SYNTHESIS OF BIOLOGICALLY ACTIVE 2-CHLORO-*N*-ALKYL/ARYL ACETAMIDE DERIVATIVES

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ABSTRACT

Medicinal chemistry plays an important role in development of drug for cure; maintain and improved health of human being. It is also equally important to design chemical entities for prevent the growth of micro-organism, which come in contact with human being in day-to-day life. We have synthesized 2-chloro-N-alkyl/aryl Acetamide derivatives with an aim as new bioactive agent, which can be used as anti microbial agents such as herbicides, antifungal, disinfectant. The present study involves the synthesis, purification and characterization of various N-substituted chloroacetamide derivatives. The chloroacetyl chloride treated with various aliphatic and aromatic amines at room temperature with stirring for few hours with monitoring reaction by thin layer chromatography gave 2-chloro-N-alkyl/aryl Acetamide as solid compounds. We checked the melting point of synthesized compounds with an open ended capillary tube method. The spectral techniques like Infra red and GC-MS have been used for characterization and establishment of structure of synthesized compounds. The antimicrobial screening of the synthesized chloroacetamides have shown excellent antibacterial and antifungal activity.

Key words: Acetamide, antibacterial, antifungal.

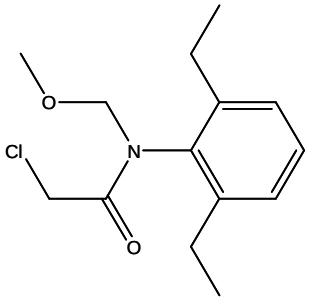
1. INTRODUCTION:

Halogenated acids and acid derivatives compounds that eliminate or inhibit the development of bacteria, fungi, parasites or viruses are called antimicrobials and respectively referred to as antibacterial, antifungal, antiprotozoal and antiviral agents **[2].** Numerous antimicrobial agents' activity they can be toxic to human beings. Antimicrobials have to be non-toxic, non-allergenic, effective and selective, chemically stable, active against possibly more than one bacterium and inexpensive **[3]**.

Antibacterial agents usually act by modifying the structure or the metabolic pathways of bacteria. Disinfectants kill bacteria but are unselective and can be toxic for mammals and are not used *in vivo*. **[4]**

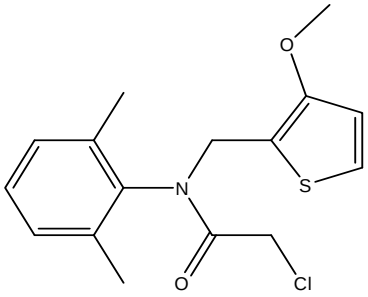
Arylacetamides acts as anti microbial agents such as herbicides, antifungal, disinfectant .Examples of 2-chloro acetamides which acts as herbicides such as 2-chloro-N-(2, 6-diethylphenyl)-N-(methoxymethyl) acetamide **(a)** and 2-chloro-N-(2, 6-dimethylphenyl)-N-[(3-methoxy-2-thienyl) methyl] acetamide **(b) (Figure 1)** are shown. **[7, 8]**

Four equivalents of chloroacetyl chloride was added drop wise over one hour to the aqueous amine solution. Then the solution was left to stir overnight. The desired reaction mixture was added to ice-cold water. Precipitate was filtered and recrystalised with ethanol**[1,5]**. 2-chloro-*N*- Alkyl or Aryl acetamide act as an intermediate which was synthesized by nucleophilic substitution reaction of chloroacetylchloride and different aqueous amines**.(Figure 2)**



2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide

(a)



2-chloro-N-(2,6-dimethylphenyl)-N-[(3-methoxy-2-thienyl)methyl]acetamide

(b)

Figure 1: Examples of 2-chloroacetamides acting as herbicides.

2. METHODS

2.1. Chemistry

All chemicals used were of Laboratory Reagent (LR) Grade. The synthesized derivatives were characterized by melting point, TLC, FT-IR, GC-MS and NMR. Thin Layer Chromatography was performed using Silica Gel G (Merck Index) coated on glass plates and the spots were visualized by exposure to iodine. Melting points were taken in open glass capillary tubes in liquid paraffin bath and were uncorrected. IR spectra were recorded on FTIR-8400S SHIMADZU spectrophotometer. GC-MS spectra & chromatogram were recorded on GCMS-QP 2010 SHIMADZU instrument.

2.1.1. Synthesis of 2-chloro-N-alkyl/aryl acetamide (3)

Four equivalents of chloroacetyl chloride (1) was added drop wise over one hour to the aqueous amine (2) solution. Then the solution was left to stir overnight. The desired product was isolated as precipitate after pouring reaction mixture to an ice-cold water. Precipitate was filtered, washed with cold water and dried. Recrystalised using 95% ethanol (Figure 1).

2.1.2. Chloroacetamide (3a)

In 100 ml RBF, a solution of aqueous amine(0.02 mol) that is ammonia in 4ml water was stirred on magnetic stirrer for 10 min. Four equivalent of Ethyl chloroacetate(0.02 mol) was added drop wise for 1 hr. Reaction mixture was kept for stirring for 24 writhe reaction mixture was cooled, poured into ice-cold water (50 ml) containing a drop of pyridine and stirred until the oil solidifies. Crude product was filtered, washed with cold water and dried. The product was recrystallised from ethanol.

Yield 83.21%, mp 118-120^{0}C, and IR 3381.33cm^{-1}(NH-stretch) , 1627.97 (C=O stretch:1° amide), 1415.80 (NH-bend:1° amide), 1286.56 (C-N stretch), 775.41 (C-Cl stretch) ,Mass spectrum [M$^+$], m/z 93 (100%) [M+1] 93,[M+2] 78,[M+3]44.

2.1.3. Phenyl Chloroacetamide (3b)

It was obtained from (1) with aniline in crystalline form. Yield 82.77%, mp 140-142^{0}C, and IR 3277.17cm^{-1}(NH-stretch: 2° amide) , 1688.48 (C=O stretch:2° amide), 1533.46 (NH-bend:2° amide), 1273.06 (C-N stretch), 744.55 (C-Cl stretch) ,Mass spectrum [M$^+$], m/z 169 (100%) ,[M+1] 169, [M+2] 120, [M+3] 93.

2.1.4. 2-chloro-N-m-tolyacetamide (3c)

It was obtained from (1) with m-toludine in crystalline form. Yield 21.55%, mp 86-91^{0}C, and IR 3294.53cm^{-1}(NH-stretch: 2° amide) , 1672.34 (C=O stretch:2° amide), 1550.82 (NH-bend:2° amide), 1417.73 (C-N stretch), 895.00 (C-Cl stretch) ,Mass spectrum [M$^+$], m/z 183(100%) [M+1] 183, [M+2] 120, [M+3] 107.

2.1.5. 2-chloro-N-(3-chlorophenyl)acetamide (3d)

It was obtained from (1) with aqueous m-chloro aniline in crystalline form. Yield 70.32%, mp 98-100^{0}C, and IR 3282.95cm^{-1}(NH-stretch: 2° amide) , 1666.55 (C=O stretch:2° amide), 1600.97 (NH-bend:2° amide), 1442.80 (C-N stretch), 1091.75 (C-Cl stretch) , 898.96(Meta-disubstituted benzene ring),Mass spectrum [M$^+$], m/z 203(100%), [M$^+$] 207, [M+1] 203, [M+2] 154, [M+4] 127.

2.1.6. 2-chloro-N-(2-chlorophenyl)acetamide (3e)

It was obtained from (1) with aqueous o-chloro aniline in crystalline form. Yield 82.14%, mp 65-67^{0}C, and IR 3259.81cm^{-1}(NH-stretch: 2° amide) , 1658.84 (C=O stretch:2° amide), 1543.10 (NH-bend:2° amide), 1435.09 (C-N stretch), 1045.45 (C-Cl stretch) , 690.54 (Ortho-disubstituted benzene ring),Mass spectrum [M$^+$], m/z 203(100%), [M$^+$] 207, [M+1]203, [M+2] 168.

2.1.7. 2-chloro-N-(2-methoxyphenyl)acetamide (3f)
It was obtained from (1) with aqueous o-methoxy aniline in crystalline form. Yield 59.62%, mp 40-42^{0}C, and IR 3271.38cm^{-1}(NH-stretch: 2° amide) , 1668.48 (C=O stretch:2° amide), 1543.10 (NH-bend:2° amide), 1475.59 (C-N stretch), 785-540 (C-Cl stretch) , 759.98 (Ortho-disubstituted benzene ring),Mass spectrum [M$^+$], m/z 199(100%), [M+1]199, [M+2] 150 [M+3] 122.
2.1.8. 2-chloro-N-(4-methoxyphenyl)acetamide (3g)
It was obtained from (1) with aqueous p-methoxy aniline in crystalline form. Yield 38.17%, mp 118-120^{0}C, and IR 3286.81cm^{-1}(NH-stretch: 2° amide) , 1658.84 (C=O stretch:2° amide), 1531.53 (NH-bend:2° amide), 1438.94 (C-N stretch), 785-540 (C-Cl stretch) , 810.13 (Para-disubstituted benzene ring), Mass spectrum [M$^+$], m/z 199(100%), [M+1] 199, [M+2] 122, [M+3] 108.

2.1.9. 2-chloro-N-methyl-N-phenylacetamide (3h)
It was obtained from (1) with N-methyl aniline in crystalline form. Yield 25.80%, mp 66-68^{0}C, and IR 1670.41 (C=O stretch:3° amide), 3049.56 (C-N stretch: 3° amide), 785-540 (C-Cl stretch) , 563.23 (Monosubstituted benzene ring), Mass spectrum [M$^+$], m/z 183(100%), [M+1] 183, [M+2] 148, [M+3] 106.

2.1.10. N-benzyl-2-chloroacetamide (3i)
It was obtained from (1) with benzylamine in crystalline form. Yield 40.77%, mp 91-93^{0}C, and IR 3275.24cm^{-1}(NH-stretch: 2° amide) , 1624.12 (C=O stretch:2° amide), 1543.10 (NH-bend:2° amide), 1438.94 (C-N stretch), 785-540 (C-Cl stretch) , 567.03 (Monosubstituted benzene ring), Mass spectrum [M$^+$], m/z 183(100%), [M+1] 183, [M+2] 148.

2.1.11. 2-chloro-N-(4-nitrophenyl)acetamide (3j)
It was obtained from (1) with aqueous p-nitro aniline in crystalline form. Yield 23.90%, mp 180-183^{0}C, and IR 3279.10cm^{-1}(NH-stretch: 2° amide) , 1670.41 (C=O stretch:2° amide), 1591.33 (NH-bend:2° amide), 1502.60 (C-N stretch), (C-Cl stretch) , 850.64 (Para-disubstituted benzene ring), 850.64 (NO_2-Asymmetric stretch), Mass spectrum [M$^+$], m/z 214(100%), [M$^+$] 216, [M+1] 214, [M+2] 166, [M+3] 138.

2.1.12. 2-chloro-N-(4-flurophenyl)acetamide (3k)
It was obtained from (1) with aqueous p-fluro aniline in crystalline form. Yield %, mp ^{0}C, and IR 3271.38 cm^{-1}(NH-stretch: 2° amide) , 1651.12 (C=O stretch:2° amide), 1535.39 (NH-bend:2° amide), 1502.60 (C-N stretch), (C-Cl stretch) , 825.56 (Para-disubstituted benzene ring), 1219.05 (C-F stretch), Mass spectrum [M$^+$], m/z 199(100%).

2.1.13. 2-chloro-N-(naphthalen-2-yl)acetamide (3l)
It was obtained from (1) with 2-naphthalene in crystalline form. Yield %, mp ^{0}C, and IR 3252.09cm^{-1}(NH-stretch: 2° amide) , 1660.77 (C=O stretch:2° amide), 1562.75 (NH-bend:2° amide), 1404.22 (C-N stretch), 752.26 (1,2 disubstituted benzene ring) , 856.42 (Meta-disubstituted benzene ring), Mass spectrum [M$^+$], m/z 147(100%) [M$^+$] 219, [M+1] 184, [M+2] 170, [M+3] 143.

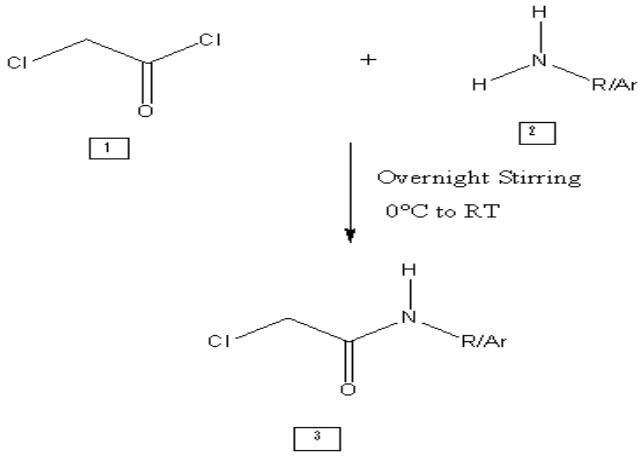


Figure 2: Synthesis of 2-chloro-N-alkyl/aryl acetamide

2.2. Biological activity

Thirteen compounds were screened in vitro for their antimicrobial activity .Samples were prepare in a 1 mg mL^{-1} solution of DMF (Dimethyl formamide) and send to BAC –test laboratory,Nashik,India. They made test against four strains of bacteria E-coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923), which shown antibacterial activity and another microorganism that is Candida sp. for antifungal activity by agar diffusion technique. The bacteria were maintained on nutrient agar ,DMF showed no inhibition zone. The agar media was inoculated with different microorganisms culture tested. After 24 hr of incubation at 30^0c, the diameter of inhibition zone (mm) was measured and Gentamicin for antibacterial activity, Amphotericin B for antifungal activity was used as references(Figure 3-5).. Acetamide derivatives have shown excellent antibacterial and antifungal activity(Figure 6,7).

3. RESULT

Figure 3: Activity of *E-coli strain* (ATCC 25922)

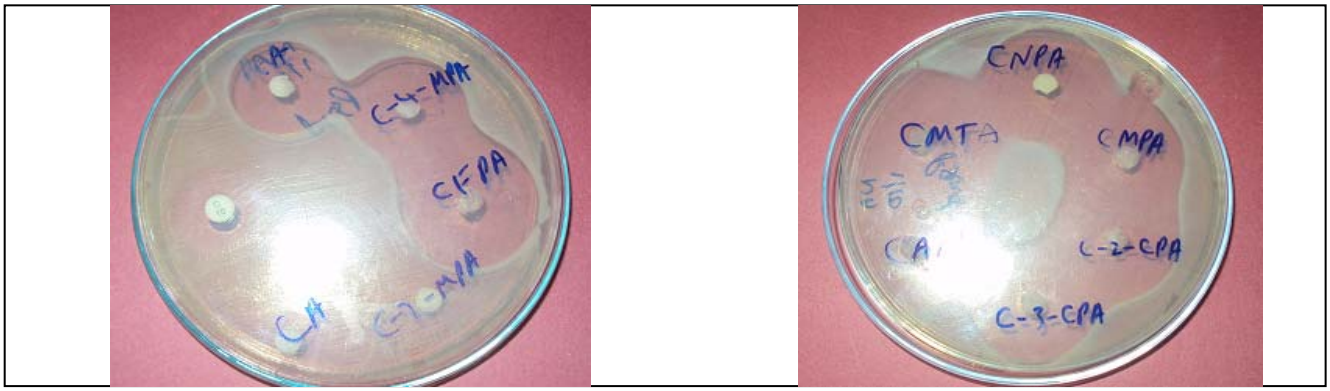


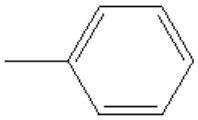
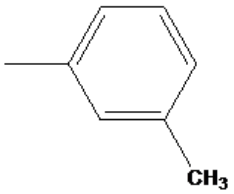
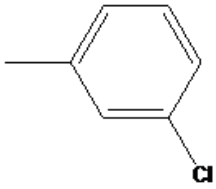
Figure 4: Activity of Pseudomonas aeruginosa (ATCC 27853)

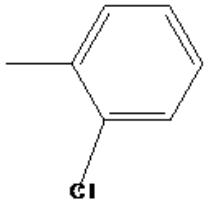
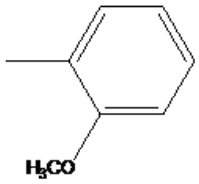
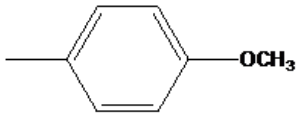
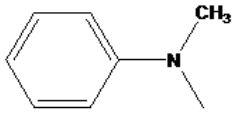
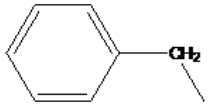
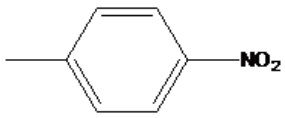
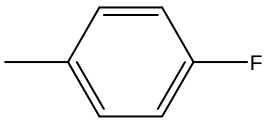
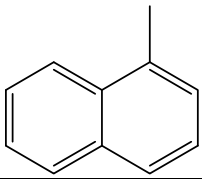
Figure 5: Activity of Staphylococcus aureus (ATCC 25923)

3.1. SYNTHETIC RESULT

The structures of the synthesized compounds were confirmed by IR, GC-MS and ^{1}H NMR. Structures, yields, melting points and R_F values of the synthesized compounds are reported. **(Table 3.1.1)**

Table 3.1.1: Physical characterization of 2-chloro-*N*- Alkyl/Aryl acetamide derivatives

No.	R	Code	Melting point (^{0}C)	Yield (%)	R_f value
1		3a	118-120	83.21%	0.65
2		3b	140-142	82.77%	0.62
3		3c	86-91	21.55%	0.63
4		3d	98-99	70.32%	0.66

5		3e	65-67	82.14%	0.59
6		3f	40-42	59.62%	0.56
7		3g	118-120	38.17%	0.71
8		3h	66-68	25.80%	0.57
9		3i	91-93	40.77%	0.72
10		3j	180-183	23.90%	0.68
11		3k	127-130	53.07%	0.52
12		3l	155-158	61.02%	0.40

Code No.	Code No.	Sample	*E.coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923
3a	1.	CA	**7 mm**	**16 mm**	No zone
3b	2.	PCA	**26 mm**	**23 mm**	**25 mm**
3c	3.	CMTA	**30 mm**	**35 mm**	**36 mm**
3d	4.	C-3-CPA	**27 mm**	**29 mm**	**30 mm**
3e	5.	C-2-CPA	**30 mm**	**30 mm**	**36 mm**
3f	6.	C-2-MPA	**7 mm**	No zone	**10 mm**
3g	7.	C-4-MPA	**26 mm**	**25 mm**	**25 mm**
3h	8.	CMPA	**28 mm**	**28 mm**	**27 mm**
3i	9.	BCA	No zone	No zone	No zone
3j	10.	CNPA	**28 mm**	**28 mm**	**35 mm**
3k	11.	CFPA	**28 mm**	**30 mm**	**35 mm**
3l	12.	CNA	**27 mm**	**29 mm**	**30 mm**
3m	13.	APC	No zone	No zone	No zone
	14.	Gentamicin	14 mm	20 mm	20 mm

3.2 PHARMACOLOGICAL RESULT

Antimicrobiological activity was performed against gram positive, gram negative bacteria and fungi.

Table 3.2.1: Antibacterial activity

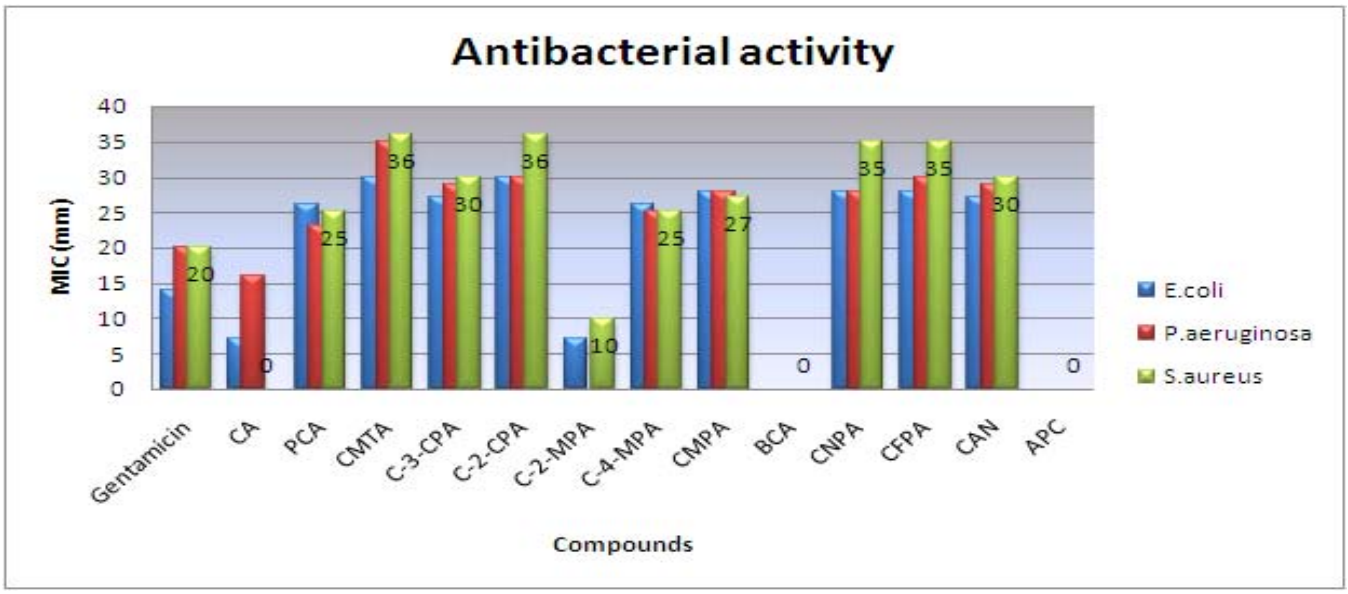


Figure 6: 2-chloro-*N*-alkyl/aryl Acetamide derivatives showing Antibacterial activity

Table 3.2.2: Antifungal activity

Code No.	No.	Sample	*Candida sp.*
3a	1.	CA	No zone
3b	2.	PCA	**14 mm**
3c	3.	CMTA	**11 mm**
3d	4.	C-3-CPA	**23 mm**
3e	5.	C-2-CPA	**21 mm**
3f	6.	C-2-MPA	**20 mm**
3g	7.	C-4-MPA	**12 mm**
3h	8.	CMPA	**29 mm**
3i	9.	BCA	No zone
3j	10.	CNPA	**15 mm**
3k	11.	CFPA	**22 mm**
3l	12.	CNA	**15 mm**
3m	13.	APC	No zone
	14.	Amphotericin B	20 mm

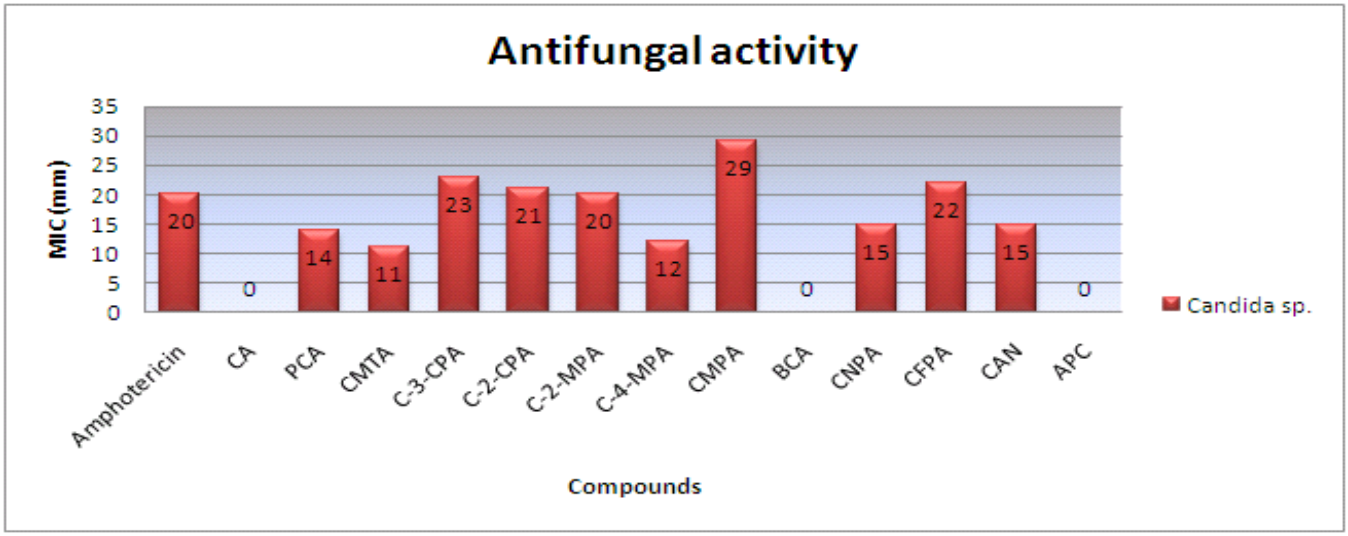


Figure 7: 2-chloro-*N*-alkyl/aryl acetamide derivatives showing Antifungal activity

4. DISCUSSION

All compounds which are synthesized from chloroacetyl chloride and aqueous amine reaction which was stirred overnight are biologically active. And they have shown good antimicrobial activity against gram positive and gram negative bacteria and fungi. A result of activity proves that Chloroacetamides can be used as Disinfectants, Surfactants, Preservatives, and Herbicides.

5. ACKNOWLEDGEMENT

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