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SYNTHESIS OF SUBSTITUTED BENZOIC ACID FROM DIBENZLIDENE ACETAMIDE AS A ANTIBACTERIAL AGENTS

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ABSTRACT

Development of newly synthesized Antibacterial agents. In the present work substituted benzoic acid hydrazides were condensed with substituted aromatic and hetroaromatic aldehydes to yield to be target products (7, 9, 12). The synthesized compounds were tested for *in vitro* Antibacterial activities against Gram Positive, Gram negative bacteria and Standard drugs (Fluconazaole). The Evaluation of antibacterial activity study in a zone of inhibition was determined by cup plate method. (Bacteria) for 24 hrs and at 25°C (fungi) for 3 days and the results were recorded Growth of microorganism.

KEYWORDS

Antibacterial agents, Chemical Synthesis and Zone of Inhibition.

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INTRODUCTION

The development of antimicrobial agents (antibacterial and antifungals) to treat infections has been one of the most notable medical achievements of the past century. The advances in medical care are threatened, however, by a natural phenomenon known as ‘‘antimicrobial resistance’’. The increased use of antibacterial and antifungal agents in recent years has resulted in the development of resistance to these drugs with important implications for morbidity, mortality and health care costs. In spite of a large number of antibiotics and chemotherapeutics available for medical use, the antimicrobial resistance created a substantial need of new class of antimicrobial agents.

Disease causing microbes that have become resistant to drug therapy are an increasing public health problem nowadays. There is a real need for discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms of actions, which are distinct from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant. Hydrazide analogues also possess other biological activities like anticonvulsant, antidepressant, anti-inflammatory, anti-malarial, anti-mycobacterial, anticancer and antimicrobial activities.

Principles of antibacterial agents

Proper selection of an antibacterial agent is based on a number of factors, including the identity of the pathogen, the site of infection, the pharmaco kinetics (PK) and pharmaco dynamics (PD) of the agent, potential toxicity to the patient, possible drug interactions, cost, and convenience of administration. The initial choice of an antimicrobial agent may be modified during the course of treatment as the patient's clinical status evolves (eg, response to therapy, function of major organ systems, and so forth) and as more information about the nature of the infection comes to light. For example, when a patient presents severely ill, with signs and symptoms suggesting over whelming bacterial infection, one must choose antimicrobial agents empirically. In such emergent circumstances, an initial regimen is selected based on the best information about the nature of the infection that can be gleaned from the available history, physical examination, and preliminary laboratory studies. Possible pathogens are identified based on these findings, and drugs are targeted against the likely culprits, based on known patterns of antimicrobial activity. Estimates of antimicrobial susceptibility of likely pathogens must take into account various factors that might predict resistance, such as the setting in which the infection was acquired (community, hospital, or nursing home); the previous use of antibiotics in the patient; and the potential of likely pathogens to produce extended-spectrum b-lactamases in the presence of b-lactam antibiotics.

Principles of PK and PD must also be considered in designing empiric regimens and in modifying those regimens in accordance with changes in the clinical condition of the patient. Patients who are severely ill almost always are treated with intravenous agents to bypass potentially slow and erratic oral absorption so that therapeutic levels are reached as soon as possible. Loading doses often are given under these circumstances to achieve a steady state more rapidly.

Combinations of antimicrobial agents may be chosen not only for breadth of spectrum, but for favourable PD effects, such as synergistic killing, in which two agents demonstrate greater than additive activity. Toxicity is important to consider in formulating an empiric regimen. This is especially true in severely ill patients in whom organ function may be tenuous already. Therapy may be modified as the clinical course evolves. When the identity of the etiologic organism has been confirmed by culture, antibacterial therapy can be refined based on precise measures of susceptibility of the strains to antimicrobial agents. Depending on the patient's clinical progress, changes in dosage, route of administration, and even class of agent may be necessary or desirable. In general, an empiric regimen ultimately should be refined to the narrowest antimicrobial spectrum, least toxicity, least invasive route of administration, and lowest cost that is effective. This article discusses the principles outlined previously, particularly those of PK, toxicity, monitoring, and related reasons for treatment failure, and briefly addresses the topics of susceptibility testing and PD. Susceptibility testing and PD are discussed in detail elsewhere in this issue.

AIM AND OBJECTIVES

Aim

Synthesis of Dibenzylideneacetamide From substituted Benzoic acid as Antibacterial agents.

Objectives

The objectives of this dissertation work are as follows:

- Develop simple reaction scheme for synthesis of Dibenzylideneacetamide.

- To elucidate structure of synthesized compounds by NMR, IR.
- Screen synthesized compounds for antibacterial activity by cup plate method.
- Characterization of synthesized compounds by different Physicochemical Properties and Analytical Techniques.
- To evaluate the targeted compounds for antibacterial activity.

MATERIAL AND METHODS[1-13]

REACTION SCHEME

Reaction procedure

Thionyl chloride 5ml was added to substituted benzoic acid 2.5gm in a round bottom flask. After addition, the mixture was refluxed for 2 hrs. The excess of thionyl chloride was removed by distillation. To the solution of substituted acid chloride 2.5ml of hydrazine hydrate. The mixture was refluxed for 30 min. Then the reaction mixture was cooled and the resultant precipitate (substituted benzoic acid hydrazide) A solution of 0.05 ml of substituted aldehyde in 15ml ethanol. The mixture was refluxed on water bath for 3 h. Then the reaction mixture was allowed to cool at room temperature and the precipitate obtained was filtered, dried and recrystallized from ethanol.

Starting materials were obtained from commercial sources for substituted Benzoic acid and aldehyde as further purification. Solvents were dried by standard procedures. Reaction progress was observed by thin layer chromatography making use of commercial silica gel plates. Melting points were determined in open capillary tubes on a sonar melting point apparatus and are uncorrected. 1H NMR (Nuclear magnetic resonance spectra in appropriate deuterated solvents and are expressed in parts per million. Infrared (IR) spectra were recorded on FTIR spectrometer.

INFRARED SPECTRA

EVALUATION ANTIBACTERIAL ACTIVITY

The antibacterial activity was performed against Gram-positive bacteria *Staphylococcus aureus*, Gram-negative bacteria *E. coli* and a agar media. The zone of inhibition was determined by cup plate method. (Bacteria) for 24 hrs and at 250 C (fungi) for 3 days and the results were recorded Growth of microorganism.

Preparation of sample

To weight accordely powder of 0.02gm of each powdered material was soaked in 15ml of ethanol for 24hrs with shaking. The resultant extracts were centrifuged at 3000 rpm for 5 min. at 4^0C. The supernatant was filtered through filter paper the residues were used for a second extraction with 10ml of ethanol. After the second extraction, the filtrates were conc. The supernatant may be used for zone of inhibition.

Preparation of suspension of microorganism

The suspension of microorganism was made by transferring the organism from culture to 5ml of 0.9 % saline solution.

Procedure of assay

1. Using pour plate technique suspension of each microorganism (1ml) was added to test tube containing sterile nutrient agar at 45^0C.
2. Medium was poured in sterilized petriplates in aseptic condition and kept aside for solidification.
3. After solidification, cups were prepared aseptically (three cups in each plate by using cork borer.
4. For each organism, 6 plates were prepared and in each plate three cups were made. In one cup, 0.1ml of standard (dibenzylidene acetamide) was placed and in six different cups, solution of the eight different test compounds (0.1ml = 100ug) were placed.
5. The plates were kept in refrigerator for 30 min for diffusion of solution and then incubated at 37^0C for 24 hrs.
6. The results was recorded by measuring the zone of inhibition (in mm) including the cup
7. Diameter, 7mm. This was subtracted from each reading to obtain the actual zone of inhibition.

RESULTS AND DISCUSSION

The reaction between substituted benzoic acid and thionyl chloride yielded corresponding acid

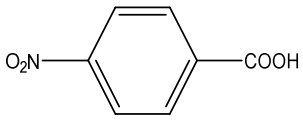
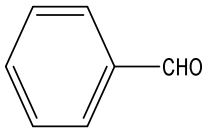
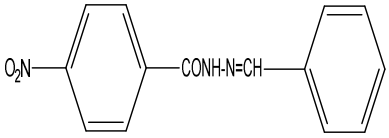
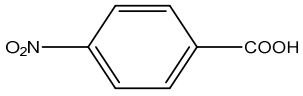
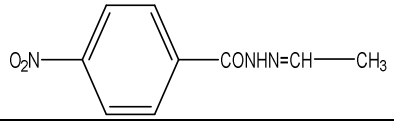
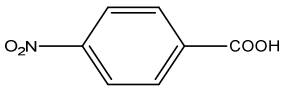
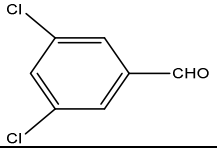
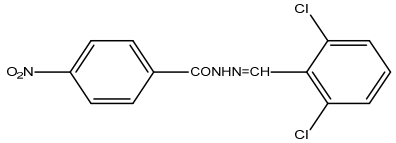
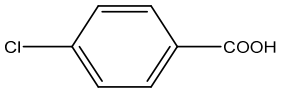
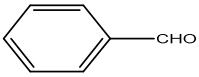
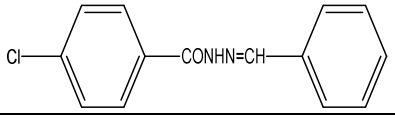
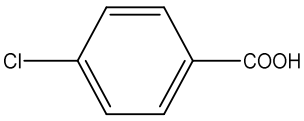
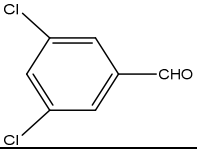
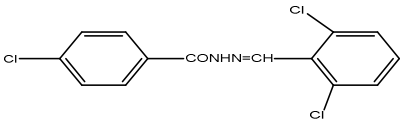
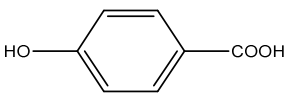
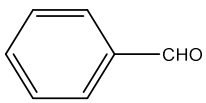
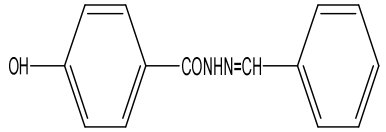
chlorides, which on reaction with hydrazine afforded the corresponding hydrazides in appreciable yield (Figure No.1). Further the hydrazides were condensed with substituted aldehydes to yield the substituted benzoic acid dibenzylydene hydrazides scheme (1) (Table No.1).

The structures of compounds 1-08 were assigned by IR and 1H-NMR spectroscopic data, (Table No.2-11) which are consistent with the proposed molecule a structures. Eight compounds were screened in vitro for their antibacterial activities against Gram positive bacteria - Staphylococcus aureus, Gram negative bacterium - Escherichia coli and a agar media and their antibacterial activity was compared with fluconazole as control drugs for activity respectively. The results of antibacterial evaluation are presented in Table No.12. The compounds showed better antibacterial activity Table No.13. The deduced patterns of antimicrobial activity of substituted hydrazides.

B.Subtilis ≤ E.coli ≤ S. aureus ≤ A. niger

The compounds 3 and 18, 10 are the most effective compounds against S. aureus with zone of inhibition are respectively. Against the gram negative Bactria is *E. coli* the 11, and 7 showed the better activity in comparison to other compounds synthesized. Emerged as most active antibacterial substituted hydazides. The compounds exhibited very weak activity against *A. niger* with a maximum of ZOFI respectively.

Table No.1: Synthesis of Substituted benzoic acid dibenzylidene acetamide

S.No	Comp.	"R"	"X"	
1	3			
2	4		CH_3CHO	
3	5			
4	7			
5	8			
6	9			

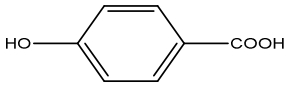
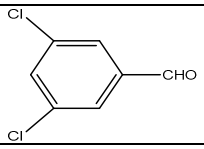
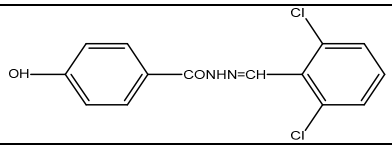
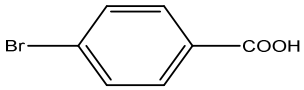
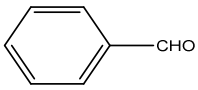
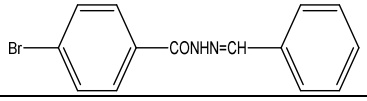
7	10			
8	12			

Table No.2: IR Interpretation of Compound 3

S.No	Observed Frequency (cm^{-1})	Standard Frequency (cm^{1})	Assignment
1	2978.09	3200-3600	C-H Stretching of Aromatic ring
2	933.55	800-950	NO_2Stretching, of Nitro
3	1303.88	1320-1210	C–O stretching
4	1589.34	1550-1650	N-H Stretching
5	2978.09	3300	CH-CH stretching in–ring

Table No.3: IR Interpretation of Compound 4

S.No	Observed Frequency (cm^{-1})	Standard Frequency (cm^{1})	Assignment
1	2978.09	3200-3600	C-H Stretching of Aromatic ring
2	933.55	800-950	NO_2Stretching, of Nitro
3	1303.88	1320-1210	C–O stretching
4	1589.34	1550-1650	N-H Stretching
5	2978.09	3300	CH-CH_3stretching in–ring

Table No.4: IR Interpretation of Compound 5

S.No	Observed Frequency (cm^{-1})	Standard Frequency (cm^{1})	Assignment
1	2978.09	3200-3600	C-H Stretching of Aromatic ring
2	933.55	800-950	NO_2Stretching, of Nitro
3	1303.88	1320-1210	C–O stretching
4	1589.34	1550-1650	N-H Stretching
5	2978.09	3300	CH-CHstretching in–ring
6	1681.93	1800	C-Cl stretching

Table No.5: IR Interpretation of Compound 7

S.No	Observed Frequency (cm^{-1})	Standard Frequency (cm^{1})	Assignment
1	2978.09	3200-3600	C-H Stretching of Aromatic ring.
2	1303.88	1320-1210	C–O stretching
3	1589.34	1550-1650	N-H Stretching
4	2978.09	3300	CH-CHstretching in–ring
5	1681.93	1800	C-Cl stretching

Table No.6: IR Interpretation of Compound 8

S.No	Observed Frequency (cm^{-1})	Standard Frequency (cm^{1})	Assignment
1	2978.09	3200-3600	C-H Stretching of Aromatic ring
2	1303.88	1320-1210	C–O stretching
3	1589.34	1550-1650	N-H Stretching
4	2978.09	3300	CH-CHstretching in–ring
5	1681.93	1800	C-Cl stretching

Table No.7: IR Interpretation of Compound 9

S.No	Observed Frequency (cm^{-1})	Standard Frequency (cm^{1})	Assignment
1	2978.09	3200-3600	C-H Stretching of Aromatic ring.
2	1303.88	1320-1210	C–O stretching
3	1589.34	1550-1650	N-H Stretching
4	2978.09	3300	CH-CHstretching in–ring
5	3703.33	3200-3700	C-OH stretching

Table No.8: IR Interpretation of Compound 10

S.No	Observed Frequency (cm^{-1})	Standard Frequency (cm^{1})	Assignment
1	2978.09	3200-3600	C-H Stretching of Aromatic ring.
2	1303.88	1320-1210	C–O stretching
3	1589.34	1550-1650	N-H Stretching
4	2978.09	3300	CH-CHstretching in–ring
5	3286.70	3200-3700	C-OH stretching
6	1681.93	1800	C-Cl stretching

Table No.9: B. NMR Spectra Compound 12

S.No	Observed Frequency (cm^{-1})	Standard Frequency (cm^{1})	Assignment
1	2978.09	3200-3600	C-H Stretching of Aromatic ring.
2	1303.88	1320-1210	C–O stretching
3	1589.34	1550-1650	N-H Stretching
4	2978.09	3300	CH-CHstretching in–ring
5	1681.93	1800+15	C-Br stretching

Table No.10: NMR Interpretation of Compound 3

S.No	δ Value (PPM)	Splitting Pattern	Assignment of Hydrogen
1	8.31	Doublet	CH (Aromatic)
2	8.11	Doublet	CH(Aromatic)
3	7.55-7.76	Multiplate	CH(Aromatic)
4	11.76	Singlet	NH
5	8.55	Singlet	CH

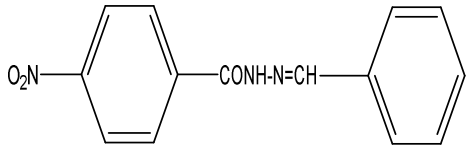
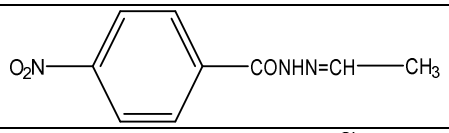
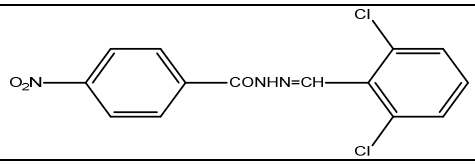
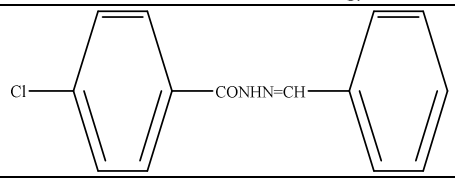
Table No.11: IR Interpretation of Compound 9

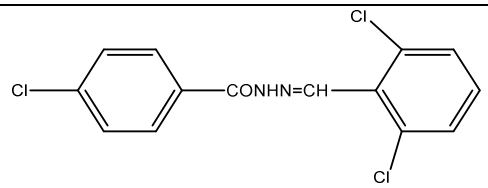
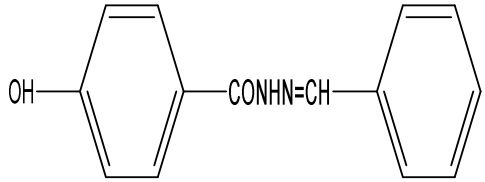
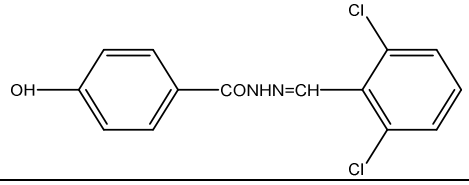
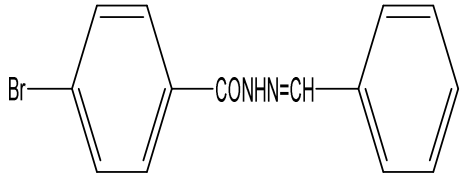
S.No	δ Value (PPM)	Splitting Pattern	Assignment of Hydrogen
1	8.37	Singlet	CH
2	8.11	Doublet	CH(Aromatic)
3	6.88, 7.76, 7.58 and 7.93	Multiplate	CH(Aromatic)
4	11.67	Singlet	NH
5	8.55	Singlet	CH

Table No.12: Antibacterial activities of substituted benzoic acid dibenzylidene acetamide

S.No	No. of Compounds	Zone of Inhibition in mm	
		(Gram Negative *E Coil*)	**(Gram Positive *Staphylococcus aureus*)**
1	3	6mm	6mm
2	4	11mm	-
3	5	-	-
4	7	7mm	-
5	8	-	6
6	9	-	18
7	10	-	9
8	12	-	8
9	Std. Fluconazole	12mm	15mm

Table No.13: Physicochemical characteristics of substituted benzoic acid dibenzylidene acetamide

S.No	Compound code and Structure	Molecular formula	Molecular weight (gm)	Yield (%)	Melting point (°C)	Rf (A)
1		$C_{14}H_{12}N_1O_3$	242.08	72.00	230	0.45
2		$C_9H_9N_3O_4$	223.00	78.48	235	0.65
3		$C_8H_{10}O_2N_2$	160	51.00%	154-156	0.62
4		$C_{15}H_{12}O_2N_2$	235.30	66.36%	135-136	0.58

5		$C_{15}H_{11}N_2O_1Cl_3$	325.02	48.08	238	0.55
6		$C_{14}H_{13}N_2O_1$	225.08	66.31	208	0.66
7		$C_{14}H_{11}N2O_2Cl_2$	279.64	49.14	210	0.58
8		$C_{14}H_{10}N_2O_1$	218.05	66.00	220	0.45

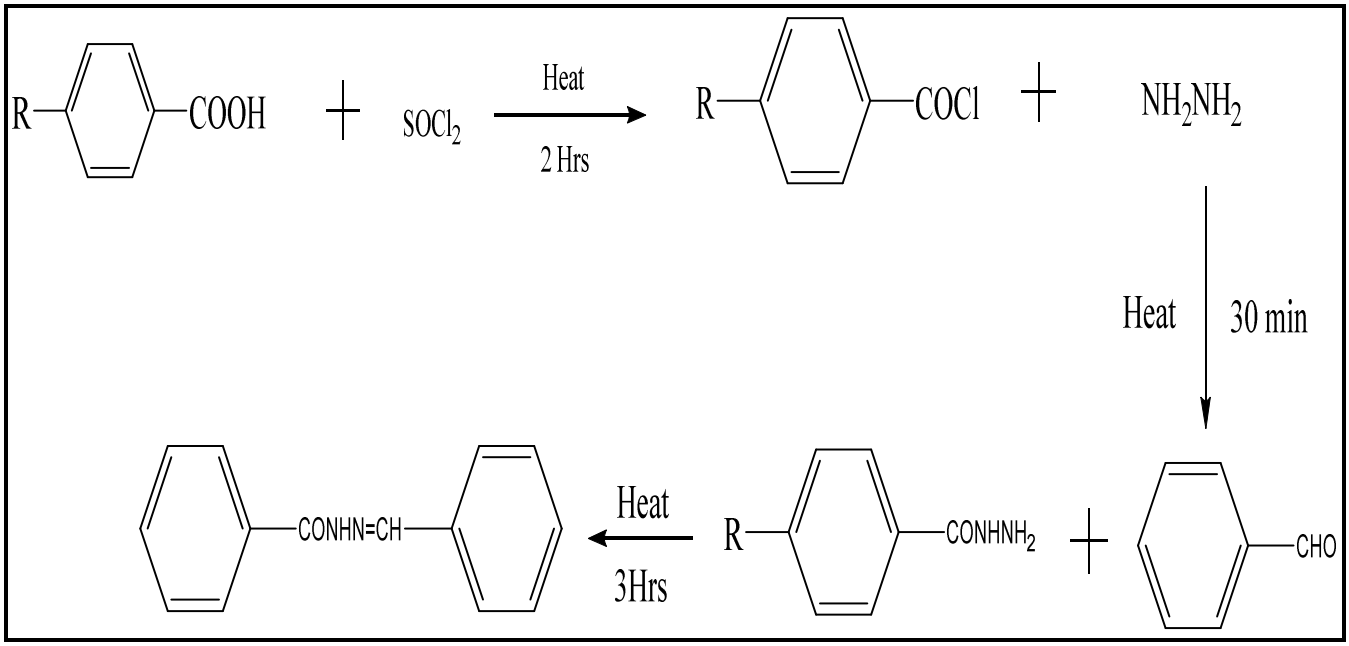


Figure No.1: General reaction

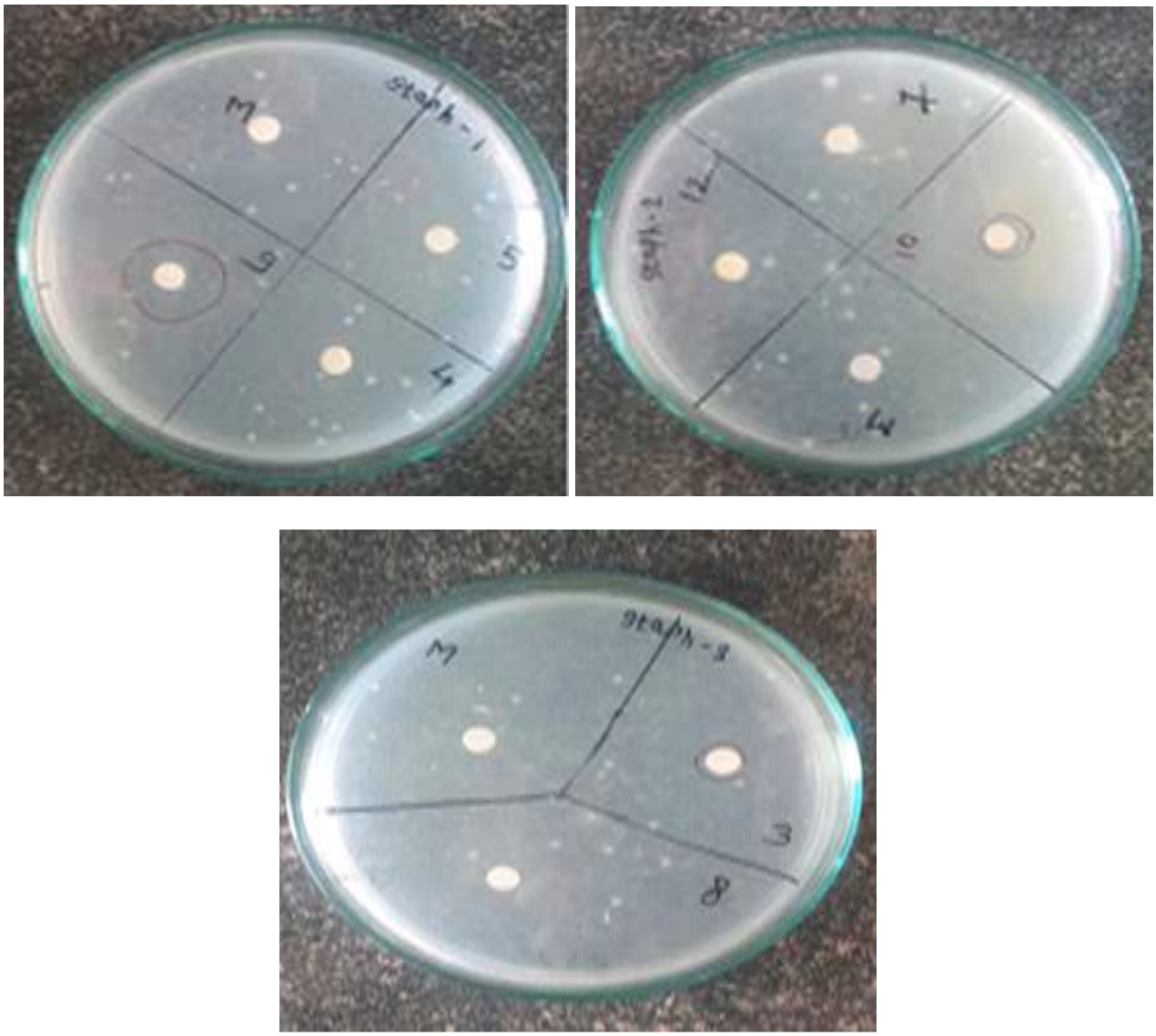


Figure No.2: Antibacterial activities of substituted benzoic acid dibenzylidene acetamide from *Staph. Aureus*

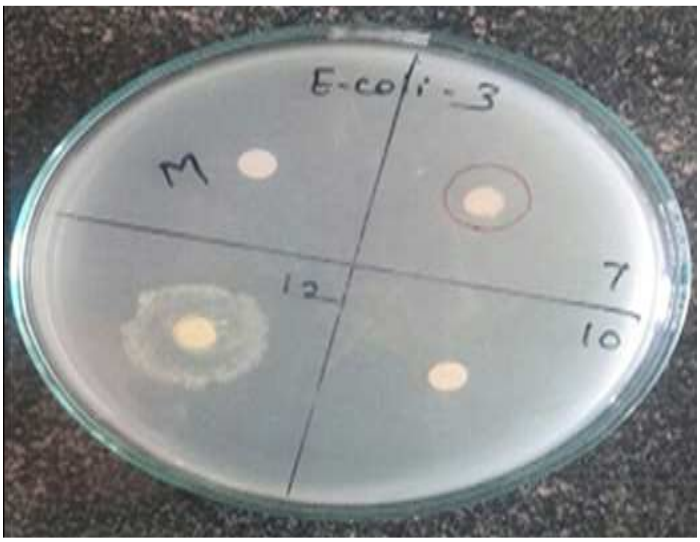


Figure No.3: Antibacterial activities of substituted benzoic acid dibenzylidene acetamide from *E. coli.*

CONCLUSION

In conclusion, a series of substituted hydrazide derivatives have been synthesized and their *in vitro* antibacterial activity was evaluated against four representative microorganisms. The results of antibacterial study indicated that the presence of in aromatic ring improved antibacterial activity, whereas the presence of nitro group improved antifungal activity of substituted hydrazides. To understand the relationship between physicochemical parameters and antimicrobial activity of substituted hydrazide derivatives in describing the antimicrobial activity of synthesized compounds.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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