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FACILE, EFFICIENT, CATALYST-FREE, MICROWAVE ASSISTED ONE-POT GREEN SYNTHESIS OF SUBSTITUTED 1, 2, 4-TRIAZOLES DERIVATIVES AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITIES

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ABSTRACT
An innovative, efficient and one-pot microwave assisted reaction has been created to produce 1, 2, 4-triazoles derivatives with high efficiency and in short reaction time. All the synthesized compounds were thoroughly identified and characterized using several spectroscopic approaches like 13C NMR; 1H NMR as well as IR also checks its antimicrobial activities against some pathogens. This environmentally friendly protocol provides numerous advantages, including simplicity of operation, cost-effectiveness, rapid reaction time, high yield and reduced waste generation, establishing the approach as both green and environment friendly.
Keywords: Green synthesis, 1,2,4-triazoles, Catalyst-free, microwave assisted, antimicrobial activities.
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INTRODUCTION

The compounds featuring a 1, 2, 4-triazole ring moiety have global research interest due to their diverse synthetic and biological activities. In recent years, there has been a notable focus on developing simple, environmentally friendly, cost-effective synthesis methods with metal or without metal catalysts and investigating the biological potential of these molecules across various applications. To make chemical techniques clean and eco-friendly, advancement of green protocols in organic chemistry have arisen as a primary driver[1].

The ring system of triazoles is an important heterocyclic moiety. It is a crucial structural framework for various agrochemicals, biological as well as pharmaceutical agents[2-4].

Traizole along with its derivatives are very vital class of heterocyclic chemicals which show an extensive range of organic characteristics like antihypertensive[5], anticancer[6], anti-inflammatory[7], anti-histamine[8], antimalarial[9], antioxidant[10], antimycobacterial[11], antiviral[12], antileishmanial[13], antimicrobial[14], antitubercular[15], antiproliferative[16], antiulcer effects[17] and antidepressant[18] effects.

Chemists have been interested in microwave radiation because it allows for the reduction of reaction times and the acceleration of product yields, which is crucial for lengthy high-temperature reaction protocol[19]. Organic reaction synthesis has made more use of microwave radiation typically a domestic microwave[20] is employed for the synthesis.

According to a review of the literature, various techniques for the synthesis of triazole moieties have been reported recently. Most of these methods carried out with various hazardous solvent and catalysts like [C_{16} M Py] $AlCl_3$ Br[17], Cu catalyst[21], PEG 400[22], sulfamic acid[23], TEA[24], p-TSA[24] and TFA[24]. The majority of these procedures also have specific disadvantages, including the utilization of expensive catalysts, hazardous solvents, severe reaction conditions, prolonged reaction times and fewer yields.

Microwave-assisted techniques have been used recently to boost product yields for a variety of heterocycles in addition to minimizing reaction time periods. We have developed a synthesis technique that is easy to use, affordable, effective and safe for the environment. In reported work, there is an acidic

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media with 80 % acetic acid are used, but here we used green acid as a citric acid which makes this protocol as a green approach to synthesis of of 1, 2, 4-triazoles derivatives.

In present work, we have developed a catalyst-free, efficient and one-pot production of 1, 2, 4-triazoles derivatives by employing microwave irradiation technique. We used aldehyde, semicarbazide, citric acid and ethanol in microwave irradiation technique to synthesized 1, 2, 4-triazoles derivatives as well as assess its antimicrobial efficacy against bacteria like *Bacillus subtilis* (Gram +) as well as *Pseudomonas aeruginosa* (Gram -).

EXPERIMENITAL

Material and Methods

Until specified differently, all reagents were bought from commercial vendors plus utilized exactly as supplied. On a silica-coated glass plate thin-layer chromatography was carried out using UV light detection. In DMSO d6 solvent, ^{13}C NMR along with ^{1}H NMR spectra has been documented utilizing tetramethylsilane as the internal standard. ^{1}H NMR spectra was acquired at 400 MHz utilizing Bruker instrument. Coupling constant (J), chemical shift, and multiplicity (m–multiplet; q–quartet; t–triplet; d–doublet; s–singlet) are given in Hz for 1H NMR data. Using KBr pellets, the IR spectra were taken utilizing a Bruker apparatus. The Systronics EQ730 Digital Melting Point Apparatus served as the instrument for documenting the melting points.

General procedure for Production of Substituted 1, 2, 4-Triazoles Derivatives

Aldehyde **1** (1mmol), semicarbazide **2** (1mmol), concentrated solution of citric acid (7-8 mL) and ethanol (5-7mL) were placed in a microwave vial. Resultant blend was exposed to microwave radiation at 300 W for about 5-6 minutes. Reaction was observed through thin layer chromatography. After finishing, this combination was extracted after concentrating utilizing ethyl acetate. Following separation of organic layer, it was subjected to drying using anhydrous sodium sulphate, after which it underwent evaporation utilizing a rotary evaporator. Crude product underwent recrystallization from ethanol to yield the product in its purest form.

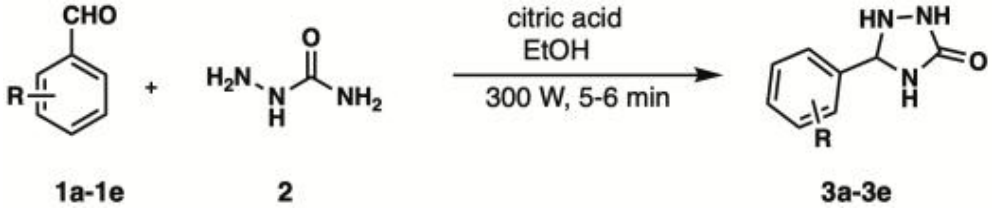

Fig.-1: Reaction Scheme: Synthetic pathway to produce substituted 1, 2, 4-triazoles derivatives

Table 1: Substrate Scope for preparing substituted 1, 2, 4-Triazoles derivatives Using Microwave Irradiation

S. No	R	Product	Time (Min.)	M.P. (^{0}C)	Colour	Yield (%)
1	4-Cl	3a	6	210-212	White	92
2	4-NO_2	3b	5	190-191	Yellow	95
3	4-F	3c	6	204-205	White	90
4	4-OMe	3d	6	196-198	White	92
5	-H	3e	5	204-205	White	90

Spectral Characterizations

5- (4-Chlorophenyl) -1, 2, 4-triazolidin-3-one (3a)

IR (KBr): 3462, 3275, 3140, 1698, 1666, 1426, 1356, 1223, 1203, 1138, 1089, 1011, 934, 858, 653, 513 cm^{-1}; ^{1}H NMR (400 MHz, DMSO- d_6): 6.53 (s, 2H, NH), 7.43-7.45 (d, J = 8.48 Hz, 2H, ArH), 7.75-7.77 (d, J=8.52 Hz, 2H, ArH), 7.83 (s, 1H, CH), 10.33 (s, 1H, NH); ^{13}CMR (100MHz, DMSOd_6): 128.64, 129.05, 133.85, 134.26, 138.48, 157.25

5-(4-Nitrophenyl) -1, 2, 4-triazolidin-3-one (3b)

IR (KBr): 3308, 3138, 2984, 1694, 1638, 1442, 1396, 1265, 1196, 1081, 1009, 926, 861, 690, 513 cm^{-1}; ^{1}H NMR (400 MHz, DMSO- d_6): 6.67 (s, 2H, NH), 7.93 (d, J = 7.8 Hz, 1H, ArH), 8.0 (d, J=7.5 Hz, 1H, ArH), 8.02 (d, J=7.1 Hz, 1H, ArH), 8.20 (d, J=6.7 Hz, 1H, ArH), 8.22 (s, 1H, CH), 10.60 (s, 1H, NH);

^{13}CMR (100 MHz, DMSO-d_6): 124.23, 127.81, 137.27, 141.83, 147.56, 156.98

5-(4-Fluorophenyl) -1, 2, 4-triazolidin-3-one (3c)
IR (KBr): 3459, 3272, 3192, 1665, 1587, 1501, 1357, 1298, 1138, 1095, 1010, 930, 867, 607, 513 cm^{-1}; ^{1}H NMR (400 MHz, DMSO- d_6): 6.49 (s, 2H, NH), 7.20-7.24 (d, J = 8.5 Hz, 2H, ArH), 7.77-7.8 (d, J=8.5 Hz, 2H, ArH), 7.83 (s, 1H, CH), 10.23 (s, 1H, NH);^{13}CMR (100 MHz, DMSO-d_6): 129.11, 131.88, 138.71, 157.38, 161.76, 164.21

5-(4-Methoxyphenyl) -1, 2, 4-triazolidin-3-one (3d)
IR (KBr): 3451, 3278, 3161, 1683, 1643, 1508, 1357, 1170, 1089, 1028, 953, 811, 662, 520 cm^{-1}; ^{1}H NMR (400 MHz, DMSO- d_6): 3.79 (s, 3H, OCH_3), 6.40 (s, 2H, NH), 6.93-6.96 (d, J = 8.7 Hz, 2H, ArH), 7.64-7.66 (d, J=8.7 Hz, 2H, ArH), 7.79 (s, 1H, CH), 10.07 (s, 1H, NH); ^{13}CMR (100 MHz, DMSO-d_6): 55.67, 114.54, 127.91, 128.50, 139.81, 157.45, 160.53

5-(phenyl) -1, 2, 4-triazolidin-3-one (3e)
IR (KBr): 3457, 3285, 3188, 1683, 1646, 1596, 1512, 1490, 1295, 1140, 1089, 1027, 990, 857, 667, 521 cm^{-1}; ^{1}H NMR (400 MHz, DMSO- d_6): 6.49 (s, 2H, NH), 7.34-7.40 (m, 5H, ArH), 7.8 (s, 1H, CH), 10.26 (s, 1H, NH); ^{13}CMR (100MHz, DMSOd_6): 127.01, 129.04, 129.49, 135.24, 139.87, 157.36

Evaluation of Antimicrobial Characteristics of Substituted 1, 2, 4-Triazoles Derivatives
Each of synthesized products underwent a thorough screening process for their antimicrobial activities by using Gram Positive test organism was *Bacillus subtilis* and Gram Negative test organism was *Pseudomonas aeruginosa.*

Anti-Microbial-Zone Inhibition Test- *Bacillus subtilis*
***Requirements**
Mueller-Hinton Agar (MHA) Plates, (SRL- Chem-Cat no.-24756)
Solvent (vehicle control) - DMSO (SRL Chem 28580), Bacterial Culture (*Bacillus subtilis,* MTCC 1133) received from Chandigarh's Microbial Type Culture Collection and Gene Bank (MTCC), Ciprofloxacin - (SRL Chem- 78079) - 2mg/ml, Amount Loaded – (10µl), Whatman No. 1 filter paper (5mm).

***Experiments**
Zone Inhibition Technique (also referred as the Kirby-Bauer approach) was utilized to evaluate antibacterial action. MHA plates were inoculated by evenly distributing 100 µl of a bacterial culture of *Bacillus subtilis*, prepared to a 0.5 McFarland Unit, achieving a cell density of 1.5 X 10^8 CFU per mL from Mueller-Hinton Broth. Subsequently, discs with 10µl of various concentrations ranging amid 0-100 mM were carefully positioned on plates. Each plate contained a disc loaded solely with solvent, serving as vehicle (control), whereas the Ciprofloxacin disc at 3 µg was regarded as positive control. For 24 hours *Bacillus subtilis* MHA plates were incubated at 37°C utilizing an incubator, then after the clear zone formed around the disc were recorded.

Table-2: Dilution Table for Samples*

Total Volume of Reaction Mixture	200	µl
Volume of Treatment Compound	200	µl
Volume to be dispensed for Treatment	10	µl

*Above dilution table same for *Bacillus subtilis* and *Pseudomonas aeruginosa.*

Table-3: Preparation of Sample Solutions

S. No.	Amount (µmol/disc)	Stock (mM)	Prev Stock (µl)	Solvent (µl)
1	0	0	0	200
2	62.5	6.25	100	100
3	125	12.5	100	100
4	250	25	100	100
5	500	50	100	100
6	1000	100	200	0
	Stock Conc.	100	mM	

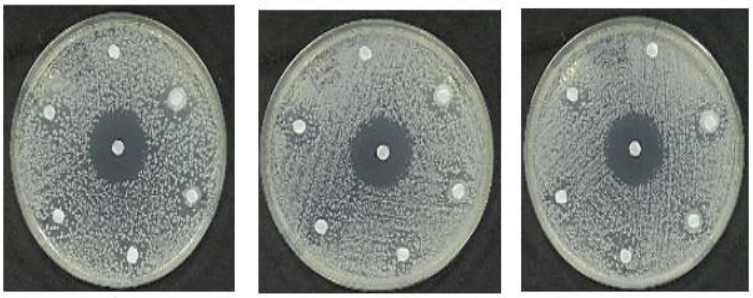

Fig.-2: Product: (3a) A B C

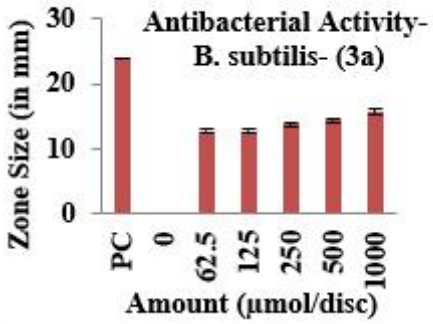

Fig.-3: Antibacterial Activity- *B. subtilis*- (3a)

Table-4: Statistical analysis for product (3a)

Amount (µmol/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	24	24	24	24	0	0
0	0	0	0	0	0	0
62.5	13	12	13	12.6667	0.57735	0.33333
125	13	12	13	12.6667	0.57735	0.33333
250	14	14	13	13.6667	0.57735	0.33333
500	15	14	14	14.3333	0.57735	0.33333
1000	16	15	16	15.6667	0.57735	0.33333

Amount present per disc in µmol, Dispensed Volume- 10µl, Positive Control (Ciprofloxacin) - 3µg, SEM- Standard Error of Mean, SD- Standard Deviation

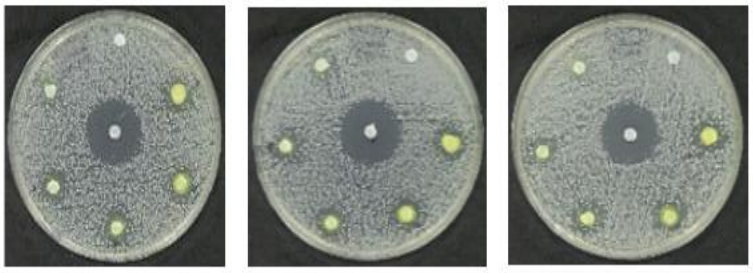

Fig.-4: Product: (3b) A B C

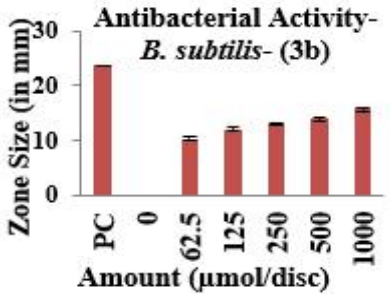

Fig.-5: Antibacterial Activity- *B. subtilis*- (3b)

Table-5: Statistical analysis for product (3b)

Amount (µmol/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	24	24	23	23.6667	0.57735	0.33333
0	0	0	0	0	0	0
62.5	10	11	10	10.3333	0.57735	0.33333
125	12	12	12	12	0	0
250	13	12	14	13	1	0.57735
500	15	13	14	14	1	0.57735
1000	16	15	16	15.6667	0.57735	0.33333

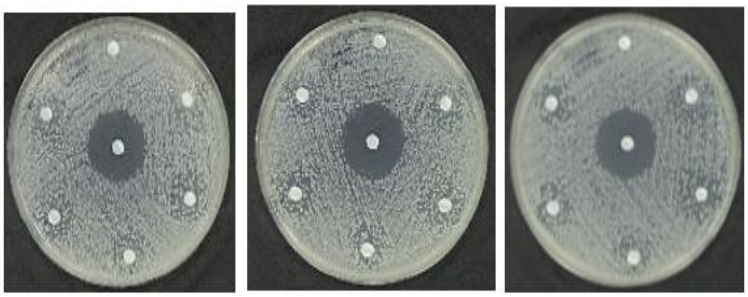

Fig.-6: Product: (3c) A B C

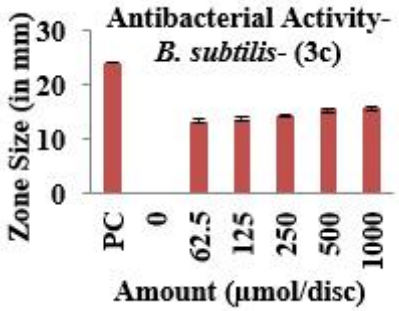

Fig.-7: Antibacterial Activity- *B. subtilis*- (3c)

Table-6: Statistical analysis for product (3c)

Amount (µmol/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	24	24	24	24	0	0
0	0	0	0	0	0	0
62.5	14	13	13	13.3333	0.57735	0.33333
125	14	13	14	13.6667	0.57735	0.33333
250	15	14	14	14.3333	0.57735	0.33333
500	15	15	16	15.3333	0.57735	0.33333
1000	16	15	16	15.6667	0.57735	0.33333

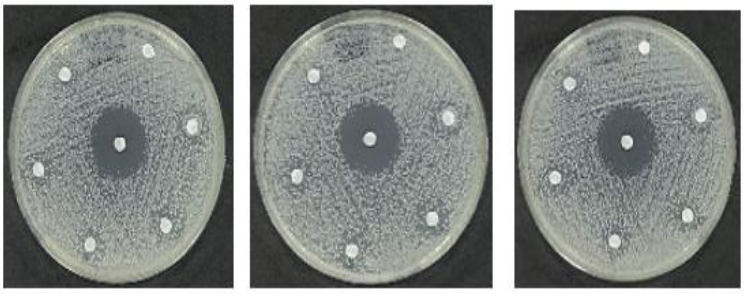

Fig.-8: Product: (3d) A B C

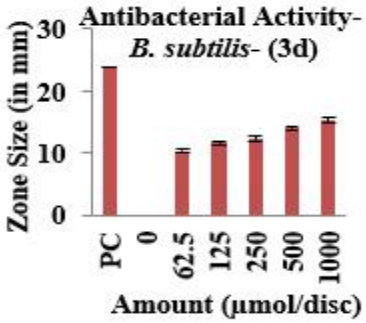

Fig.-9: Antibacterial Activity- *B. subtilis*- (3d)

Table-7: Statistical analysis for product (3d)

Amount (µmol/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	24	24	24	24	0	0
0	0	0	0	0	0	0
62.5	11	10	10	10.3333	0.57735	0.33333
125	12	12	11	11.6667	0.57735	0.33333
250	13	12	12	12.3333	0.57735	0.33333
500	15	14	13	14	1	0.57735
1000	16	14	16	15.3333	1.1547	0.66667

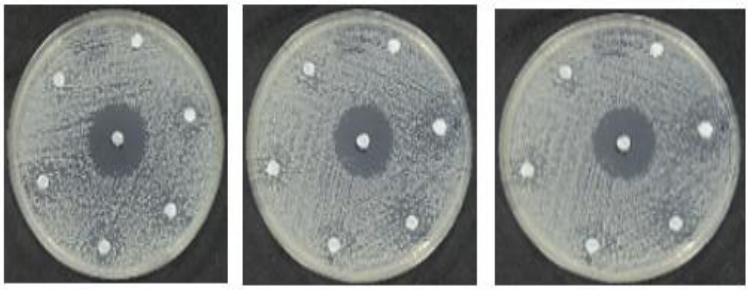

Fig.-10: Product: (3e) A B C

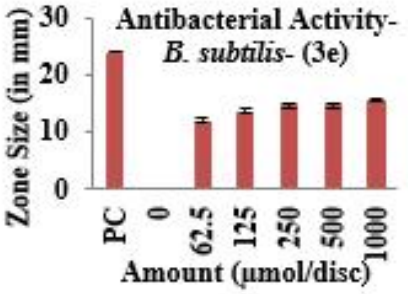

Fig.-11: Antibacterial Activity- *B. subtilis*- (3e)

Table-8: Statistical analysis for product (3e)

Amount (µmol/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	24	24	24	24	0	0
0	0	0	0	0	0	0
62.5	12	12	12	12	0	0
125	14	14	13	13.6667	0.57735	0.33333
250	15	15	14	14.6667	0.57735	0.33333
500	15	15	14	14.6667	0.57735	0.33333
1000	15	16	16	15.6667	0.57735	0.33333

Anti-Microbial-Zone Inhibition Test- *P. aeruginosa*
***Requirements:** As similar to *Bacillus subtilis*.

***Experiments:** The experimental procedure is similar as done in *Bacillus subtilis* except, Ciprofloxacin disc 10µg was utilized as positive control.

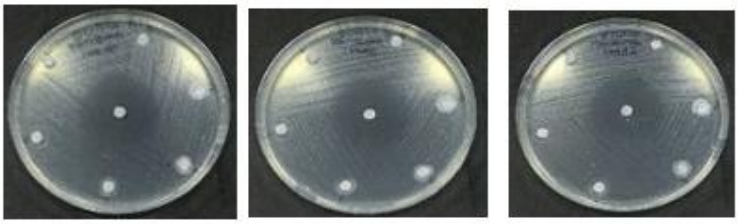

Fig.-12: Product: (3a) A B C

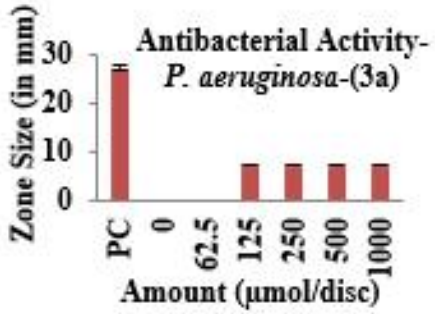

Fig.-13: Antibacterial Activity- *P. aeruginosa*-(3a)

Table-9: Statistical analysis for product *P. aeruginosa* - (3a)

Amount (µmol/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	28	27	27	27.3333	0.57735	0.33333
0	0	0	0	0	0	0
62.5	0	0	0	0	0	0
125	7	7	7	7	0	0
250	7	7	7	7	0	0
500	7	7	7	7	0	0
1000	7	7	7	7	0	0

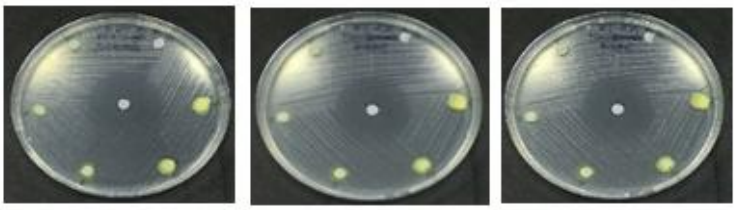

Fig.-14: Product: (3b) A B C

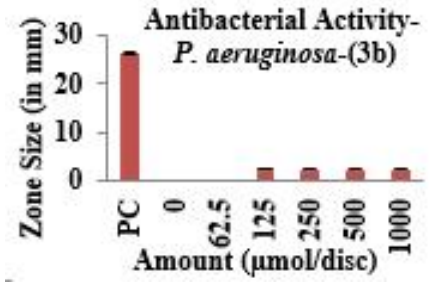

Fig.-15: Antibacterial Activity- *P. aeruginosa*-(3b)

Table-10: Statistical analysis for product *P. aeruginosa* - (3b)

Amount (µmol/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	26	26	27	26.3333	0.57735	0.3333
0	0	0	0	0	0	0
62.5	0	0	0	0	0	0
125	0	0	7	2.33333	4.04145	2.3333
250	0	0	7	2.33333	4.04145	2.3333
500	0	0	7	2.33333	4.04145	2.3333
1000	0	0	7	2.33333	4.04145	2.3333

RESULTS AND DISCUSSION

This is a modified procedure for preparation of 1, 2, 4-triazoles derivatives with the use of microwave irradiation technique. The key point of this reaction is the used of substituted aromatic aldehyde, semicarbazide and green acid as a citric acid which indicates this is a green protocol. This procedure was accelerated with the help of microwave irradiation, which provides adequate power for obtaining the desirable results. The reaction proceeded without the use of a catalyst under microwave irradiation, resulting in an excellent yield achieved within a reaction time of 5-6 minutes. This reaction time was very less as compared to various reported protocols. The Aldehyde with electron withdrawing group as nitro group show less reaction time with high yield as compared to aldehyde having electron donating group like -Cl, -F and -OMe groups (Table-1). The products were confirmed based on TLC, Melting point, FTIR, ^{13}C-NMR as well as ^{1}H-NMR data.

Table-11: Activity against *Bacillus subtilis*

S. No.	Sample No.	Efficient Quantity	Mean Zone at Efficient Quantity (in mm)
1	Ciprofloxacin (PC)	3 µg	24
2	3a	62.5 µmol	12.66
3	3b	62.5 µmol	10.33
4	3c	62.5 µmol	13.33
5	3d	62.5 µmol	10.33
6	3e	62.5 µmol	12

Table-12: Activity against *Pseudomonas aeruginosa*

S. No.	Sample No.	Efficient Quantity	Mean Zone at Efficient Quantity (in mm)
1	Ciprofloxacin (PC)	10µg	27.33
2	3a	125 µmol	7
3	3b	125 µmol	2.33
4	3c	-	-
5	3d	-	-
6	3e	-	-

Based on the results obtained against Bacillus subtilis, samples- 3a, 3b, 3c, 3d and 3e were found effective at the amount mentioned in the above Table-11. Similarly the results obtained against Pseudomonas aeruginosa, samples- 3a, 3b, were found effective at the amount mentioned in the above Table -12, whereas samples- 3c, 3d, and 3e does not seem to impose antimicrobial effect till 1000 µmol/disc.

CONCLUSION

We have created a novel green protocol for synthesizing 1, 2, 4-triazole derivatives that is substantially effective, catalysts free besides being environmentally friendly. This procedure is very helpful in balancing both industrial demands and environmental concerns. The resulting triazoles products demonstrate notable efficacy against microbes like *Bacillus subtilis* as well as *Pseudomonas aeruginosa.*

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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