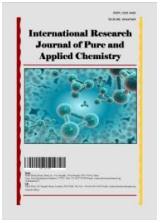

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Green Tamarind Extract Catalyzed Synthesis of 4-Amino-1,2,4-Triazole Derivatives and Their *In-vitro* Antimicrobial Activity

Diksha Verma[1*], Sunita Sharma[2], Tanvi Sahni[1] and Geetika Arora[1]

[1]Department of Chemistry, Punjab Agricultural University, Ludhiana, 141001, India.
[2]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, 141001, India.

Authors' contributions

The work was carried out in collaboration among all authors. Author DV performed the synthesis and wrote the draft of the manuscript under the supervision of authors SS, TS and GA. Author SS helps to design the study and helped in analyze the spectral data. Authors TS and GA managed the statistical analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

In several organic synthesis and chemical transformations, the use of green chemistry has decreased the reaction time and chemical waste. Due to the tremendous advantages of green chemistry, the paper presents the synthesis of benzylidene derivatives of 4-amino-1,2,4-triazole by reacting it with various substituted aldehydes/benzaldehydes using different catalytic amount(In mL) of tamarind extract/glacial acetic acid as a catalyst by conventional and microwave method. All the synthesized compounds (**1-7)** were characterized using spectroscopic techniques *viz*. UV, IR, and ^{1}H NMR. Antimicrobial activity of all the compounds was done using negative gram bacteria *i.e. Pseudomonas aeruginosa, Klebsiella* sp., and *Enterobacter* sp. against standard Ampicillin. Compound **2** containing 4-nitro substitution increased the antimicrobial activity as compared to other *Enterobacter* sp, but none of them showed better *In-vitro* antimicrobial potential than standard ampicillin. All the synthesized Schiff bases differed significantly from one another within the range of test concentrations.

**Corresponding author: E-mail: dikshaverma1343@gmail.com;*

Keywords: Benzylidene derivatives; 4-amino-1,2,4-triazole; tamarind extract; microwave irradiation; antimicrobial activity.

1. INTRODUCTION

In Organic chemistry, for the synthesis of a compound, there is the involvement of harmful and toxic chemicals like sulfuric acid [1], hydrogen bromide [2], etc. which are very dangerous to human health and also cause environmental pollution. Due to these reasons, green synthesis comes into play which involves the use of eco-friendly solvents and catalysts. For the synthesis of Schiff bases, natural acids *i.e.* fruit juices like grape juice, pomegranate juice [3], sweet lemon juice [4], and aqueous extract of unripe mango [5] instead of glacial acetic acid. Among all the natural acids, tamarind extract is one of the important natural acid catalysts which can be used for its synthesis [6]. Tamarind extract is purely acidic containing tartaric acid along with malic and citric acid. This property makes it useful as an acid catalyst for Schiff base synthesis. Green chemistry methods offer a non-toxic approach, selectivity, reduces reaction time, and simple to carry out [7]. Green methodologies involve the use of grindstone [8], water-based [9], microwave irradiation [10] and sonication methods [11] for the synthesis of benzylidene anilines. Thus, for the synthesis of organic compounds in less reaction time and with a higher yield, the green method is best in contrast to the conventional method.

Benzylidene derivatives are those compounds which are formed by the derivatization of the carbonyl group. Due to their tremendous application in various fields, they have been studied for many years. Schiff bases containing aromatic and heteroaromatic nucleus that possess biological activity. To enhance the biological potential of the compounds, the active nucleus is usually linked with another nucleus. Schiff bases containing heterocyclic rings bearing nitrogen and sulfur atom are known to show significant activity against microorganisms. However, different microbes exhibit somehow different antimicrobial activity which is assumed to be due to variation in the cell wall structure of the microbes as well as varied stability and solubility of Schiff bases [12]. Since these organic compounds contain -HC=N- moiety, they show much greater efficiency than corresponding aldehyde and amine from which they have been synthesized [13]. Nowadays, Schiff bases are very popular among scientists because of their tremendous use as an antimicrobial agent [14]. An antimicrobial agent is an agent that stops the growth of microorganisms or even kills them. The grouping of antimicrobial medicines is done according to the type of microorganism acting on them. For example, antibiotics and antifungal are used against bacteria and fungi respectively.

The present study is based on the synthesis of benzylidene derivatives of 4-amino-1,2,4-triazole using green chemistry and hence testing of synthesized compounds for microbial activity.

2. EXPERIMENTAL

2.1 Reagents and Instrumentation

All the chemicals used were of Analytical Grade and procured from Thermo Fisher Scientific, Sisco Research, Central Drug House (P), Loba Chemie, Himedia all from (India). Melting points of all the compounds were determined in open capillaries using the melting point apparatus. The purity of all the synthesized compounds was checked using thin-layer chromatographic plates with silica gel (gypsum) as adsorbent and ethanol as a developing solvent. For the visualization of the spots, iodine vapors were used as a visualizing agent. UV spectra were recorded on UV 2600 Spectrophotometer of Tech Comp Company. IR spectra were recorded on Perkin-Elmer FT IR Spectrometer and Thermo Scientific Nicolet 6700 FT- IR spectrometer in KBr pellets. ^{1}H NMR spectra were recorded on Bruker Advance II 400 Spectrometer. Tetramethylsilane (TMS) was used as an internal standard with δ value 0.0. With respect to the TMS standard in ^{1}H NMR, the chemical shifts of all the synthesized compounds were recorded. The notations used were (s) singlet, (d) doublet, (t) triple,t, and (q) quartet. All the compounds gave C, H, and, N that were recorded using Vario EL III Elementor CHNS analyzer.

2.2 General Experiments

2.2.1 Preparation of Tamarind Extract

Tamarinds were purchased from the local market. Tamarind (5.0g) was added to 25 mL of water and heated for 2 hours to form a homogeneous mixture. After that, the mixture was allowed to cool, filtered two times through muslin cloth and the filtrate was kept in a

refrigerator at 3°C for further use as a catalyst [6].

2.2.2 Synthesis of *N*-substituted benzylidene-4*H*-1,2,4-triazol-4-amines

a) Conventional method

A mixture of substituted aldehydes/benzaldehydes (0.01 mol) and 4-amino-1,2,4-triazole (0.01 mol, 0.84 g) were taken in three different 150 mL beakers. In each beaker, ethanol (15 mL) was added and the reaction mixture was stirred so that the reactants get mixed well. After that, there was the addition of tamarind extract/glacial acetic acid in variable amounts (0.5 mL, 0.75 mL, and 1.00 mL) in each beaker. The reaction mixture was then stirred until the reaction completed along with continuous monitoring to check the progress of reaction which was done by TLC. A single spot on the chromatographic plate confirmed the completion of the reaction and formation of the product. The yield and melting point of all the synthesized compounds were also recorded.

b) Microwave irradiation method

The compounds **1-7** were synthesized by reacting equimolar amount of substituted aldehydes/benzaldehydes and 4-amino -1,2,4-triazole (0.01 mol) in three different 150 mL beakers using ethanol (10 mL) as a solvent. All three reactions were irradiated at 900W for variable amounts of tamarind extract/glacial acetic acid (0.50 mL, 0.75 mL, and 1.00 mL) respectively at an interval of 15 seconds. The completion of all the reactions was monitored by TLC. The resultant solid product was then weighed to compare the yield which came out to be different from the amounts of both catalysts used.

2.3 Characterization data of Schiff Bases

Synthesis of N-(4-Bromobenzylidene)-4H-1,2,4-triazole-4-amine (1)

Color: Cream; m.p.: 228-229°C; UV (nm): 291; IR (KBr, cm^{-1}): 3120 (Ar C-H str.), 1616 (C=N str.), 1512 (N-N str.), 1487 (C=C str.), 1254 (C-N str.), 957, 825 (C=C bend.), 854 (C-N bend.), 707 (=C-H Bend.) and (C-Br str.); ^{1}H NMR (400 MHz, DMSO- d_6, 'δ'): 9.06 (s, 1H, CH=N), 8.97 (s, 2H, triazole carbons), 7.65-7.80 (m, 4H, Ar-H); Elemental analysis (%) found (Calculated) for $C_9H_7N_4Br$ C - 43.45 (43.05), H - 2.81 (2.41), N - 22.31 (22.71)

Synthesis of N-(4-Nitrobenzylidene)-4H-1,2,4-triazole-4-amine (2)

Color: Yellow; m.p:218-219°C; UV (nm): 330; IR (KBr, cm^{-1}): 3092 (Ar C-H str.), 1519 (C=N str.), 1519 (N-N str.), 1512 (C=C str.), 1347 (NO_2 asym str.),), 1315 (NO_2 symmetric str.), 1217 (C-N str.), 956, 752 (C=C bend.), 850 (C-N bend.), and 687 cm^{-1} (C-H bend.); ^{1}H NMR (400 MHz, DMSO- d_6, 'δ'): 9.28 (s, 1H, CH=N), 9.13 (s, 2H, triazole carbons), 8.11-8.39 (m, 4H, Ar-H); Elemental analysis (%) found (Calculated) for $C_9H_7N_5O_2$ C- 49.37 (49.77), H- 3.65 (3.25), N- 32.65 (32.25)

Synthesis of N-(4-Dimethylaminbenzylidene)-4H-1,2,4-triazole-4-amine (3)

Color: Yellow; m.p:210-211°C; UV (nm): 310; IR (KBr, cm^{-1}): 3007 (aromatic -C-H str.), 2957(asym), 2839(sym) (-C-H str. in methyl group), 1684 (C=N str.), 1599 (N-N str.), 1499 (C=C str.), 1455(asym), 1317(sym), (C-H bend. of methyl group), 1271 (C-N str.), 977, 825 (C=C bend.), 863 (C-N bend.), 812 (C-C str.) and 623 cm^{-1} (C-H bend.); ^{1}H NMR (400 MHz, DMSO- d_6, 'δ'): 8.21 (s, 1H, CH=N), 8.97 (s, 2H, triazole carbons), 7.66-7.68 (d, 2H, Ar-H), 6.76-6.78 (d, 2H, Ar-H), 3.05 (s, 6H, CH_3); Elemental analysis (%) found (Calculated) for $C_{11}H_{13}N_5$ C- 61.78 (61.38), H- 6.49 (6.09), N- 32.14 (32.54).

Synthesis of N-(4-Diethylaminobenzylidene)-4H-1,2,4-triazole-4-amine (4)

Color: White; m.p:160-161°C; UV (nm): 310; IR (KBr, cm^{-1}): 3101 (aromatic -C-H str.), 2975 (asym), 2930 (sym) (-C-H str. of ethyl group), 1685 (C=N str.), 1595 (N-N str.), 1495 (C=C str.), 1366 (C-H bend. of ethyl group), 1273 (C-N str.), 936, 816 (-C-C str.), 857 (C-N bend.), and 683 cm^{-1} (C-H bend.); ^{1}H NMR (400 MHz, DMSO- d_6, 'δ'): 8.38 (s, 1H, CH=N), 8.55 (s, 2H, triazole carbons), 6.71-6.73 (d, 2H, Ar-H), 7.65-7.67 (d, 2H, Ar-H), 1.21-1.24 (t, 6H, CH_3), 3.42-3.47 (q, 4H, CH_2); Elemental analysis (%) found (Calculated) for $C_{13}H_{17}N_5$ C- 64.57 (64.17), H- 7.44 (7.04), N- 28.38 (28.78).

Synthesis of N-(2,5-Dimethoxybenzylidene)-4H-1,2,4-triazole-4-amine (5)

Color: Yellow; m.p:186-187°C; UV (nm): 279; IR (KBr, cm^{-1}): 3087 (aromatic -C-H str.), 2917 (-C-H str. of methoxy group), 1696 (C=N str.), 1537 (N-N str.), 1439 (C=C str.), 1583 (N-N str.), 1241 (C-N str.), 1301 (C-O-C str.), 942, 816 (C=C

bend.), 874 (C-N bend.) and 698 cm^{-1}(C-H bend.); ^{1}H NMR (400 MHz, DMSO- d_6, 'δ'): 8.99 (s, 1H, CH=N), 8.63 (s, 2H, triazole carbons), 3.89 (s, 1H, OCH_3), 3.84 (s, 1H, OCH_3), 6.94 (s, 1H, Ar-H), 7.09-7.10 (d, 1H, Ar-H), 7.11-7.12 (s, 1H, Ar-H); Elemental analysis (%) found (Calculated) for $C_{11}H_{12}N_4O_2$ C- 56.49 (56.89), H- 5.61 (5.21), N-24.52 (24.12).

Synthesis of* N-*(Cinnambenzylidene)-4H-1,2,4-triazole-4-amine (6)

Color: White; m.p:169-170°C; UV (nm): 300; IR (KBr, cm^{-1}): 3049 (aliphatic -C-H str. in conjugation), 3006 (Ar C-H str.), 1629 (C=N str.), 1589 (aliphatic C=C str.), 1499 (N-N str.), 1453 (aromatic C=C str.), 1263 (C-N str.), 982, 841 (C=C bend.), 878 (C-N bend.) and 695 cm^{-1} (C-H bend.); ^{1}H NMR (400 MHz, DMSO- d_6, 'δ'): 8.38-8.40 (d, 1H, CH=N), 8.57 (s, 2H, triazole carbons), 7.42-7.45 (m, 3H, Ar-H), 7.55-7.57 (m, 2H, Ar-H), 7.00-7.06 (m, 1H, Ar-H), 7.26-7.27 (d, 1H, Ar-H); Elemental analysis (%) found (Calculated) for $C_{11}H_{10}N_4$ C- 67.18 (67.58); H- 6.54 (6.14), N- 25.67 (26.27).

Synthesis of* N-*(Furfuralbenzylidene)-4H-1,2,4-triazole-4-amine (7)

Color: Brown; m.p:239-240°C; UV (nm): 298; IR (KBr, cm^{-1}): 3016 (Ar C-H str.), 1619 (C=N str.), 1511 (N-N str.), 1489 (C=C str., 1224 (C-N str.), 1167 (C-O-C str.), 941, 841 (C=C bend.), 880 (C-N bend.), and 692 cm^{-1} (C-H bend.); ^{1}H NMR (400 MHz, DMSO-d_6, 'δ'): 8.94 (s, 1H, CH=N), 8.97 (s, 2H, triazole carbons), 7.14-7.15 (m, 2H, Ar-H), 6.67-6.68 (m, 1H, Ar-H); Elemental analysis (%) found (Calculated) for $C_7H_6N_4O$ C- 52.25 (51.85), H- 3.33 (3.73), N- 34.15 (34.55).

2.4 Evaluation of *In-vitro* Antimicrobial Activity

In-vitro antimicrobial activity of synthesized Schiff bases was tested against *Pseudomonas aeruginosa* (KF 853103.3), *Klebsiella* sp. (KF 424316.1), and *Enterobacter* sp. by disc plate method [15]. Stock solutions (2.00 mg/mL) of the synthesized compounds were made in DMSO and further serial dilutions of the stock solution were done. Nutrient agar was used as culture media which was poured in sterile plates after autoclaving. Plates were then allowed to solidify and were kept for 24 hours to ensure sterility. Prepared suspensions of 3-4 hours old broth of test bacteria (0.1 mL on each sterilized plate) were inoculated in the medium plates under aseptic conditions. Sterile filter paper discs (HiMedia sterile susceptibility discs) moistened with different concentrations of the test compound were placed on inoculated plates aseptically. Disc dipped in DMSO served as control. Petri plates were then incubated at 28 ± 2°C for 24 hours and diameters of growth of zone of inhibition (mm) were measured. The inhibition of bacteria by medium containing the test compound was compared with antibiotic ampicillin standard as control. All these experiments of antibacterial activity were carried out in triplicate. Statistical analysis of all the synthesized compounds was also done using CRD (Complete Randomized Design) and sin arc transformations.

3. RESULTS AND DISCUSSION

3.1 Chemistry

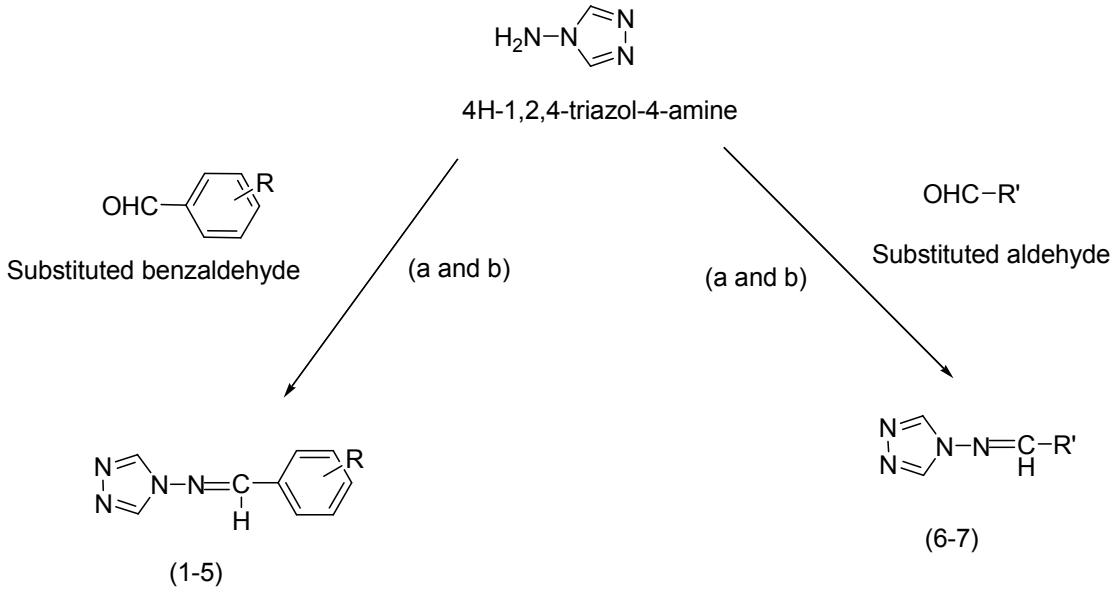
All the physical parameters of the synthesized compounds including color, melting point, molecular weight, molecular formulae, and elemental analysis are represented in Table 1. The reaction time and yield for all the synthesized Schiff bases were compared based on different methods and catalysts used shown in Tables 2-5. It showed that the compounds (**1-7**) synthesized using the microwave method were synthesized in less reaction time with higher yield in contrast to conventional methods irrespective of the catalyst used. Also, glacial acetic acid proved to be a better catalyst as compared to tamarind extract concerning reaction time and yield (Scheme 1).

All the synthesized compounds were characterized using spectroscopic techniques like UV, IR, and ^{1}H NMR. The absence of a peak at 2920 cm^{-1} and 2820 cm^{-1} in IR due to the -CH=O group and in the range of 3400 -3600 cm^{-1} due to the NH_2 group confirmed the synthesis of Schiff bases. In ^{1}H NMR, peak in the range 9-10 δ and 3-5 δ corresponding to aldehydic proton (CH=O) and amine protons (Ar-NH_2) were absent which further supported the synthesis of Schiff bases. From the IR data, it revealed that CH=N moiety gave a characteristic band in the range of 1594-1695 cm^{-1} while in ^{1}H NMR data, the one proton singlet signal for CH=N moiety ranged between 'δ' 8.21-9.28.

Table 1. Physical data of synthesized compounds (1-7)

Compound	Molecular formula	Molecular weight (g)	Color	Melting point (ºC)	R_f value	Found (Calculated) % C	H	N
1	$C_9H_7N_4Br$	251	Cream	228-229	0.86	43.45 (43.05)	2.81 (2.41)	22.31 (22.71)
2	$C_9H_7N_5O_2$	217	Yellow	218-219	0.80	49.37 (49.77)	3.65 (3.25)	32.65 (32.25)
3	$C_{11}H_{13}N_5$	215	Yellow	210-211	0.52	61.78 (61.38)	6.49 (6.09)	32.14 (32.54)
4	$C_{13}H_{17}N_5$	243	White	160-161	0.50	64.57 (64.17)	7.44 (7.04)	28.38 (28.78)
5	$C_{11}H_{12}N_4O_2$	232	Yellow	186-187	0.67	56.49 (56.89)	5.61 (5.21)	24.52 (24.12)
6	$C_{11}H_{10}N_4$	198	White	169-170	0.60	67.18 (67.58)	6.54 (6.14)	25.67 (26.27)
7	$C_7H_6N_4O$	162	Brown	239-240	0.55	52.25 (51.85)	3.33 (3.73)	34.15 (34.55)

The solvent system for TLC was Ethanol.



a = Conventional or Microwave irradiation method, b = Tamarind extract or Glacial acetic acid

Compound	R
1	4-Br
2	4-NO_2
3	4-$N(CH_3)_2$
4	4-$N(C_2H_5)_2$
5	2,5-(OCH_3)

Compound	R'
6	C_6H_5CH=CH-
7	

Scheme 1. Synthesis of *N*-substituted benzylidene-4*H*-1,2,4-triazol-4-amines (1-7)

Table 2. Comparison of yield (%) of different Schiff bases using different catalysts by the conventional method

Amount of catalyst (mL)	0.50			0.75			1.00		
Method compounds	**(A)**	**(B)**	**% decrease in yield**	**(A)**	**(B)**	**% decrease in yield**	**(A)**	**(B)**	**% decrease in yield**
1	63.72	55.96	7.76	68.17	60.01	8.16	72.58	66.53	6.05
2	77.14	69.58	7.56	83.91	76.11	7.80	93.71	85.23	8.48
3	81.48	11.62	69.86	86.10	17.69	68.41	91.18	24.23	66.95
4	66.34	54.81	11.53	69.18	57.14	12.04	72.12	60.32	11.80
5	78.20	68.42	9.78	85.10	73.13	11.97	91.71	77.74	13.97
6	84.25	82.14	2.11	88.41	87.34	1.07	94.45	91.21	3.24
7	66.37	57.32	9.05	71.27	62.87	8.40	75.17	69.21	5.96
Range	63.72-84.25	11.62-82.14	2.11-69.86	68.17-88.41	17.69-87.34	1.07-68.41	72.12-94.45	24.23-91.21	4.24-66.95
Mean	73.93	57.12	16.81	78.96	62.04	16.84	84.41	77.78	16.78

A = glacial acetic acid, B = tamarind extract

Table 3. Comparison of yield (%) of different Schiff bases using different catalysts by microwave irradiation method

Amount of catalyst (mL)	0.50			0.75			1.00		
Method compounds	**(A)**	**(B)**	**% decrease in yield**	**(A)**	**(B)**	**% decrease in yield**	**(A)**	**(B)**	**% decrease in yield**
1	84.17	83.12	1.05	88.04	86.54	1.50	95.43	89.43	6.00
2	89.54	87.21	2.33	92.11	91.42	0.69	97.41	96.01	1.40
3	88. 07	84.31	3.76	91.27	88.05	3.22	95.18	92.54	2.64
4	69.71	64.75	4.96	73.18	70.50	2.68	77.25	74.70	2.55
5	87.15	77.74	9.41	92.05	80.39	11.66	95.87	84.74	11.13
6	87.18	85.20	1.98	90.55	89.51	1.04	95.02	92.70	2.32
7	92.78	90.78	2.00	95.51	93.43	2.08	97.91	96.80	1.11
Range	69.71-92.78	64.75-90.78	1.05-9.41	73.18-95.51	70.50-93.43	0.69-11.66	77.25-97.91	74.70-96.80	1.11-11.13
Mean	85.51	81.87	3.64	88.95	85.69	3.27	93.44	89.56	3.88

A = glacial acetic acid, B = tamarind extract

Table 4. Comparison of time (min) of different Schiff bases using different catalysts by the conventional method

Amount of catalyst (mL)	0.50			0.75			1.00		
Method compounds	**(A)**	**(B)**	**Increase in time (min)**	**(A)**	**(B)**	**Increase in time (min)**	**(A)**	**(B)**	**Increase in time (min)**
1	900	1440	540	660	1200	540	420	900	480
2	840	1080	240	540	900	360	300	540	240
3	1020	1260	240	780	1080	300	480	600	120
4	1260	1740	480	1080	1500	420	840	1260	520
5	1800	1380	420	1020	1080	60	600	720	60
6	780	1200	420	600	960	360	300	720	420
7	720	1440	720	540	1080	540	240	780	550
Range	720-1800	1080-1740	240-720	540-1080	900-1500	60-540	240-840	540-1260	60-550
Mean	1045.7	1362.85	437.14	745.71	1114.28	368.57	454.28	788.57	341.42

A = glacial acetic acid, B = tamarind extract

Table 5. Comparison of time (min) of different Schiff bases using different catalysts by microwave irradiation method

Amount of catalyst (mL)	0.50			0.75			1.00		
Method compounds	**(A)**	**(B)**	**Increase in time (min)**	**(A)**	**(B)**	**Increase in time (min)**	**(A)**	**(B)**	**Increase in time (min)**
1	1.10	2.10	1.00	0.55	1.40	0.85	0.45	1.10	0.65
2	1.50	1.40	0.10	1.30	1.20	0.10	1.00	1.10	0.10
3	6.20	13.40	7.20	5.40	11.30	5.90	5.00	8.00	3.00
4	3.00	6.00	3.00	2.50	5.30	2.80	2.30	4.40	2.10
5	3.10	5.30	3.20	2.30	4.00	1.70	2.00	3.30	1.30
6	1.30	4.00	2.70	1.20	2.20	1.00	1.00	1.20	0.20
7	1.30	7.30	6.00	1.00	5.00	4.00	0.40	3.00	2.60
Range	1.10-6.20	1.40-13.40	0.10-7.20	0.55-5.40	1.20-11.30	0.10-5.90	0.40-5.00	1.10-8.00	0.10-3.00
Mean	2.50	5.64	3.31	2.03	4.34	2.34	1.74	3.15	1.42

A = glacial acetic acid, B = tamarind extract

3.2 *In-vitro* Antimicrobial Activity

Schiff bases were also evaluated for their *In-vitro* antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella* sp., and *Enterobacter* sp. All the compounds showed significant activity against all the bacteria. (Tables 6-8) showed the minimum inhibition zone of all the compounds against *Pseudomonas aeruginosa, Klebsiella* sp., and *Enterobacter* sp. respectively. Minimum inhibition concentration (MIC) of all the compounds was also calculated against all three bacteria along with standard Ampicillin.

3.2.1 *Pseudomonas aeruginosa*

From the data presented in Table 6, it was noticed that all compounds were moderately active against *Pseudomonas aeruginosa* at 2.00 mg mL^{-1}. At all respective concentrations, compound **5** was most effective having 2,5-dimethoxy substitution on the benzene ring due to the + R effect of the OCH_3 group. At higher test concentration *i.e.* at 2.00 mg mL^{-1}, none of the compounds had inhibition zone less than 13.0 mm. All the compounds came out to be effective at lower concentrations *i.e.* at 0.50 mg mL^{-1} except compound **1** and **6** having p*ara* bromo substitution on the benzene ring (-I effect) and cinnamaldehyde (-R effect) moiety respectively. Compound **1** was least effective against *Pseudomonas aeruginosa* at all concentrations. Amongst electron releasing substituents on the benzene ring, diethyl substitution at the *para* position was more promising than dimethyl substitution at the *para* position at all concentrations. None of the compounds was recorded as effective as standard Ampicillin at all test concentrations. All the compounds differed significantly in various concentrations. The MIC values of all the synthesized compounds along with standard ampicillin were also calculated and represented in Table 6 which further supported the higher efficiency of standard ampicillin followed by compound **5**. Zone of inhibition formed by ampicillin and compound **7** was also shown in Plate 1 at their respective concentrations.

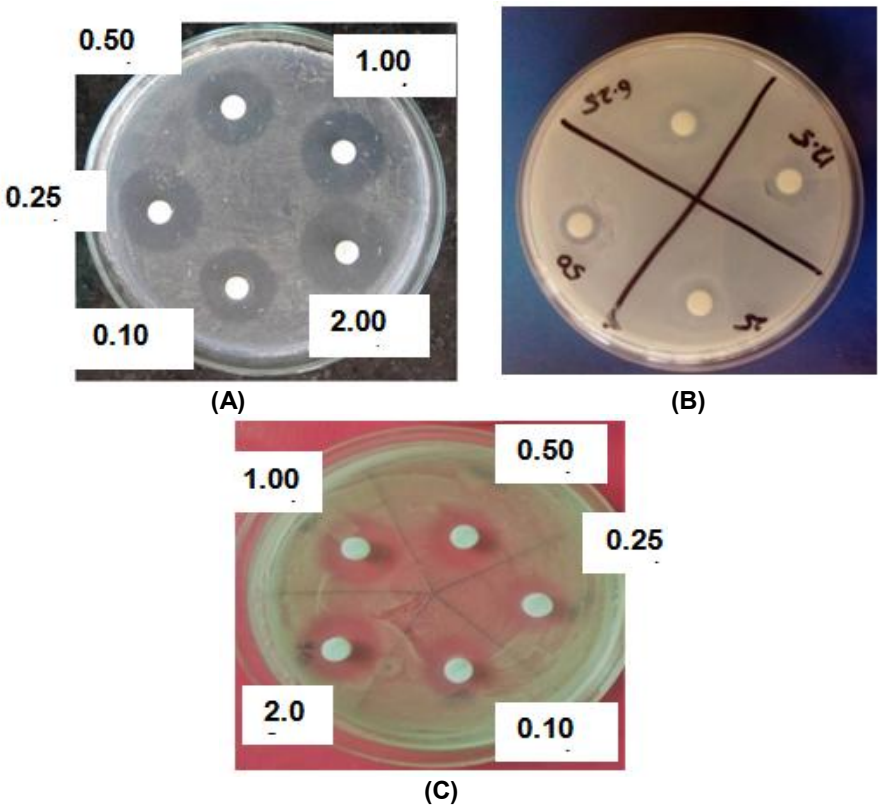


Plate 1. Zones of inhibition formed by Ampicillin (A) and (B) at 2.00, 1.00, 0.50, 0.25, 0.10, 0.00625, 0.0125, 0.025 and 0.050 mg/mL and compound 7(C) at 2.00, 1.00, 0.50, 0.25 and 0.10 mg/mL against *Pseudomonas aeruginosa*

Table 6. Effect of different Schiff bases on the growth of *Pseudomonas aeruginosa* at different concentrations

Compounds	The diameter of growth inhibition zone (mm)						MIC
	2.00 mg/mL	1.00 mg/mL	0.50 mg/mL	0.25 mg/mL	0.10 mg/mL	0.05 mg/mL	mg/mL
1	13.0 ± 0.5 (21.12)	11.5± 0.2(19.81)	10.0± 0.2(18.42)	8.0 ± 0.2(16.41)	7.0 ± 0.1 (15.32)	0(0.00)	0.08
2	17.0 ± 0.2 (24.33)	15.0± 0.6 (22.77)	14.0± 0.3 (21.96)	12.5 ± 0.1 (20.69)	11.5 ± 0.5 (19.81)	9.0 ± 0.4 (17.44)	0.03
3	14.0 ± 0.3 (21.96)	13.5± 0.1 (21.54)	12.5± 0.4 (20.69)	10.0 ± 0.4 (18.42)	9.0 ± 0.3 (17.44)	8.5 ± 0.4 (16.93)	0.06
4	18.0 ± 0.1 (25.09)	16.0± 0.1 (23.57)	15.0± 0.2 (22.77)	14.0 ± 0.2 (21.96)	12.5 ± 0.1 (20.69)	10.5 ± 0.3 (18.89)	0.025
5	19.0 ± 0.2 (25.83)	17.0± 0.3 (24.33)	15.5± 0.2 (23.17)	14.5 ± 0.6 (22.37)	13.0 ± 0.1 (21.12)	11.0 ± 0.5 (19.35)	0.02
6	13.5 ± 0.2 (21.54)	12.0± 0.2 (20.25)	11.0±0.6 (19.35)	9.0 ± 0.6 (17.44)	7.0 ± 0.4 (15.32)	0(0.00)	0.07
7	15.5 ± 0.2 (23.17)	14.5± 0.1 (22.37)	12. 5± 0.1(20.69)	11.5 ± 0.1 (19.18)	9.5 ± 0.3 (17.94)	8.0 ± 0.1 (16.41)	0.045
Ampicillin	20.0 ± 0.1(26.55)	18.0 ± 0.2(25.09)	16.5± 0.2(23.95)	15.0 ± 0.1(22.77)	14.0 ± 0.2 (21.96)	12.0 ± 0.1 (20.25)	0.012
CD (*P*= 0.05)	0.95	0.87	0.87	0.87	0.87	0.74	

**Each value is expressed as mean ± SD of three replications for the zone of inhibition;*
***Figures in the parenthesis are arc sin transformed values*

Table 7. Effect of different Schiff bases on the growth of *Klebsiella* sp. at different concentrations

Compounds	The diameter of growth inhibition zone (mm)						MIC
	2.00 mg/mL	1.00 mg/mL	0.50 mg/mL	0.25 mg/mL	0.10 mg/mL	0.05 mg/mL	mg/mL
1	18.5 ± 0.1 (25.46)	15.5 ± 0.5 (23.17)	13.0 ± 0.1 (21.12)	11.0 ± 0.3 (19.35)	8.5 ± 0.4 (16.93)	0(0.00)	0.075
2	16.0 ± 0.5 (23.57)	11.5 ± 0.1 (19.81)	10.0 ± 0.4 (18.42)	9.0 ± 0.2 (17.44)	8.0 ± 0.1 (16.41)	0(0.00)	0.09
3	22.0 ± 0.4 (27.95)	19.5 ± 0.3 (26.19)	15.5 ± 0.2 (23.17)	13.5 ± 0.1 (21.54)	9.5 ± 0.3 (17.94)	8.5 ± 0.1 (16.93)	0.025
4	23.5 ± 0.1 (28.98)	20.0 ± 0.4 (26.55)	18.0 ± 0.1 (25.09)	14.5 ± 0.2 (22.37)	10.0 ± 0.5 (18.42)	9.5 ± 0.4 (17.94)	0.015
5	20.5 ± 0.5 (26.90)	17.0 ± 0.1 (24.33)	15.0 ± 0.2 (22.77)	12.0 ± 0.1 (20.25)	10.5 ± 0.3 (17.44)	8.5 ± 0.3 (16.93)	0.04
6	21.5 ± 0.4 (27.61)	18.5 ± 0.1 (25.46)	15.0 ± 0.1 (22.77)	12.0 ± 0.5 (20.25)	9.0 ± 0.2 (17.44)	8.0 ± 0.1 (16.41)	0.03
7	20.0 ± 0.5 (26.55)	16.5 ± 0.2 (23.95)	14.5 ± 0.2 (22.37)	11.5 ± 0.1 (19.81)	8.5 ± 0.3 (16.93)	7.0 ± 0.2 (15.32)	0.09
Ampicillin	43 ± 0.1(40.95)	38 ± 0.3(38.04)	35 ± 0.4(36.25)	31 ± 0.1(33.81)	29 ± 0.1(32.56)	25 ± 0.1(29.98)	0.008
CD (*P*= 0.05)	0.87	0.95	1.65	0.87	0.87	0.74	

**Each value is expressed as mean ± SD of three replications for the zone of inhibition*
***Figures in the parenthesis are arc sin transformed values*

Table 8. Effect of different Schiff bases on the growth of *Enterobacter* sp. at different concentrations

	The diameter of growth inhibition zone (mm)						MIC
Compounds	**2.00 mg/mL**	**1.00 mg/mL**	**0.50 mg/mL**	**0.25 mg/mL**	**0.10 mg/mL**	**0.05 mg/mL**	**mg/mL**
1	18.0 ± 0.5 (25.09)	14.5 ± 0.1 (22.37)	12.5 ± 0.4 (20.69)	10.0 ± 0.1 (18.42)	8.0 ± 0.2 (16.41)	0 (0.00)	0.06
2	24.5 ± 0.2 (29.65)	22.5 ± 0.3 (28.30)	19.0 ± 0.1 (25.83)	16.0 ± 0.1 (23.5)	13.5 ± 0.3 (21.54)	9.0 ± 0.2 (17.44)	0.01
3	22.0 ± 0. 3 (27.95)	17.5 ± 0.4 (24.71)	16.0 ± 0.5 (23.57)	12.0 ± 0.2 (20.25)	9.0 ± 0.1 (17.44)	8.0 ± 0.2 (16.41)	0.02
4	24.0 ± 0.1 (29.32)	18.0 ± 0.1 (25.09)	16.0 ± 0.2 (23.57)	15.5 ± 0.5 (23.17)	11.5 ± 0.4 (19.81)	8.5 ± 0.5 (16.93)	0.015
5	18.5 ± 0.3 (25.46)	16.0 ± 0.4 (23.57)	14.5 ± 0.3 (22.37)	11.0 ± 0.4 (19.35)	8.0 ± 0.2 (16.41)	7.0 ±0.2 (15.32)	0.035
6	20.0 ± 0.5 (26.55)	17.0 ± 0.3 (24.33)	15.5 ± 0.1 (23.17)	12.0 ± 0.5 (20.25)	8.5 ± 0.1 (16.93)	7.5 ± 0.3 (15.88)	0.03
7	17.5 ± 0.1 (24.71)	13.5 ± 0.4 (21.54)	12.0 ± 0.1 (20.25)	10.5 ± 0.3 (18.89)	8.0 ± 0.3 (16.41)	00.00)	0.06
Ampicillin	26 ± 0.02(30.64)	24 ± 0.1(29.32)	20 ± 0.1(26.55)	17 ± 0.5(15.32)	15 ± 0.1(22.77)	12 ± 0.2(20.25)	0.004
CD (p=0.05)	1.02	0.87	0.95	0.87	0.87	0.74	

**Each value is expressed as mean ± SD of three replications for the zone of inhibition*
***figures in the parenthesis are arc sin transformed values*

3.2.2 *Klebsiella* sp.

Data presented in Table 7 gives information about the inhibition zone of all the synthesized Schiff bases against *Klebsiella* sp. It was observed that compound **4** was most effective at all concentrations. None of the compounds exhibited an inhibition zone of less than 16.0 at 2.00 mg mL^{-1} concentration.

Among *para*-substituted benzene ring, compound **4** having diethylamino substitution was most effective followed by compounds **3**, **1**, and **2** having dimethylamino, bromo, and nitro substitution respectively. Compound **2** was least effective at all test concentrations among all Schiff bases with complete inactivation at 0.05 mg mL^{-1}. Also, compound **1** showed inactivation at 0.05 mg mL^{-1}. None of the compounds was as active as standard ampicillin at various test concentrations.

Minimum Inhibition Concentration values of all the synthesized Schiff bases were also presented in Table 7 against *Klebsiella* sp. From MIC value also, it was revealed that compound **4** with *para* diethylamino substitution was most effective while compound **2** was least effective at any concentration. Plate 2 showed inhibitory zones of ampicillin and compound **3**.

3.2.3 *Enterobacter* sp.

The effect of Schiff bases on the growth of *Enterobacter* sp. is presented in Table (8). It was noticed that the compound **2** bearing nitro group substitution at the *para* position was most effective while compound **7** containing furfural moiety was least effective at all the concentrations. None of the compounds showed inhibition zone less than 17 mm at 2.00 mg mL^{-1} concentration. Compound **6** and compound **3**

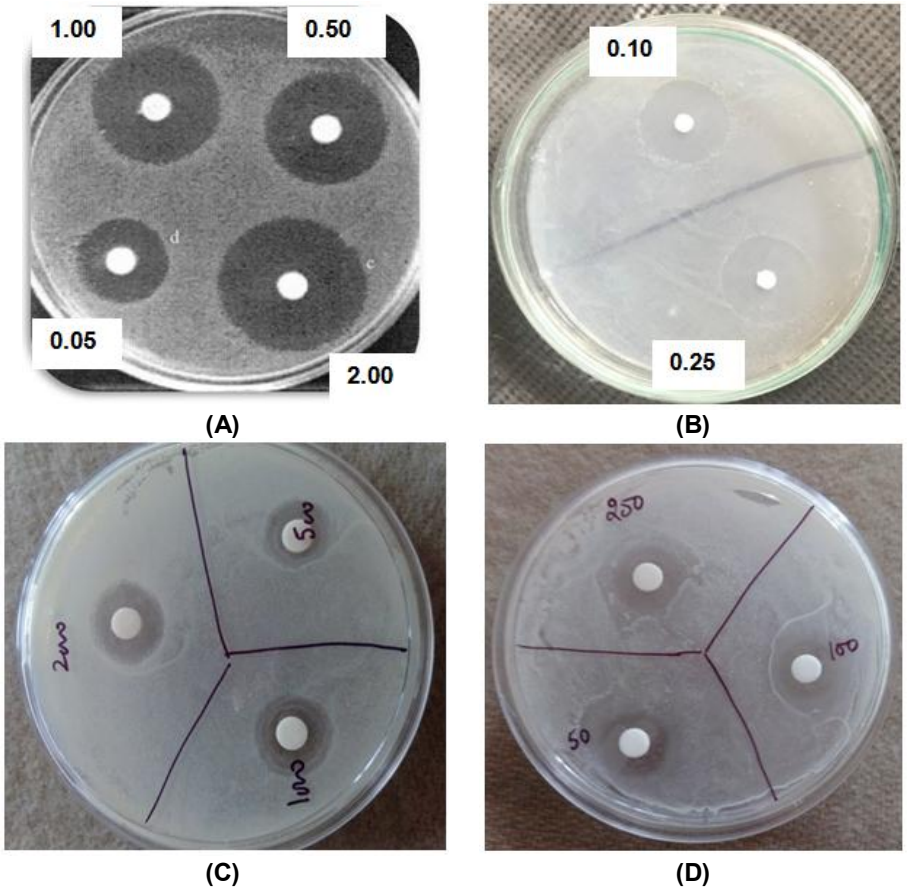


Plate 2. Zones of inhibition formed by Ampicillin (A) and (B) at 2.00, 1.00, 0.50, 0.25, 0.10 and 0.05 mg/mL and Compound 3 (C) and (D) at 2.00, 1.00 and 0.50, 0.25, 0.10 and 0.05 mg/mL against *Klebsiella* sp

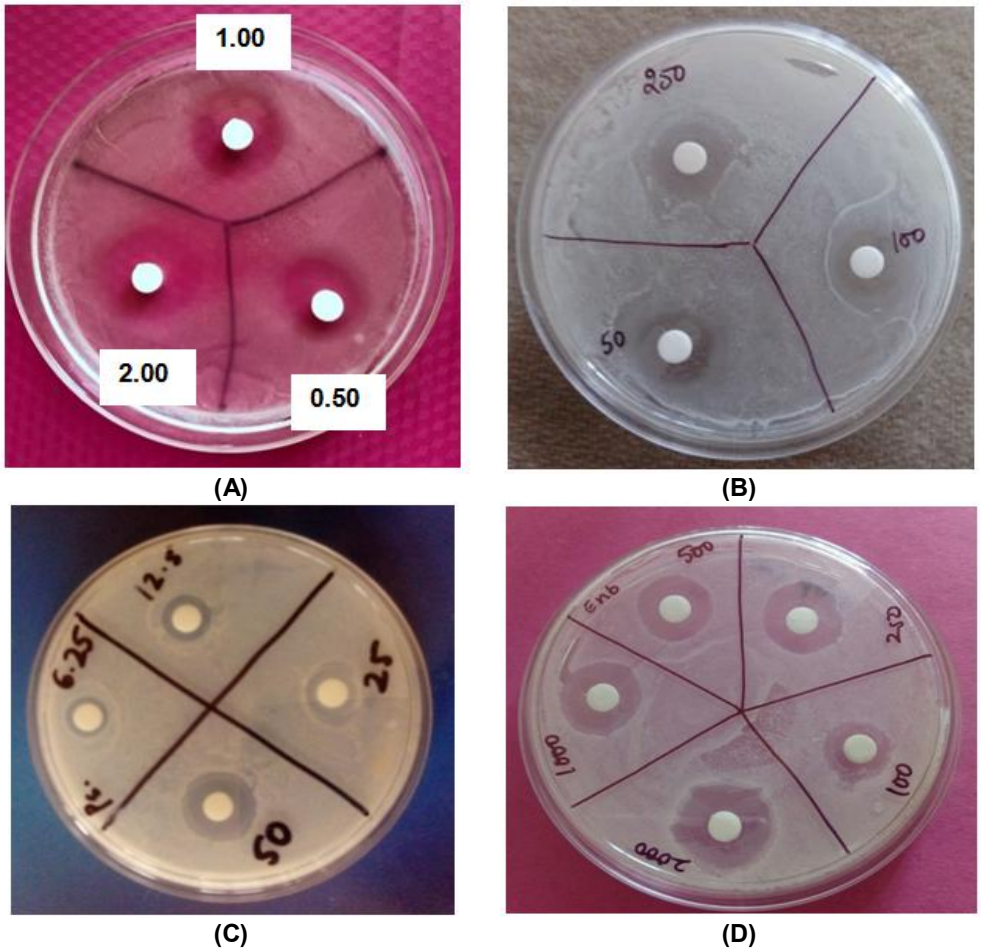


Plate 3. Zones of inhibition formed by Ampicillin (A), (B) and (C) at 2.00, 1.00, 0.50, 0,25, 0.10, 0.00625, 0.0125, 0.025 and 0.050 mg/mL and Compound 7 (D) at 2.000, 1.000, 0.500, 0.250 and 0.100 mg/mL against *Enterobacter* sp

were found to be equally active at 0.25 mg mL^{-1} while at all other concentrations, compound **3** was more effective. Compounds **3** and **4** were found to be equally effective at 0.50 mg mL^{-1} while at other concentrations, compound **4** was comparatively more active. Compound **1** and **7** were the only compounds which were not active at 0.05 mg mL^{-1}. All the compounds differed significantly at various test concentrations.

MIC values of different Schiff bases against *Enterobacter* sp. were also shown in Table 8. Compound **2** having *para* nitro substitution which was most effective exhibited least MIC value followed by compound **4** having diethylamino substitution while compound **7** showed the highest MIC value. Also, compound **1** and **7** had the same MIC value due to their equal effectiveness against *Enterobacter* sp. In Plate 3, the zone of inhibition formed by ampicillin and compound **7** was compared at their respective concentrations.

4. CONCLUSION

The Schiff bases (**1-7**) synthesized using glacial acetic acid as a catalyst were obtained in better yield and with less reaction as compared to tamarind extract which is a natural acidic catalyst. Microwave-assisted synthesis proved best in contrast to conventional method concerning (a) better yield, (b) less reaction time, (c) fewer side reactions resulting in the synthesis of pure product, and many more. Antimicrobial activity of all the compounds revealed that Compound **5** containing 2,5-dimethoxy substitution exhibited more activity against *Pseudomonas aeruginosa*. Compound **4** having

para N, N-diethyl substitution showed remarkable activity than others against *Klebsiella* sp. while compound **2** containing *para* nitro substitution increased the antibacterial activity as compared to others against *Enterobacter* sp and none of the compounds was found to be as effective as standard Ampicillin.

DISCLAIMER

All the synthesized compounds were new and not reported in literature. The compounds were tested for their utility as antimicrobial agents. The funding was done by parent university (Punjab Agricultural University).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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