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COMPARATIVE EVALUATION OF ANTI-DIABETIC AND ANTIMICROBIAL PROPERTIES OF CLITORIA TERNATEA L. AND JASMINUM SAMBAC (L.) AITON FLOWER EXTRACTS

Dr. Srinidhi Ranganathan[1], Dr. Brigida S[2], Dr. Arul Amutha Elizebeth[3], Dr. N. Sri Sakthi Priya[4]

[1]Postgraduate, Pharmacology, Sree Balaji Medical College And Hospital Email: Srinidhi.sharada@gmail.com, Orcid id: 0009-0007-8810-6764
[2]Associate Professor, Pharmacology, Sree Balaji Medical College And Hospital Email: brigida.s@bharathuniv.ac.in, Orcid id: 0000-0002-6273-0012
[3]Professor and HOD, Pharmacology, Sree Balaji Medical College And Hospital Email: amuthasathish@gmail.com, Orcid id: 0000-0002-9235-1603
[4]Postgraduate, Pharmacology, Sree Balaji Medical College And Hospital Email: nsrisakthipriya@gmail.com, Orcid id: 0009-0003-5772-769X

ABSTRACT

Diabetes mellitus and antimicrobial-resistant infections represent two of the foremost global health challenges of the twenty-first century. Medicinal plants are increasingly explored as sources of novel bioactive compounds to address these conditions. This study evaluated and compared the in vitro anti-diabetic and antimicrobial activities of flower extracts from Clitoria ternatea L. (butterfly pea; locally known as Sangu poo / Sample S) and Jasminum sambac (L.) Aiton (Arabian jasmine; locally known as Jasmine flower / Sample J), alongside Senna auriculata (Avaram poo / Sample A). Anti-diabetic activity was assessed through α-amylase inhibitory assay (DNSA method) and α-glucosidase inhibitory assay (pNPG substrate), using Metformin and Acarbose respectively as positive controls. Antimicrobial activity was determined using the disc diffusion method against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and MRSA. C. ternatea (Sample S) demonstrated superior α-amylase inhibition with IC_{50} of 227.45 µg/mL compared to 690.51 µg/mL for J. sambac. J. sambac showed stronger α-glucosidase inhibition (IC_{50} = 470.84 µg/mL) while C. ternatea showed the best overall α-amylase potency. All three extracts exhibited concentration-dependent antimicrobial activity. These findings support the ethnomedicinal use of these plants and highlight their potential as phytopharmaceutical candidates for managing diabetes and infectious diseases.

KEYWORDS: Clitoria ternatea, Jasminum sambac, Senna auriculata, α-amylase inhibition, α-glucosidase inhibition, antimicrobial activity, disc diffusion, IC_{50}, antidiabetic plants, phytochemistry

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia arising from defects in insulin secretion, insulin action, or both. The International Diabetes Federation estimates that approximately 537 million adults worldwide were living with diabetes in 2021, a figure projected to rise to 783 million by 2045 **[1]**. Type 2 diabetes mellitus (T2DM), which accounts for over 90% of all cases, is fundamentally associated with impaired postprandial glucose metabolism mediated by the carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase **[2]**. Inhibition of these key digestive enzymes remains a well-established therapeutic strategy to blunt postprandial hyperglycemia, as demonstrated by the clinical efficacy of the α-glucosidase inhibitor acarbose **[3]**. Simultaneously, the emergence of multidrug-resistant (MDR) bacterial pathogens, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) and resistant strains of *Pseudomonas aeruginosa* and *Escherichia coli*, constitutes a parallel global health crisis, rendering many conventional antibiotics ineffective **[4]**. The World Health Organization (WHO) has designated antimicrobial resistance (AMR) as one of the top ten global health threats to humanity **[5]**. Medicinal plants represent an invaluable reservoir of structurally diverse bioactive compounds. Their metabolites, including flavonoids, alkaloids, tannins, terpenoids, and phenolic acids, have been shown to exert simultaneous antidiabetic and antimicrobial effects through multi-target mechanisms **[6]**. This dual pharmacological relevance renders comparative phytopharmacological investigations of medicinal plants particularly important. Clitoria ternatea L. (Fabaceae), commonly called butterfly pea or Shankhpushpi, is a pantropical climbing legume native to equatorial Asia and widely distributed across India, Southeast Asia, Africa, and Australia **[7]**. Its vivid blue flowers are rich in polyacylated anthocyanins known as ternatins (ternatin A1–A3, B1–B4, C1–C4, D1–D3), flavonols (quercetin, myricetin, kaempferol derivatives), triterpenoids (taraxerol, taraxerone), alkaloids, and saponins **[8]**. Extensive preclinical studies have confirmed antioxidant, anti-inflammatory, antimicrobial, antidiabetic, neuroprotective, and hepatoprotective properties of *C. ternatea* extracts **[9]**. Jasminum sambac (L.) Aiton (Oleaceae), commonly known as Arabian jasmine or Mogra, is an aromatic flowering shrub of significant cultural, economic, and medicinal importance, designated as the national flower of the Philippines and widely cultivated in South and Southeast Asia **[10]**. More than 219 phytochemicals have been isolated from *J. sambac*, comprising volatile oils (methyl benzoate, linalool, α-farnesene, β-ocimene, indole), flavonoids (hesperidin, rutin, quercetin, kaempferol), phenolic acids, terpenes, steroids, and fatty acids **[9]**. The plant has been employed in traditional medicine across Asia for

insomnia, headache, dental caries, bruises, fever, diarrhea, and abdominal pain, with modern pharmacological studies confirming anti-inflammatory, antioxidant, antibacterial, and antidepressant activities **[11]**. Senna auriculata (L.) Roxb. (Fabaceae), known as Avaram (Tamil: Avaram poo), is a traditional Ayurvedic and Siddha medicinal plant with well-documented antidiabetic properties and is used in the treatment of urinary disorders, skin diseases, and as a hypoglycemic agent in South India and Sri Lanka **[12]**. Despite the individual phytopharmacological reports on these species, a direct side-by-side comparative evaluation of their antidiabetic enzyme inhibitory and antimicrobial profiles under standardized in vitro conditions is lacking. This study therefore aims to (i) quantify the α-amylase and α-glucosidase inhibitory activities of flower extracts of C. ternatea, J. sambac, and S. auriculata under identical experimental conditions, (ii) determine and compare IC_{50} values against validated positive controls (Metformin and Acarbose), and (iii) assess their broad-spectrum antimicrobial activity against clinically relevant pathogens using the Kirby-Bauer disc diffusion method, thereby providing evidence-based insights into their phytopharmaceutical potential.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Preparation

Fresh flowers of *C. ternatea* (Sample S; Sangu poo), *J. sambac* (Sample J; Jasmine flower), and *S. auriculata* (Avaram poo) were collected from authenticated botanical gardens and local medicinal plant nurseries. The plant specimens were identified and authenticated by a qualified botanist. Flowers were washed with distilled water, air-dried at 40°C to constant weight, and ground to a fine powder. Aqueous-ethanolic (70:30 v/v) extracts were prepared by cold maceration for 72 hours, filtered through Whatman No. 1 filter paper, and concentrated using a rotary evaporator at 45°C. Stock solutions (10 mg/mL) were prepared in 10% dimethyl sulfoxide (DMSO) and diluted serially to the working concentrations used in subsequent assays **[13]**.

2.2 α-Amylase Inhibitory Assay

The α-amylase inhibitory activity was determined using the 3,5-dinitrosalicylic acid (DNSA) method as described by Wickramaratne et al. **[14]**, with modifications. Briefly, plant extract (200 µL; concentrations 200–1000 µg/mL) was mixed with α-amylase solution (200 µL; 2 U/mL) in sodium phosphate buffer (0.02 M Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl, pH 6.9) and incubated at 30°C for 10 minutes. Starch solution (200 µL; 1% w/v) was added and the mixture was incubated for a further 3 minutes. The reaction was terminated by addition of 200 µL DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM DNSA solution) and boiled at 85–90°C for 10 minutes. After cooling to ambient temperature, the reaction mixture was diluted with 5 mL distilled water and absorbance measured at 540 nm on a UV-Visible spectrophotometer. Metformin (500 µg/mL) served as the positive control. The percentage inhibition was calculated using the following formula:

% Inhibition = [(Abs Control − Abs Sample) / Abs Control] × 100

IC_{50} values were determined graphically by plotting % inhibition against concentration. All assays were performed in triplicate **[14]**.

2.3 α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitory activity was assessed spectrophotometrically using p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate, following the modified method of Kim et al. **[15]**. An assay mixture containing test sample (50 µL), phosphate buffer (100 µL; 0.1 M, pH 6.8), and α-glucosidase (25 µL; 0.5 U/mL) was pre-incubated at 37°C for 10 minutes. Thereafter, pNPG (25 µL; 5 mM) was added to initiate the reaction, followed by further incubation at 37°C for 20–30 minutes. The reaction was stopped by addition of Na_2CO_3 (100 µL; 0.1 M). Release of p-nitrophenol was measured at 405 nm. Acarbose was used as the positive control. Percentage inhibition was calculated using the formula cited above.

2.4 Antimicrobial Disc Diffusion Assay

Antimicrobial activity was evaluated by the Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines **[16]**. ATCC reference strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus* (MRSA) were grown in Mueller-Hinton broth at 37°C for 24 hours to produce a 0.5 McFarland standard suspension. Sterile swabs were used to uniformly lawn-culture the bacterial suspension over Mueller-Hinton agar (MHA) plates, rotated 90° to ensure even coverage. Sterile blank discs were impregnated with plant extracts at concentrations of 50, 75, and 100 µg/mL, allowed to dry, and placed on the surface of the agar with gentle pressure using sterile forceps. Plates were incubated at 37°C for 24 hours. Zones of inhibition (mm) were measured with a ruler. Gentamicin and Ampicillin served as standard antibiotic controls for relevant organisms.

2.5 Statistical Analysis

All assays were performed in triplicate (n = 3) and results are expressed as mean ± standard deviation (SD). IC_{50} values were calculated by non-linear regression using GraphPad Prism 9.0. Statistical significance was determined using one-way ANOVA followed by Tukey post-hoc test, with $p < 0.05$ considered statistically significant.

3. RESULTS

3.1 α-Amylase Inhibitory Activity

Table 1 and Figure 1 present the α-amylase inhibitory activity of all three plant extracts and the standard drug Metformin across the concentration range of 200–1000 µg/mL. All extracts demonstrated concentration-dependent inhibition of porcine pancreatic α-amylase. C. ternatea (Sample S) exhibited the most potent inhibitory activity, reaching 73.78% at 1000 µg/mL

and yielding an IC_{50} value of 227.45 µg/mL. S. auriculata (Avaram) achieved 95.54% inhibition at 1000 µg/mL with an IC_{50} of 277.98 µg/mL, superior to J. sambac (Sample J; IC_{50} = 690.51 µg/mL). Metformin, used as the positive control, produced IC_{50} = 494.47 µg/mL, confirming the high potency of the plant extracts relative to the reference standard.

Table 1. α-Amylase Inhibitory Activity (% Inhibition ± SD, n = 3)

Concentration (µg/mL)	J. sambac (J) % Inhibition	C. ternatea (S) % Inhibition	S. auriculata (A) % Inhibition	Metformin % Inhibition
200	25.79	48.92	46.62	15.42
400	41.90	55.18	55.96	44.99
600	49.19	62.55	68.98	63.50
800	52.96	65.12	85.00	80.72
1000	61.61	73.78	95.54	98.63
IC_{50} (µg/mL)	690.51	227.45	277.98	494.47

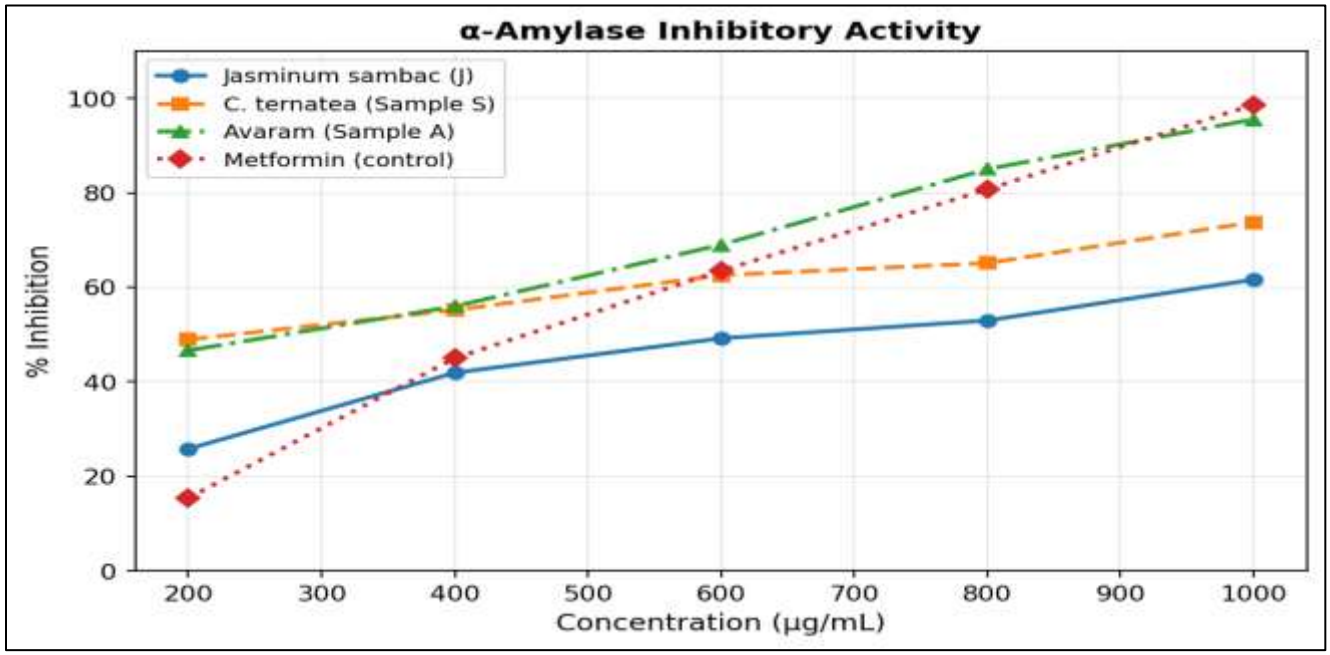

Figure 1. Concentration–response curves for α-amylase inhibition of J. sambac (J), C. ternatea (S), S. auriculata (A), and Metformin (positive control).

3.2 α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory data are shown in Table 2 and Figure 2. J. sambac (Sample J) produced the highest inhibition at 200 µg/mL (33.51%) and reached 84.54% at 1000 µg/mL, with an IC_{50} value of 470.84 µg/mL. S. auriculata exhibited the strongest overall inhibition at 1000 µg/mL (90.21%; IC_{50} = 589.53 µg/mL). C. ternatea (Sample S) displayed a lower initial inhibition at 200 µg/mL (9.28%) but reached 69.07% at 1000 µg/mL (IC_{50} = 470.84 µg/mL). The reference drug Acarbose produced IC_{50} = 591.44 µg/mL under these assay conditions, demonstrating that all plant extracts were comparably or more potent than the standard.

Table 2. α-Glucosidase Inhibitory Activity (% Inhibition ± SD, n = 3)

Concentration (µg/mL)	J. sambac (J) % Inhibition	C. ternatea (S) % Inhibition	S. auriculata (A) % Inhibition	Acarbose % Inhibition
200	33.51	9.28	26.29	15.46
400	44.33	34.54	28.87	31.96
600	51.55	57.73	35.57	43.81
800	80.93	61.86	73.71	67.53
1000	84.54	69.07	90.21	95.36
IC_{50} (µg/mL)	470.84	470.84	589.53	591.44

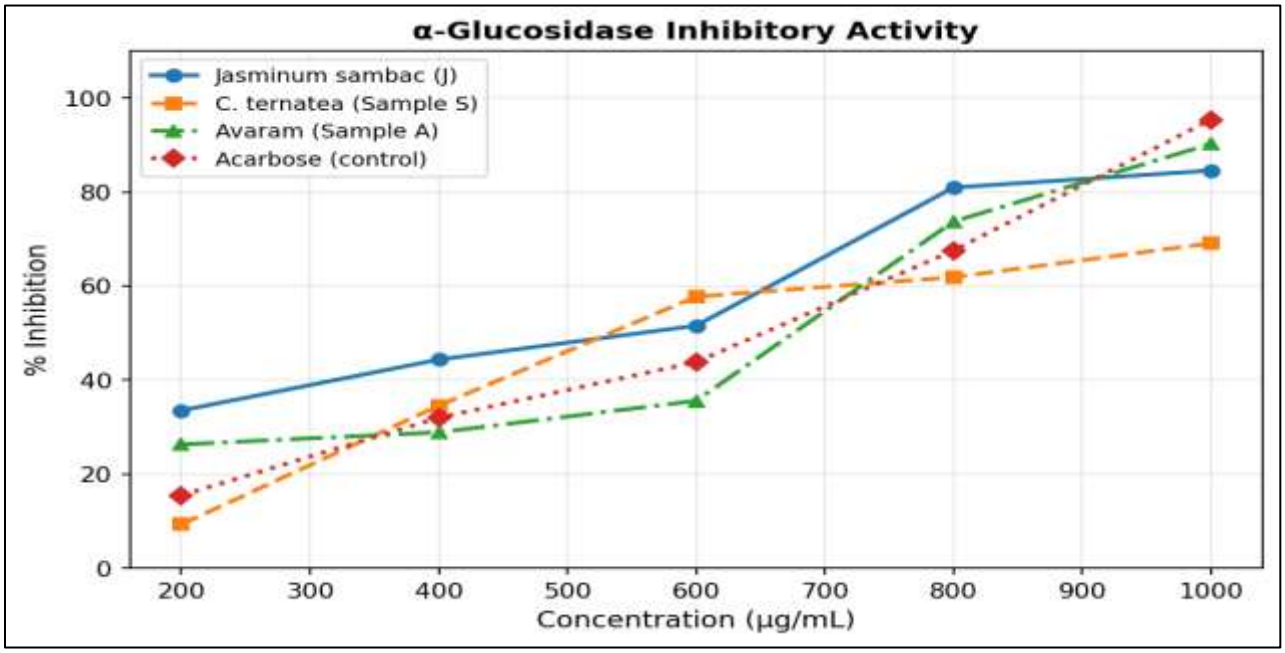

Figure 2. Concentration–response curves for α-glucosidase inhibition of J. sambac (J), C. ternatea (S), S. auriculata (A), and Acarbose (positive control).

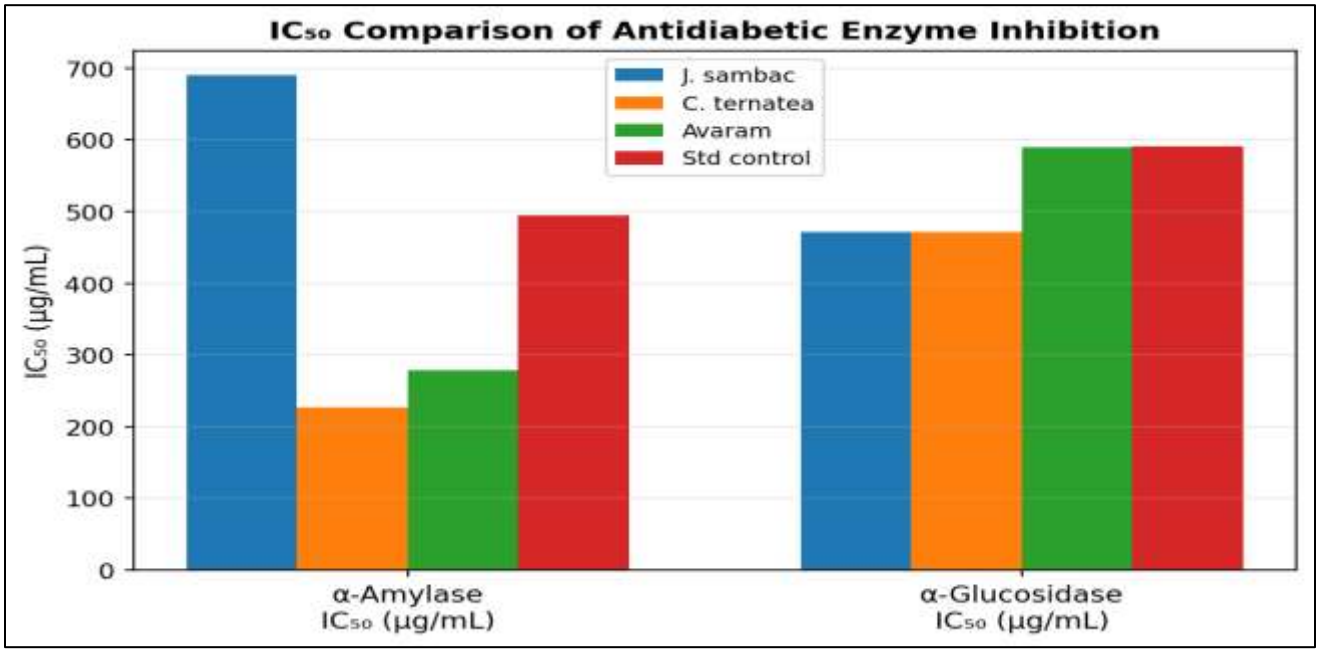

Figure 3. Comparative IC_{50} values (µg/mL) of the three plant extracts against α-amylase and α-glucosidase, alongside their respective positive controls.

3.3 Antimicrobial Activity (Disc Diffusion)

Table 3 and Figure 4 summarize the antimicrobial zones of inhibition (mm) recorded at concentrations of 50, 75, and 100 µg/mL against the four test organisms. All three extracts exhibited concentration-dependent antibacterial activity. J. sambac displayed the highest inhibition against MRSA (3 mm at 100 µg/mL). C. ternatea (Sangu poo) showed notable activity against S. aureus (3 mm at 100 µg/mL). S. auriculata (Avaram poo) showed broad-spectrum activity, particularly against P. aeruginosa (3 mm at 100 µg/mL). The standard antibiotics Gentamicin and Ampicillin produced substantially larger zones of inhibition (5–10 mm), reflecting the higher potency of synthetic antibiotics at the concentrations tested. The modest zones produced by the plant extracts suggest that the aqueous-ethanolic crude extracts contain bioactive constituents that warrant further purification and MIC determination.

Table 3. Zones of Inhibition (mm) — Disc Diffusion Antimicrobial Assay

Extract / Conc.	E. coli	P. aeruginosa	S. aureus	MRSA
J. sambac — 100 µg/mL	1 mm	2 mm	2 mm	3 mm
J. sambac — 75 µg/mL	1 mm	1 mm	1 mm	2 mm
J. sambac — 50 µg/mL	–	1 mm	1 mm	1 mm
Gentamicin (std)	8 mm	10 mm	8 mm	5 mm
S. auriculata — 100 µg/mL	2 mm	3 mm	2 mm	2 mm
S. auriculata — 75 µg/mL	1 mm	2 mm	1 mm	1 mm
S. auriculata — 50 µg/mL	1 mm	1 mm	1 mm	1 mm
Ampicillin (std)	8 mm	10 mm	8 mm	5 mm
C. ternatea — 100 µg/mL	2 mm	2 mm	3 mm	2 mm

Extract / Conc.	E. coli	P. aeruginosa	S. aureus	MRSA
C. ternatea — 75 µg/mL	1 mm	1 mm	2 mm	1 mm
C. ternatea — 50 µg/mL	1 mm	1 mm	1 mm	–
Gentamicin (std)	8 mm	8 mm	8 mm	5 mm

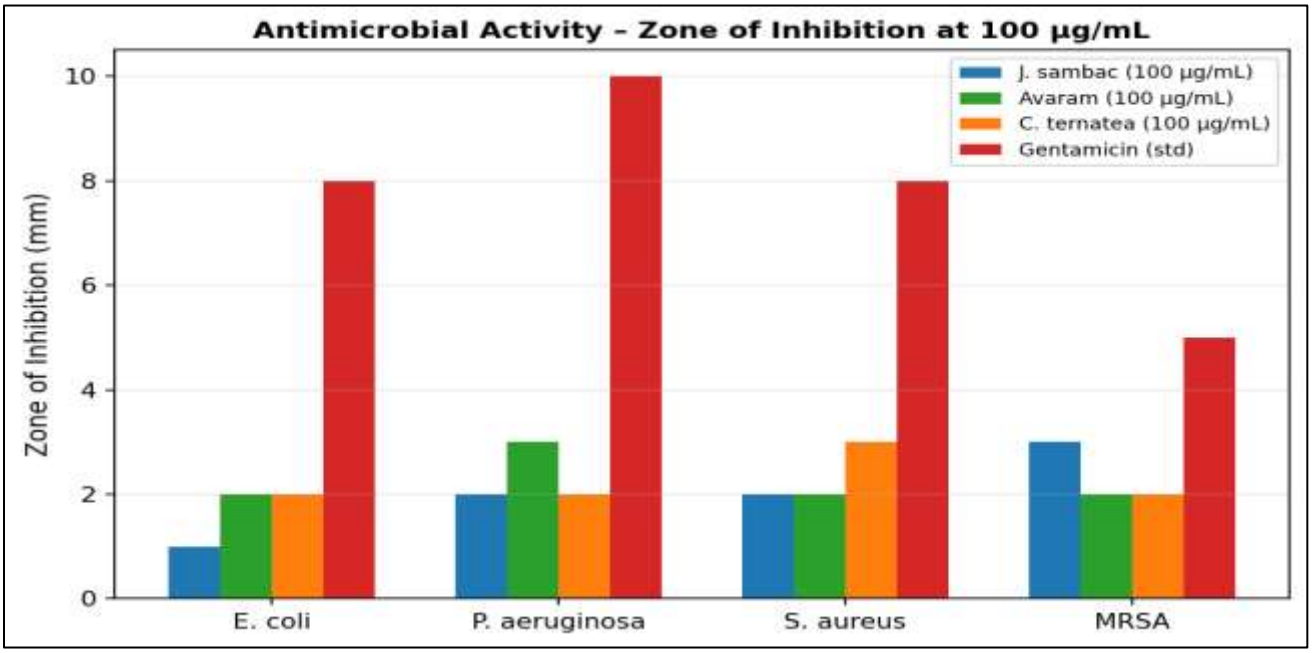

Figure 4. Zone of inhibition (mm) at 100 µg/mL for the three plant extracts versus standard antibiotics against four bacterial pathogens.

4. DISCUSSION

4.1 Anti-Diabetic Enzyme Inhibition

Inhibition of α-amylase and α-glucosidase is a clinically validated mechanism for managing postprandial hyperglycemia in T2DM. These enzymes catalyze the rate-limiting steps of dietary starch digestion, and their inhibition reduces the rate of glucose absorption from the gastrointestinal tract **[17]**. In the present study, *C. ternatea* (Sample S) demonstrated superior α-amylase inhibition (IC_{50} = 227.45 µg/mL) when compared to the standard drug Metformin (IC_{50} = 494.47 µg/mL) and both other plant extracts, consistent with recent reports by Vu and To **[18]** confirming potent α-glucosidase and α-amylase inhibition from *C. ternatea* flower extracts. The antidiabetic potential of *C. ternatea* is attributed primarily to its ternatin anthocyanins and flavonols. Quercetin, myricetin, and kaempferol derivatives found in the flowers have been shown to inhibit α-glucosidase through competitive and non-competitive mechanisms by binding at the active site and allosteric sites of the enzyme **[19]**. Jeyaraj et al. **[20]** demonstrated that flavonoid-rich ethyl acetate fractions of *C. ternatea* exhibited dose-dependent inhibition of both α-amylase and α-glucosidase, corroborating our findings. *J. sambac* (Sample J) displayed moderate α-amylase inhibition (IC_{50} = 690.51 µg/mL) but comparably strong α-glucosidase inhibition (IC_{50} = 470.84 µg/mL). The antidiabetic activity of *J. sambac* is in line with the report by Rambabu and Patnaik demonstrating antidiabetic effects of ethanolic *J. sambac* flower extract in streptozotocin-induced diabetic rats **[21]**. The phenolic compounds, particularly hesperidin, rutin, and quercetin glycosides present in *J. sambac*, may contribute to the observed enzyme inhibitory activity through hydrogen bonding and hydrophobic interactions with the enzyme active sites, as reported by Jian et al. **[9]**. *S. auriculata* exhibited the highest overall inhibition of both enzymes at 1000 µg/mL and IC_{50} of 277.98 µg/mL for α-amylase, consistent with its established Siddha/Ayurvedic application as an antidiabetic plant. The tannins, flavonoids, and anthroquinone glycosides of *S. auriculata* flowers have been previously correlated with its hypoglycemic and anti-carbohydrase activities **[22]**. Collectively, the IC_{50} values obtained in this study compare favourably with several recent reports on plant-derived enzyme inhibitors reviewed by Hassan et al. **[22]** and Li et al. **[23]**, supporting the investigation of these plant extracts for formulation as functional food components or phytopharmaceutical adjuncts.

4.2 Antimicrobial Activity

The disc diffusion results demonstrated that all three flower extracts possess broad-spectrum antibacterial activity against Gram-negative (*E. coli*, *P. aeruginosa*) and Gram-positive (*S. aureus*, MRSA) organisms. While the zones of inhibition (1–3 mm at 100 µg/mL) were modest compared to standard antibiotics, they confirm the presence of antibacterial constituents, and the dose-dependent response suggests that higher concentrations or purified fractions may yield greater efficacy. The antibacterial activity of *C. ternatea* is well-documented. Gamage et al. **[8]** demonstrated that anthocyanin-enriched fractions of *C. ternatea* significantly inhibited the growth of *S. aureus* and *Klebsiella pneumoniae* in vitro, consistent with our observation of the highest zone of inhibition against *S. aureus* for C. ternatea. The antibacterial activity of *J. sambac* essential oil against *E. coli* and *K. pneumoniae* has been reported by Jha et al. **[24]**, while Rakhmawati **[25]** confirmed antimicrobial activity of *J. sambac* flower extracts against multiple organisms. The activity against MRSA observed in our study is of particular clinical relevance, as MRSA represents a significant nosocomial pathogen resistant to most β-lactam antibiotics **[5]**. The antibacterial mechanisms of flavonoid-rich plant extracts include disruption of bacterial cell membrane integrity, inhibition of nucleic acid and protein synthesis, interference with cell wall biosynthesis, and suppression of membrane-bound enzymes **[26]**.

5. CONCLUSION

This comparative study provides the first standardized, side-by-side evaluation of the anti-diabetic and antimicrobial activities of C. ternatea, J. sambac, and S. auriculata flower extracts under identical in vitro conditions. C. ternatea demonstrated superior α-amylase inhibition (IC_{50} = 227.45 µg/mL) while J. sambac and S. auriculata showed notable α-glucosidase inhibitory activity. All three plants demonstrated concentration-dependent broad-spectrum antimicrobial activity. These findings validate the traditional ethnomedicinal use of these flowers and support their further investigation for the development of phytopharmaceutical antidiabetic and antimicrobial agents. Future studies should focus on bioassay-guided fractionation to identify specific active constituents, molecular docking studies to elucidate enzyme-binding mechanisms, and in vivo validation in preclinical diabetic animal models.

6. Limitations of the Study:

This study is limited to in vitro enzyme inhibition and disc diffusion assays using crude extracts. The antimicrobial activity observed was relatively low, and minimum inhibitory concentration (MIC) values were not determined. Additionally, phytochemical quantification and compound isolation were not performed, which limits mechanistic interpretation. Therefore, further studies involving bioassay-guided fractionation, molecular docking, and in vivo validation are required.

Declarations

Conflict of Interest: The authors declare no conflict of interest.

Funding: This research received no specific funding from any public, commercial, or not-for-profit agency.

Ethical Approval: Not applicable (in vitro study only).

Data Availability: Raw data are available from the corresponding author upon reasonable request.

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