

Synthesis and evaluation of vanillin derivatives as antimicrobial agents

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Abstract

The present work involves the synthesis of novel acetyl vanillin derivatives and evaluation for their anti-microbial potential against *Escherichia coli*. The titled compounds were prepared by reacting acetyl nitro vanillin with different substituted amines (Schiff base). The starting material acetyl nitro vanillin was synthesized by reacting acetyl vanillin with fuming nitric acid which in turn was prepared from 4-hydroxy-3-methoxybenzaldehyde (vanillin). The chemical structures of the synthesized compounds were confirmed on the basis of their spectral data (FT-IR, UV/Visible, ¹H-NMR, Mass spectra). All the synthesized compounds were tested *in vitro* for antimicrobial activity and compound **F** & **I** showed significant activity as compared to the standard drug.

Keywords: Vanillin, Antimicrobial activity, Schiff bases, Ciprofloxacin

Introduction

Antimicrobial agents like penicillins, cephalosporins, tetracyclines, nitrobenzene derivatives, macrolide antibiotics (erythromycin, roxithromycine etc.), polypeptide

antibiotics (polymixin –B, Bacitracin etc.), nicotinic acid derivatives derived from the various sources are currently available in the market. But microbial resistance in most of the pathogenic bacteria to these drugs repeatedly appears. In order to check this consequential medical problem, the extension of newer varieties of antimicrobial agents is a prominent task [1]. Vanillin (4-hydroxy-3-methoxybenzaldehyde), a dietary flavoring agent, has been reported to show antioxidant and anti-mutagenic activities, and has also been proved to be an anti-carcinogen against a variety of chemical and physical agents [2].

Vanillin is considered to be one of the most widely appreciated flavoring compounds. In spite of its flavor qualities, vanillin exhibits the antimicrobial potential and has been used as a natural food preservative [4]. Many natural compounds, including phenolic compounds derived from plants, exhibit strong antimicrobial properties and therefore have the potential to be applied to food products as novel preservatives [5]. It is investigated that vanillin primarily affects the cytoplasmic membrane of the food-related bacteria *E. coli*, *L. plantarum* and *L. innocua*. The antimicrobial activity of vanillin and related derivatives was found to be dependent on the time of exposure, concentration and the target organism. The inhibitory action of vanillin is more effective against non-lactic Gram-positive than Gram-negative bacteria [6]. The MIC of vanillin is bacteriostatic in contrast to the more potent phenolic antimicrobials such as carvacrol and thymol, which are bactericidal [7, 8].

The mode of action of nearly all antimicrobials can be classed into one or more of the following groups: (a) reaction with the cell membrane, (b) inactivation of essential enzymes, or (c) destruction or inactivation of genetic material [9]. Phenolic compounds are hydrophobic in nature and are therefore regarded as membrane active [10].

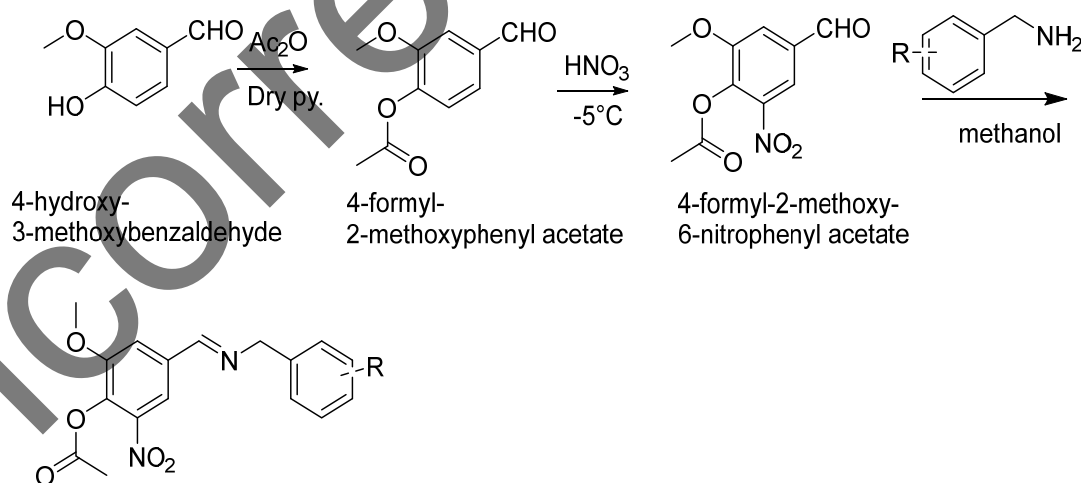
On the basis of these observations we tried our efforts to synthesize some nitro vanillin derivatives, starting with vanillin, which possess minimum microbial resistance and better activity. We now report the synthesis of these new compounds and resulting structure activity relationships for zone inhibition.

MATERIALS AND METHOD

All reagents and chemicals were used as obtained from the commercial supplier without further purification. All moisture sensitive reactions were carried out under dry nitrogen atmosphere. Column chromatography was carried out using silica gel 60-120 mesh size and distilled solvents. Thin layer chromatography (TLC) analyses were performed on precoated silica gel plates (GF254, Merck). Infrared (IR) spectra were recorded on BRUKER ALPHA using ATR technique. Nuclear magnetic resonance ($^1\text{H-NMR}$) spectra were recorded on a BRUKER AVANCE II 400 MHz spectrometer in deuterated solvents. The chemical shifts are reported in δ (ppm) relative to internal standard tetramethylsilane (TMS) and coupling constants J are given in Hertz. Mass spectrometry was conducted using Agilent 1100 LC has been coupled with Bruker mass spectrometer model Esquire 3000.

RESULTS AND DISCUSSION

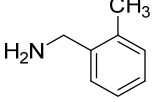
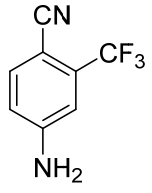
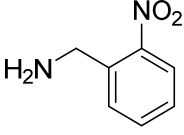
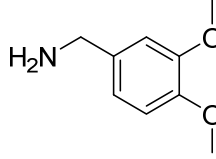
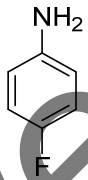
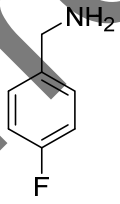
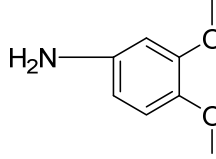
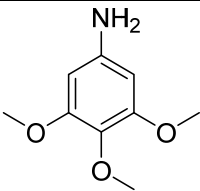
The designed compounds on the basis of various literature reports were synthesized according to the following synthetic scheme- 1. The starting material vanillin was used to afford the targeted compounds A-J, with a series of reaction. The completion of reaction was monitored with the help of TLC at each step. The compounds were characterized for physical parameters and tabulated in table 1.

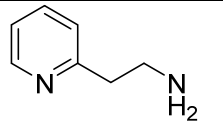
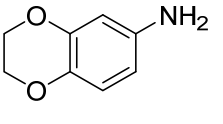


Scheme-1

The chemical structures of the synthesized compounds were confirmed using various spectral techniques such as IR, NMR, Mass etc. are mentioned in the experimental part.

Table 1: Physical constant of Schiff bases

S. No.	Code	R=Amine	M.F.	M. Wt. g/mol	M.P.	Yield
1	A		$C_{18}H_{18}N_2O_5$	342.35	Oily mass	60 %
2	B		$C_{18}H_{12}F_3N_3O_5$	407.31	Oily mass	40 %
3	C		$C_{17}H_{15}N_3O_7$	373.09	Oily mass	35 %
4	D		$C_{19}H_{20}N_2O_7$	388.38	Oily mass	52 %
5	E		$C_{16}H_{13}FN_2O_5$	332.29	Oily mass	47 %
6	F		$C_{17}H_{15}FN_2O_5$	346.31	Oily mass	34 %
7	G		$C_{18}H_{18}N_2O_7$	374.35	Oily mass	58 %
8	H		$C_{19}H_{20}N_2O_8$	404.38	Oily mass	53 %

9	I		$C_{17}H_{17}N_3O_5$	343.34	Oily mass	37 %
10	J		$C_{18}H_{16}N_2O_7$	372.33	Oily mass	46 %

The acetylation of vanillin was confirmed with presence of carbonyl peak in IR at $\sim 1695\text{ cm}^{-1}$ and resonance of methyl protons at $\sim 2.29\text{ ppm}$ in proton-NMR. Further nitration of compounds was confirmed by appearance of IR peak at $\sim 1540\text{ cm}^{-1}$ in various derivatives (A-J). Substitution of various amines was confirmed by the presence of IR peak at $\sim 1370\text{ cm}^{-1}$ whereas proton resonance at $\sim 8.5\text{ ppm}$ in all the synthesized compounds.

Although the compounds showed significant activity as compared to the standard but compounds F (fluorine substituted) and I (pyridine substituted) showed similar inhibition ($25\text{ }\mu\text{g/ml}$) as compared to ciprofloxacin ($10\text{ }\mu\text{g/ml}$). This study revealed that there must be proper electron withdrawing substituents at *para* position of the substituted amines to have potent activity.

Experimental work

General method for preparation of acetyl vanillin

Vanillin (3.2 mmol) was dissolved in dichloromethane ($5\text{-}6\text{ ml}$) and acetic anhydride (3.84 mmol) and dry pyridine (3.84 mmol) were added to this solution under anhydrous conditions. This mixture was stirred for $3\text{-}4\text{ hrs}$ at room temperature. Completion of reaction is detected by TLC in $1:1$ Hexane: Ethyl acetate. The solvent DCM was evaporated and the mixture was poured on crushed ice. Precipitates obtained were filtered and rinsed with water, dried the precipitate and re-crystallized from 95% ethanol.

General method for preparation of acetyl nitro vanillin

Acetyl vanillin (2.59 mmol) was dissolved in 5 ml of dichloromethane (DCM). This mixture was kept in -5 to $-10\text{ }^\circ\text{C}$ ice bath and stirred. When clear solution is

obtained, 2 ml of fuming nitric acid was added to this reaction mixture then stirred for 1-2 hr at room temperature. For work up add ice cold water was added, the precipitate was obtained, this obtained precipitate filtered washed and dried. TLC taken in 1:1 Hexane: Ethyl acetate.

General method for preparation of Schiff bases

Equimolar mixture of acetyl nitro vanillin (0.418 mmol) was dissolved in 25 ml of ethanol and then various amines (**a-j**) (0.428 mmol) were added to the mixture and stirred for 2-3 hrs. at ambient temperature. The completion of reaction was detected by the TLC in 1:1 Hexane: Ethyl Acetate. Solvent evaporated and oily compounds were collected.

2-Methoxy-4-(((2-methylbenzyl)-imino)-methyl)-6-nitrophenyl acetate (A)

¹H-NMR: δ 2.29 (s, 3H, CH₃), 2.38 (s, 3H, CH₃), 3.83 (s, 3H, O-CH₃), 4.87 (s, 2H, CH₂-N), 7.16- 7.10 (tt, $J=$ 5.2, 5.0, 5.0, 5.2 3H, CH₂-Ar), 7.34 (d, $J=$ 7.2, 1H, CH-Ar), 8.05 (s, 2H, CH-Ar) and 8.65 (s, 1H, CH=N-) ppm.

ESI-MS (m/z): 342

IR: 1467, 1643, 1695, 1019, 1538, 1371, 1597.

4-(((4-Cyano-3-(trifluoromethyl)-phenyl)-imino)-methyl)-2-methoxy-6-nitrophenyl acetate (B)

¹H-NMR: δ 2.18 (s, 3H, CH₃), 3.73 (s, 3H, O-CH₃), 6.27 (s, 2H, CH-Ar), 7.19 (s, 1H, CH-Ar), 7.48 (s, 1H, CH-Ar), 8.08 (s, 1H, CH-Ar) and 8.11 (s, 1H, CH=N-) ppm.

ESI-MS (m/z): 407

IR: 2218, 1643, 1696, 1021, 1541, 1359, 1609, 1171.

2-Methoxy-6-nitro-4-(((2-nitrobenzyl)-imino)-methyl)-phenyl acetate (C)

¹H-NMR: δ 2.23 (s, 3H, CH₃), 3.61 (s, 3H, O-CH₃), 4.80 (s, 2H, CH₂-N), 7.16- 7.10 (tt, $J=$ 5.2, 5.0, 5.0, 5.2 3H, CH₂-Ar), 7.31 (d, $J=$ 7.2, 1H, CH-Ar), 8.15 (s, 2H, CH-Ar) and 8.23 (s, 1H, CH=N-) ppm.

ESI-MS (m/z): 373

IR: 1467,1592,1651,1025,1524,1343.

4-(((3,4-Dimethoxybenzyl)-imino)-methyl)-2-methoxy-6-nitrophenyl acetate (D)

¹H-NMR: δ 2.28 (s, 3H, CH₃), 3.83 (s, 9H, O-CH₃), 4.87 (s, 2H, CH₂-N), 6.68(d, $J= 7.2$, 1H, CH-Ar), 6.76 (d, $J= 8.8$, 1H, CH-Ar), 6.90(s, 1H, CH-Ar), 8.05 (s, 2H, CH-Ar), 8.74 (s, 1H, CH=N-).

ESI-MS (m/z): 388.

IR: 1467, 1591, 1654, 1022, 1541, 1336.

4-(((4-Fluorophenyl)-imino)-methyl)-2-methoxy-6-nitrophenyl acetate (E)

¹H-NMR: δ 2.28 (s, 3H, CH₃), 3.83 (s, 3H, O-CH₃), 7.24(d, $J= 7.6$, 2H, CH₂-Ar), 7.32 (d, $J= 6.2$, 2H, CH-Ar), 8.05 (s, 2H, CH-Ar), 8.66 (s, 1H, CH=N-).

ESI-MS (m/z): 332.

IR: 1593, 1658, 1006, 1545, 1384, 1609, 1154.

4-(((4-Fluorobenzyl)-imino)-methyl)-2-methoxy-6-nitrophenyl acetate (F)

¹H NMR: δ 2.28 (s, 3H, CH₃), 3.83 (s, 3H, O-CH₃), 4.87 (s, 2H, CH₂-N), 7.17(d, $J= 7$, 2H, CH₂-Ar), 7.22 (d, $J= 6$, 2H, CH-Ar), 8.05 (s, 2H, CH-Ar), 8.65 (s, 1H, CH=N).

ESI-MS (m/z): 346

IR: 1507, 1652, 1090, 1062, 1546, 1385, 1438, 1120.

4-(((3,4-Dimethoxyphenyl)-imino)-methyl)-2-methoxy-6-nitrophenyl acetate (G)

¹H NMR: δ 2.28 (s, 3H, CH₃), 3.83 (s, 9H, O-CH₃), 6.78-6.76(d,s, $J= 4.8$, 2H, CH₂-Ar), 7.88 (d, $J= 10.0$, 1H, CH-Ar), 8.05 (s, 2H, CH-Ar), 8.66 (s, 1H, CH=N).

ESI-MS (m/z): 374

IR: 1467, 1655, 1593, 1024, 1545, 1384, 1609.

2-Methoxy-6-nitro-4-(((3,4,5-trimethoxyphenyl)-imino)-methyl)-phenyl acetate (H)

¹H NMR: δ 2.28 (s, 3H, CH₃), 3.83 (s, 12H, O-CH₃), 6.63 (s, 2H, CH-Ar), 8.05 (s, 2H, CH-Ar), 8.66 (s, 1H, CH=N-).

ESI-MS (m/z): 404

IR: 1650, 1721, 1005, 1545, 1385, 1596.

2-Methoxy-6-nitro-4-(((2-(pyridin-2-yl)-ethyl)-imino)-methyl)-phenyl acetate (I)

¹H NMR: δ 2.29 (s, 3H, CH₃), 3.26 (t, $J = 7.0$, 2H, N-CH₂CH₂), 3.83 (s, 3H, O-CH₃), 3.87 (t, $J = 7.1$, 2H, CH₂-N), 7.25-7.20 (m, $J = 4.7, 8.0, 1.4, 4.7$, 2H, CH-Ar), 7.58 (t, $J = 4.5, 6.1$, 1H, CH-Ar), 8.05 (s, 2H, CH-Ar), 8.44 (d, $J = 9.8$, 1H, CH-Ar), 8.65 (s, 1H, CH=N-).

ESI-MS (m/z): 343

IR: 1655, 1590, 1001, 1538, 1368, 1435.

4-(((2, 3-Dihydrobenzo-[b][1,4]-dioxin-6-yl)-imino)-methyl)-2-methoxy-6-nitro-phenyl acetate (J)

¹H NMR: δ 2.28 (s, 3H, CH₃), 3.83 (s, 3H, O-CH₃), 4.28 (s, 4H, CH₂-O), 6.78 (d, $J = 5.8$, 2H, CH-Ar), 6.89 (d, $J = 9.1$, 1H, CH-Ar), 8.05 (s, 2H, CH-Ar), 8.66 (s, 1H, CH=N-).

ESI-MS (m/z): 372

IR: 1656, 1597, 1017, 1535, 1426.

Antimicrobial activity

Bacterial cultures

Bacterial cultures were procured from microbial culture collection, National Centre for Cell Science, Pune, Maharashtra, India. The lyophilized cultures of bacterial strains upon culturing in nutrient broth for 24-48 hours at 37 °C in an incubator resulted into turbid suspension of activated live bacterial cell ready to be used for

microbiological study. The *E. coli* (Bact-1) used for antimicrobial studies was found to be active using well diffusion method (100 µl). From the broth of respective revived cultures of bacteria, loop full of an inoculum was taken and streaked on to the nutrient agar medium and incubated again at same culture conditions and duration that yielded the pure culture colonies on to the surface of the agar culture that are successfully stored in refrigerated conditions at 4 °C as stock culture to be used for further experimentation.

Anti-biogram studies

The antimicrobial activity of the synthesized compound against bacterial pathogens was used under present study. The synthesized compounds (A-J) were prepared in various concentrations (100, 50 and 25 µg/ml) and applied on to the test organism using well diffusion method. Results of the experiment are being concluded in the table no. 2, which clearly shows the anti-microbial activity of synthesized compound out of the bacterial strains used in the present work (figure 1).

Table no. 2: Antimicrobial activity of ciprofloxacin and synthesized compounds (A-J) on *E. coli*

S. No.	Micro-organism → Sample	<i>E coli</i>
		Zone of inhibition Mean (mm)
1	Ciprofloxacin (µg/ml)	
	30	20±0.15
	20	18±0.13
2	10	15±0.19
	Drug -A (µg/ml)	
	100	10± 0.04
3	50	8± 0.11
	25	6± 0.08
	Drug -B (µg/ml)	
4	100	6± 0.11
	50	6± 0.16
	25	6± 0.02
5	Drug -C (µg/ml)	
	100	6± 0.19
	50	6± 0.11
6	25	6± 0.05
	Drug -D (µg/ml)	
7	100	16± 0.11

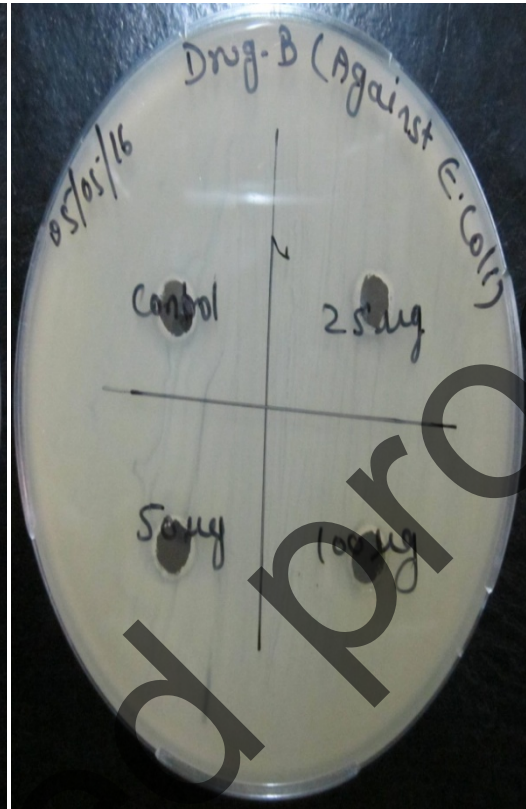
	50	10± 0.03
	25	08± 0.01
6	Drug -E (µg/ml)	
	100	20± 0.14
	50	15± 0.13
	25	7± 0.08
7	Drug -F (µg/ml)	
	100	15± 0.03
	50	13± 0.13
	25	11± 0.05
8	Drug -G (µg/ml)	
	100	15± 0.01
	50	8± 0.17
	25	6± 0.09
9	Drug -H (µg/ml)	
	100	10± 0.06
	50	8± 0.05
	25	6± 0.10
10	Drug -I (µg/ml)	
	100	15± 0.11
	50	13± 0.16
	25	10± 0.02
11	Drug -J (µg/ml)	
	100	14± 0.04
	50	11± 0.11
	25	6± 0.08

Figure 1: Antimicrobial activity of synthesized compounds (A-J) against *E. coli*





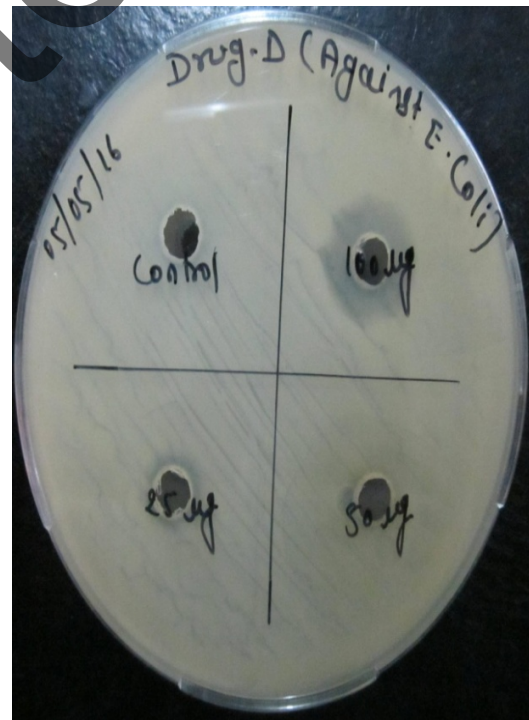
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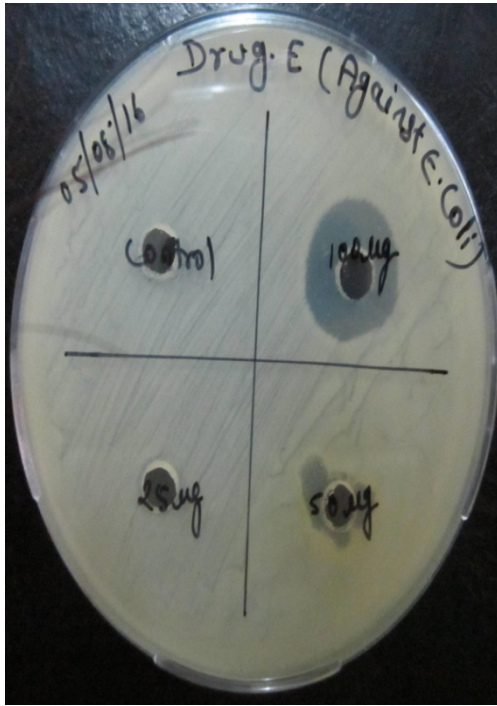
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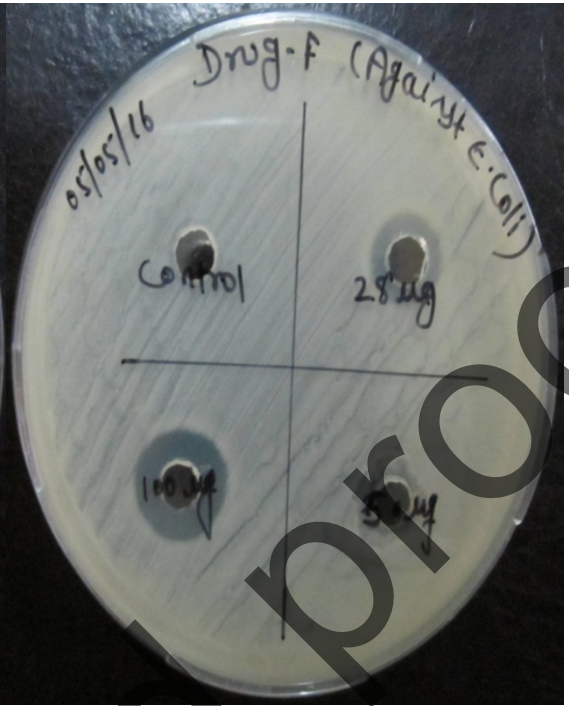
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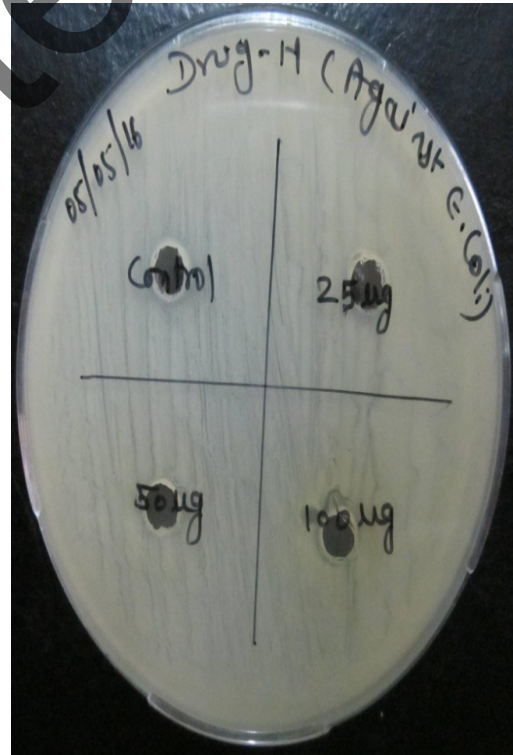
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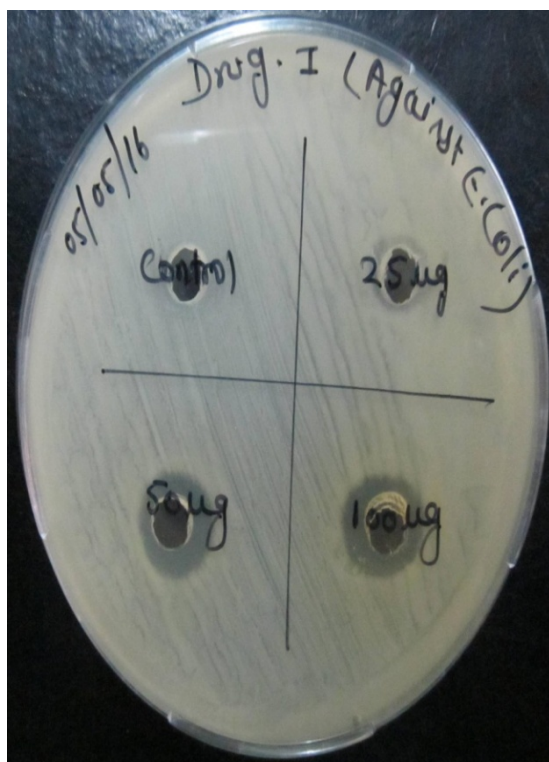
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CONCLUSIONS

In conclusion, we have reported a simple synthetic method of vanillin related derivatives. The reactions occurred very fast, in mild condition, using reasonable reagents and solvents with moderate yield. The antimicrobial activity of the synthesized compounds was effectively screened against *E. coli*. The compounds bearing electron withdrawing substituents (F & I) showed significant activity as compared to standard. The outcome of the work will be valuable for further studies in term of toxicity effect and quantity structure activity relationship (QSAR) to improve their biological and pharmacological properties.

ACKNOWLEDGEMENTS

Authors are thankful to the Vice-chancellor, Banasthali Vidyapith, Banasthali, India for providing constant support for research work. The financial assistance from DST-CURIE and MHRD, New Delhi is duly acknowledged.

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