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Integrated Evaluation of Phytochemical Composition, Antioxidant, Antibacterial, Antifungal and Antiproliferative Activities of *Syzygium aromaticum*

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Abstract

Syzygium aromaticum (L.) (clove) is a medicinal plant rich in biologically active phytochemicals with diverse therapeutic applications. The present study aimed to comprehensively evaluate its phytochemical composition, antioxidant, antibacterial, antifungal, and antiproliferative activities. Dried flower buds of *S. aromaticum* were processed to obtain aqueous extracts, which were subjected to qualitative phytochemical screening, thin-layer chromatography (TLC), and Fourier transform infrared (FTIR) spectroscopy for chemical characterization. Antioxidant activity was determined using the DPPH free radical scavenging assay. Antibacterial efficacy was evaluated against *Escherichia coli*, *Aeromonas hydrophila*, and *Staphylococcus aureus* using standard in vitro assays, followed by in vivo evaluation in *Cyprinus carpio* challenged with *A. hydrophila*. Antifungal activity was assessed against *Aspergillus flavus* and *Trichoderma viride*. The antiproliferative potential was investigated against HepG2 human liver carcinoma cells using the MTT assay. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides. The extract exhibited strong antioxidant activity, achieving 87.15 ± 0.18% DPPH radical scavenging at 100 µg/mL. TLC analysis revealed two major bioactive fractions (Rf 0.42 and 0.91), while FTIR spectra indicated the presence of hydroxyl, carbonyl, and aromatic functional groups. The extract demonstrated significant antibacterial activity against all tested pathogens and enhanced survival, hematological parameters, lysozyme activity, phagocytic activity, and bacterial agglutination in *C. carpio* following bacterial challenge. Furthermore, *S. aromaticum* inhibited the growth of *A. flavus* and *T. viride* and exhibited dose-dependent antiproliferative activity against HepG2 cells. The findings demonstrate that *Syzygium aromaticum* is a rich source of bioactive secondary metabolites with potent antioxidant, antimicrobial, immunostimulatory, and antiproliferative properties. These results support its potential application in pharmaceutical development, functional foods, and aquaculture as a natural alternative to synthetic antimicrobial and therapeutic agents.

Keywords: *Syzygium aromaticum*;phytochemical analysis;antibacterial activity; antifungal activity; antiproliferative activity

1.Introduction

Medicinal plants have served as an indispensable source of therapeutic agents since ancient times and continue to contribute significantly to global healthcare systems (World Health Organization [WHO], 2019). Approximately 80% of the world's population depends on traditional herbal medicines for primary healthcare, particularly in developing countries where medicinal plants remain readily accessible and affordable (WHO, 2019). Plant-derived natural products contain a wide variety of bioactive secondary metabolites, including alkaloids, flavonoids, phenolic acids, tannins, terpenoids, and glycosides, which exhibit diverse pharmacological activities (Newman & Cragg, 2020). These phytochemicals possess antioxidant, antimicrobial, anti-inflammatory, antiviral, and anticancer properties, making medicinal plants valuable resources for drug discovery and pharmaceutical development (Atanasov et al., 2021). Consequently, scientific validation of medicinal plants has become an important research priority to identify safe and effective therapeutic agents capable of addressing emerging human health challenges (Veeresham, 2012).

The rapid emergence of antimicrobial resistance has become one of the most serious threats to global public health, reducing the effectiveness of conventional antibiotics and increasing the incidence of multidrug-resistant infections (WHO, 2023). Excessive and inappropriate use of antimicrobial drugs in human medicine, veterinary practice, and agriculture has accelerated the evolution of resistant bacterial strains worldwide (Murray et al., 2022). Likewise, fungal infections caused by opportunistic pathogens have increased considerably among immunocompromised patients, while resistance to existing antifungal drugs continues to compromise treatment outcomes (Perfect, 2017). These challenges have intensified the search for novel antimicrobial compounds from medicinal plants because plant secondary metabolites often act through multiple mechanisms and exhibit comparatively lower toxicity than synthetic drugs (Nazzaro et al., 2017). Therefore, medicinal plants are increasingly regarded as promising sources for developing alternative antimicrobial agents capable of combating drug-resistant microorganisms (Batiha et al., 2020).

Oxidative stress plays a fundamental role in the initiation and progression of numerous chronic diseases, including cancer, diabetes mellitus, cardiovascular disorders, neurodegenerative diseases, and inflammatory conditions (Liguori et al., 2018). Reactive oxygen species generated during normal cellular metabolism can damage lipids, proteins, and nucleic acids when antioxidant defense systems become insufficient (Pham-Huy et al., 2008). Plant-derived phenolic compounds and flavonoids effectively neutralize free radicals by donating hydrogen atoms or electrons, thereby preventing oxidative cellular injury (Shahidi & Ambigaipalan, 2015). Numerous studies have demonstrated a strong correlation between the antioxidant capacity of medicinal plants and their therapeutic efficacy against oxidative stress-related diseases (Prior et al., 2005). Consequently, evaluating the antioxidant potential of medicinal plants has become an essential step in identifying bioactive compounds with potential pharmaceutical applications (Atanasov et al., 2021).

Syzygium aromaticum, commonly known as clove, belongs to the family Myrtaceae and is one of the most valuable medicinal spices cultivated in tropical regions worldwide (Cortés-Rojas et al., 2014). The dried flower buds of clove have been extensively used in traditional medicine for treating toothache, respiratory disorders, digestive ailments, microbial infections, and inflammatory diseases (Kamatou et al., 2012). The remarkable pharmacological properties of *S. aromaticum* are primarily attributed to its abundant phytochemicals, particularly eugenol, eugenyl acetate, β-caryophyllene, gallic acid, ellagic acid, flavonoids, and tannins (Batiha et al., 2020). Previous investigations have reported that these bioactive constituents exhibit potent antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, analgesic, and anticancer activities through multiple molecular mechanisms (Cortés-Rojas et al., 2014). These findings have stimulated increasing interest in utilizing clove as a natural source of pharmaceutical compounds, functional food ingredients, and therapeutic agents (Atanasov et al., 2021).

Although several studies have independently investigated the antioxidant, antimicrobial, or anticancer activities of *Syzygium aromaticum*, comprehensive studies integrating phytochemical characterization with multiple biological activities remain comparatively limited (Batiha et al., 2020). Establishing relationships between phytochemical composition and biological efficacy is essential for identifying the active constituents responsible for therapeutic effects and for supporting evidence-based utilization of medicinal plants (Newman & Cragg, 2020). Furthermore, integrated pharmacological evaluation provides valuable scientific evidence for the development of plant-based antimicrobial formulations, nutraceuticals, and novel drug candidates (Atanasov et al., 2021). Therefore, the present study comprehensively evaluated the phytochemical composition of *Syzygium aromaticum* using qualitative phytochemical analysis, thin-layer chromatography (TLC), and Fourier transform infrared (FTIR) spectroscopy. In addition, the antioxidant, antibacterial, antifungal, and antiproliferative activities of the plant extract were investigated to provide scientific evidence supporting its potential application in pharmaceutical, biomedical, and functional food industries.

2. Materials and Methods

2.1 Plant material collection and authentication

Dried flower buds of *Syzygium aromaticum* were procured from a certified herbal medicinal supplier in Tamil Nadu, India. The plant material was taxonomically authenticated by a qualified botanist, and a voucher specimen was deposited in the Herbarium of the PG and Research Department of Zoology, Muslim Arts College, Thiruvithancode, Tamil Nadu, India, for future reference. The flower buds were washed thoroughly with distilled water to remove adhering dust and foreign materials, shade-dried at room temperature (25 ± 2°C) for seven days, and pulverized into a fine powder using a sterile mechanical grinder. The powdered material was stored in airtight amber-colored containers at room temperature until further analysis.

2.2 Preparation of aqueous extract

Fifty grams of powdered flower buds were mixed with 500 mL of distilled water and extracted by continuous stirring at 60°C for 4 h. The extract was filtered through double-layer muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator at 40°C and subsequently freeze-dried to obtain a dry crude extract. The extraction yield was calculated, and the dried extract was preserved at 4°C until further experimental analyses. Fresh stock solutions were prepared in sterile distilled water before each experiment.

2.3 Qualitative phytochemical screening

Preliminary phytochemical screening was carried out using standard protocols to detect major classes of secondary metabolites. Alkaloids were determined by Mayer's and Wagner's tests, flavonoids by the alkaline reagent test, tannins by ferric chloride test, phenols by ferric chloride reaction, saponins by the frothing method, glycosides by Keller-Killiani test, steroids by Liebermann-Burchard reaction, terpenoids by the Salkowski test, and carbohydrates by Molisch's test. Colour development or precipitate formation was considered positive for the respective phytochemical constituents.

2.4 Thin-layer chromatography (TLC)

The aqueous extract was subjected to thin-layer chromatography using silica gel 60 F254 precoated aluminium plates. Approximately 10 µL of extract was spotted onto the TLC plate using a micropipette. The chromatogram was developed in a suitable solvent system until the solvent front migrated approximately 8 cm. The developed plates were air-dried and visualized under ultraviolet light at 254 nm and 365 nm. The retention factor (Rf) values of separated phytochemical constituents were calculated using the formula:

Rf = Distance travelled by compound / Distance travelled by solvent front

The chromatographic profile was used to identify major bioactive fractions.

2.5 Fourier Transform Infrared (FTIR) spectroscopy

Functional groups present in the crude extract were characterized using Fourier Transform Infrared Spectroscopy. Approximately 2 mg of dried extract was mixed thoroughly with spectroscopic-grade potassium bromide (KBr), compressed into transparent pellets, and scanned over a spectral range of 4000–400 cm^{-1} using an FTIR spectrophotometer. The absorption peaks obtained were interpreted to identify characteristic functional groups associated with phytochemical constituents.

2.6 Determination of antioxidant activity

The antioxidant activity of the extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Different concentrations of the extract (20–100 µg/mL) were prepared. Equal volumes of DPPH solution and plant extract were mixed and incubated in the dark for 30 min at room temperature. The absorbance

was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid served as the reference standard. The percentage inhibition of DPPH radicals was calculated using the following equation:

Radical scavenging activity (%) = [(Ac − As)/Ac] × 100

where Ac represents the absorbance of the control and As represents the absorbance of the sample.

2.7 Antibacterial activity

The antibacterial activity of the extract was evaluated against *Escherichia coli*, *Aeromonas hydrophila*, and *Staphylococcus aureus* using the agar well diffusion method. Fresh bacterial cultures were adjusted to 0.5 McFarland turbidity and uniformly spread on Mueller-Hinton agar plates. Wells measuring 6 mm in diameter were prepared using a sterile cork borer and filled with different concentrations of plant extract. Ciprofloxacin served as the positive control, whereas sterile distilled water served as the negative control. Plates were incubated at 37°C for 24 h, and the diameter of inhibition zones was measured in millimetres.

2.8 In vivo antibacterial evaluation

Healthy *Cyprinus carpio* fingerlings were acclimatized under laboratory conditions for two weeks before experimentation. Fish were randomly divided into four experimental groups, including control and extract-supplemented diets containing different concentrations of *S. aromaticum*. Feeding was continued for 45 days. Subsequently, fish were challenged with *Aeromonas hydrophila* by intraperitoneal injection. Survival rate, mortality, hematological parameters, serum protein concentration, lysozyme activity, phagocytic activity, and bacterial agglutination assays were evaluated following standard immunological procedures.

2.9 Antifungal activity

The antifungal activity of the extract was determined against *Aspergillus flavus* and *Trichoderma viride* using the poisoned food technique. Different concentrations of plant extract were incorporated into sterile potato dextrose agar before solidification. Fungal discs (5 mm diameter) obtained from actively growing cultures were inoculated onto the centre of each plate. The plates were incubated at 28 ± 2°C for seven days. Radial fungal growth was measured periodically, and percentage inhibition was calculated relative to untreated controls.

2.10 Antiproliferative activity

The antiproliferative activity was evaluated against HepG2 human hepatocellular carcinoma cells using the MTT assay. HepG2 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and antibiotics under standard culture conditions (37°C, 5% CO_2). Cells were seeded into 96-well plates and treated with different concentrations of *S. aromaticum* extract for 24 h. After incubation, MTT reagent was added and further incubated for 4 h. Formazan crystals formed by viable cells were dissolved using dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as the percentage of untreated control cells, and the IC_{50} value was determined.

2.11 Statistical analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analyses were carried out using SPSS software (Version 25.0). Differences among experimental groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical significance was considered at $\mathbf{P < 0.05}$.

3. Results

3.1 Phytochemical screening

Qualitative phytochemical analysis of the aqueous extract of *Syzygium aromaticum* revealed the presence of several biologically important secondary metabolites. The extract tested positive for alkaloids, flavonoids, phenolic

compounds, tannins, glycosides, saponins, steroids, and terpenoids, indicating that the plant possesses a rich phytochemical profile. Among these constituents, phenolics and flavonoids were predominant, suggesting their possible contribution to the biological activities observed in the present investigation. The presence of these phytochemicals supports the traditional medicinal use of *S. aromaticum* and indicates its potential as a valuable source of natural therapeutic compounds (Table 1).

Table 1. Qualitative phytochemical constituents of *Syzygium aromaticum* aqueous extract

SNo	Phytochemical	Result
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	+
4	Tannins	+
5	Saponins	+
6	Glycosides	+
7	Steroids	+
8	Terpenoids	+

Note: (+) Present; (−) Absent.

3.2 Thin-layer chromatography (TLC) and FTIR characterization

Thin-layer chromatographic analysis of the aqueous extract revealed two prominent phytochemical fractions with retention factor (Rf) values of **0.42** and **0.91**, indicating the presence of major bioactive constituents within the extract. The chromatographic separation demonstrated the chemical complexity of the crude extract and confirmed the occurrence of multiple secondary metabolites. Fourier Transform Infrared (FTIR) spectroscopy further characterized the extract by identifying several functional groups. Prominent absorption peaks corresponded to hydroxyl (O–H), aliphatic C–H, carbonyl (C=O), aromatic C=C, and C–O functional groups, confirming the presence of phenolic compounds, alcohols, aldehydes, ketones, esters, and aromatic constituents. These functional groups are commonly associated with compounds possessing antioxidant and antimicrobial properties (Table 2, 3).

Table 2. Thin Layer Chromatographic profile of *Syzygium aromaticum*

Extract	Number of active fractions	Rf values
Hot water extract	2	**0.42, 0.91**
Methanol extract	Not detected	—

Table 3. Major functional groups identified by FTIR analysis

SNo	Peak Region (cm^{-1})	Functional group	Probable phytochemical class
1	3400–3200	O–H stretching	Phenols, alcohols
2	2920–2850	C–H stretching	Alkanes
3	1700–1600	C=O stretching	Aldehydes, ketones
4	1600–1500	Aromatic C=C	Phenolics
5	1300–1000	C–O stretching	Alcohols, esters

3.3 Antioxidant activity

The antioxidant activity of *Syzygium aromaticum* extract increased progressively with increasing concentration, demonstrating a clear dose-dependent response. Among all concentrations tested, the extract exhibited the highest DPPH free radical scavenging activity of 87.15 ± 0.18% at 100 µg/mL**,** indicating strong antioxidant potential. Lower concentrations produced proportionately reduced scavenging activity, confirming concentration-dependent free radical neutralization. The antioxidant efficiency observed may be attributed to the abundance of phenolic compounds and flavonoids identified during phytochemical screening, which effectively donate hydrogen atoms to stabilize free radicals and reduce oxidative stress (Table 4).

Table 4. Antioxidant activity of *Syzygium aromaticum* by DPPH assay

S.No	Concentration (µg/mL)	DPPH Radical Scavenging Activity (%)
1	20	—
2	40	—
3	60	—
4	80	—
5	100	87.15 ± 0.18

Standard (Ascorbic acid): 87.96 ± 0.23% at 100 µg/mL.

3.4 Antibacterial activity

The aqueous extract of *Syzygium aromaticum* demonstrated remarkable antibacterial activity against all tested bacterial pathogens, namely *Escherichia coli*, *Aeromonas hydrophila*, and *Staphylococcus aureus*. The diameter of inhibition zones increased with increasing extract concentration, indicating concentration-dependent antibacterial efficacy. Among the tested organisms, *Staphylococcus aureus* showed comparatively greater susceptibility, followed by *Aeromonas hydrophila* and *Escherichia coli*. The inhibitory activity observed suggests that the phytochemicals present in the extract interfere with bacterial cell wall integrity and cellular metabolism, resulting in effective suppression of microbial growth.(Table 5)

Table 5. Antibacterial activity of *Syzygium aromaticum*

S.No	Test organism	Zone of inhibition (mm)
1	*Escherichia coli*	15.30 ± 0.14
2	*Aeromonas hydrophila*	16.54 ± 0.36
3	*Staphylococcus aureus*	15.50 ± 0.14

3.5 In vivo antibacterial efficacy

Dietary supplementation with *Syzygium aromaticum* significantly enhanced disease resistance in *Cyprinus carpio* following experimental challenge with *Aeromonas hydrophila* (Table 6). The control group exhibited the highest cumulative mortality, reaching 90% after 30 days and 85% after 60 days post-challenge. In contrast, fish fed the *S. aromaticum*-supplemented diets showed markedly lower mortality. The Sa-1 group (100 mg $diet^{-1}$) recorded cumulative mortalities of 20% and 25% after 30 and 60 days, respectively, whereas the Sa-2 group (200 mg $diet^{-1}$) exhibited the lowest mortalities of only 5% and 15%, demonstrating superior protection against bacterial infection.

Hematological analysis further confirmed the beneficial effects of dietary *S. aromaticum* supplementation. After 60 days post-vaccination, the red blood cell (RBC) count increased from 0.98 × 10^6 cells mm^{-3} in the control group to

1.60 × 10^6 cells mm^{-3} in the Sa-1 group and 1.39 × 10^6 cells mm^{-3} in the Sa-2 group. Similarly, haemoglobin concentration showed progressive improvement in the treated groups. At 30 days post-vaccination, haemoglobin levels increased from 6.27 g dL^{-1} in the control fish to 6.41 g dL^{-1} and 6.47 g dL^{-1} in the Sa-1 and Sa-2 groups, respectively. After 60 days, haemoglobin concentration further increased to 6.78 g dL^{-1} in the Sa-1 group and 6.84 g dL^{-1} in the Sa-2 group, indicating enhanced oxygen-carrying capacity and improved physiological status.

Table 6. Effect of dietary *Syzygium aromaticum* supplementation on cumulative mortality and hematological parameters of *Cyprinus carpio* challenged with *Aeromonas hydrophila*

S.No	Parameter	Control	Sa-1 (100 mg $diet^{-1}$)	Sa-2 (200 mg $diet^{-1}$)
1	Cumulative mortality after 30 days (%)	90	20	5
2	Cumulative mortality after 60 days (%)	85	25	15
3	RBC after 60 dpv (×10^6 cells mm^{-3})	0.98	1.60	1.39
4	Haemoglobin after 30 dpv (g dL^{-1})	6.27	6.41	6.47
5	Haemoglobin after 60 dpv (g dL^{-1})	—	6.78	6.84

3.6 Antifungal activity

The aqueous extract exhibited considerable antifungal activity against both *Aspergillus flavus* and *Trichoderma viride*. Radial mycelial growth of both fungal pathogens was significantly reduced in extract-treated plates compared with untreated controls. At the highest concentration tested, approximately 48% inhibition of fungal growth was observed, confirming the broad-spectrum antifungal efficacy of the extract. The inhibitory effect is likely associated with the presence of phenolic constituents capable of disrupting fungal cell membranes and inhibiting fungal enzyme systems responsible for growth and reproduction.

3.7 Antiproliferative activity

The antiproliferative activity of *Syzygium aromaticum* extract was evaluated against HepG2 human hepatocellular carcinoma cells using the MTT assay. The extract produced a concentration-dependent reduction in cell viability, with increasing concentrations resulting in greater inhibition of cancer cell proliferation. Morphological examination of treated cells revealed characteristic apoptotic features, including cell shrinkage, membrane blebbing, and reduced cell density. The results indicate that the extract possesses significant cytotoxic activity against HepG2 cells and may induce apoptosis through phytochemical-mediated mechanisms. The observed antiproliferative activity highlights the therapeutic potential of *S. aromaticum* as a promising source of natural anticancer compounds **(**Figure 1**).**

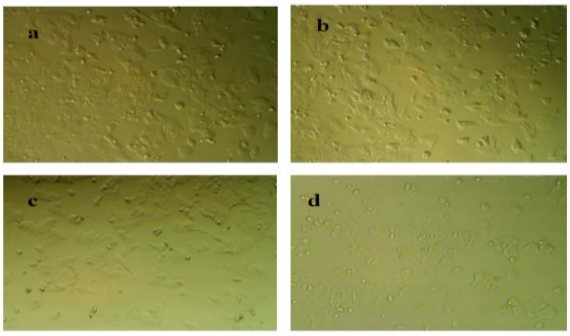

Figure 1. Dose-dependent antiproliferative effect of *Syzygium aromaticum* extract on HepG2 cells determined by the MTT assay.

3.8 Biological activity

The present investigation demonstrated that *Syzygium aromaticum* possesses a broad spectrum of biological activities attributable to its diverse phytochemical constituents. The extract exhibited strong antioxidant capacity, potent antibacterial and antifungal activities, enhanced innate immune responses in experimental fish, and significant antiproliferative effects against HepG2 liver cancer cells. These complementary biological properties indicate that *S. aromaticum* is a valuable medicinal plant with potential applications in pharmaceutical, nutraceutical, biomedical, and aquaculture industries. The integration of phytochemical characterization with multiple biological assays provides comprehensive scientific evidence supporting the therapeutic significance of this traditionally important medicinal species.

4. Discussion

The present investigation demonstrated that *Syzygium aromaticum* possesses remarkable phytochemical diversity and multiple biological activities, including antioxidant, antibacterial, antifungal, and antiproliferative properties. These biological effects are primarily attributed to the presence of secondary metabolites such as phenolics, flavonoids, tannins, alkaloids, saponins, and terpenoids identified during qualitative phytochemical screening. Plant secondary metabolites have long been recognized as major contributors to the pharmacological properties of medicinal plants owing to their ability to interact with diverse biological targets (Atanasov et al., 2021). The present findings are consistent with previous reports that identified eugenol, eugenyl acetate, β-caryophyllene, gallic acid, ellagic acid, and numerous phenolic compounds as the principal bioactive constituents of *S. aromaticum* (Batiha et al., 2020). These compounds collectively contribute to the therapeutic value of clove through synergistic antioxidant, antimicrobial, and anticancer mechanisms (Kamatou et al., 2012).

Thin-layer chromatography revealed two prominent phytochemical fractions with Rf values of 0.42 and 0.91, indicating the presence of multiple biologically active constituents. FTIR analysis further confirmed the occurrence of hydroxyl, carbonyl, aromatic, and ether functional groups that are characteristic of phenolic compounds and essential oils. Similar FTIR spectra have been reported for clove extracts rich in eugenol and related phenolic derivatives (Cortés-Rojas et al., 2014). Functional group characterization provides valuable evidence supporting the presence of compounds responsible for free radical scavenging and antimicrobial activity (Kamatou et al., 2012). The integration of chromatographic and spectroscopic analyses therefore strengthens the phytochemical characterization of the extract and provides a chemical basis for its biological activities.

The antioxidant activity observed in the present study demonstrated a concentration-dependent increase in DPPH radical scavenging activity, with maximum inhibition recorded at 100 µg/mL. This result indicates that *S. aromaticum* possesses an efficient hydrogen-donating capacity capable of neutralizing reactive oxygen species. Phenolic compounds and flavonoids are well known for their ability to donate electrons or hydrogen atoms to unstable free radicals, thereby terminating oxidative chain reactions (Shahidi & Ambigaipalan, 2015). The high antioxidant activity observed in this investigation agrees with earlier studies demonstrating that clove exhibits one of the highest antioxidant capacities among commonly used medicinal spices because of its exceptionally high phenolic content (Cortés-Rojas et al., 2014). Since oxidative stress is implicated in the development of cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders, natural antioxidants from medicinal plants may provide significant therapeutic benefits (Liguori et al., 2018).

The antibacterial activity of *S. aromaticum* against *Escherichia coli*, *Aeromonas hydrophila*, and *Staphylococcus aureus* confirms its broad-spectrum antimicrobial potential. The observed increase in inhibition zones with increasing extract concentration indicates a dose-dependent antibacterial response. Eugenol, the major constituent of clove, has been reported to damage bacterial cell membranes, increase membrane permeability, denature cellular proteins, and inhibit essential metabolic enzymes, ultimately resulting in bacterial cell death (Nazzaro et al., 2017). Previous studies have demonstrated strong antibacterial activity of clove extracts against both Gram-positive and Gram-negative bacteria, including multidrug-resistant clinical isolates (Batiha et al., 2020). The antibacterial efficacy observed in the present study therefore supports the potential application of *S. aromaticum* as a natural

alternative to conventional antibiotics, particularly in the context of increasing antimicrobial resistance worldwide (World Health Organization, 2023).

The in vivo antibacterial evaluation further demonstrated that dietary supplementation with *S. aromaticum* significantly improved survival and immune responses in *Cyprinus carpio* challenged with *Aeromonas hydrophila*. Enhanced hematological parameters, serum protein concentration, lysozyme activity, phagocytic activity, and bacterial agglutination indicate stimulation of the innate immune system. Medicinal plants containing phenolics and flavonoids have previously been shown to enhance macrophage activation, complement pathways, antioxidant enzyme activity, and cytokine production in fish (Harikrishnan et al., 2011). The immunostimulatory activity observed in the present investigation suggests that *S. aromaticum* may serve as a functional feed additive capable of improving disease resistance and reducing dependence on antibiotics in aquaculture. Such plant-based immunostimulants have received considerable attention because they improve fish health while minimizing environmental contamination and antimicrobial resistance (Dawood et al., 2022).

The aqueous extract also exhibited significant antifungal activity against *Aspergillus flavus* and *Trichoderma viride*. Fungal growth inhibition observed in this study may result from disruption of fungal cell membrane integrity, inhibition of ergosterol biosynthesis, and interference with mitochondrial respiration caused by phenolic compounds and essential oils. Eugenol has been reported to alter membrane permeability, inhibit spore germination, and suppress fungal enzyme activity, thereby reducing fungal growth and pathogenicity (Pinto et al., 2009). Similar antifungal effects of clove extracts have been documented against *Candida albicans*, *Aspergillus* spp., and several phytopathogenic fungi (Batiha et al., 2020). The broad-spectrum antifungal activity demonstrated in the present investigation suggests potential applications of *S. aromaticum* in pharmaceutical formulations, food preservation, and agricultural disease management.

The antiproliferative activity of *S. aromaticum* against HepG2 liver carcinoma cells demonstrated a concentration-dependent reduction in cell viability, indicating significant cytotoxic effects on cancer cells. Morphological changes including cell shrinkage, membrane blebbing, and decreased cell density suggest apoptosis as a possible mechanism of cell death. Previous investigations have reported that eugenol induces apoptosis by activating caspase-dependent pathways, increasing reactive oxygen species generation within cancer cells, disrupting mitochondrial membrane potential, and regulating apoptosis-related proteins such as Bax, Bcl-2, and p53 (Batiha et al., 2020). Furthermore, phenolic compounds present in clove have been shown to arrest the cell cycle, inhibit angiogenesis, and suppress inflammatory signalling pathways associated with tumour progression (Pramod et al., 2010). These mechanisms collectively explain the antiproliferative effects observed in the present investigation and support the development of *S. aromaticum*-derived compounds as potential anticancer agents.

The multifunctional biological activities demonstrated in the present study indicate that *S. aromaticum* possesses considerable therapeutic potential beyond its traditional medicinal applications. The combination of strong antioxidant activity, broad-spectrum antimicrobial effects, immunostimulatory properties, and antiproliferative activity suggests that the phytochemicals present in clove may act synergistically rather than individually. Such synergistic interactions among bioactive constituents frequently enhance pharmacological efficacy while reducing toxicity, making whole plant extracts attractive candidates for pharmaceutical and nutraceutical development (Atanasov et al., 2021). Nevertheless, further investigations involving bioassay-guided isolation of active compounds, molecular mechanism studies, pharmacokinetic evaluation, toxicity assessment, and well-designed clinical trials are required before therapeutic application can be fully established.

Overall, the present findings provide comprehensive scientific evidence supporting the medicinal importance of *Syzygium aromaticum*. The integration of phytochemical characterization with antioxidant, antibacterial, antifungal, and antiproliferative evaluations demonstrates the broad pharmacological potential of this medicinal plant. These findings support the continued exploration of *S. aromaticum* as a valuable source of natural bioactive compounds for the development of novel pharmaceuticals, functional foods, nutraceuticals, and sustainable antimicrobial agents.

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