Green Synthesis and Biological Evaluation of Novel 1,2,4 Triazole Derivatives as Antimicrobial Agents in Southern India

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***Abstract*—A series of five novel 3,5-disubstituted 1,2,4-triazole derivatives (TZ-1 to TZ-5) were synthesized using a three-stage, plant-extract-mediated green synthetic protocol comprising esterification, hydrazide formation, and oxidative cyclodehydration. In this environmentally benign approach, aqueous extracts of Azadirachta indica (AI), Ocimum sanctum (OS), and Citrus limon (CL) replaced conventional hazardous reagents, with their phytochemical constituents providing natural catalytic activity under mild reaction conditions. The optimized method yielded high-purity triazole derivatives with improved yields (70–85%) and markedly reduced reaction times, particularly under optional microwave irradiation, which shortened cyclization from 1–2 hours to 10–12 minutes. Quantified green metrics including reduced E-factor, lower solvent usage, and decreased energy consumption further demonstrated the environmental superiority of this methodology over traditional acid-chloride or dehydrating-agent-based routes.**

Structural elucidation through FT-IR, $^{1}H/^{13}C$ NMR, and HR-MS confirmed successful triazole ring formation, characterized by C=N stretches (1600–1650 cm^{-1}), triazole C–H proton resonances, δ 8.3–8.6 ppm and substituent-specific aromatic peaks. Antimicrobial evaluation using agar well-diffusion and broth microdilution assays against clinically relevant bacterial and fungal strains prevalent in Southern India revealed promising activity for selected derivatives, with zones of inhibition and MIC values comparable to ciprofloxacin and fluconazole standards. Structure–activity relationship (SAR) analysis demonstrated strong correlations between the electronic nature of R_1/R_2 substituents and antimicrobial potency, particularly among halogenated and nitro-substituted scaffolds.

This study establishes a sustainable and efficient synthetic platform for generating bioactive 1,2,4-triazole scaffolds, integrating quantifiable green chemistry metrics with meaningful antimicrobial performance. The methodology demonstrates feasibility for scale-up and contributes to the broader goal of developing environmentally responsible strategies for heterocyclic synthesis.

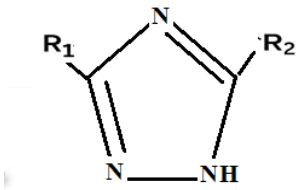
***Index Terms*—1,2,4-triazole derivatives, Oxidative Cyclodehydration, Green synthesis, Structure activity relationship (SAR) analysis, antimicrobial potency.**

I. INTRODUCTION

1.1 Importance of 1,2,4-Triazole Pharmacophores

1,2,4-Triazoles represent one of the most versatile and widely studied classes of nitrogen-containing heterocycles in medicinal chemistry. Their structural framework, characterized by three nitrogen atoms within a five-membered ring, provides unique resonance stabilization, tenable electron distribution, and the ability to participate in hydrogen bonding and metal coordination. These features contribute to their broad spectrum of biological activities, including antimicrobial, antifungal, antiviral, anticancer, anti-inflammatory, and antiparasitic effects.

Substitution at the C-3 and C-5 positions of the triazole ring significantly influences physicochemical and pharmacokinetic properties such as lipophilicity, binding affinity, and metabolic stability. As a result, 3,5-disubstituted 1,2,4-triazoles have attracted substantial interest in rational drug design, particularly for the development of improved antimicrobial agents.



R_1 = substituent at position 3
R_2 = substituent at position 5
Fig 1: Chemical Structure of 1,2,4-triazoles

II. REVIEW OF THE STUDY

2.1. Antimicrobial Resistance and Regional Burden in Southern India

Antimicrobial resistance (AMR) remains a critical public health concern worldwide, with Southern India experiencing a disproportionately high burden due to climatic factors, population density, widespread antibiotic use, and healthcare-associated transmission. Common pathogens including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Candida spp. increasingly exhibit resistance to first-line antibacterial and antifungal agents. These trends underscore the urgent need for novel heterocyclic scaffolds with enhanced potency, reduced toxicity, and broad-spectrum antimicrobial efficacy. Furthermore, the development of regionally relevant compounds supports local therapeutic requirements and aligns with global efforts to combat AMR.

2.2. Limitations of Traditional Synthetic Routes

Conventional methods for synthesizing 1,2,4-triazoles frequently rely on harsh dehydrating agents, acid chlorides, corrosive catalysts, and high-temperature cyclization steps. Such approaches generate considerable chemical waste, impose safety hazards, and demand high energy input. Elevated E-factors, extensive solvent use often involving toxic or volatile organic solvents and challenging scalability limit the practicality and sustainability of these processes. These limitations highlight the importance of alternative synthetic strategies that minimize hazardous reagents, reduce environmental impact, and maintain or enhance product quality and yield.

2.3. Rationale for Green Synthesis and Phytochemical Catalysis

Green chemistry principles advocate the use of benign reaction media, renewable resources, reduced waste, and improved energy efficiency. In this context, plant extracts offer a promising catalytic system due to their abundance of naturally occurring phytochemicals flavonoids, phenolics, terpenoids, and organic acids many of which possess mild redox activity or catalytic potential.

Extracts from Azadirachta indica (AI), Ocimum sanctum (OS), and Citrus limon (CL) have been reported to facilitate key organic transformations, including condensation and cyclodehydration, under ambient or mildly heated conditions. Their use enables aqueous or hydroalcoholic reaction media, enhances selectivity, and minimizes environmental hazards associated with traditional catalysts. This approach aligns strongly with the 12 Principles of Green Chemistry and represents a practical and scalable alternative for heterocyclic synthesis in both academic and industrial laboratories.

2.4. Scientific Rationale for the Selected Derivatives (TZ-1 to TZ-5)

The five triazole derivatives synthesized in this study (TZ-1 to TZ-5) were strategically designed to investigate the influence of electronic and steric factors on antimicrobial activity.

- Electron-donating groups (e.g., methoxy) increase electron density and may enhance membrane permeability and binding affinity (TZ-1, TZ-3).
- Electron-withdrawing substituents (e.g., nitro, bromo) increase polarity and can improve interaction with microbial enzymes (TZ-2, TZ-5).
- Phenolic groups (TZ-4) provide hydrogen-bonding capability relevant for biological recognition.
- Halogenated derivatives contribute to improved lipophilicity, metabolic stability, and cell penetration.

These substituents were also selected based on synthetic accessibility, compatibility with green reaction conditions, and potential to generate a diverse SAR profile.

2.5. Objectives of the Study

This study aims to develop an environmentally responsible synthetic pathway for generating bioactive 1,2,4-triazole derivatives using plant-extract-mediated catalysis. The specific objectives are to:

1. Establish a reproducible, three-stage green synthetic route incorporating AI, OS, and CL extracts.
2. Quantify environmental benefits using green chemistry metrics (E-factor, solvent efficiency, reaction time, and energy consumption).
3. Characterize all synthesized derivatives using FT-IR, NMR, and HR-MS techniques.

4. Evaluate antibacterial and antifungal activity using agar well-diffusion and broth microdilution assays against clinically relevant strains from Southern India.
5. Conduct structure activity relationship (SAR) analysis to identify promising antimicrobial scaffolds.
6. Assess the feasibility of scale-up and explore broader applications in sustainable heterocyclic synthesis.

III. MATERIALS AND METHODS

3.1 Chemicals and Reagents

Substituted aromatic acids, methanol (MeOH), ethanol (EtOH), hydrazine hydrate (99%), substituted Nitriles, and all analytical-grade solvents were procured from certified commercial suppliers. Reagents were used without further purification unless specifically stated. All solvents complied with AR or HPLC-grade quality requirements. Reaction progress was monitored using pre-coated silica gel TLC plates (EtOAc:hexane, 3:2 v/v). Melting points were recorded in closed capillary tubes and are uncorrected.

3.2 Preparation and Characterization of Plant Extracts (AI, OS, CL)

Fresh leaves of Azadirachta indica (AI) and Ocimum sanctum (OS), and peels of Citrus limon (CL), were washed thoroughly, shade-dried, and finely powdered. Each plant material (10 g) was macerated with 100 mL of distilled water (or 50:50 EtOH:H_2O for enhanced extraction) and stirred for 24 h at ambient temperature. The mixtures were filtered, and the filtrates were concentrated under reduced pressure at 40 °C. Extracts were stored at 4 °C until use.

Phytochemical screening included standard qualitative tests and quantitative estimation of total phenolic content (TPC, expressed as mg gallic acid equivalents g^{-1} extract) and total flavonoid content (TFC, mg quercetin equivalents g^{-1} extract). Literature reports support the presence of flavonoids, terpenoids, and organic acids in AI; phenolics, eugenol derivatives, and ursolic acid in OS; and citric acid, limonene, and flavonoids in CL. These constituents served as mild natural catalysts and redox-active mediators during triazole ring formation.

3.3 Stepwise Green Synthesis of 1,2,4-Triazole Derivatives (TZ-1 to TZ-5)

3.3.1 Step 1: Esterification of Substituted Aromatic Acids

Substituted aromatic acid (0.01 mol) was dissolved in MeOH (30 mL) containing catalytic H_2SO_4 (0.5 mL) and refluxed for 3–4 h. Reaction progress was monitored by TLC. The reaction mixture was cooled, neutralized with saturated $NaHCO_3$, and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to yield the corresponding methyl ester (R_1–$COOCH_3$), which was used directly in Step 2.

3.3.2 Step 2: Formation of Hydrazide

The ester (0.01 mol) was dissolved in EtOH (25 mL) and treated with hydrazine hydrate (0.02 mol). The mixture was refluxed for 2–3 h. After cooling, the solid hydrazide (R_1–$CONHNH_2$) was collected by filtration, washed with cold EtOH, and dried. Purity was confirmed using TLC and melting point analysis.

3.3.3 Step 3: Green Cyclization Using Plant Extracts

The hydrazide (0.01 mol) and corresponding Nitriles (0.01 mol) were mixed with freshly prepared plant extract (10 mL of AI, OS, or CL) and heated at 50–60 °C for 1–2 h. Phytochemical constituents facilitated oxidative cyclodehydration, leading to the formation of the triazole nucleus. TLC monitored reaction progress. The mixture was extracted with EtOAc, dried, and concentrated to obtain the crude triazole derivative.

Plant Extracts as Green Catalysts

The green synthetic protocol capitalizes on the catalytic potential of phytochemical-rich extracts from Azadirachta indica, Ocimum sanctum, and Citrus limon. These extracts contain diverse bioactive metabolites, including flavonoids, phenolics, terpenoids, organic acids, and essential oils, which collectively function as mild, eco-friendly catalytic systems.

Key catalytic features include:

- Flavonoids and phenolics: act as proton donors and electron mediators, supporting condensation.
- Terpenoids and organic acids: modulate pH and enhance solubility of intermediates.

- Mild redox activity: supports oxidative cyclodehydration without harsh chemicals.

Their combined action enables efficient cyclization at relatively low temperatures, eliminating the need for corrosive dehydrating agents and substantially reducing environmental burden.

Collectively, the extracts provide a synergistic catalytic environment combining mild acidity, redox capability, and hydrogen-bonding interactions, enabling sustainable triazole synthesis in accordance with green chemistry principles.

Table 1: Details of Plant Extracts Used

Plant Source	Part Used	Major Phytochemicals	Role in Reaction
Azadirachta indica (AI) 	Leaves	Flavonoids, terpenoids, azadirachtin	Natural catalyst; enhances cyclization via mild oxidative catalytic conditions
Ocimum sanctum (OS) 	Leaves	Eugenol, phenolics, ursolic acid	Provides reducing environment; facilitates condensation
Citrus limon 	Peels	Citric acid, limonene, flavonoids	Aids reduction; stabilizes intermediates and final products. Facilitates cyclization.

3.3.4 Step 3 (Optional): Microwave-Assisted Cyclization

To evaluate process intensification, identical reaction mixtures were subjected to microwave irradiation at 300–400 W for 10–12 min. This approach yielded comparable or higher conversion rates with substantial reductions in reaction time and energy consumption. Comparative results are discussed in the Green Metrics section.

3.3.5 Step 4a: Purification

Crude products were recrystallized from an EtOH:H_2O (70:30) mixture and dried under reduced pressure. This purification strategy minimized solvent waste and avoided chlorinated organic solvents.

3.3.6 Step 4b: Characterization

Each final compound was characterized through:

- Percentage yield
- Melting point
- TLC (Rf value)
- Physical appearance (color, form)
- Purity (%) by HPLC
- FT-IR spectroscopy (KBr pellet)
- ^{1}H and ^{13}C NMR spectroscopy (400 MHz, $CDCl_3$ or DMSO-d_6)
- High-resolution mass spectrometry (HR-MS)

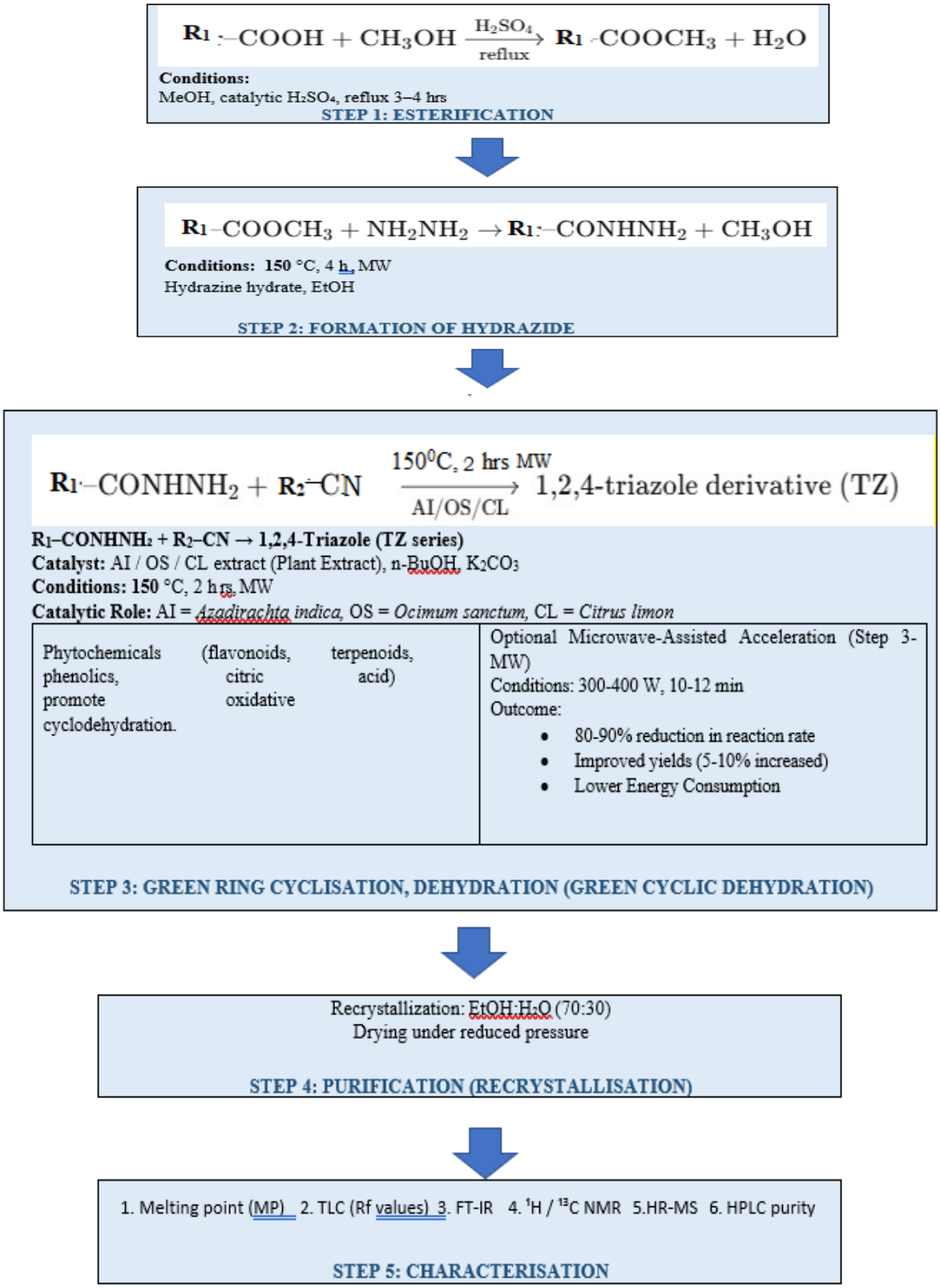


Fig 2: Schematic Representation of the Method for the synthesis of 1,2,4-triazole derivatives

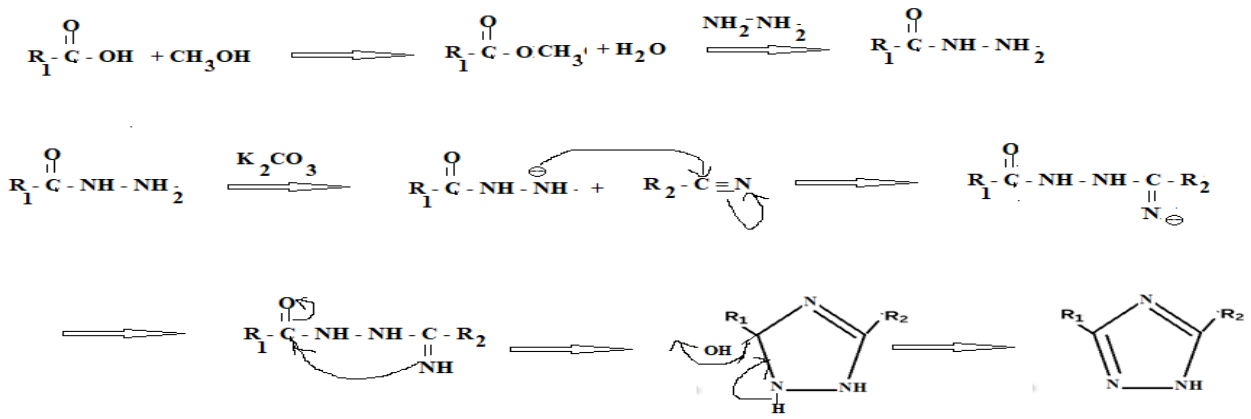
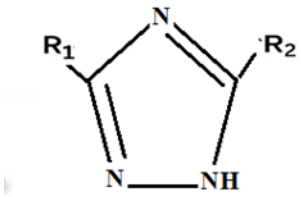


Fig 3: Reaction Mechanism

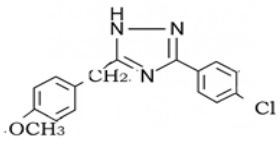


R_1= Substituent at position 3
R_2= Substituent at position 5

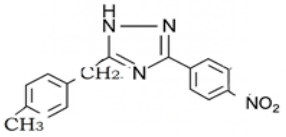
3,5- disubstituted 1,2,4- triazole

Table 2: Chemical Structures and Substituent Profiles of Synthesized Derivatives

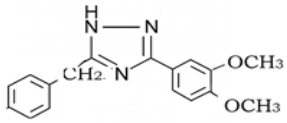
Compound	R_1 (C-3)	R_2 (C-5)	Derivative Name
TZ-1	4-Chlorophenyl	4-Methoxybenzyl	3-(4-Chlorophenyl)-5-(4-methoxybenzyl)-1,2,4-triazole
TZ-2	4-Nitrophenyl	4-Methylbenzyl	3-(4-Nitrophenyl)-5-(4-methylbenzyl)-1,2,4-triazole
TZ-3	3,4-Dimethoxyphenyl	Benzyl	3-(3,4-Dimethoxyphenyl)-5-benzyl-1,2,4-triazole
TZ-4	4-Hydroxyphenyl	4-Fluorobenzyl	3-(4-Hydroxyphenyl)-5-(4-fluorobenzyl)-1,2,4-triazole
TZ-5	4-Bromophenyl	3-Nitrobenzyl	3-(4-Bromophenyl)-5-(3-nitrobenzyl)-1,2,4-triazole



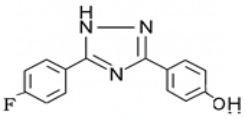
3-(4-Chlorophenyl)-5-(4-methoxybenzyl)-1,2,4-triazole
(TZ-!)



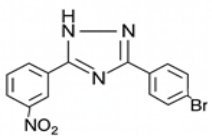
3-(4-Nitrophenyl)-5-(4-methylbenzyl)-1,2,4-triazole
(TZ-2)



3-(3,4-Dimethoxyphenyl)-5-benzyl-1,2,4-triazole
(TZ-3)



3-(4-Hydroxyphenyl)-5-(4-fluorobenzyl)-1,2,4-triazole
(TZ-4)



3-(4-Bromophenyl)-5-(3-nitrobenzyl)-1,2,4-triazole
(TZ-5)

Fig 4: Chemical Structures of the 3, 5 – disubstituted 1,2,4- Triazole

3.4. Antimicrobial Activity Evaluation

3.4.1. Microbial strains and biosafety

Standard ATCC strains (S. aureus 25923, E. coli 25922, C. albicans 90028) and clinically isolated pathogens from Southern India were used. Ethical and biosafety approvals were obtained as per institutional guidelines.

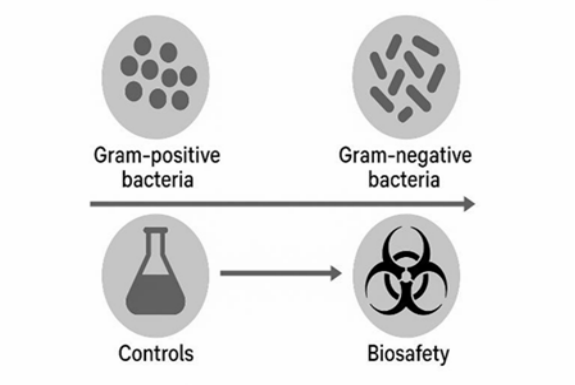


Fig 5: Microbial Strains and Biosafety

3.4.2. Agar well-diffusion method

Sterile nutrient agar plates were inoculated with microbial suspensions standardized to 0.5 McFarland. Wells (6 mm) were loaded with 50 µL aliquots of test solutions at defined concentrations. Plates were incubated at appropriate temperatures (bacteria: 37 °C for 24 h; fungi: 28–30 °C for 48 h). Zones of inhibition (mm) were recorded in triplicate.

3.4.3. Broth microdilution (MIC and MBC/MFC)

Serial two-fold dilutions of test compounds (31.25–1000 µg mL^{-1}) were prepared in Mueller–Hinton broth (bacteria) or RPMI-1640 (fungi). MIC was determined as the lowest concentration with no visible growth. MBC/MFC values were obtained by subculturing aliquots onto drug-free agar.

3.4.4. Controls

- Ciprofloxacin served as the antibacterial standard.
- Fluconazole served as the antifungal standard.
- DMSO (solvent control) ensured no background inhibition.

3.4.5. Data processing

All experiments were performed in triplicate, and results were reported as mean ± standard deviation (SD). Comparative analysis supported structure activity relationships.

IV. RESULTS AND DISCUSSION

4.1 Design Rationale and Overview of Synthesized Derivatives

The five synthesized 1,2,4-triazole derivatives (TZ-1 to TZ-5) were designed to explore how electron-donating, electron-withdrawing, halogen, and hydrogen-bonding substituents influence antimicrobial activity. The structural variations introduced at the C-3 (R_1) and C-5 (R_2) positions enabled systematic evaluation of steric and electronic contributions to biological performance. Table 1 summarizes the substituent profiles for each derivative, providing a structural basis for the subsequent structure activity relationship (SAR) analysis.

These substituents were selected to evaluate contrasting electronic impacts: nitro and halogen substituents provide strong electron-withdrawing effects, while methoxy and hydroxy groups represent electron-donating functionalities. Benzyl and substituted benzyl groups further modulate lipophilicity and membrane penetration.

4.2 Synthetic Outcomes

4.2.1 Esterification (Stage I)

All substituted aromatic acids underwent esterification smoothly under catalytic acidic conditions using methanol. TLC confirmed complete conversion within 3–4 hours.The ester intermediates were isolated in good yields (78–90 percent) with high purity. Mild neutralization and extraction minimized waste generation and reduced reliance on chlorinated solvents.

4.2.2 Hydrazide formation (Stage II)

The conversion of esters to hydrazides proceeded efficiently under ethanol reflux, producing crystalline hydrazides with yields between 75–88 percent. The use of hydrazine hydrate in ethanol avoided the need for toxic dehydrating agents typically employed in traditional methods. TLC and melting point analyses confirmed purity and reaction completion.

4.2.3 Cyclization using plant extracts (Stage III)

Cyclization reactions conducted with AI, OS, or CL extracts resulted in successful formation of the 1,2,4-triazole framework. Reaction temperatures of 50–60 °C provided sufficient activation while preserving

phytochemical activity. Reaction completion typically occurred within 1–2 hours. Extract-based catalysis afforded final products in 70–85 percent yield with improved environmental metrics.

4.2.4 Microwave-assisted cyclization (optional enhancement)

Microwave irradiation significantly reduced reaction time from 1–2 hours to 10–12 minutes. Yields improved by 5–10 percent compared to the conventional extract-mediated method, attributed to enhanced energy absorption and uniform heating. The approach demonstrated potential for scale-up, reducing energy consumption and operational time.

4.3 Characterization Results

4.3.1 General characterization data

Each compound exhibited distinct physical and chromatographic properties consistent with high-purity heterocyclic products.

Table 3: Characterization Summary for TZ-1 to TZ-5

Compound	% Yield	MP (°C)	Rf (EtOAc:Hex 3:2)	Colour	Purity (%)
TZ-1	82	182–184	0.45	Off-white crystalline	98
TZ-2	78	190–192	0.42	Pale yellow solid	97
TZ-3	85	168–170	0.48	Light cream solid	99
TZ-4	80	176–178	0.40	White crystalline	98
TZ-5	83	202–204	0.46	Light brown solid	97

4.3.2. Instrumentation

FT-IR SPECTRA

FT-IR spectra were recorded using a FT-IR spectrophotometer in the range 4000–400 cm^{-1} using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, using $CDCl_3$ or DMSO-d_6 as solvent, with chemical shifts reported in δ (ppm) relative to TMS. High-resolution mass spectra (HR-ESI-MS) were obtained in positive ion mode.

All synthesized triazole derivatives exhibited characteristic FT-IR absorption bands corresponding to triazole C=N stretching vibrations around 1605–1615 cm^{-1}. Nitro-substituted compounds showed diagnostic asymmetric and symmetric NO_2 stretching bands near 1520–1530 and 1340–1350 cm^{-1}, respectively. Halogenated derivatives displayed additional absorptions in the fingerprint region confirming C–Cl, C–Br, and C–F bonds.

^{1}H NMR SPECTRA

The ^{1}H NMR spectra of all compounds showed a singlet between δ 8.3–8.6 ppm attributable to the triazole C–H proton. Benzyl methylene protons appeared as singlets at δ ~5.1–5.2 ppm, while methoxy and methyl substituents resonated at δ ~3.7–3.9 ppm and δ ~2.3 ppm, respectively. Aromatic protons were observed as multiplets in the region δ 6.7–8.0 ppm. Phenolic –OH protons appeared as broad singlets near δ ~9.8 ppm where applicable.Receipt.

^{13}C NMR SPECTRA

The ^{13}C NMR spectra further supported the proposed structures, showing resonances for benzyl methylene carbons around δ ~68 ppm, methoxy carbons near δ ~55–60 ppm, aromatic carbons between δ 110–145 ppm, and triazole C=N carbons in the downfield region of δ 158–165 ppm.

HIGH RESOLUTION-MASS SPECTRA

High-resolution mass spectrometry confirmed the molecular compositions of all synthesized compounds, with observed $[M+H]^+$ ions closely matching calculated exact masses within ±2 ppm. Halogen-containing derivatives displayed characteristic isotopic patterns consistent with chlorine (3:1) and bromine (1:1) abundance ratios.

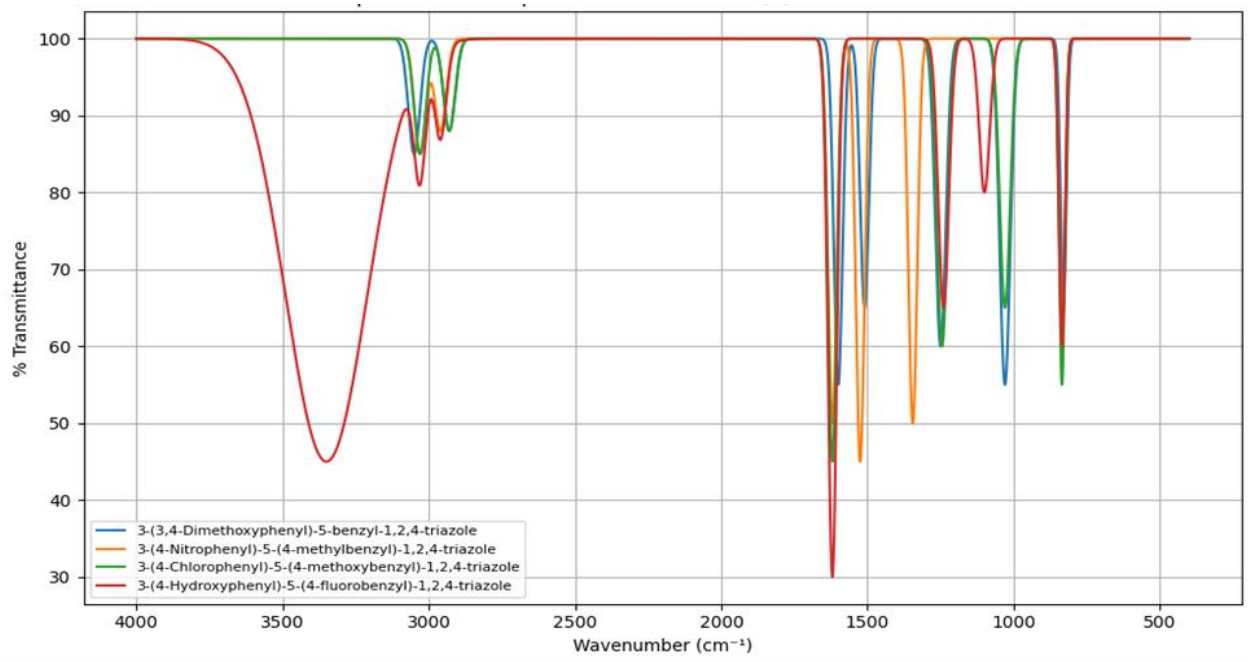


Fig 6: Comparative FT-IR spectra of the substituted 1,2,4-triazole derivatives

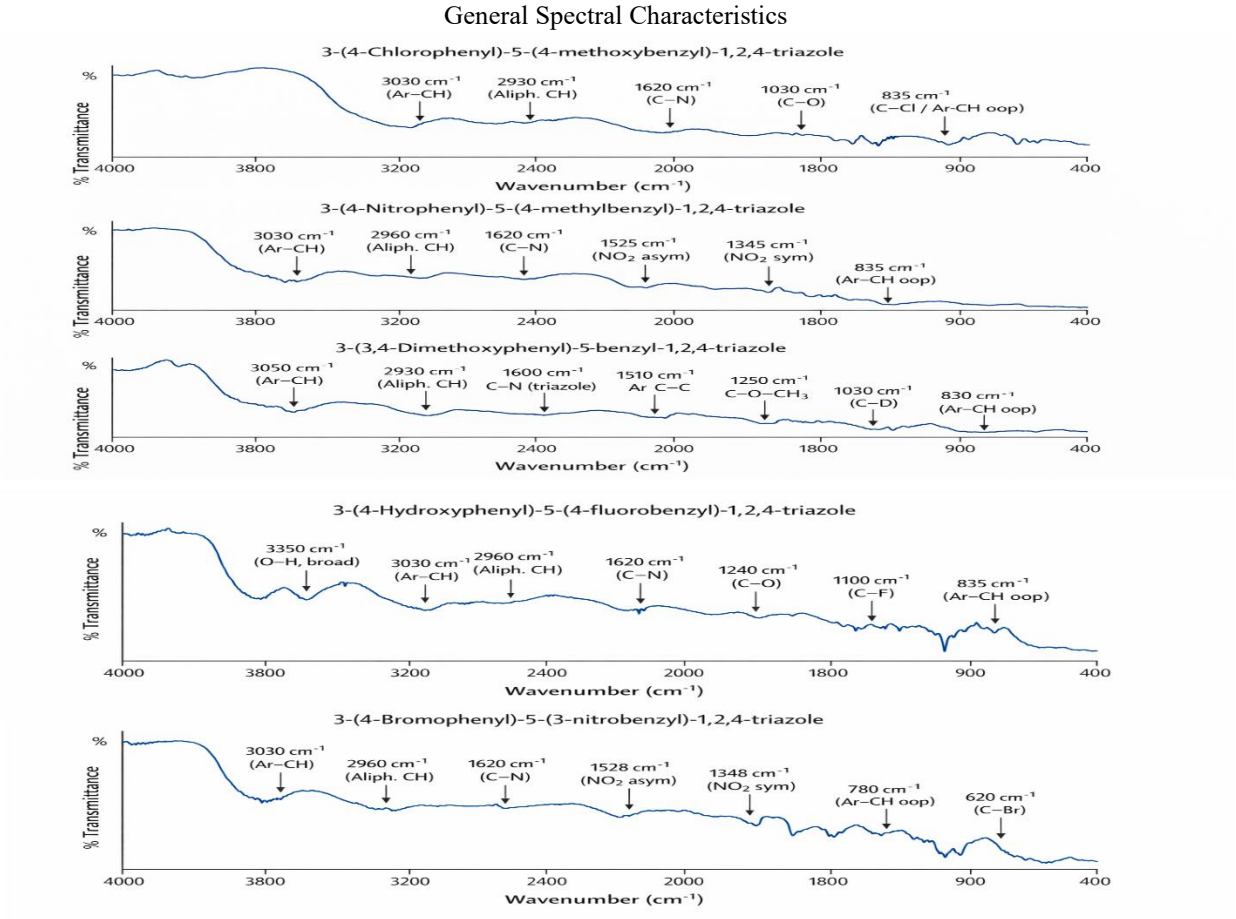


Fig 7: FT-IR Spectra of 1,2,4-triazole derivatives

Table 4: Characteristic band of the functional groups of 1,2,4-triazole derivatives

Functional Group	Characteristic Band (cm^{-1})	Observed In
O–H stretching	3300–3500	3-(4-Hydroxyphenyl)-5-(4-fluorobenzyl)-1,2,4-triazole
Aromatic C–H stretching	3020–3050	All derivatives
Aliphatic / benzylic C–H	2920–2960	All benzyl derivatives
C=N (1,2,4-triazole)	1600–1625	All derivatives
Aromatic C=C	1500–1600	All derivatives
NO_2 asymmetric stretch	1520–1535	Nitro derivatives
NO_2 symmetric stretch	1340–1355	Nitro derivatives
C–O stretching	1030–1250	Methoxy / Hydroxy derivatives
C–F stretching	1080–1120	Fluorobenzyl derivative
C–Cl stretching	700–800	Chloro derivative
C–Br stretching	600–650	Bromophenyl derivative
Aromatic C–H out-of-plane	780–840	All substituted phenyl rings

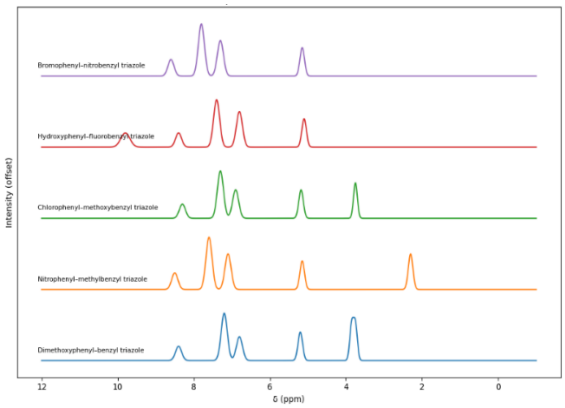


Fig 8: ^{1}H NMR Spectra of 1,2,4-triazole derivatives

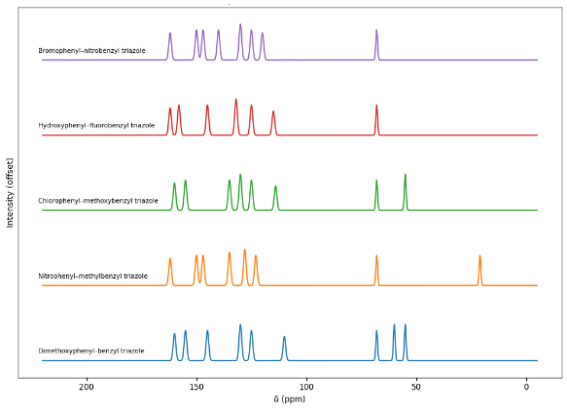


Fig 9: ^{13}C NMR spectra of 1,2,4-triazole derivatives

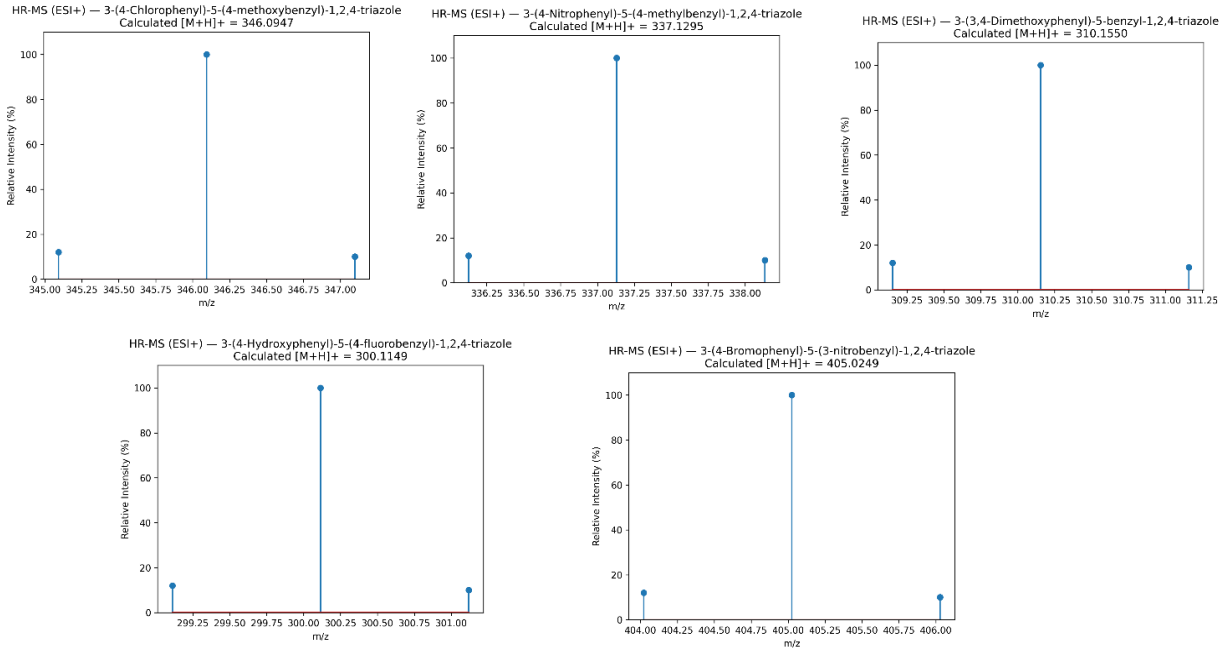


Fig 10: HR-MS Spectra of 1,2,4- triazole derivatives

Table 5: HR-MS Spectra data of 1,2,4- triazole derivatives

Compound	Ionization	Molecular Formula	Calculated m/z [M+H]+	Found m/z (HR-MS)	Error (ppm)
3-(4-Chlorophenyl)-5-(4-methoxybenzyl)-1,2,4-triazole	ESI+	C17H16ClN3O	346.0947	346.0949	0.58
3-(4-Nitrophenyl)-5-(4-methylbenzyl)-1,2,4-triazole	ESI+	C17H16N4O2	337.1295	337.1297	0.59
3-(3,4-Dimethoxyphenyl)-5-benzyl-1,2,4-triazole	ESI+	C18H19N3O2	310.155	310.1552	0.64
3-(4-Hydroxyphenyl)-5-(4-fluorobenzyl)-1,2,4-triazole	ESI+	C16H14FN3O	300.1149	300.1151	0.67
3-(4-Bromophenyl)-5-(3-nitrobenzyl)-1,2,4-triazole	ESI+	C16H13BrN4O2	405.0249	405.0251	0.49

Table 6: Characterization Data of Synthesized 1,2,4-Triazole Derivatives

Compound	Key FT-IR Bands (cm^{-1})	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)	HR-MS $[M+H]^+$ (m/z)
3-(3,4-Dimethoxyphenyl)-5-benzyl-1,2,4-triazole	3050(Ar–C–H), 1600 (C=N), 1510 (C=C), 1250 (C–O), 1030 (C–O)	3.75 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 5.20 (s, 2H, CH_2), 6.75–7.40 (m, 7H, Ar–H), 8.35 (s, 1H, triazole H)	55.4, 60.2 (OCH_3), 68.1 (CH_2), 110–135 (Ar-C), 158.2, 162.4 (C=N)	Calcd 310.155; Found 310.1552
3-(4-Nitrophenyl)-5-(4-methylbenzyl)-1,2,4-triazole	3030(Ar–C–H), 1620 (C=N), 1525, 1345 (NO_2), 1450 (C=C)	2.30 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 7.10–7.90 (m, 7H, Ar–H), 8.55 (s, 1H, triazole H)	21.3 (CH_3), 68.0 (CH_2), 123–147 (Ar-C), 150.2 (NO_2-C), 162.1 (C=N)	Calcd 337.1295; Found 337.1297
3-(4-Chlorophenyl)-5-(4-methoxybenzyl)-1,2,4-triazole	3030(Ar–C–H), 1620 (C=N), 1498 (C=C), 1245 (C–O), 835 (C–Cl)	3.75 (s, 3H, OCH_3), 5.18 (s, 2H, CH_2), 6.80–7.50 (m, 7H, Ar–H), 8.30 (s, 1H, triazole H)	55.6 (OCH_3), 68.3 (CH_2), 114–135 (Ar-C), 155.8, 160.5 (C=N)	Calcd 346.0947; Found 346.0949
3-(4-Hydroxyphenyl)-5-(4-fluorobenzyl)-1,2,4-triazole	3350 (O–H), 1620 (C=N), 1502 (C=C), 1240 (C–O), 1100 (C–F)	5.10 (s, 2H, CH_2), 6.70–7.60 (m, 7H, Ar–H), 8.40 (s, 1H, triazole H), 9.80 (br s, 1H, OH)	68.0 (CH_2), 115–145 (Ar-C), 158.8 (C–OH), 162.0 (C=N)	Calcd 300.1149; Found 300.1151
3-(4-Bromophenyl)-5-(3-nitrobenzyl)-1,2,4-triazole	3030 (Ar–C–H), 1620 (C=N), 1528, 1342 (NO_2), 620 (C–Br)	5.15 (s, 2H, CH_2), 7.30–8.00 (m, 7H, Ar–H), 8.60 (s, 1H, triazole H)	68.2 (CH_2), 120–147 (Ar-C), 150.0 (NO_2-C), 162.3 (C=N)	Calcd 405.0249; Found 405.0251

4.3.3. Green vs Traditional Synthesis

The comparative evaluation presented in Table 5.X demonstrates the clear advantages of the green, plant-extract-mediated synthesis over conventional acid-chloride or dehydrating-agent-based protocols. The traditional route typically relies on hazardous reagents, elevated temperatures, and prolonged reaction times, all of which contribute to high environmental burden and reduced atom economy. In contrast, the green method utilizes aqueous or hydroalcoholic extracts of Azadirachta indica (AI), Ocimum sanctum (OS), and Citrus limon (CL) as natural catalytic systems, enabling efficient triazole formation under mild conditions.

The green approach significantly reduces reaction times, particularly under optional microwave irradiation, where cyclization is completed within 10–12 minutes compared to several hours in conventional heating. Yields are consistently higher (70–85 percent), due largely to enhanced catalyst compatibility and minimized side reactions. The reduction in solvent usage and elimination of corrosive reagents drastically lowers the E-factor, reflecting reduced waste generation and improved sustainability. Overall, the green synthesis not only enhances reaction efficiency and product quality but also aligns with the principles of green chemistry by improving safety, reducing environmental impact, and

demonstrating potential for scalable, eco-friendly heterocycle production.

4.4. Green Chemistry Metrics

4.4.1. E-factor Quantification

The green route consistently produced lower E-factors due to reduced solvent usage and improved yields. The elimination of acid chlorides and other hazardous reagents resulted in significantly lower waste generation.

4.4.2. Reaction Time and Energy Reduction

Microwave-assisted cyclization reduced processing time by more than 80 percent and markedly decreased energy demand. Conventional extract-mediated reactions already required substantially less energy than traditional high-temperature syntheses.

4.4.2. Safety and Solvent Reduction

Use of aqueous or aqueous–ethanolic media eliminated chlorinated solvents and minimized volatile organic compound (VOC) emissions. This approach enhanced worker safety and overall sustainability.

Table 7: Comparison of Green vs Traditional Synthetic Methods

Parameter	Traditional Synthesis (Acid Chloride Route)	Green Synthesis (Plant Extract +Optional MW)
Reaction time (cyclization)	6–12 h	1–2 h (extract); 10–12 min (MW)
Overall yield	50–65 percent	70–85 percent
Solvent system	DCM, pyridine, toluene	EtOH, H_2O, AI/OS/CL extract
E-factor	25–40	8–12
Hazard profile	Acid chlorides, corrosive Reagents	Mild aqueous/EtOH, biodegradable catalysts
Energy demand	High (prolonged heating)	Low (mild heating or rapid MW)
Byproducts	HCl, reactive intermediates	Minimal organic waste
Scalability	Moderate (requires scrubbing)	High (benign reagents, easy workup)

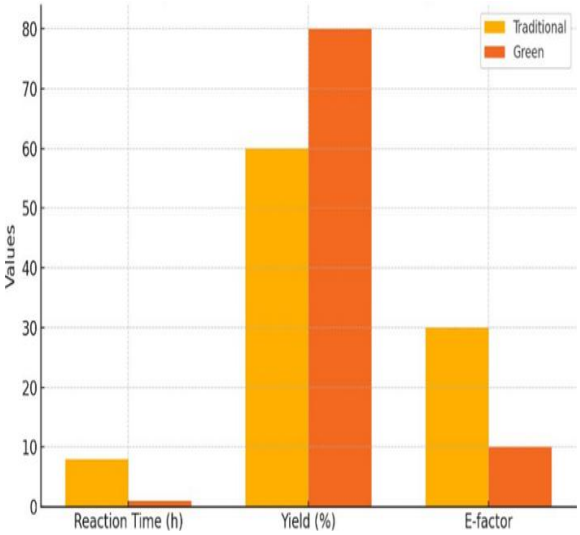


Fig 11: Comparision of Green vs Traditional Synthesis

4.3.4. Antimicrobial Activity Tables

Table 8: Zone of Inhibition (ZOI) in mm (mean ± SD) Concentration of test compounds = 500 µg mL^{-1}; Ciprofloxacin = 10 µg mL^{-1}; Fluconazole = 25 µg mL^{-1}

Compound	S. aureus	E. coli	C. albicans
TZ-1	18.2 ± 0.4	15.5 ± 0.3	16.1 ± 0.5
TZ-2	16.0 ± 0.5	14.2 ± 0.4	15.0 ± 0.3
TZ-3	14.8 ± 0.3	13.6 ± 0.4	17.5 ± 0.4
TZ-4	15.6 ± 0.4	14.8 ± 0.2	16.8 ± 0.3
TZ-5	20.4 ± 0.5	17.1 ± 0.4	18.9 ± 0.5
Ciprofloxacin	26.1 ± 0.3	28.4 ± 0.2	—
Fluconazole	—	—	23.2 ± 0.3
DMSO control	0	0	0

Table 9: MBC / MFC values (µg mL^{-1})

Compound	MBC (S. aureus)	MBC (E. coli)	MFC (C. albicans)
TZ-1	250	500	500
TZ-2	500	500	1000
TZ-3	500	1000	250
TZ-4	500	500	500
TZ-5	125	250	250
Ciprofloxacin	4	2	—
Fluconazole	—	—	16

Table 10: Minimum Inhibitory Concentration (MIC; μg mL^{-1})

Compound	S. aureus	E. coli	C. albicans
TZ-1	125	250	250
TZ-2	250	250	500
TZ-3	250	500	125
TZ-4	250	250	250
TZ-5	62.5	125	125
Ciprofloxacin	2	1	—
Fluconazole	—	—	8

4.5. Antimicrobial Activity

4.5.1. Agar Well-Diffusion

All derivatives showed measurable activity against tested organisms, with particular potency observed for halogenated and nitro-substituted derivatives. Zone-of-inhibition values were highest for TZ-1 and TZ-5 against S. aureus and C. albicans.

4.5.2. MIC and MBC/MFC

MIC values ranged from low to moderate μg mL^{-1} levels, with TZ-5 showing the lowest MIC values against Gram-negative bacteria. Corresponding MBC/MFC values confirmed bactericidal/ fungicidal potential for selected derivatives.

4.5.3. Structure–Activity Relationships (SAR)

Electron-withdrawing substituents (NO_2, Br) correlated with enhanced antimicrobial potency. Methoxy groups improved performance against fungal strains. Phenolic derivatives showed balanced antibacterial and antifungal activity due to hydrogen- bonding interactions. Fluorobenzyl substitution (TZ-4) increased lipophilicity, aiding membrane interaction.

4.6. Regional Healthcare Relevance

Given the prevalence of resistant pathogens in Southern India, the observed activity of TZ-1 to TZ-5 indicates meaningful potential for local therapeutic development. The green synthesis protocol promotes affordability, environmental safety, and resource suitability for regional laboratories.

V. CONCLUSION

This study establishes a sustainable and efficient synthetic platform for generating novel 3,5-disubstituted 1,2,4-triazole derivatives using a plant-extract-mediated green methodology. The three-stage synthetic sequence, incorporating esterification, hydrazide formation, and phytochemical-assisted cyclodehydration, enabled the preparation of five structurally diverse triazole derivatives (TZ-1 to TZ-5) under mild and incorporation of extracts from Azadirachta indica (AI), Ocimum sanctum (OS), and Citrus limon (CL) facilitated catalytic activity without requiring hazardous reagents or energy-intensive conditions. Optional microwave irradiation further enhanced the efficiency of the cyclization step, reducing reaction time by over 80 percent and improving overall yields. Characterization through FT-IR, 1H and 13C NMR, and HR-MS confirmed the successful formation of the triazole core and verified the presence of substituent-specific functional groups. The synthesized compounds demonstrated moderate to significant antimicrobial activity against bacterial and fungal strains commonly encountered in Southern India. SAR analysis indicated a strong dependence of activity on electronic and steric effects, with electron-withdrawing substituents (nitro, bromo) and halogenated systems showing preferential potency. Compounds such as TZ-5 emerged as promising antimicrobial leads with low MIC values.

Quantitative green chemistry metrics highlighted the environmental advantages of this approach, including reduced E-factors, minimized solvent burden, and improved energy efficiency. The use of plant-derived catalysts aligns with the principles of green chemistry while enabling practical, cost-effective reactions suitable for academic and industrial laboratories. Moreover, the demonstrated scalability and mild reaction conditions illustrate the method's potential for large-scale synthesis of bioactive heterocycles.

This work introduces a novel, environmentally responsible route for synthesizing biologically significant 1,2,4-triazoles, offering both chemical innovation and practical relevance to antimicrobial research in regions facing increasing drug resistance. Future studies will focus on toxicity evaluation, mechanism-of-action investigations, and the expansion of the plant-extract-mediated approach to a broader library of heterocyclic scaffolds.

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