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ORIGINAL ARTICLE **OPEN ACCESS**

Co-culturing *Actinomycetes* and *Candida*: Unlocking Bioactive Metabolites through Microbial Interactions

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ABSTRACT
Co-culturing, the simultaneous cultivation of two or more microorganisms, can activate silent biosynthetic gene clusters and stimulate the production of novel secondary metabolites through nutrient competition, signalling exchange, and physical interactions. In this study, Actinomycetes and Candida species isolated from rhizosphere soil were co-cultivated in Modified Yeast Glucose Medium (MYGM) at 28 °C for 12 days to evaluate their bioactive potential. The co-culture displayed distinct growth dynamics, including early Candida biofilm formation, increased Actinomycetes proliferation, and medium colour change, indicating metabolic interplay. Ethyl acetate extracts from the co-culture were screened for antioxidant, anti-inflammatory, antimicrobial, anti-quorum sensing, anti-biofilm, anticancer, and cytotoxic activities. The extract showed enhanced antimicrobial efficacy against various bacterial and fungal pathogens, along with significant quorum sensing inhibition and biofilm suppression. GC–MS analysis revealed unique metabolites absent in monocultures, suggesting activation of previously silent biosynthetic pathways. These findings underscore the metabolic synergy between Actinomycetes and Candida, positioning co-culture as a promising strategy for discovering novel bioactive compounds with pharmaceutical relevance.
Keywords: *Co-culture, Streptomyces sp., Candida sp., secondary metabolites, antimicrobial activity, quorum sensing inhibition, biofilm suppression, GC–MS analysis.*

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INTRODUCTION

Antimicrobial resistance (AMR) is definitely a growing crisis that is causing an uproar leading to permanent damages or lethality. The existing antibiotics are used against the pathogenic microbes extensively that is resulting in the resistance towards the same antibiotic. While the antibiotic itself takes a toll on the human metabolism, the stronger the medicine is the greater its effects are. Whether the antibiotic is synthetic or natural, when it is exhausted the microbial metabolism finds loopholes and find the escape mechanism. [1] And scientists predict that from the era where discovery of an antibiotic is a need, we will escalate to an era where discovery of antibiotic will be mandatory. "AMR directly caused 1.27 million deaths" says GRAM (Global Research on Anti-Microbial resistance). [2] Many researchers have predicted the same and have proposed synthetic and natural methods in obtaining antibiotics. Synthetic methods are promising but is bound to have side effects on regular intake and ultimately, AMR. Natural methods may be primitive but it is promising. But it may also end up in similar issue. [3] But what if an antibiotic can be specifically produced for a particular microorganism?

One such method is co-culturing. Co-cultivation also referred to as mixed fermentation, microbial consortium, or microbial blend - is a technique that involves cultivating multiple microbial species simultaneously under controlled conditions. This method simulates natural microbial ecosystems, encouraging interspecies interactions that can trigger otherwise dormant biosynthetic gene clusters. In such dynamic microbial communities, bacteria and fungi engage in complex relationships that influence their survival, adaptability, and pathogenic potential, ultimately shaping the ecological balance of their environment. [4]

Cultivating diverse microbial species can stimulate the synthesis of novel metabolites and activate previously silent biosynthetic gene clusters. This approach mirrors natural microbial ecosystems, where interspecies interactions often lead to chemical innovation. A classic example is the serendipitous discovery of penicillin. More recently, enhanced Istamycin production was observed when *Streptomyces tenjimariensis* was co-cultured with a consortium of twelve marine bacterial strains, demonstrating the power of microbial interplay in unlocking new bioactive compounds. [5]

A key aspect of these interactions is quorum sensing, a cell density-dependent process where microbes communicate and coordinate behavior by releasing signaling molecules like auto-inducers. [6] Microorganisms release quorum-sensing molecules (QSMs) to regulate their biological functions and enable cell-to-cell communication. In fungi, common QSMs include farnesol, tyrosol, phenylethanol, and tryptophol. [7] One of the widely explored communities in co-culture systems, *Actinomyces* (or *Actinobacteria*), a gram-positive group of *Actinomycetes* with fungus-like branching filaments, play a pivotal role due to their prolific production of secondary metabolites such as antibiotics, antifungals, and anticancer agents. These microbes harbour numerous silent biosynthetic gene clusters (BGCs) that are often activated under stress or interspecies interactions. [8] Notably, co-culturing Streptomyces sp. 2-85 with Cladosporium sp. 3-22 enhanced borrelidin production [9], while *Streptomyces lividans* with *Fusarium tricinctum* induced novel naphthoquinone dimers [10].

Alongside, *Candida*—a genus of yeast-like fungi commonly found in diverse environments including the human microbiome—contributes to co-culture dynamics. *Candida albicans*, a prominent species, is known for biofilm formation and secreting farnesol, a quorum sensing molecule with interkingdom signalling roles [11]. Its interactions influence virulence and antimicrobial resistance, mediated by signalling molecules like N-acyl homoserine lactones (AHLs) in bacteria and farnesol in fungi. [12] These microbial interactions, driven by nutrient competition, signalling exchange, and physical proximity, are expected to activate silent BGCs in Actinomyces and modify *Candida's* metabolic profile, potentially leading to novel antimicrobial or antifungal compounds.

Antimicrobial resistance is emerging as a major threat, driven by the widespread misuse of antibiotics, chemicals, detergents, cleansing agents and self-medication [13]. Co-culturing microbes offers a promising solution by unlocking silent biosynthetic gene clusters and generating chemically diverse metabolites. This study explores how co-culture conditions enhance secondary metabolite production, aiming to identify compounds with antimicrobial, antifungal, or quorum-sensing modulatory effects. The findings could reveal novel bioactive molecules and deepen our understanding of microbial interactions, with potential applications in medicine and biotechnology.

MATERIAL AND METHODS

Standard laboratory glassware and analytical-grade reagents were employed throughout the study, with a detailed list provided in Appendix A. Rhizosphere soil samples were collected from various garden locations using sterile containers to prevent contamination. The samples were promptly transported to the laboratory under controlled conditions for further microbial isolation and analysis.

Microbial strains used in this study were isolated from soil using standard enrichment and plating techniques. The *Actinomycete* isolate was cultured on starch casein agar, while the *Candida* species was maintained on Sabouraud dextrose agar. Pure cultures were obtained through repeated streaking and incubated under optimal conditions for each organism.

APPENDIX A: MATERIALS USED

Glassware:

- Sterile test tubes
- Sterile Conical Flasks
- Sterile Beakers
- Clean and Sterile Grease free Glass Slides
- Sterile Pippetes
- Glass Funnels
- L – Rod

Other Requirements:

- Inoculation loop
- Centrifuge tubes
- Rotary shaker
- UV Spectrophotometer
- Incubator
- Hot Air Oven
- Micropipette
- Whatman No.1 filter paper

- Sterile forceps
- Anticoagulated Human Blood
- Distilled Water

Media:

- Nutrient Agar
- Starch Casein Agar (SCA)
- Potato Dextrose Broth
- Potato Dextrose Agar
- Modified Yeast Extract Glucose Broth
- Modified Yeast Extract Glucose Agar
- Muller Hinton Agar (MHA)
- Muller-Hinton broth (MHB)
- Sabouraud Dextrose broth (SDB)
- Sabouraud Dextrose agar (SDA)
- Dulbecco's Modified Eagle Medium (DMEM)

Cultures:

- **Bacterial:**
    - *Escherichia coli*
    - *Proteus mirabilis*
    - *Staphylococcus aureus*
    - *Salmonella sp.*
    - *Klebsiella pneumoniae*
    - *Pseudomonas aeruginosa*
- **Fungal:**
    - *Aspergillus niger*
    - *Penicillium sp.*
    - *Trichoderma sp.*
    - *Mucor sp.*
    - *Trichophyton rubrum*
    - *Trichophyton mentagrophytes*
    - *Candida albicans*
- **Cell Lines**
    - MCF – 7 (Michigan Cancer Foundation - 7) Cell Lines
    - VERO (Verda Reno) Cell Lines

Reagents:

- Gram Staining Reagents: Crystal Violet, Gram's Iodine, Ethanol, Saffranine
- Lactophenol Cotton Blue Stain
- Ethyl Acetate
- 30% Acetic Acid
- 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)
- Dimethyl sulfoxide (DMSO)
- Methanol
- Bovine Serum Albumin (BSA)
- Fetal Bovine Serum (FBS)
- 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT)

APPENDIX B: LIST OF CULTURE MEDIA USED AND COMPOSITION

Composition of MYGM Broth:

Ingredients	Gms/Litre
Yeast Extract	1
Glucose	10
Peptone	2
Magnesium sulphate heptahydrate	0.2
Potassium dihydrogen phosphate	1
Dipotassium hydrogen phosphate	1
Final pH (at 25°C)	6.8 to 7.2

Composition of Potato Dextrose Broth:

Ingredients	Gms/Litre
Potato Infusion	4
Dextrose	20
Final pH (at 25°C)	5.6 ± 0.2

Preliminary identification was performed based on colony morphology, microscopic examination, and conventional biochemical tests. The *Actinomycete* exhibited filamentous growth with characteristic pigmentation and aerial mycelia, while *Candida* showed creamy, smooth colonies with budding yeast cells under microscopy. (Figure 1,2,3 & 4)

Figure 1 - *Actinomycetes* on SCA Under 100X

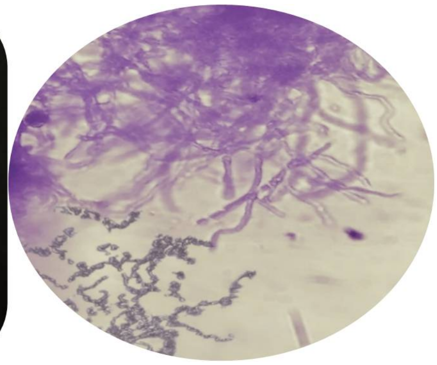


Figure 2 - Gram Staining of the isolated *Actinomycetes*

Figure 3 - Isolation of *Candida* sp. on SDA

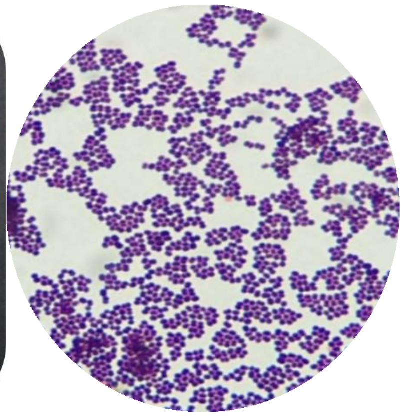


Figure 4 - Gram Staining of *Candida sp.* Under 100X

Co-culture of Bacteria and Fungi

A suitable nutrient medium that equally supports both the bacteria and fungi was selected. Modified Yeast Glucose Medium (MYGM) was prepared in 50 mL volumes under sterile conditions, following standard protocols. [12] (Refer Appendix B for Composition) A 100 µL aliquot of 24-hour cultures of Bacterial Isolate and Fungal Isolate was inoculated into sterile MYGM broth. The co-culture was incubated at 28 °C for 12 days with regular monitoring. [14] Viable Plate Count (VPC) was performed post-incubation to assess microbial viability. Serial dilutions (10^{-2} to 10^{-7}) of the co-culture broth were plated on MYGA, followed by incubation at 28 °C for 48–72 hours. Colony morphology was examined to confirm isolate viability. [15], [16]

Mono-Culturing of Bacteria and Fungi

MYGM was prepared in 50 mL volumes under sterile conditions. 100 µL of 24-hour culture of Bacterial Isolate was inoculated into MYGM broth and incubated at 28 °C for 12 days with regular observation. [14] 100 µL of 24-hour culture of Fungal Isolate was inoculated into MYGM broth and incubated at 28 °C for 12 days with regular observation. [14]

Extraction of Metabolites

Culture broths from both co-cultures and monocultures were aseptically filtered using sterile Whatman No. 1 filter paper. The filtrates were extracted with an equal volume of ethyl acetate (50 mL per flask) to isolate polar bioactive compounds. [9] The organic phase was evaporated under sterile conditions at 40 °C with intermittent stirring. The resulting crude extracts were dried and stored for subsequent bioassays.

Biological Activity Assays

The antioxidant activity of the extract was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay [17] [18]. The anti-inflammatory activity of the extracts was evaluated using the inhibition of protein denaturation assay, following the method of Mizushima and Kobayashi [19]. Antimicrobial activity was assessed using the agar well diffusion method [20]. Wells (5 mm diameter) were punched into agar plates inoculated with standardized microbial suspensions, and 20 μL of coculture extract were introduced into each well. Zones of inhibition were measured after incubation. Anti-quorum sensing activity was evaluated using the minimum inhibitory concentration (MIC) assay against Serratia marcescens [21]. Biofilm inhibition was assessed using the crystal violet staining method[22]. The haemolytic activity of the extracts was evaluated [23]. Anticancer activity was assessed using the MTT assay on MCF-7 cell lines [24]. Cytotoxicity was evaluated using the MTT assay on VERO cell lines [24].

GC–MS analysis was performed using an Agilent 7890A GC/240-MS/4000 system equipped with a Centroid Filtered capillary column. The oven temperature was programmed from 50 °C to 310 °C. Metabolites were identified by comparing mass spectra with entries in the NIST and WILLY libraries.

RESULTS

The Colonies exhibiting desired morphological traits were aseptically isolated and streaked onto Sabouraud Dextrose Agar (SDA) for *Candida sp.* and Starch Casein Agar (SCA) for *Actinomycetes sp.* [25]

Co-culture Dynamics

The co-culture of *Actinomycetes sp*. and *Candida sp*. was incubated at 28 °C for a period of 12 days. During the initial phase, *Candida sp*. exhibited dominant growth, with prominent biofilm formation observed by day 3. By day 5, the biofilm began to dissolve, coinciding with increased turbidity and visible sedimentation at the base of the culture flask. By day 12, the medium had developed a distinct golden yellow coloration, suggestive of active metabolic interactions between the two organisms. (Figure 11) Viability assessment confirmed the presence of both isolates after 3 days, with distinct colony morphologies. (Figure 5 & 6)

Figure 5 - Viability test on MYGM agar showing two distinct colonies

Figure 6 - Viability test plate showing toffee peach coloured colonies and White creamy colonies

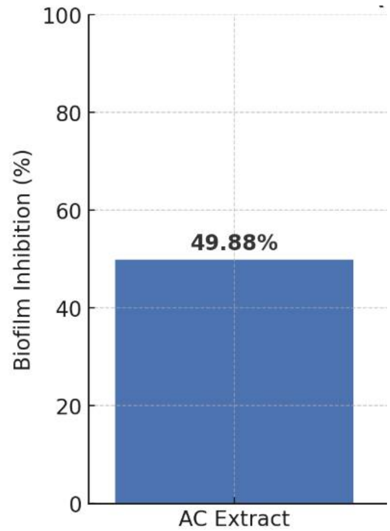


Figure 7 – A bar graph on Biofilm Inhibition of AC Extract

Figure 8 - Anti-QS Test for AC Extract

Mono-culture Observations

In MYGM broth, *Actinomycetes* sp. showed delayed growth, with turbidity by day 3 and golden yellow coloration with dense mycelia by day 12. (Figure 9) *Candida* sp. exhibited early biofilm formation by day 3, intensifying to a creamy medium by day 5, and forming a settled biofilm mat by day 8, with no major changes thereafter. (Figure 10) Polar bioactive compounds were extracted using ethyl acetate, and the concentrated phase was stored in sterile Eppendorf tubes at room temperature for analysis.

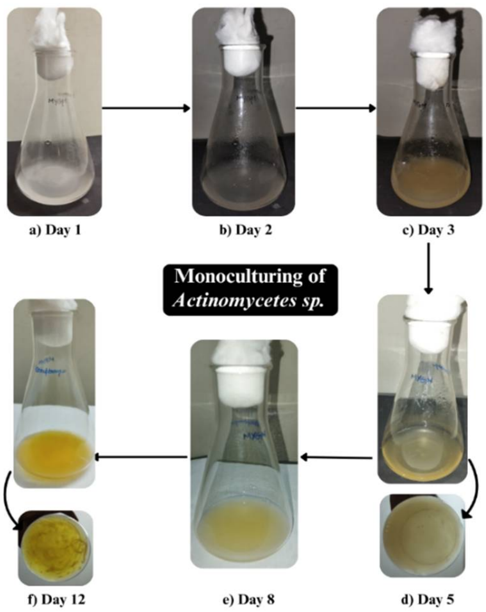


Figure 9 – Culturing of *Actinomycetes* sp.

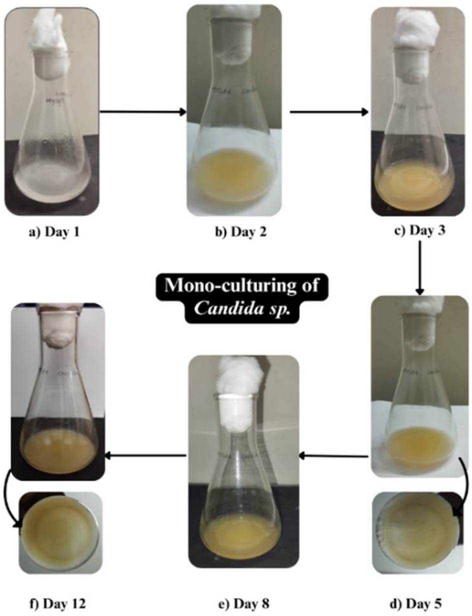


Figure 10 – Culturing of Candida sp.

Biological Activity Assays

The co-culture extract demonstrated notable biological activities across multiple assays. The co-culture extract exhibited dose-dependent antioxidant activity, reaching 37.73% DPPH scavenging at 1000 µg/mL. The extract demonstrated potent anti-inflammatory potential, achieving 91.20% inhibition of protein denaturation at 100 µg/mL. A clear concentration-dependent trend was observed, with activity increasing steadily from 39.01% at 20 µg/mL. (Table 3)

The extract also showed strong anti-quorum sensing activity, reducing prodigiosin production in *Serratia marcescens* even at 15.625 µg/mL. (Figure 8 & Table 4) It also showed effective biofilm inhibition, achieving 49.88% reduction in biomass compared to the control (OD = 2.101). (Figure 7) It exhibited mild haemolysis, with 13.38% lysis compared to the positive control (OD = 2.183) which is within acceptable safety limits, indicating low cytotoxicity toward human erythrocytes and supporting the extract's biocompatibility.

The extract exhibited significant cytotoxicity against MCF-7 breast cancer cells, as determined by the MTT assay. Cell viability decreased progressively with increasing concentration, reaching 26.15% at 1000 µg/mL. The calculated IC_{50} value was 83.36 µg/mL, indicating effective inhibition of cancer cell proliferation. The AC extract exhibited low cytotoxicity toward VERO cell lines, maintaining over 50% cell viability even at the highest tested concentration (1000 µg/mL). Cell viability gradually increased with decreasing concentration, reaching 95.03% at 7.8 µg/mL. The calculated IC_{50} value was 1302.54 µg/mL, indicating a favourable safety profile for potential therapeutic applications.

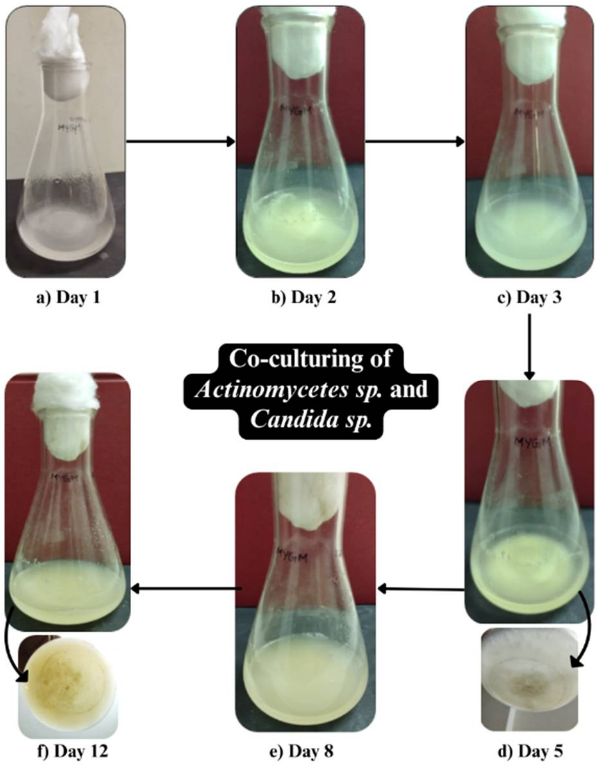


Figure 11 – Co-culturing of *Actinomycetes sp.* and *Candida sp.*

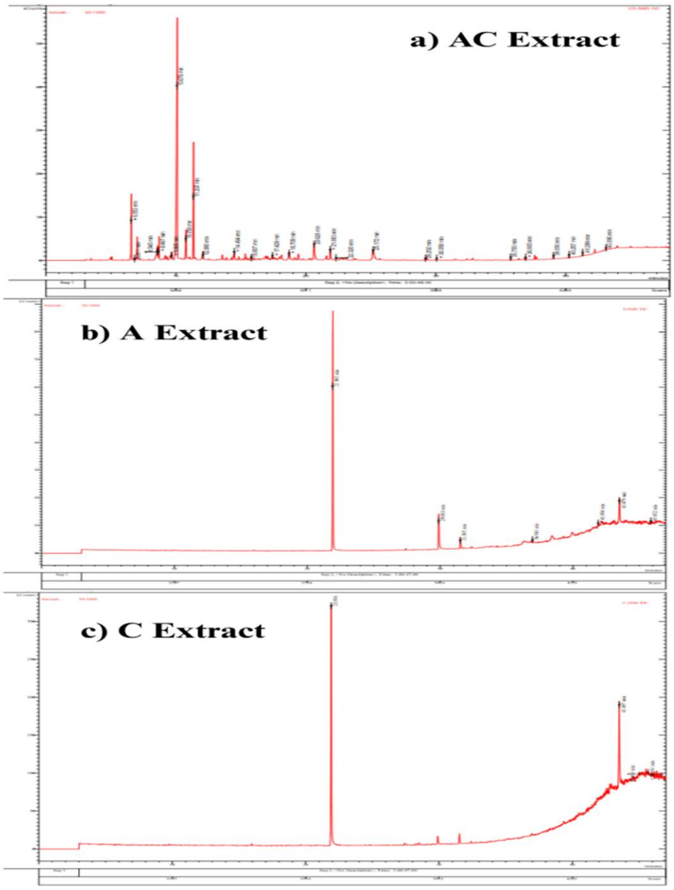


Figure 12 - Comparison of Extract Chromatograms of AC Coculture and Monocultures of A and C

a) Actinomycetes sp. & Candida sp. coculture

b) Actinomycetes sp. extract (A)

c) Candida sp. extract (C)

Antimicrobial Assay

The extract showed progressive efficacy for antibacterial activity, with inhibition zones increasing across concentrations. At 1000 µg/mL, notable zones of inhibition were observed against *Pseudomonas aeruginosa* (20 mm), *Staphylococcus aureus* (19 mm), and *Salmonella sp.* (12 mm), indicating broad-spectrum efficacy. Moderate activity was also recorded against *Escherichia coli*, *Proteus mirabilis,* and *Klebsiella sp*., with inhibition zones ranging from 9–12 mm. (Table 1)

It also exhibited consistent antifungal activity across all tested strains, with inhibition zones increasing proportionally with concentration. At 1000 µg/mL, *Candida albicans* (25 mm), *Mucor sp.* (20 mm), and *Aspergillus niger* (19 mm) showed the highest susceptibility, indicating strong antifungal efficacy. Moderate inhibition was observed against *Trichoderma sp.* (15 mm), *Rhizopus sp.* (13 mm), and *Trichophyton mentagrophytes* (12 mm), while *Penicillium sp*. and *Trichophyton rubrum* exhibited comparatively lower sensitivity. (Table 2)

The observed inhibition of *Candida sp.* by the co-culture extract suggests that a bioactive metabolite targeting this organism was likely produced as a result of microbial interaction within the co-culture environment. Hence, co-culturing presents a promising strategy for the targeted inhibition of specific microbes through induced metabolite production.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–MS analysis of the AC Extract revealed a diverse profile of secondary metabolites. Major compounds identified included α-Terpineol, known for antimicrobial and antioxidant properties, and 2-Heptenoic acid, phenyl ester, associated with pathogen inhibition. The presence of Cyclotetrasiloxane, octamethyl suggests a role in anti-biofilm activity through disruption of microbial adhesion. (Table 5)

Comparison between monoculture and co-culture extracts showed enhanced metabolite diversity and concentration in the co-culture system. (Figure 12) Unique compounds were detected only in the co-cultured extract, indicating activation of silent biosynthetic pathways through microbial interaction. These findings underscore the metabolic synergy between *Actinomycetes sp.* and *Candida sp.,* validating co-culturing as a promising strategy for bioactive compound discovery.

Table 1 – Antibacterial Activity of the Extract

AC Extract	Zone of Inhibition in mm			
Bacteria / Conc.	**C**	**500**	**750**	**1000**
Escherichia coli	15	5	6	9
Proteus mirabilis	15	5	6	10
Staphylococcus aureus	22	5	7	19
Salmonella sp.	14	7	8	12
Klebsiella sp.	20	5	7	12
Pseudomonas aeruginosa	21	5	7	20

Table 2 – Antifungal Activity of the Extract

AC Extract	Zone of Inhibition in mm			
Fungi / Conc.	**C**	**500**	**750**	**1000**
Aspergillus niger	9	7	12	19
Penicillium sp.,	12	5	8	9
Mucor sp.,	8	5	7	20
Trichoderma sp.	8	5	8	15
Trichophyton rubrum	16	4	6	8
Trichophyton mentagrophytes	10	6	8	12
Candida albicans	*8*	*6*	*8*	*25*
Rhizopus sp.	7	5	8	13

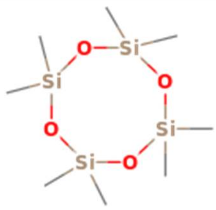
Table 3– Anti-inflammatory assay results for AC Extract

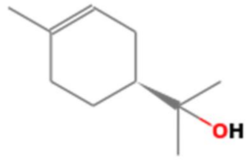
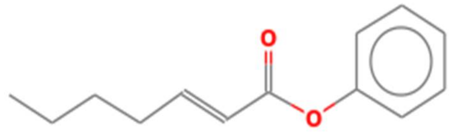
S.No	Concentration (µg/ml)	AC Extract	
		Absorbance O.D	**% Inhibition**
1	20	0.111	**39.01**
2	40	0.084	**53.84**
3	60	0.060	**67.03**
4	80	0.037	**79.67**
5	100	0.016	**91.20**
6	Control O.D.	**0.106**	

Table 4 – Anti-Quorum Sensing Assay for AC

S.No	Concentration (µg/ml)	AC extract
		Absorbance O.D
1	15.625	0.983
2	31.25	0.895
3	62.5	0.734
4	125	0.629
5	250	0.434
6	500	0.403
7	1000	0.386
6	Control O.D.	1.282

Table 5 – GCMS Compound Identification from GCMS

Compound Name	Molecular Formula	Molecular Structure
AC Extract		
Cyclotetrasiloxane, octameth	$[(CH3)_2SiO]_4$	

L-α-Terpineol	$C_{10}H_{18}O$	
2-Heptenoicacid, phenyl ester	$C_{13}H_{16}O_2$	

DISCUSSION

This study investigated the potential of bacterial–fungal co-cultures, specifically *Actinomycetes* sp. with *Candida* sp. (AC), for bioactive metabolite production. The findings highlighted enhanced metabolite diversity and bioactivity in the co-culture system, validating microbial interactions as crucial drivers of biosynthetic innovation. GC–MS analysis revealed a variety of compounds with therapeutic relevance, including α-Terpineol and 2-Heptenoic acid, phenyl ester, as well as Cyclotetrasiloxane, octamethyl—each associated with antimicrobial, antioxidant, and anti-biofilm activities. Antimicrobial assays demonstrated strong antibacterial and antifungal activity, with significant zones of inhibition against pathogens such as *Pseudomonas aeruginosa, Salmonella sp., Staphylococcus aureus*, *Candida albicans*, *Mucor sp.*, *Trichophyton mentagrophytes,* and *Aspergillus niger*. Notable compounds like α-Terpineol, widely reported for its antimicrobial properties [26], [27], were identified as key contributors, disrupting microbial cell walls and membranes.

Quorum-sensing inhibition was also observed, with reduced prodigiosin production in Serratia marcescens, aligning with findings by Jiang et al, [28]. The inhibition of *Candida albicans* by the AC extract suggests that targeted metabolites were produced through microbial interaction, consistent with antagonistic trends reported by Espinosa-Ortiz et al. [29]. The extract also exhibited strong antioxidant and anti-inflammatory activities, supported by compounds such as Cyclotetrasiloxane, octamethyl and α-Terpineol, known for mitigating oxidative stress and inflammation [17]. Anticancer activity was confirmed via MTT assay on MCF-7 cells, with an IC_{50} of 83.36 µg/mL, while cytotoxicity analysis on VERO cells showed an IC_{50} of 1302.54 µg/mL, indicating selective toxicity and therapeutic safety. Additionally, biofilm inhibition was evident, with siloxane derivatives disrupting microbial adhesion and matrix formation. Compared to previous literature focused on monoculture systems [30], [31], this study demonstrates the unique metabolic synergy of co-culturing *Actinomycetes* sp. and *Candida* sp., reinforcing its potential. The identification of unique metabolites, further highlights the novelty of this approach. Thus all assays and methodology performed justifies the efficacy of the extracts, offering their capabilities as a therapeutic.

CONCLUSION

With the rising demand for effective antibiotics and the alarming increase in resistance to existing ones, the discovery of novel antimicrobial compounds has become imperative. Co-culturing, a naturally occurring ecological interaction, offers a promising strategy for unlocking hidden biosynthetic potential. When carefully controlled, this approach can serve as a powerful tool in the fight against pathogenic microbes.

Author Declaration

This manuscript is based on research conducted as part of my postgraduate studies at Valliammal College for Women.

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. A. K. Jayaprakasan, A. Ghosh, B. Banerjee, M. M. Singh, and H. K. Solanki, (2022). 'Growing burden of antimicrobial resistance and the need for effective stewardship: An Indian perspective', *One Health Bulletin*, p. 10.4103/ohbl.ohbl_10_25, doi: 10.4103/ohbl.ohbl_10_25.
2. C. J L Murray, '(2024). Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050', *Lancet*, vol. 404, no. 10459, pp. 1199–1226. doi: 10.1016/S0140-6736(24)01867-1.
3. S. SeyedAlinaghi *et al.*, (2025). 'A systematic review on natural products with antimicrobial potential against WHO's priority pathogens', *European Journal of Medical Research*, vol. 30, no. 1, p. 525. doi: 10.1186/s40001-025-02717-x.
4. M. Sanitá Lima and R. Coutinho De Lucas, (2022). 'Co-cultivation, Co-culture, Mixed Culture, and Microbial Consortium of Fungi: An Understudied Strategy for Biomass Conversion', *Front. Microbiol.*, vol. 12, p. 837685. doi: 10.3389/fmicb.2021.837685.
5. M. Slattery, I. Rajbhandari, and K. Wesson, (2001). 'Competition-mediated antibiotic induction in the marine bacterium Streptomyces tenjimariensis', *Microb Ecol*, vol. 41, no. 2, pp. 90–96. doi: 10.1007/s002480000084.
6. P. Albuquerque and A. Casadevall, (2012). 'Quorum sensing in fungi – a review', *Med Mycol*, vol. 50, no. 4, pp. 337–345. doi: 10.3109/13693786.2011.652201.
7. T. Wongsuk, P. Pumeesat, and N. Luplertlop, (2016). 'Fungal quorum sensing molecules: Role in fungal morphogenesis and pathogenicity', *J. Basic Microbiol.*, vol. 56, no. 5, pp. 440–447., doi: 10.1002/jobm.201500759.
8. R. Baltz, (2008). 'Renaissance in antibacterial discovery from actinomycetes', *Current Opinion in Pharmacology*, vol. 8, no. 5, pp. 557–563. doi: 10.1016/j.coph.2008.04.008.
9. T. Liu, X. Gui, G. Zhang, L. Luo, and J. Zhao, (2024). 'Streptomyces-Fungus Co-Culture Enhances the Production of Borrelidin and Analogs: A Genomic and Metabolomic Approach', *Marine Drugs*, vol. 22, no. 7, p. 302. doi: 10.3390/md22070302.
10. M. Moussa *et al.*, (2019). 'Co-culture of the fungus *Fusarium tricinctum* with *Streptomyces lividans* induces production of cryptic naphthoquinone dimers', *RSC Adv.*, vol. 9, no. 3, pp. 1491–1500. doi: 10.1039/C8RA09067J.
11. T. Li *et al.*, (2023). 'The fungal quorum-sensing molecule, farnesol, regulates the immune response of vaginal epithelial cells against Candida albicans', *BMC Microbiol*, vol. 23, no. 1, p. 251. doi: 10.1186/s12866-023-02987-7.
12. D. A. Hogan, (2006). 'Talking to Themselves: Autoregulation and Quorum Sensing in Fungi', *Eukaryot Cell*, vol. 5, no. 4, pp. 613–619. doi: 10.1128/EC.5.4.613-619.2006.
13. L. S. Tironi, L. B. Carletto, E. O. Silva, J. Schripsema, and J. H. H. Luiz, (2024). 'Endophytic Fungi Co-Culture: An Alternative Source of Antimicrobial Substances', *Microorganisms*, vol. 12, no. 12, p. 2413, doi: 10.3390/microorganisms12122413.
14. M. Moussa, W. Ebrahim, R. Kalscheuer, Z. Liu, and P. Proksch, (2020). 'Co-culture of the bacterium Pseudomonas aeruginosa with the fungus Fusarium tricinctum induces bacterial antifungal and quorum sensing signaling molecules', *Phytochemistry Letters*, vol. 36, pp. 37–41. doi: 10.1016/ j.phytol.2020.01.013.
15. J. Reynolds,(2025). 'Serial Dilution Protocols', Serial Dilution Protocols. Accessed: Mar. 23. [Online]. Available: https://asm.org:443/Protocols/Serial-Dilution-Protocols
16. K. Wise, (2025). 'Preparing Spread Plates Protocols', Preparing Spread Plates Protocols. Accessed: Mar. 23, [Online]. Available: https://asm.org:443/Protocols/Preparing-Spread-Plates-Protocols
17. A. A. Hamed *et al.*, (2024). 'Induction of antimicrobial, antioxidant metabolites production by co-cultivation of two red-sea-sponge-associated Aspergillus sp. CO2 and Bacillus sp. COBZ21', *BMC Biotechnol*, vol. 24, no. 1, p. 3. doi: 10.1186/s12896-024-00830-z.
18. P. Molyneux,(2003). 'The use of the stable radical Diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity', Songklanakarin J. Sci. Technol. vol. 26, 2: 211-219.
19. Y. Mizushima and M. Kobayashi, (1968). 'Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins', *J Pharm Pharmacol*, vol. 20, no. 3, pp. 169–173. doi: 10.1111/j.2042-7158.1968.tb09718.x.
20. C. Rodriguez, W. Pauli, and P. Bazevque, (2025). 'An antibiotic assay by the agar well diffusion method', 1990. Accessed: Mar. 23. [Online]. Available: https://www.semanticscholar.org/paper/An-antibiotic-assay-by-the-agar-well-diffusion-Rodriguez-Pauli/f1b56c366cbafee034f420309622e6f55a66878d
21. Vishali. S and Soundhari. C, (2019). 'Inhibition of quorum sensing mediated pigment production in Serratia marcescens by secondary metabolite of Aspergillus sp. identified by metagenomic studies', *Pharma Innovation*, vol. 8, no. 6, pp. 290–295.
22. M. Carducci *et al.*, 'Development and characterization of a hemolysis inhibition assay to determine functionality of anti-Streptolysin O antibodies in human sera', *J Immunol Methods*, vol. 526, p. 113618, Mar. 2024, doi: 10.1016/j.jim.2024.113618.
23. T. Mosmann, (1983). 'Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays', *J Immunol Methods*, vol. 65, no. 1–2, pp. 55–63.doi: 10.1016/0022-1759(83)90303-4.
24. M. Goodfellow *et al.*, Eds, *Bergey's Manual® of Systematic Bacteriology*. New York, NY: Springer New York, 2012. doi: 10.1007/978-0-387-68233-4.
25. Q. Kong *et al.*, (2019). 'Antifungal mechanisms of α-terpineol and terpene-4-alcohol as the critical components of Melaleuca alternifolia oil in the inhibition of rot disease caused by Aspergillus ochraceus in postharvest grapes', *J Appl Microbiol*, vol. 126, no. 4, pp. 1161–1174. doi: 10.1111/jam.14193.
26. X. Yang *et al.*, (2023). 'Antibacterial activity and mechanisms of α-terpineol against foodborne pathogenic bacteria', *Appl Microbiol Biotechnol*, vol. 107, no. 21, pp. 6641–6653. doi: 10.1007/s00253-023-12737-4.

27. Q. Jiang, J. Chen, C. Yang, Y. Yin, and K. Yao, (2019). 'Quorum Sensing: A Prospective Therapeutic Target for Bacterial Diseases', *Biomed Res Int*, vol. p. 2015978, Jan. 2019, doi: 10.1155/2019/2015978.
28. E. J. Espinosa-Ortiz, E. R. Rene, and R. Gerlach, (2022). 'Potential use of fungal-bacterial co-cultures for the removal of organic pollutants', *Critical Reviews in Biotechnology*, vol. 42, no. 3, pp. 361–383, Apr. doi: 10.1080/07388551.2021.1940831.
29. J. Wakefield, H. M. Hassan, M. Jaspars, R. Ebel, and M. E. Rateb, (2017). 'Dual Induction of New Microbial Secondary Metabolites by Fungal Bacterial Co-cultivation', *Front. Microbiol.*, vol. 8, p. 1284, doi: 10.3389/fmicb.2017.01284.
30. A. Sung, S. Gromek, and M. Balunas, (2017). 'Upregulation and Identification of Antibiotic Activity of a Marine-Derived Streptomyces sp. via Co-Cultures with Human Pathogens', *Marine Drugs*, vol. 15, no. 8, p. 250, Aug. doi: 10.3390/md15080250.

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