cytoantioxidant potential and disruption of microbial DNA and proteins. Eugenol is present in significant amount in the extracts of numerous medicinal herbs so it has fascinated the attention of several researchers and opened up the gateway of research regarding its utilization as a medicine to cure various diseases (Khalil et al., 2017). Eugenol is avowed to possess certain pharmacological properties including anaesthetic activity, antioxidant potential, antimicrobial role, anti-inflammatory action, anti-carcinogenic effects, neuroprotective ability, hypolipidemic efficiency and antidiabetic effectiveness. Eugenol is declared as GRAS (generally recognized as safe) by World Health Organization (WHO) and is considered as non-mutagenic (Cohen et al., 2021).

Despite this wide range of reported benefits, several gaps remain. Much of the available literature on eugenol focuses on clove essential oil obtained by steam or hydrodistillation, whereas solvent extraction of locally available cloves, and the combined use of simple colour tests, spectroscopic techniques and antibacterial assays to characterize the extract, have received comparatively little attention, particularly in resource-limited laboratories. The composition of cloves and clove products also varies with origin, storage and processing, so the presence and quality of eugenol in a given sample cannot be assumed. At the same time, increasing resistance of pathogenic bacteria such as Staphylococcus aureus and Escherichia coli to conventional antibiotics has created a need for safe, affordable antimicrobial agents of natural origin.

Soxhlet extraction with ethanol is a simple, low-cost and reproducible method that requires only basic laboratory equipment, and combining it with colour tests, Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC-MS) allows the extract to be examined by complementary approaches. Testing its activity against a Gram-positive (S. aureus) and a Gram-negative (E. coli) bacterium further indicates its potential value as a natural antimicrobial. The present study therefore aimed to extract eugenol from clove buds by Soxhlet extraction, determine the percentage yield, characterize the extract using colour tests, FTIR and GC-MS, and evaluate its antibacterial activity against S. aureus and E. coli. The whole and ground clove buds used in this study are shown in Figure 1.



Figure 1. Whole dried clove buds (left) and ground clove powder (right) of Syzygium aromaticum.

MATERIALS

The materials used to conduct the research: Beaker, Test tube, Measuring cylinder, Round flask, Porcelain test well, Reagent bottles, Soxhlet extractor, Rotary evaporator, Petri dish, Drying oven, Weighing balance, Mettle.

Methods

Reagents Preparation

1. Marquis Reagent Marquis reagent was prepared by mixing 10mL of formaldehyde (40% by volume) in 90mL of concentrated sulphuric acid (DeFrancesco, 2021).

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2. Mecke Reagent Mecke reagent was prepared by mixing 1 g of selenious acid in 100mL of concentrated sulphuric acid (DeFrancesco, 2021).

3. Ferric chloride Reagent Ferric Chloride reagent was prepared by mixing 10 g of anhydrous ferric chloride (which can be substituted with 16.5 g of the hexahydrate) in 100mL of DI water (DeFrancesco, 2021).

Sample Collection

Cloves were purchased from a local market (Birnin Kebbi) as dried flowering bud and ground to a 20-mesh powder with a coffee bean grinder to maximize surface area and increase extraction efficiency. And it was identified at the Botany Unit of the Department of Biological Science, Usmanu Danfodiyo University Sokoto.

Extraction Procedure

The sample was placed in a soxhlet extractor. Ethanol (90%) was placed in a round flask along with the boiling chip granules. The extraction process continued 6-7 hours at the temperature of 70-80oC. The mixture was then filtered, and the solvent was evaporated using a rotary vacuum evaporator to obtain the crude extract (CMFRI, 2008). The extract was weighed and stored in a vial for further analysis.

Percentage Yield Determination

The yield of the extract was determined based on the final extract weight over the dried powder. It was obtained from the equation below:

$$\% \text{ Yield } = \frac{\text{Weight of extract (g)}}{\text{Weight of dried clove powder (g)}} \times 100$$

where the weight of extract is the mass recovered after evaporation of the solvent and the weight of dried clove powder is the mass of sample loaded into the Soxhlet extractor.

Quantification of eugenol using FTIR, GC-MS and colour testing

1. Colour Test

Colour testing was performed by placing a few drops of the test reagent into a white porcelain test well, followed by addition of one drop of sample solution into the well. In this order of addition, placing the test reagent in the well first is important to ensure that an initial negative response is obtained prior to addition of the sample, which indicates an uncontaminated well (Jungreis, 2006).

2. GC-MS

Total ion chromatograms and mass spectra were obtained on an Agilent 7890A/5975C fitted with a HP-5 column (30 m long x 0.320 mm diameter x 0.250mcm film thickness), a helium carrier gas flow of 1.3mL/min (constant flow mode), inlet temperature of 275 deg C, MS transfer line temperature of 280 deg C, oven program of 60-320 deg C at a 30 deg/min ramp with a 2 minute hold and solvent delay at 60 deg C and a 3 minute hold at 320 deg C. The inlet split was 50:1. The quadruple was set to scan 40-550 Daltons. The MS source temperature was 230 deg C and the MS quadruple temperature was 150oC (DeFrancesco, 2021).

3. FTIR

Infrared spectra were acquired on a Perkin-Elmer Spectrum 100 series Fourier Transform Infrared Spectrophotometer fitted with a Universal Attenuated Total Reflectance Sampling accessory containing a ZnSe crystal. Spectra were recorded in % transmittance and scanned from 4000 to 650 cm-1 for four scans per spectrum at a resolution of 2 cm-1. Spectrum for the alcoholic cloves extract was obtained for the residue by placing several drops of the extract onto the ATR window and allowing the solvent to evaporate (Redondo-Cuenca et al., 2021).

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Test for Organism

The test for this organism (Escherichia coli and Staphylococcus aureus) was carried out in department of microbiology, Usmanu Danfodiyo University Sokoto.

RESULTS

The percentage yield of the extract is presented in Table 1, the zones of inhibition against the test organisms in Table 2, and the colour test results in Table 3. The FTIR and GC-MS spectra of the extract are shown in Figures 2 and 3, respectively.

Table 1. Percentage yield of the crude ethanolic clove extract.

Solvent	**Mass of dried clove powder (g)**	**Mass of crude extract (g)**	**Yield (%)**
Ethanol (90%)	12.5	9.7	77.6

Table 2. Zone of inhibition (mm) of the clove extract against the test organisms.

Test organism	**Concentration of extract (mg/cm^3)**		
	30	**60**	**90**
Staphylococcus aureus	15	R	25
Escherichia coli	18	22	28

Values are zones of inhibition in mm. R = resistant (no zone of inhibition).

Table 3. Colour test results of the clove extract.

Sample	**Marquis reagent**	**Mecke reagent**	**$FeCl_3$**
Clove extract	Red	Green to black	Green

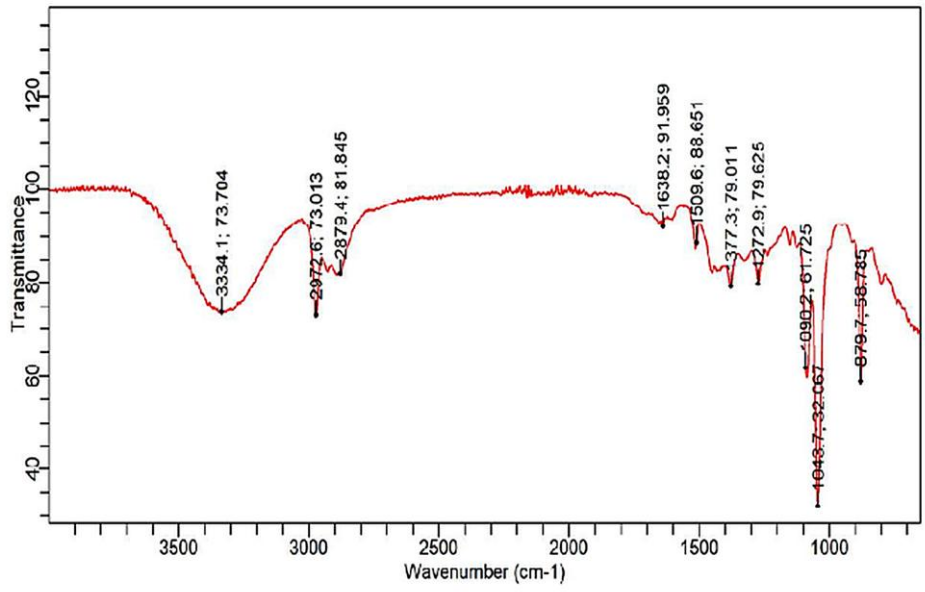


Figure 2. FTIR spectrum of the clove extract.

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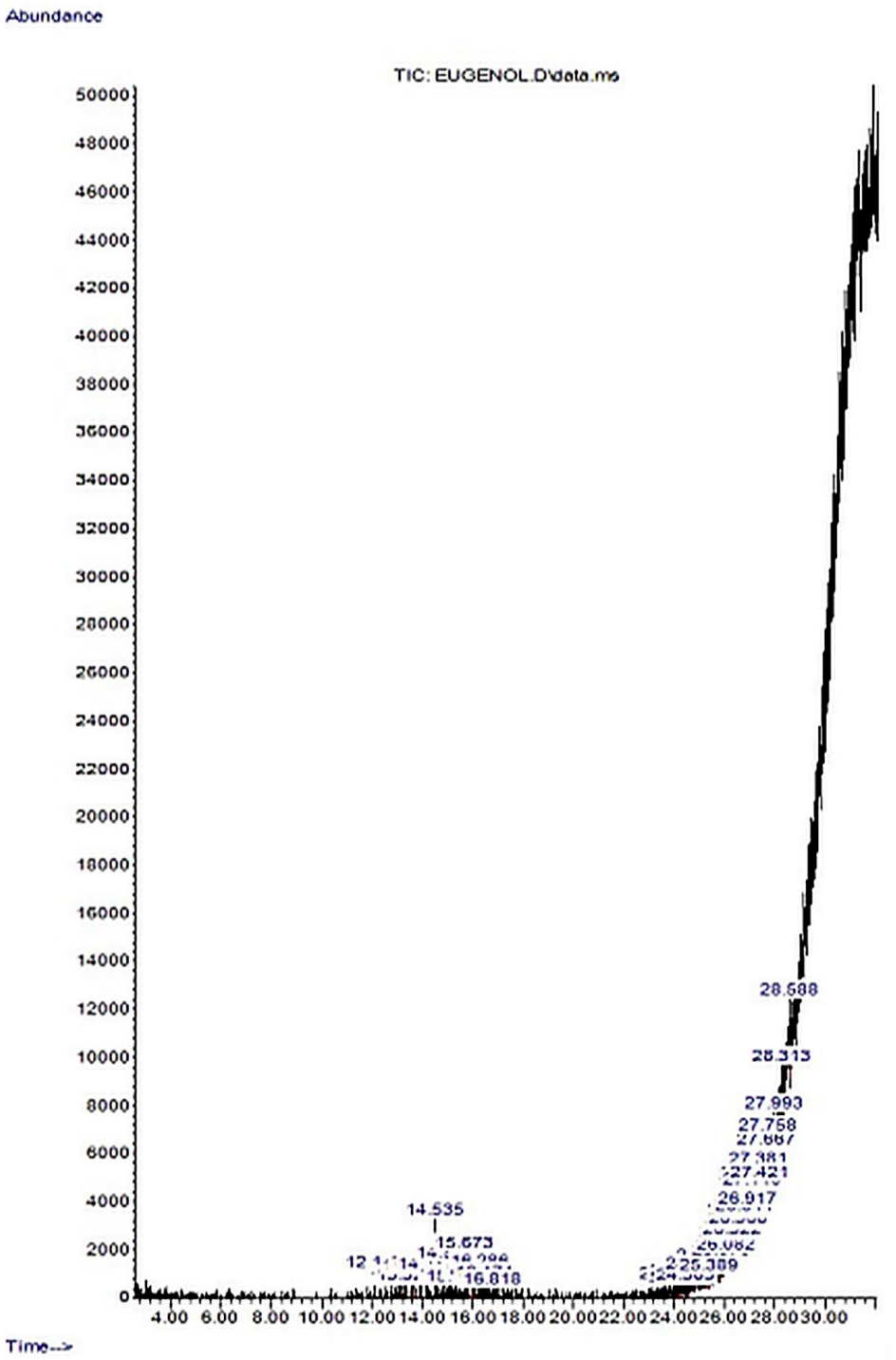


Figure 3. GC-MS total ion chromatogram of the clove extract (abundance versus retention time, min).

DISCUSSIONS

The extraction and analysis of eugenol from clove were achieved using various techniques, including FTIR, GC-MS, Colour tests and antibacterial evaluation.

The percentage yield obtained was 77.6%, calculated from 9.7 g of extract recovered from 12.5 g of dried clove powder (Table 1). This value is much higher than the yields commonly reported for clove bud oil obtained by steam or hydrodistillation. The difference is expected because the yield reported here is that of the crude ethanolic extract and not of pure eugenol. A polar solvent such as ethanol, used for 6–7 hours at 70–80 °C in a Soxhlet system, removes not only eugenol and other volatile components but also non-volatile constituents such as tannins, flavonoids, sugars and resins (Cortés-Rojas et al., 2014), and residual solvent or moisture can add to the weight of the extract. The yield therefore reflects the extraction efficiency of the

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method and should not be interpreted as the eugenol content of the cloves, which would have to be determined by a calibrated quantitative method such as GC or HPLC.

The FTIR spectrum of the extracted compound revealed characteristic absorption bands corresponding to eugenol's functional group (O-H, C=C, C-H and C-C). The bands at 3334, 2972 and 2879cm-1 ascribed to O-H, C-H and =C-H stretching, respectively. The peaks at 1638 and 1509cm-1 originated from C=C stretching vibration of the aromatic part, whereas the peaks at 1377 cm-1 and 1272 cm-1 specified to C-H band and C-O stretching. The spectral signal obtained at the frequencies of 1090, 1043 and 879 cm-1 can be attributed to the presence of CH2 deformation vibration, CH out-of-plane, and ring deformation, respectively.

The observed absorption bands are consistent with the expected functional groups of eugenol. The O-H stretching vibration at 3334cm-1 is comparable to the literature value of 3432 cm-1 reported by (Olga popovska, 2023). Similarly, the stretching vibrations at 2972 cm-1 align with the range of 2920- 3071 cm-1 reported by (Olga popovska, 2023).

Also, the C=C stretching vibration at 1638 cm-1 is slightly higher than the literature value of 1637-1606 cm-1 (Olga popovska, 2023). This discrepancy may be attributed to the differences in the extraction method or calibration. In comparison to other studies, the C-O stretching vibration at 1272cm-1 is consistent with the value reported (Chaieb et al., 2007) for eugenol extracted from clove buds. The FTIR confirms the extraction of eugenol from clove, with the characteristic absorption bands matching the literature values.

The broad O-H band at 3334 cm-1 is typical of the phenolic hydroxyl group of eugenol, and its position at a lower wavenumber than the literature value of 3432 cm-1 is consistent with intermolecular hydrogen bonding in the extract residue analysed by ATR. The bands at 1638 and 1509 cm-1 indicate the allyl double bond and the aromatic ring, while the strong bands near 1272 and 1043 cm-1 are associated with C-O stretching of the phenolic and methoxy groups (Figure 2). It should be noted, however, that ethanol also absorbs strongly near 3300, 1090, 1043 and 879 cm-1, so traces of residual solvent in the extract may have contributed to some of the bands observed in this region, particularly the intense band at 1043 cm-1. For this reason, the FTIR result is best regarded as evidence that the extract contains a phenolic compound with the functional groups of eugenol, rather than as proof of its purity.

The GC-MS analysis of the sample revealed a complex mixture of compounds with a various retention time as shown in Figure 3. Among the detected peaks, diethyl bis- (trimethylsilyl) ester was identified as the major constituent, with a peak area percentage of 3.49%. This compound was detected at a retention time of 27.140 and exhibited a molecular ion peak at 281.0 m/z.

The high-quality match 74% of diethyl bis (trimethylsilyl) ester with the reference spectrum in the NIST library suggests that this compound is a significant component of the sample. The presence of this compound may be attributed to the transformation or degradation of eugenol during extraction, storage, and analysis or even the sample contamination. The unexpected identification of diethyl bis- (trimethylsilyl) ester as the major constituent of the sample, instead of eugenol requires further investigation.

One of the observed compound 3-(-4-methoxy-phenylimino) -1-phenyl-propen-1-ol with the retention time of 12.114, area 1.26%, and quality of 4% bears structural similarity to eugenol, suggesting potential degradation.

Literature reports typically identify eugenol as the major constituent of clove essential oil, ranging from 70-80%. However, some studies have reported the presence of other compounds, such as B-caryophyllene and a-humulene (DeFrancesco, 2021).

The GC-MS result does not support eugenol being the dominant component of the extract. The compound with the highest peak area (3.49%) was a trimethylsilyl ester, and the NIST library match of 74% is only moderate, so this identification should be treated as tentative. Trimethylsilyl-containing compounds often originate from silylating reagents, septa or the polysiloxane stationary phase of the column (column bleed) rather than from the plant material. The molecular ion at m/z 281 reported for this compound is also one of the ions commonly associated with polysiloxane column bleed, and the total ion chromatogram in Figure 3 shows a steeply rising baseline at retention times above about 26 min, which is typical of column bleed at the high oven temperatures

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(up to 320 °C) used in the method. Such artefacts would also make it difficult to match the low-abundance peaks reliably, including the compound with a library quality of only 4%.

Eugenol (molecular weight 164 g/mol) would be expected to show a molecular ion at m/z 164 with characteristic fragments such as m/z 149, 131, 103 and 77. A comparison of the sample spectrum with these ions, and with an authentic eugenol standard run under the same conditions, is needed to confirm or rule out its presence. The rapid temperature ramp (30 °C/min) used here favours speed over resolution and may have limited the separation of components in the extract. Future analyses should use a slower temperature programme, a more dilute sample, an authentic standard to establish the retention time and mass spectrum of eugenol, and a solvent blank to identify column-bleed and reagent artefacts.

The Antibacterial activity of the extracted compound from clove was evaluated against Staphylococcus aureus and Escherichia coli using the agar well diffusion method. The zones of inhibition and the effective concentrations are comparable to the literature report for eugenol (Inem Ode et al., 2023). This antibacterial activity of eugenol against S. Aureus is consistent with previous studies by (Inem Ode et al., 2023) who reported MIC values ranging from 20- 50mg/mL (Inem Ode et al., 2023). Similarly, the activity against E. coli is in the agreement with report of MIC value between 20-100mg/mL. The antibacterial evaluation confirms the potential of the extracted compound from clove as a natural antimicrobial agent.

The zones of inhibition show that the extract was active against both the Gram-positive S. aureus and the Gram-negative E. coli (Table 2). For E. coli, the zone increased steadily from 18 mm at 30 mg/cm3 to 22 mm at 60 mg/cm3 and 28 mm at 90 mg/cm3, a clear concentration-dependent response. For S. aureus, the zone increased from 15 mm to 25 mm between 30 and 90 mg/cm3, although no zone was recorded at 60 mg/cm3. This irregular result may reflect experimental variation, such as uneven diffusion or well preparation, and should be verified by repeating the assay in triplicate and reporting the mean ± standard deviation. The activity against E. coli is noteworthy because the outer membrane of Gram-negative bacteria generally limits the penetration of hydrophobic compounds. The antibacterial action of eugenol has been attributed mainly to disruption of the cytoplasmic membrane, which increases its permeability and causes leakage of intracellular contents, and to inhibition of essential enzymes (Hu et al., 2018). This is consistent with activity against both types of bacteria. However, because the extract is crude and contains other phenolic compounds, part of the activity observed may be due to these constituents or to synergistic effects rather than to eugenol alone. The inclusion of a standard antibiotic as a positive control and the solvent as a negative control, together with determination of the minimum inhibitory concentration, would allow the potency of the extract to be compared directly with that of established antimicrobial agents.

The results of colour test for the extracted compound from clove shown in Table 3. The observed colour reactions are consistent with the expected results for phenolic compounds. The green colouration with FeCl3 indicates the presence of phenolic group, similar to the literature reported by (DeFrancesco, 2021) for eugenol. The red and black colouration with marquis and Mecke reagents suggests the presence of the conjugated double bonds, which in the literature reported by (DeFrancesco, 2021) was light green. The colour test results support the presence of phenolic groups and conjugated double bonds, consistent with the expected structure of eugenol.

Colour tests are presumptive and non-specific. The green colour with FeCl3 arises from the formation of a coloured complex between Fe3+ and the phenolic hydroxyl group, so it would also be given by other phenolics in the crude ethanolic extract, such as tannins and flavonoids, while the Marquis and Mecke reagents react with a broad range of aromatic compounds. The colour tests therefore indicate that phenolic compounds, of which eugenol is the principal one in cloves, are present, but they cannot by themselves confirm eugenol.

Taken together, the high yield, the phenolic colour reactions, the FTIR bands and the antibacterial activity indicate that the ethanolic clove extract is rich in phenolic constituents with the functional groups and biological activity expected of eugenol. The GC-MS result, however, did not provide independent confirmation, and the identity and purity of eugenol in the extract remain to be established. Further purification of the extract (for example by column chromatography or liquid–liquid extraction), optimised GC-MS analysis

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with an authentic eugenol standard, and replicated antibacterial assays with proper controls would strengthen these findings.

CONCLUSION

This study extracted clove buds by Soxhlet extraction with ethanol, characterized the extract using colour tests, FTIR and GC-MS, and evaluated its antibacterial activity. Extraction of 12.5 g of dried clove powder gave 9.7 g of crude extract, a percentage yield of 77.6%, showing that the method is efficient for recovering clove constituents. The Marquis, Mecke and FeCl3 colour tests gave red, green-to-black and green colours, respectively, indicating the presence of phenolic compounds. The FTIR spectrum showed bands for the O-H, C-H, aromatic C=C and C-O groups that agree with literature values for eugenol, whereas GC-MS showed a complex mixture in which eugenol was not clearly identified, so its presence is supported mainly by the colour tests and FTIR. The extract inhibited both S. aureus and E. coli, with zones of inhibition of up to 25 mm and 28 mm, respectively, at 90 mg/cm3.

These findings imply that locally available cloves are a simple and low-cost source of a phenolic extract with antibacterial activity, which could be developed as a natural antimicrobial for pharmaceutical, biomedical and food-preservation applications and may reduce reliance on synthetic antimicrobials. They also show that colour tests and FTIR alone are not sufficient to confirm the identity and purity of eugenol, which is essential for pharmaceutical and industrial use. Further work should focus on purification of eugenol from the crude extract, confirmation against an authentic standard by optimised GC-MS or HPLC, determination of the minimum inhibitory and bactericidal concentrations with positive controls, and toxicity evaluation before any application.

Conflict of Interest

The author declares that there is no conflict of interest.

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