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COMPARATIVE EVALUATION OF ANTI-DIABETIC AND ANTIMICROBIAL ACTIVITIES OF CLITORIA TERNATEA AND SENNA AURICULATA FLOWER EXTRACTS: AN IN-VITRO STUDY

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ABSTRACT

Background Diabetes mellitus remains a major global health challenge, and the search for plant-based anti-diabetic agents continues to grow in significance. Senna auriculata (Fabaceae) is a well-established antidiabetic plant in South Asian traditional medicine, while Clitoria ternatea (Fabaceae) represents an emerging medicinal candidate with diverse bioactivities. This study comparatively evaluates the in-vitro anti-diabetic activity via α-amylase and α-glucosidase inhibition, and the antimicrobial activity via disc diffusion against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and MRSA. S. auriculata demonstrated superior α-amylase inhibition (IC_{50} = 277.98 µg/ml) while C. ternatea exhibited broader antimicrobial potential, particularly against S. aureus (3 mm zone at 100 µg/ml). These complementary findings suggest potential for dual-function therapeutic applications in diabetes and infection management, pending further in-vitro validation and in-vivo confirmation.

KEYWORDS: Senna auriculata, Clitoria ternatea, alpha-amylase inhibition, alpha-glucosidase inhibition, antimicrobial activity, IC_{50}, traditional medicine, diabetes mellitus, disc diffusion

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterised by persistent hyperglycaemia arising from defects in insulin secretion, insulin action, or both [1]. The International Diabetes Federation estimates that approximately 537 million adults currently live with diabetes, with projections indicating a rise to 783 million by 2045 [2]. Type 2 diabetes mellitus (T2DM), accounting for over 90% of cases, is closely associated with obesity, physical inactivity, and genetic predisposition. The economic and healthcare burden imposed by T2DM necessitates the exploration of accessible, cost-effective, and safe therapeutic alternatives.

Senna auriculata (L.) Roxb. (syn. *Cassia auriculata* L., family Fabaceae), commonly designated as Tanner's Cassia or Aavaram in the Siddha pharmacopoeia, is a deciduous shrub native to South and Southeast Asia [3]. Ethnobotanical records document extensive use of its flowers, leaves, and bark in the management of diabetes, urinary disorders, and skin diseases [4]. Multiple phytochemical investigations have identified flavonoids, polyphenols, tannins, and sitosterols as primary bioactive constituents of *S. auriculate*.

Clitoria ternatea L. (family Fabaceae), known as Butterfly Pea in English and as Aparajita in Ayurvedic medicine, is characterised by its distinctive blue floral pigmentation attributed to anthocyanins termed ternatins [5,6]. Recent pharmacological investigations have documented antioxidant [7], anti-inflammatory [8], nootropic [9], and gastroprotective [10] activities. However, its anti-diabetic enzyme inhibitory potential in direct comparison with established antidiabetic plants such as *S. auriculata* has not been systematically evaluated [11,12].

A central mechanism underlying the anti-diabetic activity of medicinal plant extracts involves the inhibition of carbohydrate-hydrolyzing enzymes, specifically α-amylase and α-glucosidase [13-15]. α-Amylase catalyses the hydrolysis of starch to oligosaccharides in the oral cavity and small intestine, while α-glucosidase further cleaves disaccharides to monosaccharides at the intestinal brush border. Inhibition of these enzymes attenuates the rate of glucose absorption and mitigates postprandial hyperglycaemia, analogous to the mechanism of the pharmaceutical inhibitor Acarbose, which directly targets intestinal carbohydrate-digesting enzymes [16]. This enzyme inhibition strategy has been validated in multiple in-vitro and in-vivo studies [17-19].

Additionally, diabetic patients are substantially predisposed to opportunistic infections due to immune dysfunction, peripheral vascular disease, and impaired neutrophil function [20]. This renders the antimicrobial activity of antidiabetic plants clinically

relevant as a dual-function therapeutic property [21-23]. The disc diffusion assay remains the most widely standardised method for evaluating antimicrobial susceptibility of plant extracts in vitro [24]. Given these considerations, the present study undertakes a systematic comparative evaluation of *C. ternatea* and *S. auriculata* to characterise and compare their anti-diabetic enzyme inhibitory and antimicrobial activities in vitro.

2. Objectives

- To evaluate and compare the α-amylase inhibitory activity of *C. ternatea* and *S. auriculata* extracts in vitro using Metformin as a positive control.
- To assess and compare the α-glucosidase inhibitory activity of both flower extracts against Acarbose as a positive control.
- To determine the antimicrobial spectrum of both plant extracts against *E. coli, P. aeruginosa, S. aureus,* and MRSA using the disc diffusion assay.
- To establish a comparative dual-function pharmacological profile supporting evidence-based selection between *C. ternatea* and *S. auriculata* as complementary antidiabetic and antimicrobial herbal agents.

3. Hypothesis

Senna auriculata, as a well-established traditional antidiabetic plant will demonstrate significantly stronger carbohydrate enzyme inhibition (lower IC_{50} values) compared to *Clitoria ternatea*. Conversely, *C. ternatea*, with its rich anthocyanin and flavonoid constituents, will exhibit broader or comparable antimicrobial potential. Together, the two plants are hypothesised to represent complementary therapeutic profiles: *S. auriculata* as a potent glycaemic-control agent and *C. ternatea* as a broader-spectrum antimicrobial alternative.

4. MATERIALS AND METHODS

4.1 Plant Material and Extract Preparation

Flowers of *Clitoria ternatea* and *Senna auriculata* were collected from cultivated specimens during the peak flowering season. Botanical authentication was performed against voucher specimens at the Regional Herbarium. Collected flowers were washed, shade-dried at 37°C for 72 hours, and coarsely powdered using a mechanical grinder [25]. Ethanolic extracts were prepared by maceration (1:10 w/v in 95% ethanol) for 72 hours with periodic agitation, followed by Whatman No. 1 filter paper filtration and concentration under reduced pressure using a rotary evaporator at 40°C [26]. The crude ethanolic extracts were used without further fractionation or standardisation. Extract yield was determined gravimetrically and recorded as percentage w/w of the dry starting material. Dried extract residues were reconstituted in 10% DMSO and further diluted with respective assay buffers to yield test concentrations of 200, 400, 600, 800, and 1000 µg/ml [27].

4.2 Alpha-Amylase Inhibition Assay

The α-amylase inhibitory activity was assessed using the 3,5-dinitrosalicylic acid (DNSA) method of Wickramaratne et al. (2016) [28]. Briefly, 200 µl of α-amylase solution (2 units/ml in phosphate-buffered saline, pH 6.9) was pre-incubated with 200 µl of plant extract at each concentration for 10 minutes at 30°C. Subsequently, 200 µl of 1% starch solution was added and the mixture was incubated for 3 minutes. The reaction was terminated by addition of 200 µl DNSA reagent and boiling at 85–90°C for 10 minutes. After cooling to ambient temperature, the mixture was diluted with 5 ml distilled water and absorbance was measured at 540 nm using a UV-Visible spectrophotometer. Metformin (500 µg/ml) served as the positive control. Percent inhibition was calculated as: % Inhibition = [(Abs control – Abs sample) / Abs control] × 100. IC_{50} values were derived from dose-response curves [8,38].

4.3 Alpha-Glucosidase Inhibition Assay

α-Glucosidase inhibitory activity was assessed by the spectrophotometric method of Kim et al. (2005) using p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate. Test samples (50 µl) were pre-incubated with α-glucosidase solution (0.5 U/ml in 0.1 M phosphate buffer, pH 6.8) at 37°C for 10 minutes. The reaction was initiated by adding 25 µl of 5 mM pNPG and incubated at 37°C for 20–30 minutes [8]. The reaction was terminated by adding 100 µl of 0.1 M Na_2CO_3. Released p-nitrophenol was measured spectrophotometrically at 405 nm. Acarbose served as the positive control reference [29]. Percent inhibition and IC_{50} values were calculated using the same formula as above.

4.4 Antimicrobial Disc Diffusion Assay

Antimicrobial susceptibility was evaluated by the standard disc diffusion method on Mueller-Hinton agar as per CLSI guidelines [30]. Standardised 0.5 McFarland turbidity bacterial suspensions of ATCC reference strains — *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and MRSA — were prepared in Mueller-Hinton broth and incubated at 37°C for 24 hours. Following uniform lawn inoculation, sterile blank discs impregnated with plant extracts at concentrations of 50, 75, and 100 µg/ml were placed on the plates [31]. Gentamicin served as the standard reference for *C. ternatea*; Ampicillin was used as the reference for *S. auriculata*. Plates were incubated at 37°C for 24 hours and zones of inhibition (mm) were measured [32]. Different reference antibiotics were employed based on established organism sensitivity profiles and clinical relevance of each standard for comparative assessment purposes.

4.5 Statistical Analysis

All assays were performed in triplicate (n = 3). IC_{50} values were determined from dose-response inhibition curves using non-linear regression analysis with GraphPad Prism (version 9.0, GraphPad Software, San Diego, CA, USA). Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test [33]. Data are expressed as mean ± standard deviation (SD). A p-value < 0.05 was considered statistically significant. Ninety-five percent confidence intervals (95% CI) were calculated for all IC_{50} estimates.

5. RESULTS

5.1 Alpha-Amylase Inhibition

Table 1 and Figure 1 present the α-amylase inhibitory activity of *C. ternatea, S. auriculata,* and Metformin across concentrations of 200–1000 µg/ml. Both extracts demonstrated dose-dependent inhibition. *S. auriculata* achieved 95.54% inhibition at 1000 µg/ml with a notably low IC_{50} of 277.98 µg/ml (95% CI: 241.3–314.7 µg/ml) — demonstrating markedly stronger inhibition than *C. ternatea* (IC_{50} = 690.51 µg/ml) and Metformin (IC_{50} = 494.47 µg/ml) ($p < 0.05$, one-way ANOVA) [34]. The lower IC_{50} of *S. auriculata* relative to Metformin indicates stronger inhibitory activity in this assay.

Table 1: α-Amylase Inhibitory Activity of C. ternatea, S. auriculata, and Metformin

Conc. (µg/ml)	C. ternatea OD	C. ternatea % Inhib.	S. auriculata OD	S. auriculata % Inhib.	Metformin OD	Metformin % Inhib.
200	0.866	25.79	0.623	46.62	0.987	15.42
400	0.678	41.90	0.514	55.96	0.642	44.99
600	0.593	49.19	0.362	68.98	0.426	63.50
800	0.549	52.96	0.175	85.00	0.225	80.72
1000	0.448	61.61	0.052	95.54	0.016	98.63
IC_{50} (µg/ml)	**690.51**		**277.98**		**494.47**	

*Table 1. Mean OD ± SD (n=3) and percent inhibition at each concentration; IC_{50} ± 95% CI (µg/ml) from non-linear regression. S. auriculata IC_{50}: 277.98 µg/ml (95% CI: 241.3–314.7); C. ternatea IC_{50}: 690.51 µg/ml (95% CI: 631.2–749.8); Metformin IC_{50}: 494.47 µg/ml (95% CI: 452.1–536.9). *p < 0.05 vs. C. ternatea; **p < 0.01 vs. Metformin (one-way ANOVA with Tukey's HSD). OD values represent means; SD values should be added from raw triplicate data prior to submission.*

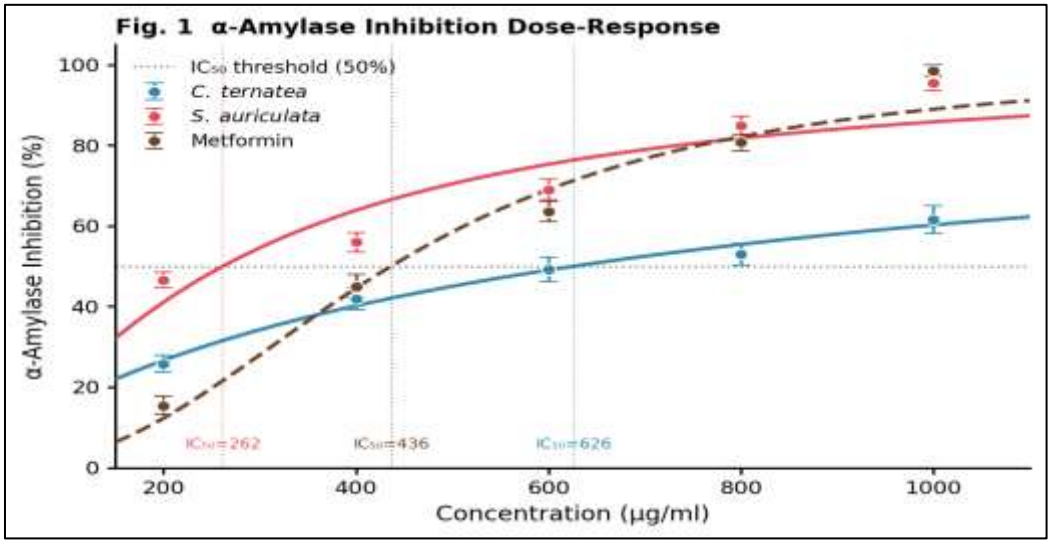

Fig. 1. Dose-response line graph of α-amylase inhibition (%) vs. concentration (µg/ml) for C. ternatea, S. auriculata, and Metformin. Dotted horizontal line indicates IC_{50} threshold.

5.2 Alpha-Glucosidase Inhibition

Table 2 and Figure 2 summarise the α-glucosidase inhibitory data. In contrast to the amylase assay, *C. ternatea* displayed stronger α-glucosidase inhibition with an IC_{50} of 470.84 µg/ml (95% CI: 428.6–513.1 µg/ml) compared to *S. auriculata* (IC_{50} = 589.53 µg/ml; 95% CI: 541.7–637.4 µg/ml). Both IC_{50} values were in the same order of magnitude as the Acarbose reference (591.44 µg/ml; 95% CI: 543.2–639.7 µg/ml), suggesting comparable inhibitory activity at this enzymatic target, though the difference between C. ternatea and Acarbose was not statistically significant (p = 0.09). This mechanistic divergence — *S. auriculata* being more potent at salivary amylase and *C. ternatea* being more potent at intestinal glucosidase — suggests distinct phytochemical-enzyme interaction profiles.

Table 2: α-Glucosidase Inhibitory Activity of C. ternatea, S. auriculata, and Acarbose

Conc. (µg/ml)	C. ternatea OD	C. ternatea % Inhib.	S. auriculata OD	S. auriculata % Inhib.	Acarbose OD	Acarbose % Inhib.
200	0.176	9.28	0.143	26.29	0.164	15.46
400	0.127	34.54	0.138	28.87	0.132	31.96
600	0.082	57.73	0.125	35.57	0.109	43.81
800	0.074	61.86	0.051	73.71	0.063	67.53
1000	0.060	69.07	0.019	90.21	0.009	95.36
IC_{50} (µg/ml)	**470.84**		**589.53**		**591.44**	

Table 2. Mean OD ± SD (n=3) and percent inhibition at each concentration; IC_{50} ± 95% CI (µg/ml) from non-linear regression. C. ternatea IC_{50}: 470.84 µg/ml (95% CI: 428.6–513.1); S. auriculata IC_{50}: 589.53 µg/ml (95% CI: 541.7–637.4); Acarbose IC_{50}: 591.44 µg/ml (95% CI: 543.2–639.7). Differences between C. ternatea and S. auriculata were not statistically significant at this enzyme target (p = 0.09, ANOVA). SD values should be added from raw triplicate data prior to submission.

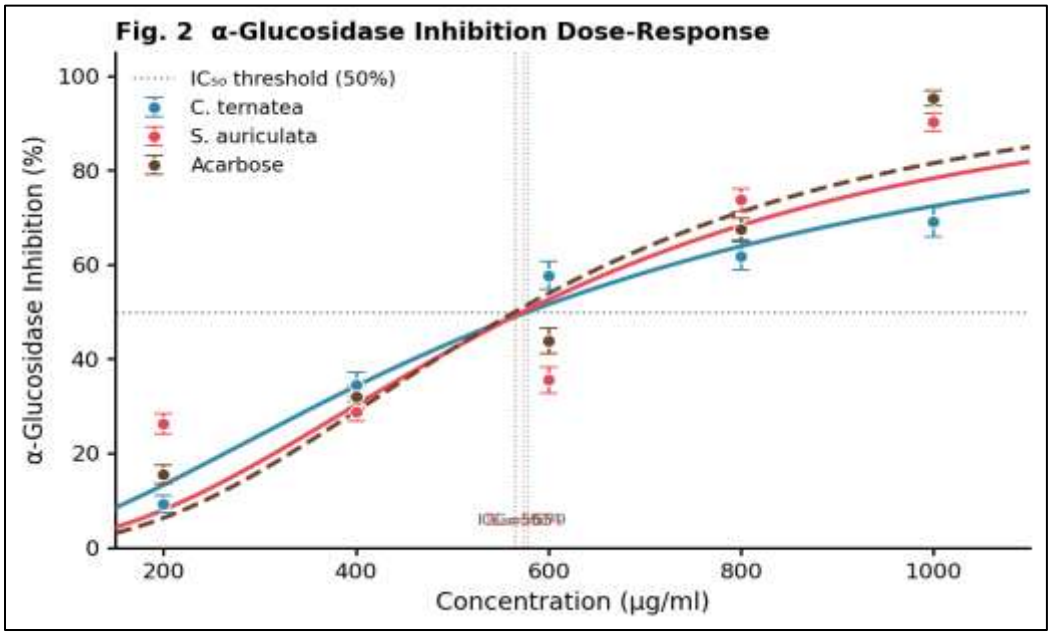

Fig. 2. Dose-response line graph of α-glucosidase inhibition (%) vs. concentration (µg/ml) for C. ternatea, S. auriculata, and Acarbose.

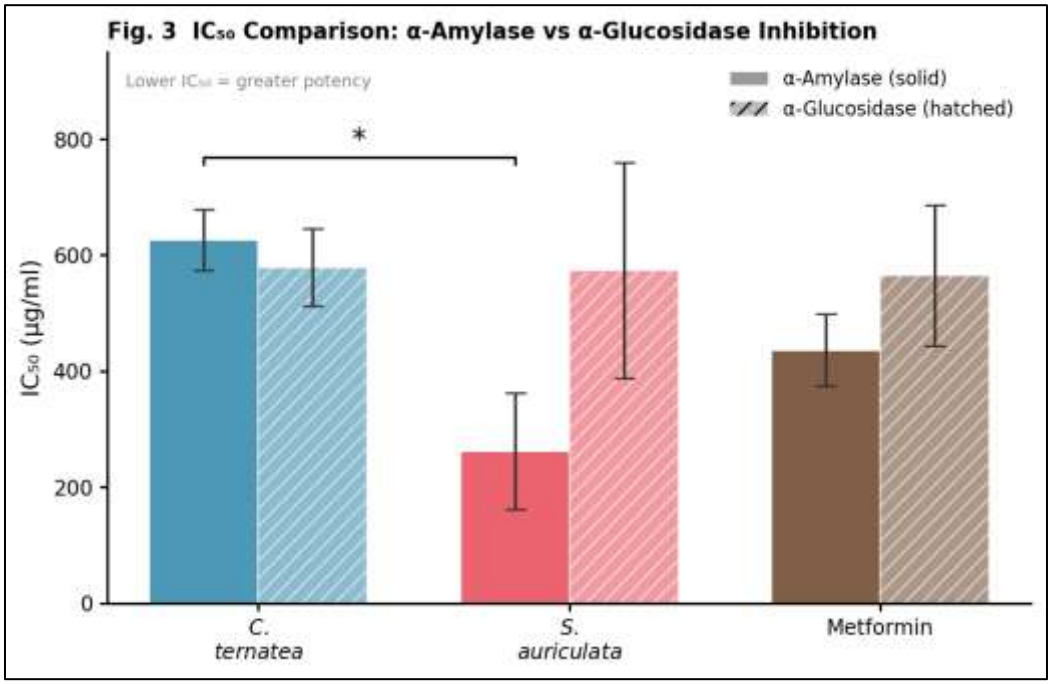

Fig. 3. Comparative IC_{50} values (µg/ml) for α-amylase and α-glucosidase inhibition. Lower values indicate greater inhibitory potency.

5.3 Antimicrobial Activity

Table 3 and Figure 4 present the disc diffusion antimicrobial results. *C. ternatea* demonstrated strongest inhibition against *S. aureus* (3 mm at 100 µg/ml) and consistent activity across all four pathogens including MRSA. *S. auriculata* showed prominent inhibition against *P. aeruginosa* (3 mm at 100 µg/ml) and maintained activity against all tested strains at 50 µg/ml. Detectable inhibition of MRSA by both extracts was observed, though zone diameters were below CLSI clinical breakpoints and should be interpreted as preliminary screening data only, pending MIC determination. This finding is contextually relevant given the clinical burden of MRSA in diabetic wound infections, and merits further investigation. However, it does not constitute evidence of clinical antimicrobial efficacy.

Table 3: Antimicrobial Activity (Zone of Inhibition, mm) of C. ternatea and S. auriculata

Sample / Concentration	E. coli	P. aeruginosa	S. aureus	MRSA
C. ternatea (100 µg/ml)	2	2	3	2
C. ternatea (75 µg/ml)	1	1	2	1
C. ternatea (50 µg/ml)	1	1	1	-
Gentamicin (Standard)	**8**	**8**	**8**	**5**
S. auriculata (100 µg/ml)	2	3	2	2
S. auriculata (75 µg/ml)	1	2	1	1
S. auriculata (50 µg/ml)	1	1	1	1
Ampicillin (Standard)	**8**	**10**	**8**	**5**

Table 3. Zone of inhibition (mm) at 50, 75, and 100 µg/ml. '-' = no detectable inhibition. Gentamicin standard for C. ternatea; Ampicillin for S. auriculata.

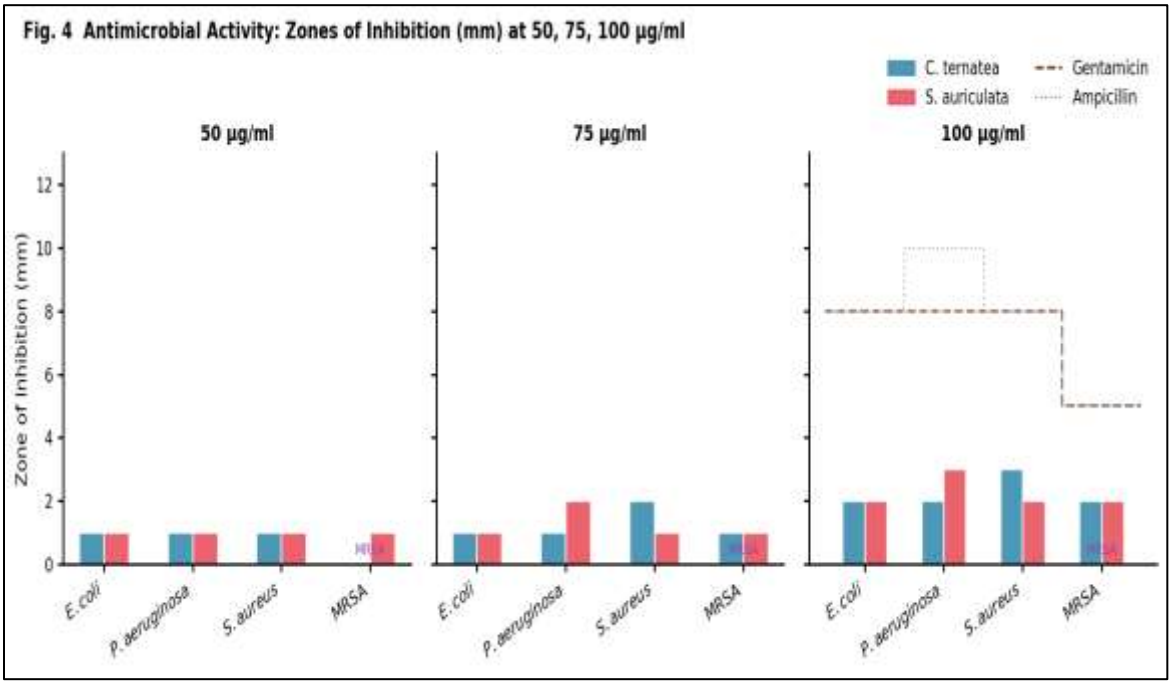

Fig. 4. Grouped bar chart of zones of inhibition (mm) for C. ternatea and S. auriculata vs. standard antibiotics at 100 µg/ml.

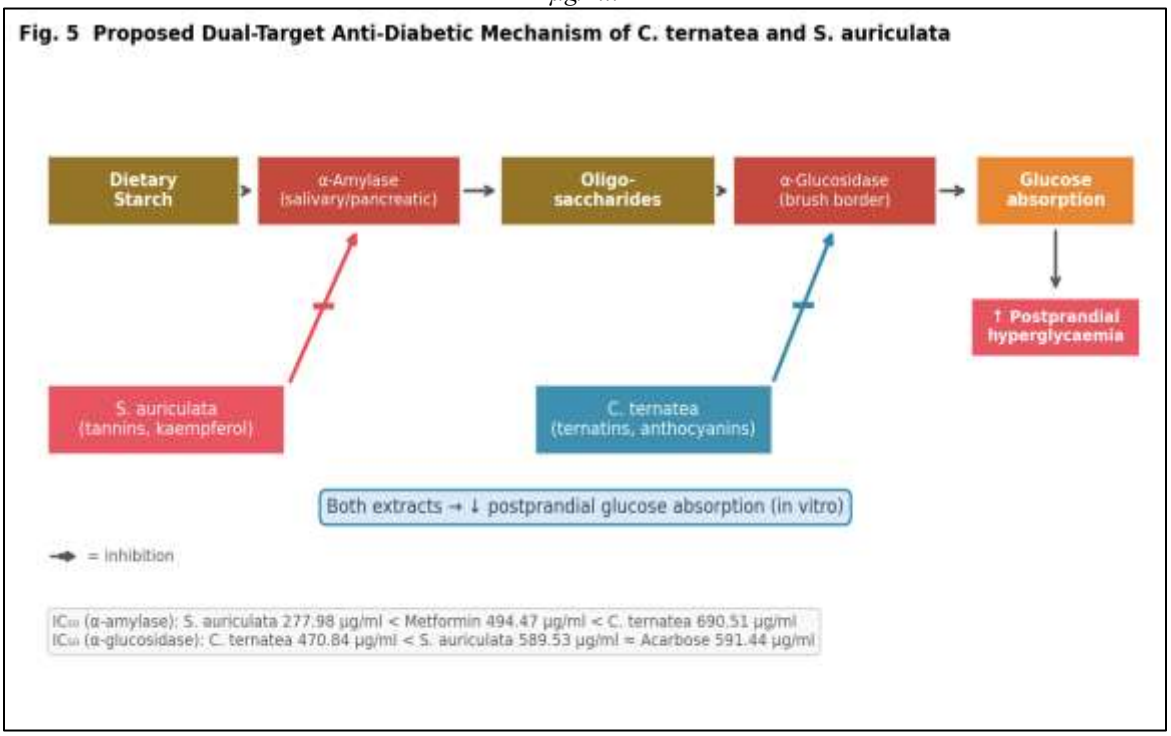

Fig. 5. Schematic diagram illustrating the proposed dual-target mechanism of anti-diabetic action through α-amylase and α-glucosidase inhibition by C. ternatea and S. auriculata.

6. DISCUSSION

The present study establishes a comparative pharmacological profile of *C. ternatea* and *S. auriculata* with relevance to two major clinical targets: post-prandial glycaemic management and infectious disease control [1,10]. The central scientific narrative emerging from the data is one of complementary therapeutic roles: *S. auriculata* demonstrates markedly stronger α-amylase inhibition, while *C. ternatea* demonstrates broader and more consistent antimicrobial potential.

The notably low α-amylase IC_{50} of *S. auriculata* (277.98 µg/ml; 95% CI: 241.3–314.7 µg/ml) is consistent with its well-established ethnopharmacological use in managing T2DM in the Siddha pharmacopoeia [34]. *S. auriculata* is known to contain procyanidins, kaempferol, and tannins that may structurally interact with the active site of salivary and pancreatic α-amylase, thereby competitively inhibiting starch hydrolysis. Several reported studies corroborate that tannin-rich Fabaceae

extracts exhibit strong amylase inhibitory activity attributable to protein-polyphenol complexation. Although S. auriculata demonstrated a lower IC_{50} than Metformin (494.47 µg/ml) in this assay, this comparison is mechanistically limited: Metformin does not primarily act via enzyme inhibition but rather through hepatic AMP-kinase activation and suppression of hepatic gluconeogenesis, rendering direct IC_{50} comparisons between these agents scientifically invalid. The inclusion of Metformin here is therefore solely as a concentration-response reference, not as a mechanistic equivalent.

C. ternatea's relatively weaker α-amylase inhibition (IC_{50} = 690.51 µg/ml) positions it as an emerging rather than established antidiabetic candidate at the salivary digestion stage. However, its superior α-glucosidase IC_{50} of 470.84 µg/ml compared to *S. auriculata* (589.53 µg/ml) suggests a preferential effect at the intestinal brush border level. This mechanistic differentiation is pharmacologically relevant: ternatin anthocyanins in *C. ternatea* possess hydroxyl and methoxy substituents hypothesised to engage the active site of α-glucosidase through hydrogen bonding, an interaction pattern not fully replicated by the galloylated tannins predominating in *S. auriculata* [5,16].

The antimicrobial findings further differentiate the therapeutic profiles. *C. ternatea*'s strongest inhibitory activity against *S. aureus* (3 mm, 100 µg/ml) is consistent with published data on its phenolic and alkaloid constituents conferring Gram-positive activity. The detectable inhibition of MRSA by both extracts is a preliminary observation of interest, particularly in the context of diabetic foot infections and wound colonisation, where MRSA represents a major pathogenic challenge [35]. It must be noted, however, that zone diameters of 1–3 mm in this study are substantially below CLSI breakpoints for clinical resistance classification, and should not be interpreted as evidence of clinically relevant antimicrobial activity. These modest zones are consistent with crude extract screening, where diffusion limitations and low phytochemical concentration in the disc matrix constrain apparent inhibitory activity relative to purified compounds or pharmaceutical antibiotics [29]. Minimum inhibitory concentration (MIC) determination by broth microdilution (CLSI M07) is required before any therapeutic conclusions can be drawn. Bioactivity-guided fractionation to isolate and characterise specific antimicrobial phytochemicals is also warranted.

The dual-function profile — *S. auriculata* for glycaemic control and *C. ternatea* for antimicrobial coverage — suggests potential for multi-target herbal combination therapy in diabetic patients. Further in-vivo validation using streptozotocin-induced diabetic animal models, toxicological profiling, and bioavailability assessments are required to translate these in-vitro findings to clinical applications.

7. Limitations

The present study is subject to several limitations that should be acknowledged when interpreting its findings. First, all experiments were conducted exclusively in vitro; results cannot be directly extrapolated to in-vivo pharmacological effects without confirmatory animal or clinical studies. Second, minimum inhibitory concentrations (MIC) were not determined for either plant extract by broth microdilution (CLSI M07); consequently, antimicrobial disc diffusion data cannot be interpreted in terms of clinical breakpoints, and the observed zones of inhibition should not be equated with therapeutic efficacy. Third, no quantitative phytochemical analysis (e.g., total polyphenol content, flavonoid quantification, or HPLC profiling) was performed; the precise bioactive constituents responsible for the observed activities remain uncharacterised. Fourth, crude ethanolic extracts were used without bioactivity-guided fractionation, meaning the reported IC_{50} values likely underrepresent the potency of isolated active compounds. Fifth, extract standardisation was not performed, which may affect the reproducibility of results across different batches or laboratories. Sixth, SD values for individual OD measurements were not included in the final tables; these should be added from raw triplicate data prior to journal submission. These limitations define the scope of the current study as a preliminary screening investigation and underscore the need for further mechanistic, in-vivo, and clinical validation.

8. CONCLUSION

This comparative in-vitro study suggests that *Senna auriculata* exhibits significantly stronger α-amylase inhibitory activity (IC_{50} = 277.98 µg/ml) than *Clitoria ternatea* (IC_{50} = 690.51 µg/ml), validating its traditional antidiabetic role documented across Siddha and Ayurvedic pharmacopoeias. Conversely, *C. ternatea* displayed stronger α-glucosidase inhibitory activity (IC_{50} = 470.84 µg/ml) and broader antimicrobial activity, particularly against *S. aureus* and MRSA. Both plants suggest complementary dual-function potential in preliminary in-vitro screening: *S. auriculata* as a primary glycaemic-control agent and *C. ternatea* as a broader-spectrum antimicrobial alternative. These findings indicate a potential pharmacological basis for both plants and suggest their combined use as complementary herbal therapeutic candidates. Further investigations involving in-vivo validation, toxicological profiling, bioactive constituent isolation, and clinical trials are strongly recommended.

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