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NOVEL N-HETEROCYCLES ACTING AS GENOMIC MUTAGENS

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ABSTRACT:

Heterocyclic chemistry is an important branch of organic and medicinal chemistry. The knowledge of heterocyclic chemistry is useful in biosynthesis and drug metabolism. In the present investigation, Novel N-Heterocycles viz. Quinolones, Pyrazolones, Schiff bases, showing efficient biocidal activities were examined for action at target site. PCR studies, DNA melting point determination studies led to the conclusion that the antimicrobial compound would bring about mutation in the DNA. The composition of DNA can be described by the proportion of its bases i.e. G+C, which ranges from 26 percent to 74 percent for different species decreased G+C content from above said results such as 22.94 percent, 29.04 percent, 13.79 percent, 4.633 percent could be suggestive of mutations in DNA after treatment with antimicrobial agent. Mutations herein suggests the decreased G:C content with the decrease in Tm of the DNA after treatment with antimicrobial agent.

Keywords: G:C content, Melting point, PCR,Mutation,Quinolones,Pyrazolones,Schiff bases

INTRODUCTION

A large no of drugs acting as gene function receptors are used as chemotherapeutic agents eg. Antibiotics, fungicides, antimalarials etc. Supressors of gene function can act either as inhibitors of nucleic acid biosynthesis or protein synthesis.These agents may act by interfering with the polymerization of nucleotides in nucleic acid (Tyagi, O.D. and Yadav, M. 1990) viz, nalidixic acid selectively inhibits DNA synthesis of pathogenic microorganisms. (Powar, C. B. and Daginawala, H. F. 1985) Nalidixic acid inhibits DNA synthesis which is due to inhibition of bacterial gyrase. (Topoisomerase II) an enzyme responsible for introducing negative supercoils into circular duplex DNA (Shen, L. L. *et al* 1989).

Heterocyclic chemistry is an important branch of organic and medicinal chemistry. The knowledge of heterocyclic chemistry is useful in the biosynthesis and drug metabolism; as well nucleic acids are important in biological processes of heredity and evolution (Bansal, R. K. 2005).

The exposed nature of 'N' atom in cyclic amines means that N- heterocycles are very frequently encountered in drug molecules, particularly those operating on central nervous system viz. cocaine, heroin and morphine, all containing nitrogen hetero atom as do cocoine and many transquillizers such as valium (Clayden, *et al.*, 2001).

Amongst the heterocyclic compounds condensed 'N' containing heterocyclic compounds viz. quinolones, pyrazolones, schiff bases, pyrroles, hydrazines because of their effectiveness in many pharmaceutical areas have been largely investigated. Due to unique physical and chemical properties, their derivatives possess a great number of biological activities such as antibacterial, antifungal, anti-inflammatory, antiparasitic, antitumour, antiviral and antihypertensive effects.

The rapid rise in the bacterial resistance to the traditional antibiotics, such as penicillins, tetracycline led to a continuing search for new classes of compounds with novel modes of antimicrobial activity. Because of broad spectrum, *in vitro* and *in vivo* chemotherapeutic efficacy, quinolone antibiotics have emerged as an area of immense interest.

Pyrozolones find wide applications as it exerts paralytic action on sensory and motor nerves resulting in some anesthesia and vasoconstriction.

Schiff bases, a class of heterocyclic compounds, are important pharmaceuticals possessing antibacterial, antifungal, anticancer, antitubercular, anti-inflammatory and herbicidal activities. Biocidal activities of schiff bases have been attributed to the C=N linkages in them.

Chemotherapeutic agents inhibiting the proliferation of infective organism, act by interfering with metabolism of microbes, there by producing the cidal or lethal effect without producing like effect in host cell (Cappucino, 1999).

Some of the standard antimicrobial drugs of heterocyclic nature are nalidixic acid, norfloxacin, ciprofloxacin, nitrofurans, amphotericin B, griseofulvin, nystatine, isoniazid, and ethambutol.

The inhibitors which block the synthesis of nucleotides prevent the growth of bacterial and tumor cells more drastically than the host organism. This partical selective toxicity of such inhibitors is the basis for their use in chemotherapy. (Powar, C. B. & Daginawala, H. F. 1985).

LITERATURE REVIEW

Certain N-hetercycles viz, nitrofurans, metronidazole, quinolones, pyrazolones, Schiff bases, pyroles and hydrazines have useful antimicrobial activity for the treatment of local, systemic and urinary tract infections. The rapid rise in the bacterials & fungal resistance to the tradifional antibiotics viz. penicillin (Nathwani, D and Wood, M. H. 1993) and tehracyclins. (Schappinger, D. & Hillen, W. 1996) has encouraged a continuing search for new classes of compounds with novel mode of antimicrobial activity.

The emergence of drug resistant bacteria is posing a major problem in antimicrobial therapy (Cruick shank, R. *et al,* 1975).

Carbazole is an N-heterocyclic compound derived from cresote, cruide oil & shale oil. It is known to be both mutagenic and toxic but has been used as an industrial raw material for the production of dyes, medicines and plastics (Inoue, K., 2014).

The interaction of many naturally occurring and synthetic ligands. With DNA is an active area of research at the interface of chemistry and biology. Many of these compounds bind noncovalently to ds DNA duplexes by intercalation a process by which the planar aromatic rings of a compound are inserted between adjacent DNA base pairs. The medical importance of intercalators as causative agents in DNA mutagenesis and as potent anticancer agents has provided a major inoetus to study structure / activity relationship & to determine the mode of action of such compounds. These studies are ultimately directed towards developing compounds that are clinically superior to those currently available and show fewer detrimental side effects. (Pasic, Lejla, *et al* 2001)

DNA gyrase and Topoisomerase IV are two important enzymes involved in bacterial DNA replication. Quinolones bind to gyrase/sopoisomerose IV DNA complexes and inhibit DNA replication. This action is responsible for the bacteriostatic & bactericidal property of guinolones. (Ngoi, S.T. & Kwai, C.T. 2014).

The change in the structure of DNA after formations of DNA – antibiotic complex has been shown by studies of crystal structure of DNA bound to anticancer drugs and other antibiotics. Scientists studied the structure bound to the sequence d (CGTACG) and found that the drug intercalates twice within the sequence at both CpG steps.

Sherman, *et. al* (1985) found that cisplastin bound to a single stranded dinucleaotide CpG is responsible for the ability of the drug to kill cancer cells. Quigley *et al* (1986) studied the complex of antibiotic and A and d^1 (GCGTACGC) and found that the drug unwinds the DNA helix by about 50 ^{0}C, leaving a weakly twisted ladder.

DNA denaturation was monitored by measuring the absorbance of UV light passed through a solution of DNA. DNA maximally absorbs UV light at a wavelength of about 260 nm. It is the bases that are principally responsible for this absorption. When the temperature of a solution of DNA raised near to the boiling point of water, the

optical density called absorbance, at 260 nm markedly increases, a phenomenon is known as hyperchromicity. Duplex DNA absorbs less UV light by about 40% than do individual DNA chains. This hyperchromicity is due to base stacking which diminishes the capacity of the bases in duplex DNA to absorb UV light. If we plot the graph of optical density of DNA as a function of temperature, we observe that the increase in absorption occurs abruptly over a peak at a relatively narrow temperature range. The midpoint of graph transition is the melting point or Tm.

The Tm is a characteristic of DNA that is lastly determined by G : C content of the DNA and the ionic strength of this solution. The greater the % of G : C base pairs in the DNA (hence lower the content of A : T), the higher the melting point. G : C base pairs contribute more to the stability due to the greater number of the hydrogen bonds but also importantly because stacking interactions of G : C base pairs with adjacent base pairs are more favorable than the corresponding interactions of A : T base pairs with their neighboring base pairs.

Devappa, S. L. *et al.,* 2008 studied the interaction of antimicrobial compounds with calf thymus DNA (CT-DNA) by electronic spectra, viscosity measurement as well thermal denaturation studies. On binding to DNA the absorption spectrum underwent bathochromic and hypochromic shifts. Viscosity measurement indicated that the viscosity of the sonicated rod like DNA fragments increased. Compounds have been screened for antibacterial and antifungal activities.

In the work, it was stated that there were strong interactions between the chromophore of MPTQ and the base pairs of DNA of calf thymus DNA, *Micrococcus lysodeikticus* DNA and *C. perfringens* DNA. Compared with the changes of absorption spectra, the strong interaction should be considered as the intercalative binding of drug with DNA. DNA melting studies were carried out on the basis that the intercalation of small molecules into the double helix is known to increase the DNA melting temperature (Tm), at which the double helix denatures into single stranded DNA, to the increased stacking of the helix in the presence of an intercalator. Melting of the helix leads to an increase in the absorbance at 260 nm. For comparision the DNA melting studies were carried out with calf thymus DNA in the absence and presence of MPTQ. These various DNA melting experiments strongly supported the intercalation of MPTQ into the double helix DNA (Gopal, M. *et al.,* 2002).

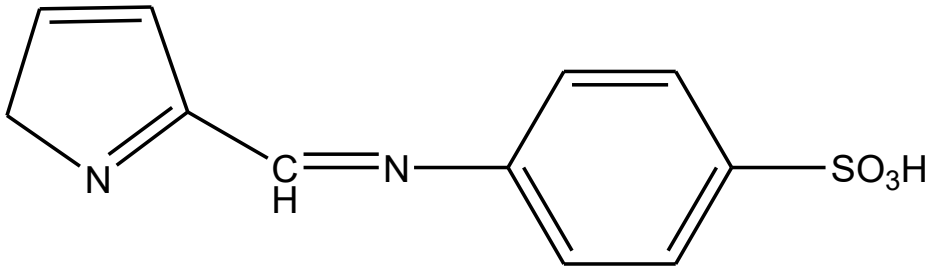
OBJECTIVE OF THE STUDY

The Present Investigation Aims To Explore The Posibal Use Of Novel N-Heterocycles As Biocidal Agents Having Significant Therapeutic Value By Assesment Of Reactivity Between Pathogenic Bacteria And Efficient Compounds Under Study.

MATERIALS AND METHODS

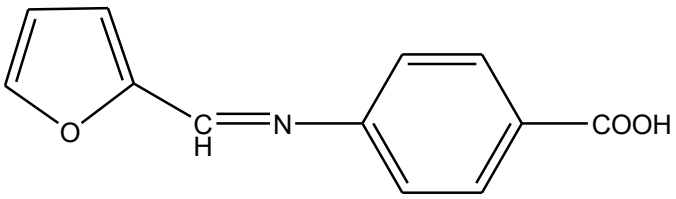
NITROGEN HETEROCYCLES UNDER STUDY

S-PCD-5



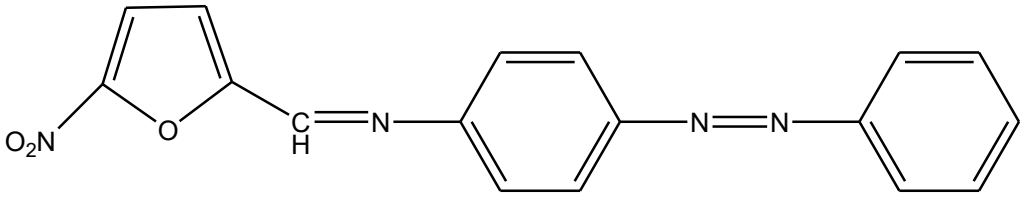
4-((5*H*-pyrrol-2-yl)methyleneamino)benzenesulfonic acid

S-FD-1



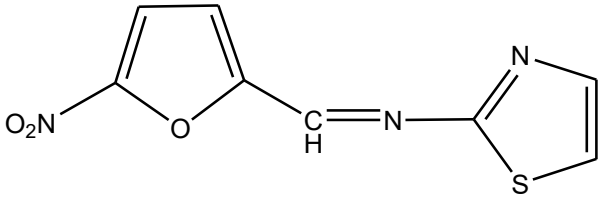
4-((furan-2-yl)methyleneamino)benzoic acid

S-NFD-1



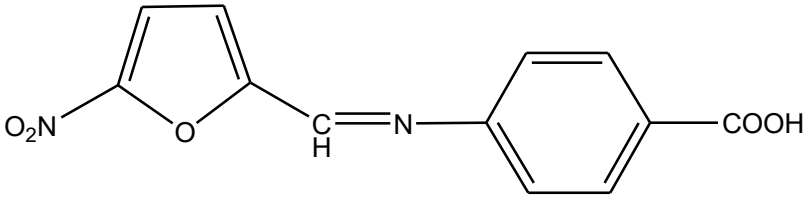
4-(2-phenyldiazenyl)-*N*-((5-nitrofuran-2-yl)methylene)benzenamine

S-NFD-2



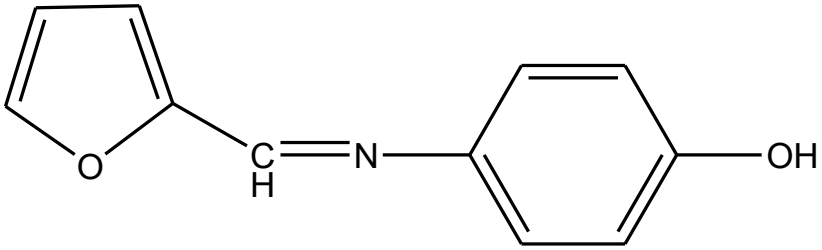
N-((5-nitrofuran-2-yl)methylene)thiazol-2-amine

S-NFD-3



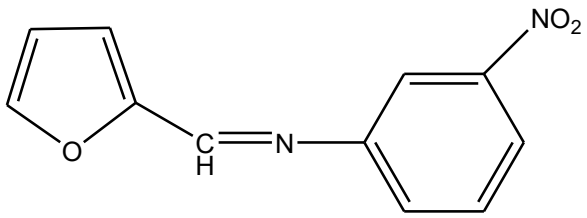
4-((5-nitrofuran-2-yl)methyleneamino)benzoic acid

S-FD-5



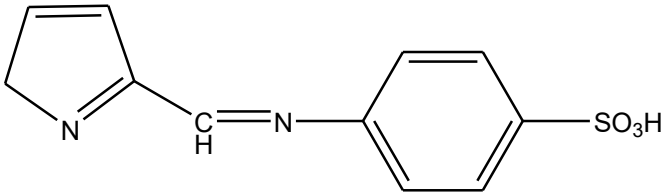
4-((furan-2-yl)methyleneamino)phenol

S-FD-7



N-((furan-2-yl)methylene)-3-nitrobenzenamine

S-PCD-5



4-((5*H*-pyrrol-2-yl)methyleneamino)benzenesulfonic acid

EXPERIMENTAL SECTION

Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD - PCR) Analysis:
For the present investigation, RAPD PCR was carried out of various untreated and treated DNA samples isolated from bacteria and fungi to determine the changes in molecular weight of genomic DNA after treatment with antimicrobial compounds.

RAPD PCR was carried out using RAPD primer OPA - 13. The amplification reaction was performed in a volume of 25 µl. The reaction was carried out using a Bio-Rad thermal cycler. Reaction mixture was prepared with 2 µl (10 ng/µl) DNA and 2.5 µl of 10X PCR buffer with MgCl2, 200 µM deoxyribonucleoside tri-phosphates and 1 µM primer. PCR programme was set with initial denaturaton at 94^{0}C for seven minutes, followed by 35 cycles of 45 sec at 94 ^{0}C, one minute at 36 ^{0}C, 1 minute at 72 ^{0}C and a final extension at 72 ^{0}C for 10 minutes. The PCR amplified product was separated on 1.5 % agarose gel by electrophoresis at 50 V for 2 hours. The bands were scored for analysis. Comparing with the standard DNA marker the change in the band pattern was examined. The effective changes in the DNA were assessed by determining the DNA melting profile of untreated and treated bacterial and fungal cultures.

- **Determination of DNA Melting Profile (Tm):**

DNA melting temperature (Tm) is the temperature at which half of the DNA helical structure is lost. The two strands of DNA helix are held together by H-bonds. Denaturation of DNA involves disruption of H-bonds by change in pH or increasing temperature resulting in separation of polynucleotide strands. Loss of helical structure can be measured by increasing absorbance at 260 nm.

Since G-C base pairs are more stable than A-T base pairs, the Tm is greater for DNA with higher G – C content. Thus the Tm is 65 ^{0}C for 35 % G- C content while it is 70 ^{0}C for 50% G - C content. Chemical agent, formamide destabilizes H - bonds of base pairs and therefore lowers Tm (Satyanarayan, U., 2006).

The associated DNA from untreated and treated samples of bacteria and fungi viz, *S. aureus, E. coli* and *C. clbicans* were checked for purity by ultraviolet spectrophotometer. Ratio of 1.7 to 1.8 (260 nm /280nm) is a characteristics of pure DNA. Further concentration of all samples was adjusted to 100 µg/ml using 1X standard saline citrate buffer and further samples were used for determination of DNA melting pattern (Gopal, M. R., Shahabuddin, M. S. and Inamdar, S. R., 2002).

To determine (Tm) melting pattern of DNA, DNA samples under study were kept at increased temperature (rise by 5 ^{0}C at every step) i.e. from 30 ^{0}C up to 100 ^{0}C using water bath and absorbance was read at 260 nm. Observations were recorded and graph was plotted for temperature versus optical density. From the graphs, melting points of sample (Tm) were deduced.

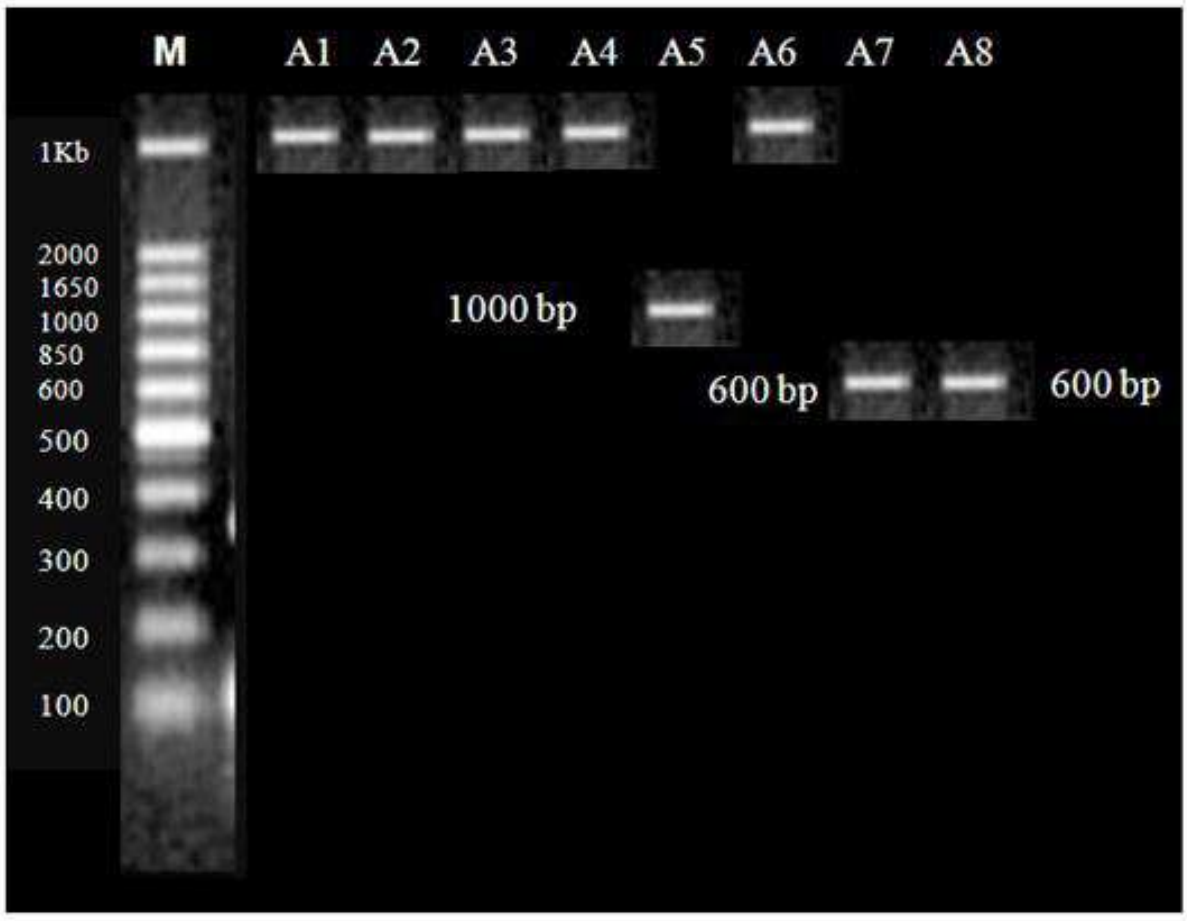


Fig. 36: RAPD PCR analysis of treated and untreated sample of *S.aureusC1*

Where M - DNA marker of 1Kb

A1 - DNA of untreated *S. aureus*

A2 - DNA of treated *S. aureus* with Q-1

A3 - DNA of treated *S. aureus* with Q-2

A4 - DNA of treated *S. aureus* with C-Pyz-2

A5 - DNA of treated *S. aureus* with S-FD-1

A6 - DNA of treated *S. aureus* with S-NFD-1

A7 - DNA of treated *S. aureus* with S-NFD-3

A8 - DNA of treated *S. aureus* with S-PCD-5

Fig. 37: RAPD PCR analysis of treated and untreated sample of E.*coliC1*

Where M - DNA marker of 1Kb

B1 - DNA of untreated E.*coli*

B2 - DNA of treated E.*coli* with Q-1

B3 - DNA of treated E.*coli* with Q-2

B4 - DNA of treated E.*coli* with C-Pyz-2

B5 - DNA of treated E.*coli* with S-FD-5

B6 - DNA of treated E.*coli* with S-NFD-1

B7 - DNA of treated E.*coli* with S-FD-1

B8 - DNA of treated E.*coli* with S-PCD-5

B9 - DNA of treated E.*coli* with S-NFD-3

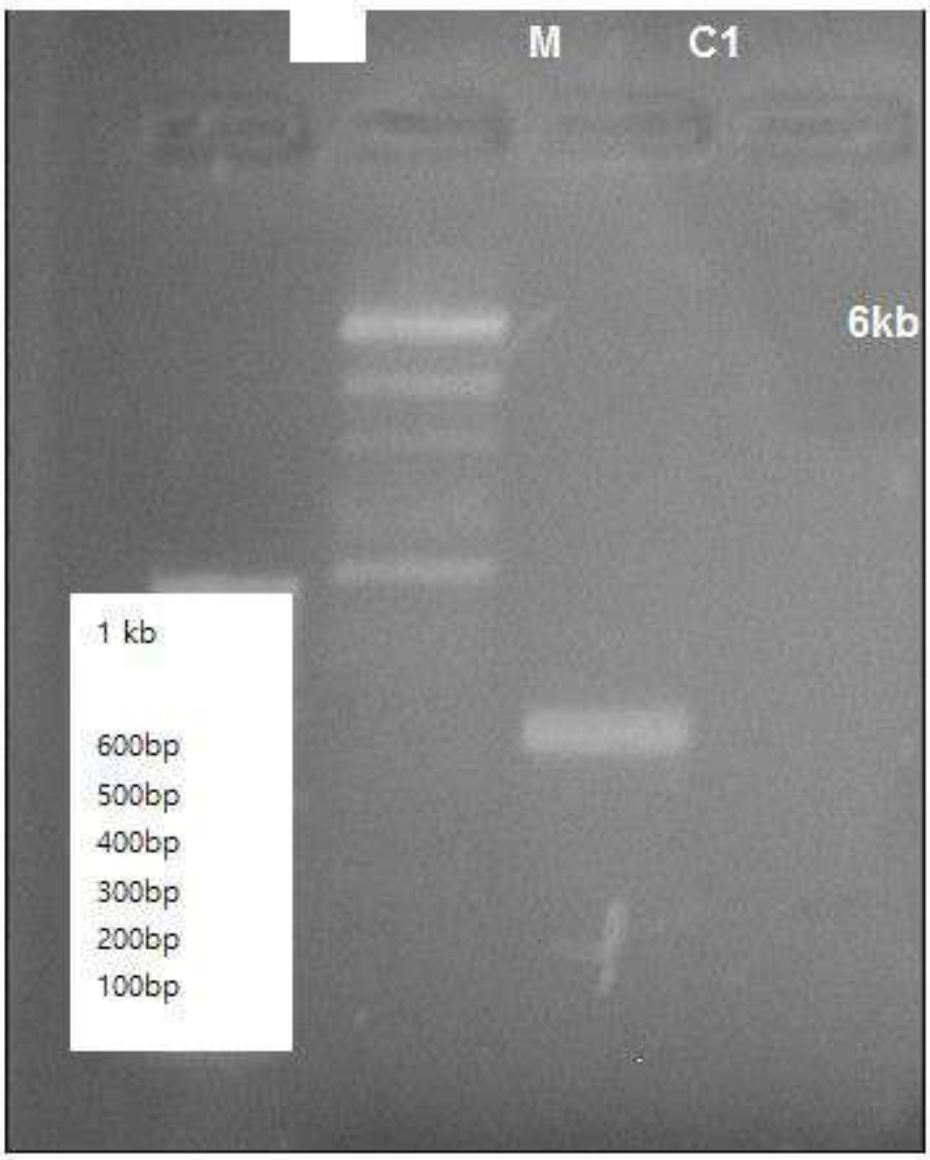


Fig. 38 : RAPD PCR analysis of treated and untreated sample of *C. albicansC1*

M : DNA Marker of 1Kb

C1 : DNA of untreated *C. albicans*.

C2 : DNA of treated *C. albicans* with S-NFD-3

C3 : No bands of DNA of treated *C. albicans* with S-FD-7

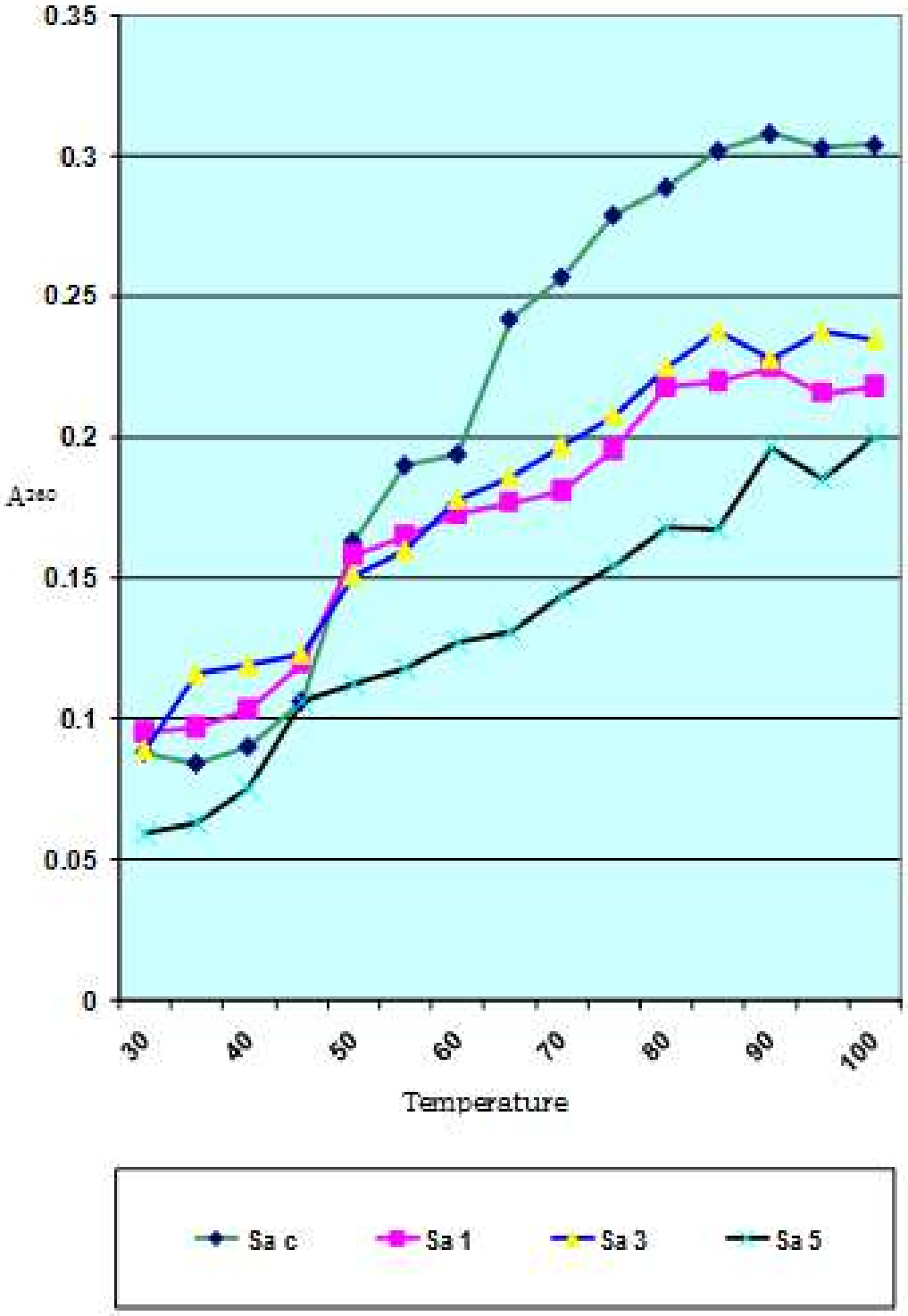


Fig. 39: Tm graph for *S. aureus*

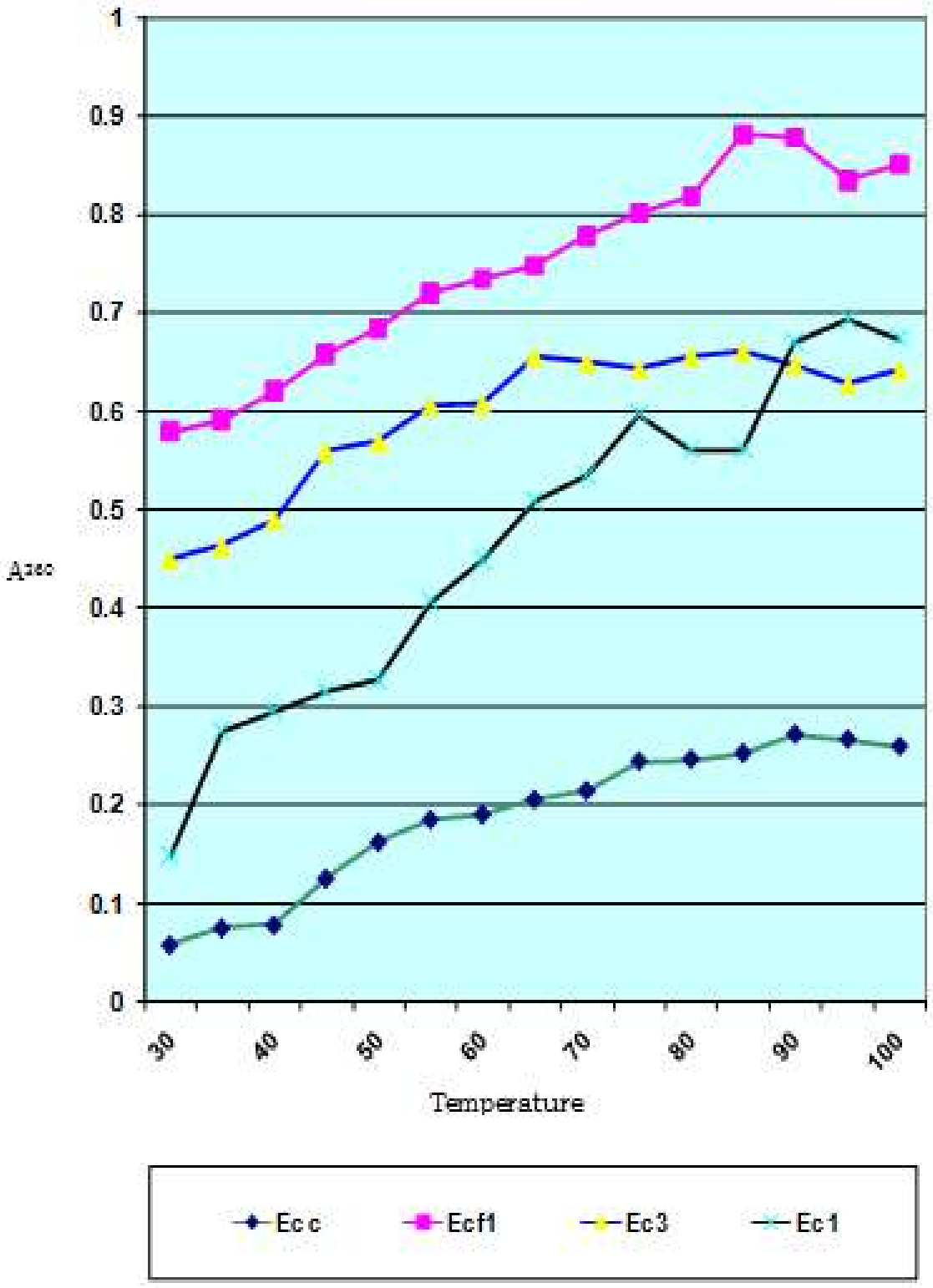


Fig. 40: Tm graph for *E.coli*

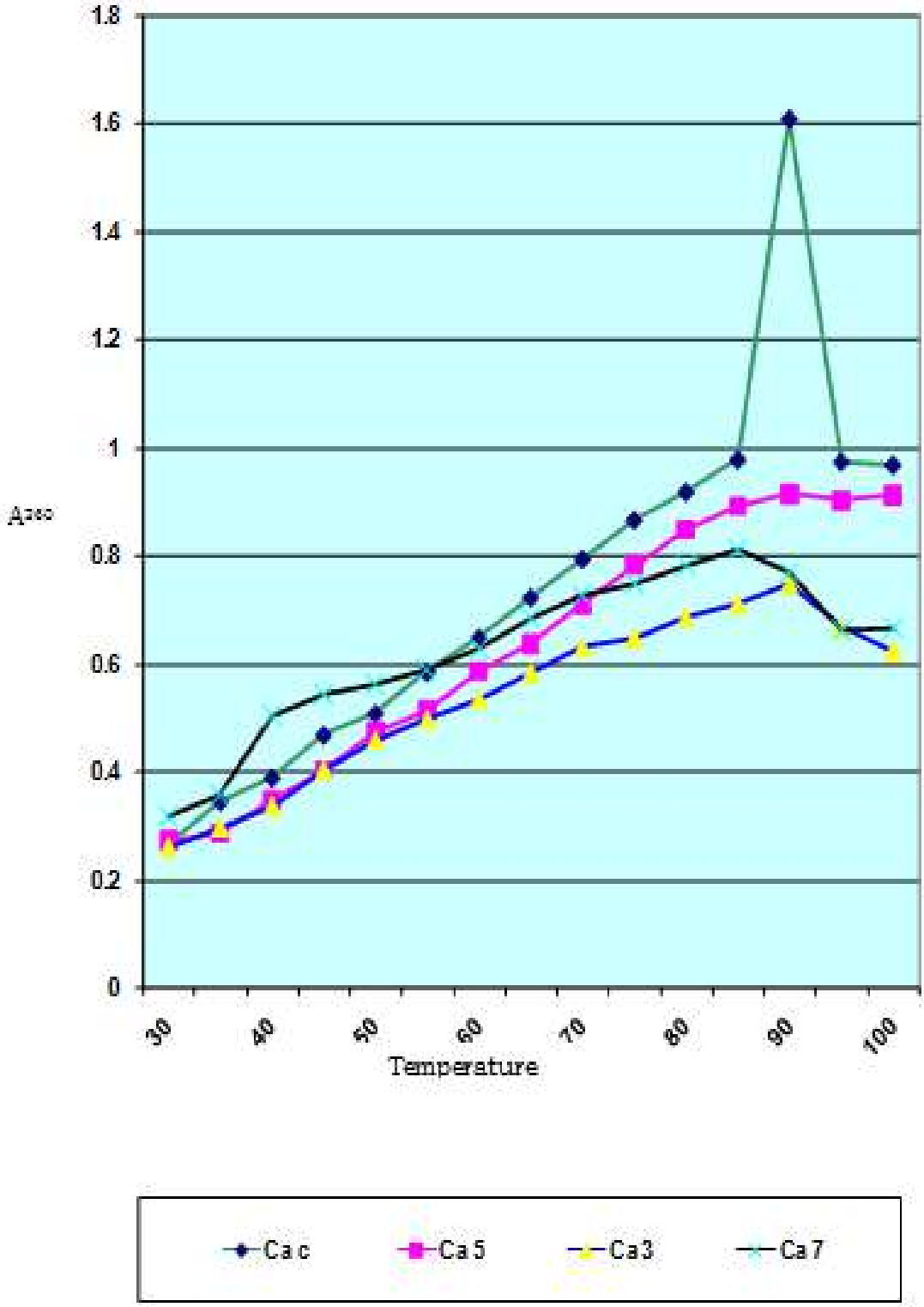


Fig. 41: Tm graph for *C.albicans*

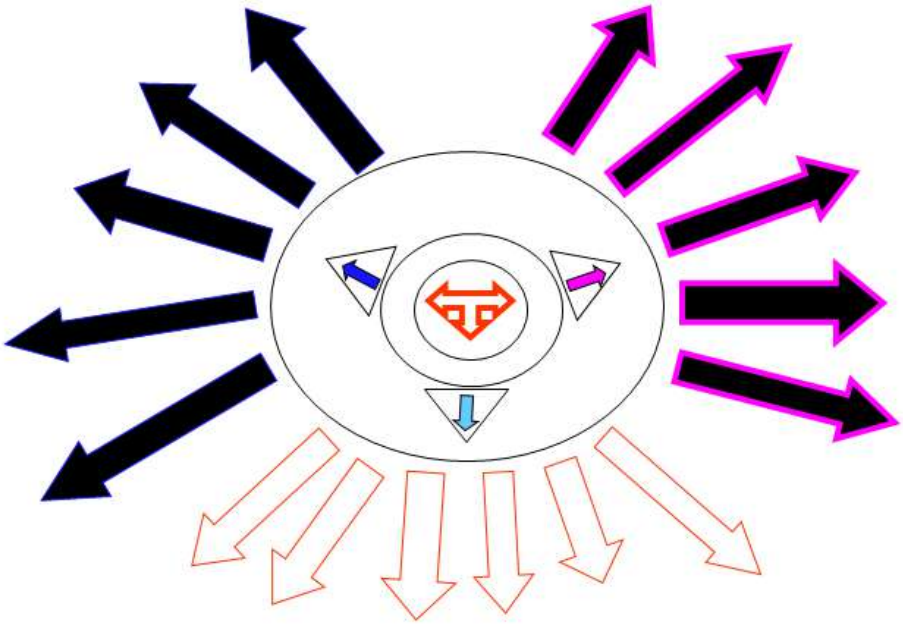


Fig. 3: Associated microflora and heterocyclic compounds

Table – 26: Showing culture codes for untreated and treated samples

Sr.	Culture	Code
1	Untreated Culture of *S.aureus*	Sa_c
1a	Culture + SFD-1	Sa_1
1b	Culture + SNFD-2	Sa_2
1c	Culture + SPCD-5	Sa_5
2	Untreated culture of *E.coli*	Ec_c
2a	Culture + SFD-1	Ec_5
2b	Culture + SNFD-3	Ec_3
2c	Culture + SNFD-1	Ec_1
3	Untreated Culture of Candida *albicans*	Ca_C
3a	Culture + SFD-5	Ca_5
3b	Culture + SNFD-3	Ca_3
3c	Culture + SFD-7	Ca_7

Table – 27: Showing purity of the isolated DNA

Sr. No.	Culture Code	A_{260}	A_{280}	Concentration (µg / ml of culture)	Ratio
1	Sa_c	0.568	0329	284.0	1.72
1a	Sa_1	0.691	0421	345.5	1.64
1b	Sa_2	0.468	0.263	234.0	1.77
1c	Sa_5	0.599	0.336	299.5	1.78
2	Ec_c	0.692	0.399	346.0	1.73
2a	Ec_{f1}	0.688	0.414	344.0	1.66
2b	Ec_3	0.574	0.319	287.0	1.79
2c	Ec_1	0.689	0.378	343.0	1.81
3	Ca_C	0.196	0.117	4.9	1.67

3a	*Ca* $_5$	0.174	0.106	4.35	1.64
3b	*Ca* $_3$	0.249	0.147	6.22	1.69
3c	*Ca* $_7$	0.206	0.128	5.15	1.60

Table – 28: Showing absorbance A $_{260}$ values for *S. aureus* isolated DNA (Untreated and Treated Sample)

Temperature (°C)	**A_{260}**			
	Sa $_c$	*Sa* $_1$	*Sa* $_2$	*Sa* $_5$
30	*0.088*	0.095	0.089	0.059
35	*0.084*	0.097	0.116	0.063
40	*0.09*	0.103	0.119	0.075
45	0.106	0.12	0.123	0.106
50	0.163	0.158	0.151	0.112
55	0.19	0.165	0.16	0.118
60	0.194	0.173	0.178	0.127
65	0.242	0.177	0.186	0.131
70	0.257	0.181	0.197	0.144
75	0.279	0.196	0.208	0.154
80	0.289	0.218	0.225	0.168
85	0.302	0.22	0.238	0.167
90	0.308	0.225	0.228	0.196
95	0.303	0.216	0.238	0.185
100	0.304	0.218	0.235	0.20

Table – 29: Showing absorbance A $_{260}$ values for *E.coli* isolated DNA (Untreated and Treated Sample)

Temperature (°C)	**A260**			
	Ec $_c$	Ec $_{f1}$	Ec $_3$	*Ec* $_1$
30	0.058	0.579	0.45	0.148
35	0.075	0.591	0.463	0.275
40	0.078	0.62	0.49	0.295
45	0.125	0.658	0.56	0.315
50	0.162	0.684	0.57	0.327
55	0.185	0.72	0.606	0.406
60	0.19	0.734	0.608	0.449
65	0.205	0.748	0.656	0.509
70	0.714	0.778	0.65	0.534
75	0.244	0.801	0.643	0.597
80	0.246	0.818	0.656	0.561
85	0.252	0.881	0.661	0.561
90	0.271	0.878	0.648	0.67
95	0.266	0.835	0.628	0.694
100	0.259	0.851	0.642	0.674

Table – 30 : Showing absorbance A $_{260}$ values for *C. albicans* isolated DNA (Untreated and Treated Sample)

Temperature (°C)	**A_{260}**			
	Ca $_C$	***Ca*** $_5$	***Ca*** $_3$	***Ca*** $_7$
30	0.264	0.271	0.259	0.317
35	0.345	0.285	0.296	0.356
40	0.389	0.346	0.336	0.503

45	0.468	0.402	0.403	0.542
50	0.503	0.474	0.459	0.561
55	0.584	0.514	0.497	0.589
60	0.648	0.586	0.534	0.628
65	0.723	0.637	0.584	0.684
70	0.794	0.71	0.631	0.726
75	0.866	0.783	0.648	0.749
80	0.918	0.849	0.687	0.781
85	0.978	0.893	0.712	0.812
90	1.069	0.916	0.748	0.769
95	0.974	0.903	0.668	0.661
100	0.968	0.912	0.624	0.664

Table – 31: Showing values of Tm (^{0}C) & (G+C) content

Sr.No.	Culture Code	Tm ^{0}c	Total G+C content
1	Sa c	66.007	33.184
2	Sa 1	62.50	22.94
3	Sa 2	65.00	29.04
4	Sa 5	65.00	29.04
5	Ec c	65.00	29.04
6	Ec f1	58.75	13.79
7	Ec 3	57.50	10.74
8	Ec 1	55.00	4.636
9	Ca c	60.00	16.836
10	Ca 5	60.00	16.836
11	Ca 3	57.5	10.736
12	Ca 7	57.5	10.736

G+C (percent) of genomic DNA was calculated according to Pariza and Indolo (1974). Percent G/C = (Tm – 53.1) x (2.44)

The melting point of treated and untreated samples of *S.aureus, E. coli* and *C. albicans* were studied. Accordingly G + C (percent) of genomic DNA was calculated in case of *S. aureus* Tm of untreated sample was found to be 66.007 ^{0}C where as Tm of the treated samples was found to be decreased i.e. 62.5 ^{0}C, 65.0 ^{0}C indicating decrease in the G + C (percent) of genomic DNA. The Tm of untreated *E. coli* DNA was found to be 65 ^{0}C whereas for treated samples it was found to be 58.75 ^{0}C, 57.5 ^{0}C, and 55.0 ^{0}C indicating decreased G + C (percent) of genomic DNA. Tm of untreated C. albicans DNA was found to be 60.0 ^{0}C whereas in treated samples Tm was found to be decreased at 57.5 ^{0}C. The above said studies laid to the conclusion that the antimicrobial compound would bring about mutation in the DNA. The composition of DNA can be described by the proportion of its bases i.e. G+C, which ranges from 26 percent to 74 percent for different species decreased G+C content from above said results such as 22.94 percent, 29.04 percent, 13.79 percent, 4.633 percent could be suggestive of mutations in DNA after treatment with antimicrobial agent. Mutations herein suggests the decreased G:C content with the decrease in Tm of the DNA after treatment with antimicrobial agent.

DISCUSSION

Presence of azomethine linkage, -nitro and acidic group might enhance the biological activity. Structure and activity relationship shows that in the above schiff bases carboxylic acid group, sulfuric acid, nitro group, diazo group and hydrogen enhances the biological activity of compounds under study.

The presence of *gyrA, gyrB* and *parC* mutations, differences in the ciprofloxacin uptake and OMP profile of 12 clinical isolates of *C. freundii* have been analyzed. In agreement with previously published results, all the nalidixic acid resistant clinical isolates included in this study showed mutation which could affect the nature of the polypeptide chain. The results suggested that in *C. freundii*, as in *E. coli* the primary quinolone acquisition point is located in *gyrA* (Margarita, M. N. *et al*., 1999).

Bactericidal activity against MRSA (1.3 – 3.5 hrs) including EMRSA – 16 genomic DNA (50 μM) with 20 μM ELB that PBD dimmers exert their antibacterial effect by cross linking of two DNA strands.

The novel antimicrobial heterocyclic compounds are known to possess DNA binding activity, this could bring about mutations in microbial DNA. Treatment of antimicrobial compounds produced low molecular weight DNA revealed by RAPD PCR studies. The efficient strains of bacteria viz. *S. aureus, E. coli* and fungus viz. *C. albicans* were treated with significant antimicrobial compounds at the MIC value and after incubation for 6 hours DNA isolated was subjected to RAPD PCR. After electrophoresis altered band pattern of DNA was observed. The fact was supported by DNA melting temperature studies. The melting point of treated and untreated DNA samples of *E. coli, S. aureus and C. albicans* was studied and accordingly G+C content of genomic DNA was reported. Decreased melting temperature of above said DNA samples was observed and decreased G+C content was recorded. Above said results concluded the changes in molecular weight of DNA after treatment with antimicrobial compounds.

CONCLUSION

The results of the present investigation regarding the melting temperature of antimicrobial compound treated DNA samples concluded that binding of antimicrobial test compounds with bacterial and fungal DNA decreases Tm, lowering the G : C content of the DNA.

Mutations in DNA after treatment with antimicrobial agents were studied by RAPD PCR and determination of melting temperature of DNA after treatment with antimicrobial compounds. For studies representative of each group i.e. Gram positive viz. *S. aureus*, Gram negative viz. *E. coli* and yeast viz. *C. albicans* were selected.

RAPD PCR studies have shown that *S. aureus, E. coli* and *C. albicans* on treatment with significant active schiff bases produced low molecular weight DNA.

Decrease in Tm indicated that in the antimicrobial agent-treated DNA sample, Tm was lowered suggesting that G : C content was decreased as compared to that of untreated DNA sample. Lowering the G : C content after treatment supported the fact that such schiff bases could act against microorganisms by affecting DNA synthesis. Such compounds could act as DNA targeting drugs. It can be concluded that such compounds might act on a particular site on the DNA which can be confirmed by molecular studies viz. DNA sequencing. To understand the exact mechanism of mode of action of these antimicrobials, additional experiments are needed to be carried out.

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