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Fischer's Indolisation of Novel [b] Indole Derivatives and Their Antimicrobial Evaluation

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Abstract

Carbazole derivatives play a vital role in the metabolism of living cell. Many of the [b] indole derivatives found to have pharmacological activity and medical uses. The chemistry of heterocyclic compounds have been attractive area of study of long time, especially nitrogen containing heterocyclic compounds have received special attention in pharmaceutical chemistry due to their diverse therapeutic applications. Compound (**A**) 5,11-dihydro diindolo(3,2-b) carbazole and compound **(B)** 9-hydro-4H-furo diindolo (3,2-b) carbazole were synthesized with suitable solvent by Fischer's method. 1,2,3,8-tetrahydropyrrolo(2,3-b) indole**(C)** and 3-H-thiazolo(4,5-b) indol-2(4H)-one**(D)** have also been synthesized in good yield by Fischer's synthesis with suitable solvent like methanol, ethanol or acetic acid. The synthesized compounds were identified and confirmed using various instrumental techniques Viz, FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. The synthesized compounds were subjected to antimicrobial activity. Evaluation of antimicrobial activity of the synthesized compounds exhibit good antibacterial activity than antifungal activity.

Keywords
5,11-dihydro diindolo(3,2-b) carbazole, Phenylhydrazine, Acetic acid, Ciprofloxacin, Amphotericin-B

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INTRODUCTION

Heterocyclic chemistry is the branch of chemistry dealing with synthesis, properties, and applications of heterocycles. Heterocyclic structures were found in many natural products. A heterocyclic compound is a cyclic compound that has atoms of at least two different elements in its ring. [1] An indole is characterized as a benzene ring fused to a nitrogen-containing five membered heterocylic ring. [2] Substituents may be added anywhere on the above molecule to create an indole derivative. Several thousand indole derivatives appear annually in chemical literature. [3] The Fischer indole synthesis is the most widely used and versatile method for indole synthesis.

Most generally applicable synthetic methods for the indole moiety involve ring closure to form the pyrrole ring of indole. Substituents are often introduced prior to the synthesis of the ring system. The classical Fischer method has maintained its prominent role as the most common synthetic route to indole derivatives for more than a century. On the other hand, cyclisation reaction has been reported including the Bischler synthesis, palladium catalysed cyclisation. Photocyclisation and radical cyclical cyclisation. The detailed indole cyclisation reactions have been reviewed elsewhere. [4-5]

Due to their interesting biological activity induce the researchers to synthesis carbazole. Carbazole have been found to possess a wide spectrum of biological activity such as antibacterial [6], antirheeumatoid arthritis [7], antitubercular [8], antiviral [9], antiepileptic [10], anti-inflammatory [11], and anti-cancer [12] activities. Further, carbazole are a large and interesting group of organic compounds active among which one can find dyes stuffs [13] , and plastics [14], carbazole constitute an important class of naturally occurring heterocycles with interesting biological activities including their special affinity towards DNA. [15] Encouraged by these observations and search for antimicrobial active molecules, it has been decided to synthesis some indole derivatives in order to evaluate their antimicrobial activities

EXPERIMENTAL SECTION

METHODS AND MATERIALS

The chemicals used for the synthesis of carbazole derivatives were purchased from Merck, Avra synthesis Pvt.Ltd. , Hyderabad.

The Melting points of synthesized compounds were determined by open capillary tubes using an X-5A Melting point instrument and were uncorrected. FTIR Spectra were recorded on a Alpha Bruker FTIR Spectrometer using KBr pellets. The ^{1}H NMR Spectra were measured on a Bruker proton NMR-Advance 300MHz with chemical shift expressed in ppm downfield from TMS as internal standard in DMSO(d^{6}). The ^{13}C NMR Spectra were determined at 300MHz with a Bruker Advance Spectrometer. Mass Spectra were recorded on GC-MASS Spectrometer using methanol as a solvent.

SYNTHESIS OF 2-BENZYL-2,3,4,9 TETRAHYDRO-1, H-PYRIDO (2,4, -b) INDOLE

A mixture of benzyl-4-pipridone(11mmol) and glacial acetic acid (10ml) were taken in a round bottom flask fitted with reflux condenser. Phenyl hydrazine (11mmol) was added slowly through the top of the condenser with constant stirring. The reaction mixture was refluxed for about 4 hours. The disappcarance of oil layer indicated the completion of the reaction and the hot mixture was poured into ice. The content was then stirred vigorously for about 30 minutes. The crude solid was filtered, washed with water and dried. The crude product was recrystallized from hot methanol ensured a black powder, which was further purified by column chromatography with a solvent system of hexane: ethyl acetate (8:2) mixture to give black powder.

SYNTHESIS OF 9-HYDRO-4H-FURO-DIINDOLO (2,3-B) CARBAZOLE]

A mixture of 1,3 cyclo hexanendione (11mmol) and glacial acetic acid 10ml were taken in a round bottom flask fitted with reflux condenser phenyl hydrazine (11mmol) was added slowly through the top of the condenser with constant stirring. The reaction mixture was refluxed for about 3 hours. The disappearance of oil layer indicated the completion of the reaction and the hot mixture was poured into ice the content was then stirred vigorously for about 30 minutes. The crude solid was filter washed with water and dried. The crude product was recrystallized from hot methanol ensured a black powder which was further purified by column chromatography with a solvent system of hexane: ethyl acetate (8:2) mixture to give black powder.

SYNTHESIS OF 5,11-DIHYDRO INDOLO (3,2-b) CARBOAZOLE

A mixture of Glutaric anhydride (18mmol) and glacial acetic acid 10 ml were taken in a round bottom flask fitted with reflux condenser. Phenyl hydrazine (18mmol) was added slowly through the top of the condenser with constant stirring. The reaction mixture was refluxed for oily layer indicated the completion of the reaction and the hot mixture was poured into watch glass. The mixture is dried in room temperature. The crude product is light yellow

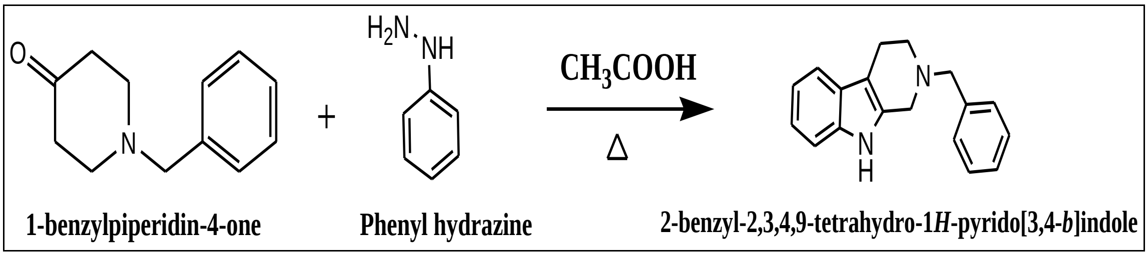
SYNTHESIS OF 3H-THIAZOLO(4,5-B) INDOL-2(4H)-ONE

A mixture of 2,4 thiazolidinedione (1.4g) and glacial acetic acid 10ml were taken in a round bottom flask fitted with reflux condenser. Phenyl hydrazine (2.72ml) was added slowly through the top of the condenser. The reaction mixture was refluxed for about 2hours. The disappearance of oily layer indicated the completion of the reaction and the hot mixture was poured to in watch glass. The mixture is dried in room temperature. The crude product is orange crystal. The crude product was recrystallized from hot methanol.

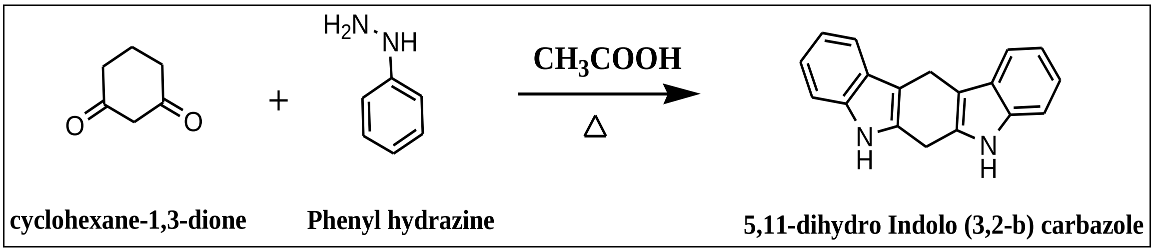
EVALUATION OF ANTIMICROBIAL ACTIVITIES

AGAR WELL DIFFUSION METHOD

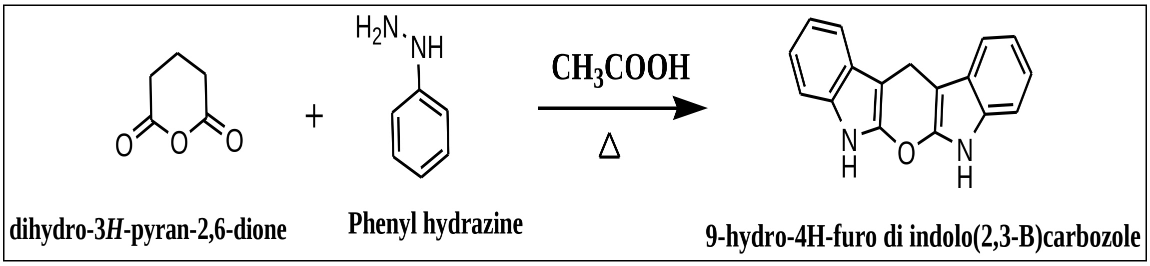
Antimicrobial analysis was followed using standard agar well diffusion method to study the antibacterial and antifungal activity of compounds. The test organisms were flood-inoculated onto the surface of BHI agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 30 µL of the sample solution were poured into the wells. The plates were incubated for 18 h at 37°C for bacteria and at room temperature for fungi. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition in mm against the test microorganisms. DMSO was used as solvent control. Ciprofloxacin was used as reference antibacterial agent. Amphotericin B was used as reference antifungal agent. The tests were carried out in triplicate. Upon incubation the zone of clearance around the wells were measured. The zone of inhibition diameter in mm as measured.



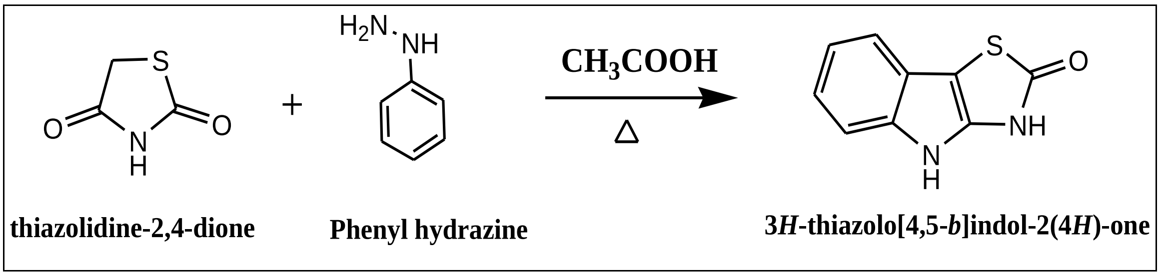
Scheme1: Synthesis of 2-benzyl-2,3,4,9-tetrahydro-1H-pyrido [3,4-b] indole.



Scheme 2: Synthesis of 5,11-dihydroindolo [3,2-b] carbazole.



Scheme 3: Synthesis of 9-hydro-4H-furo diindolo [2,3-b] carbozole.



Scheme 4: Synthesis of 3H-thiazolo[4,5-b] indol-2(4H)-one.

RESULT AND DISCUSSION

COMPOUND – A

The FTIR spectrum of the compound A have shown in Figure **1**. The sharp intense band at 3751 cm^{-1} was observed due to the N-H stretching vibration. The band at 3421 cm^{-1} was assigned to the aromatic =C-H stretching vibration. The sharp band appeared at 2854 cm^{-1} and 2924cm^{-1} was associated the aliphatic C-H stretching vibration. The ^{1}H-NMR spectrum of 2-benzyl-1,3,4 tri hydro (3,2-b) carbazole have shown in Figure **2**. A singlet peak at 9.92 ppm was due to N-H proton and one doublet peak appeared at 8.32 ppm corresponds to '**a'** proton. The multiplet signal at 7.23 - 7.34 ppm for aromatic '**b'** protons. The doublet signal appeared at 4.57 ppm for '**c'** protons. The doublet signal appeared at 3.37 ppm for 'd' protons. The singlet signal appeared at 2.49 ppm for methylene protons.

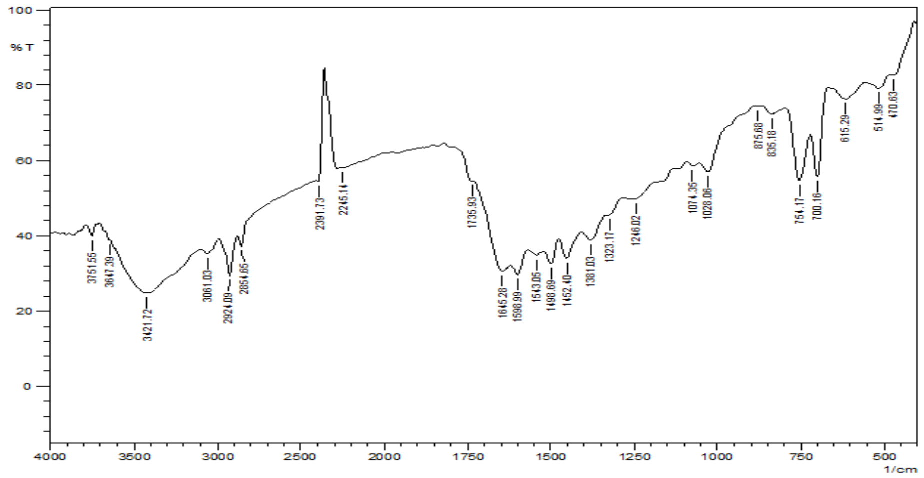


Figure 1. FT-IR spectrum of 2-benzyl-2,3,4,9 tetrahydro-1, H-pyrido(2,4,-b) indole.

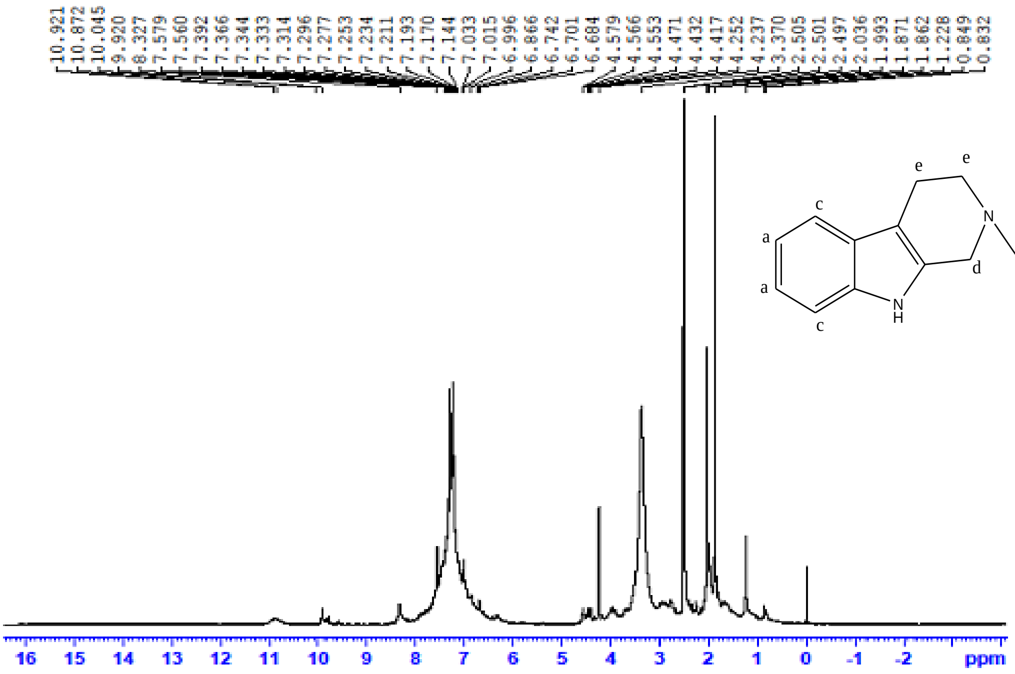


Figure 2.^{1}H NMR of 2-benzyl-2,3,4,9 tetrahydro-1, H-pyrido(2,4,-b) indole.

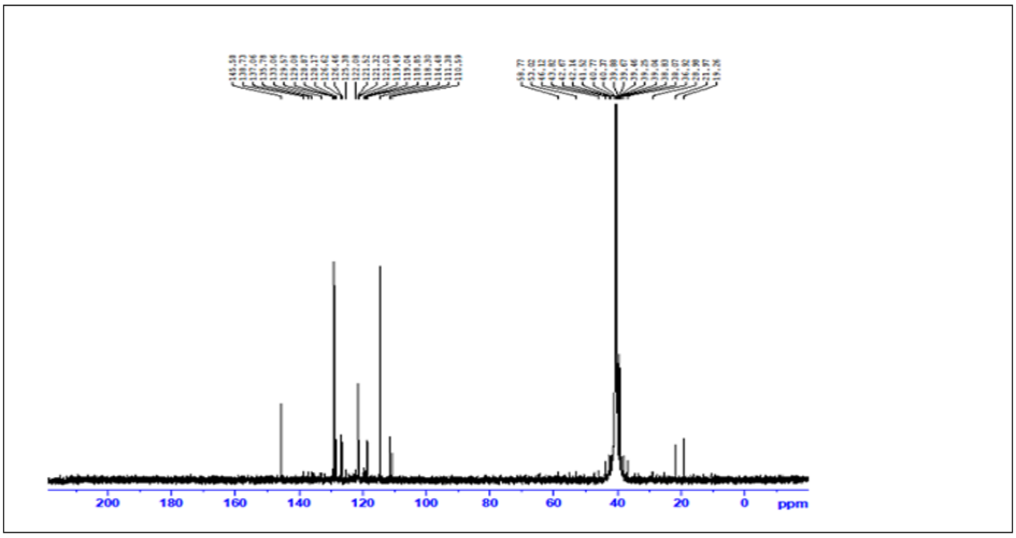


Figure 3.^{13}C NMR of 2-benzyl-2,3,4,9 tetrahydro-1, H-pyrido(2,4,-b) indole.

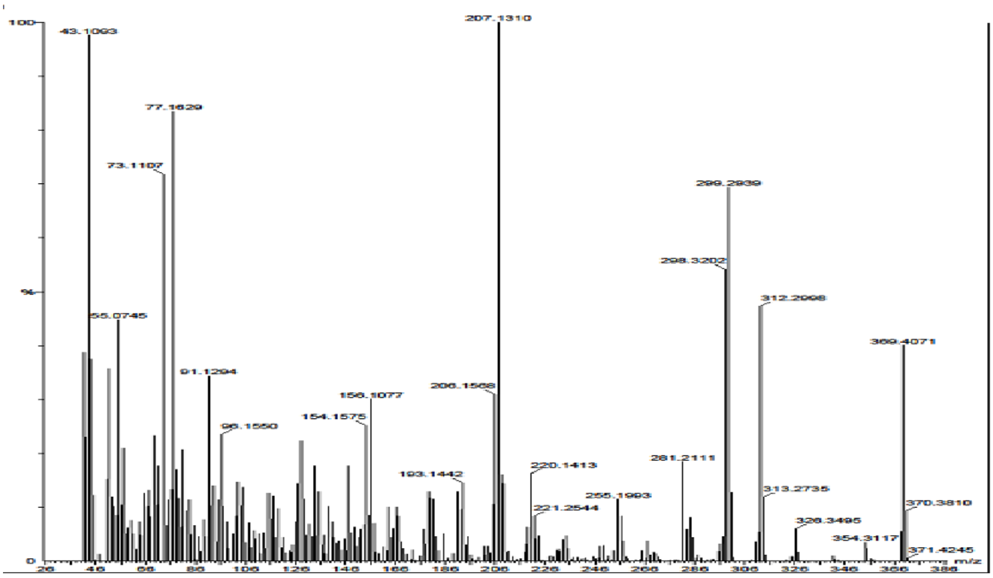


Figure 4. GC MASS spectrum of 2-benzyl-2,3,4,9 tetrahydro-1,H-pyrido(2,4,-b) indole.

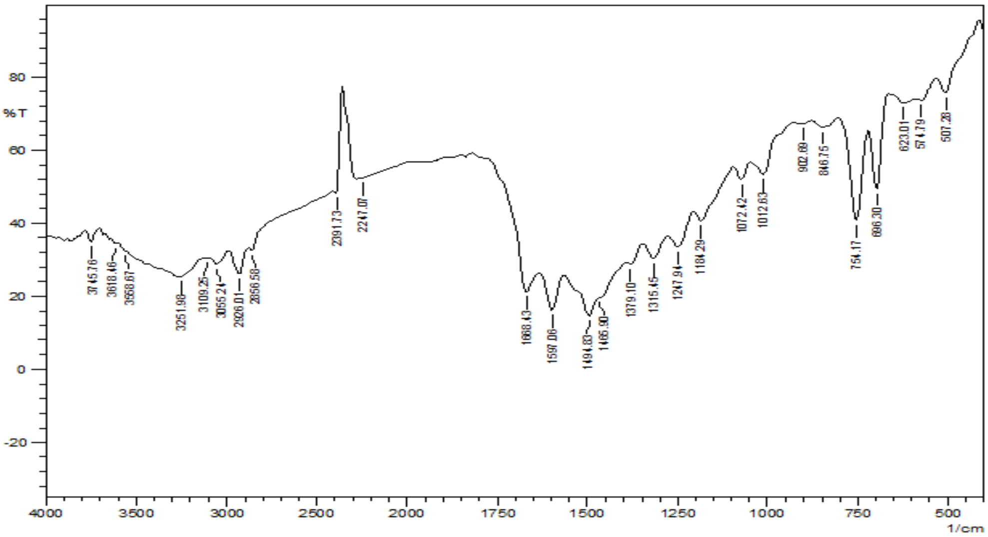


Figure 5. FT-IR spectrum of 5,11- dihydro indolo (3,2-b) carboazole.

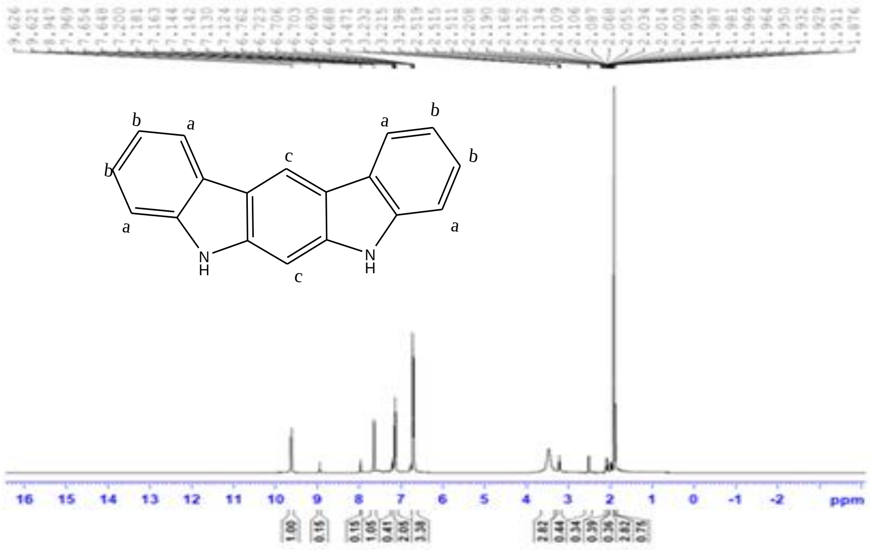


Figure 6.^{1}H NMR of 5,11- dihydro indolo (3,2-b) carboazole.

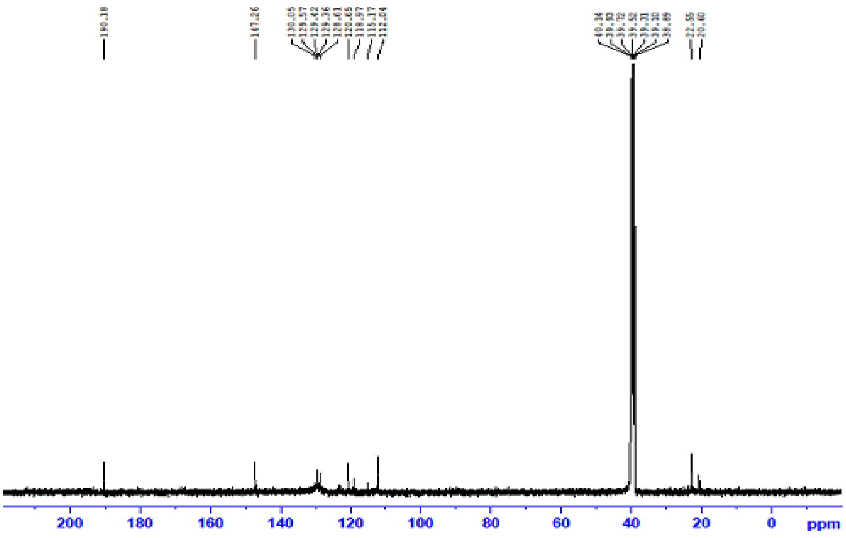


Figure 7.^{13}C NMR of 5,11- dihydro indolo (3,2-b) carboazole.

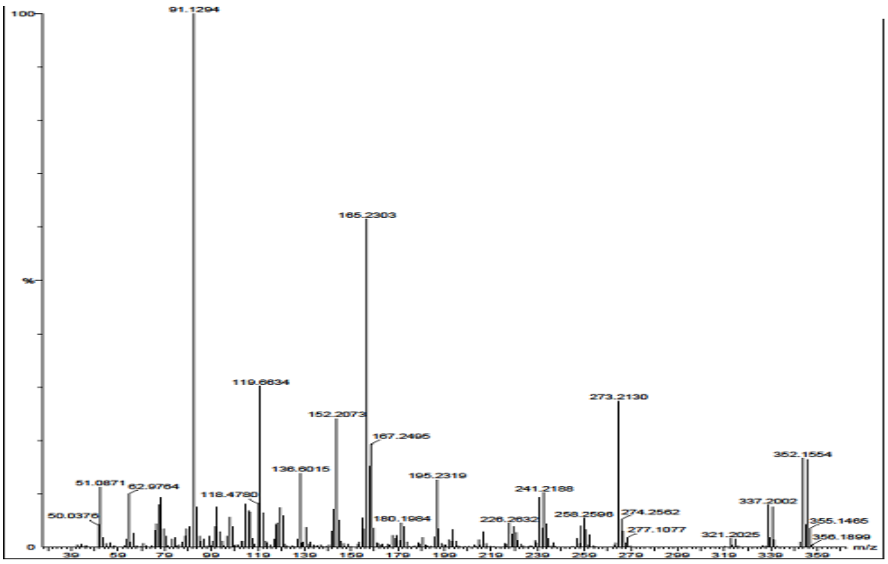


Figure 8. GC MASS spectrum of of 5,11- dihydro indolo (3,2-b) carboazole.

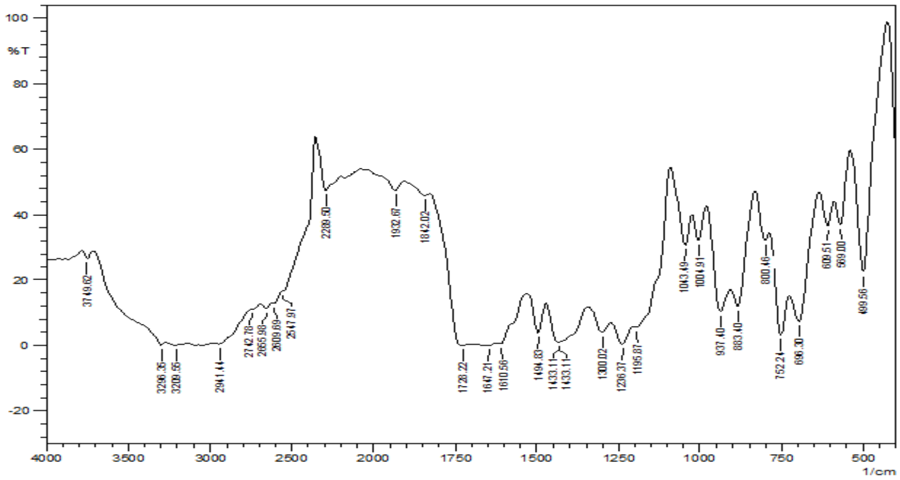


Figure 9. FT-IR spectrum of 9-hydro-4H-furo-diindolo (2,3-b) carbazole.

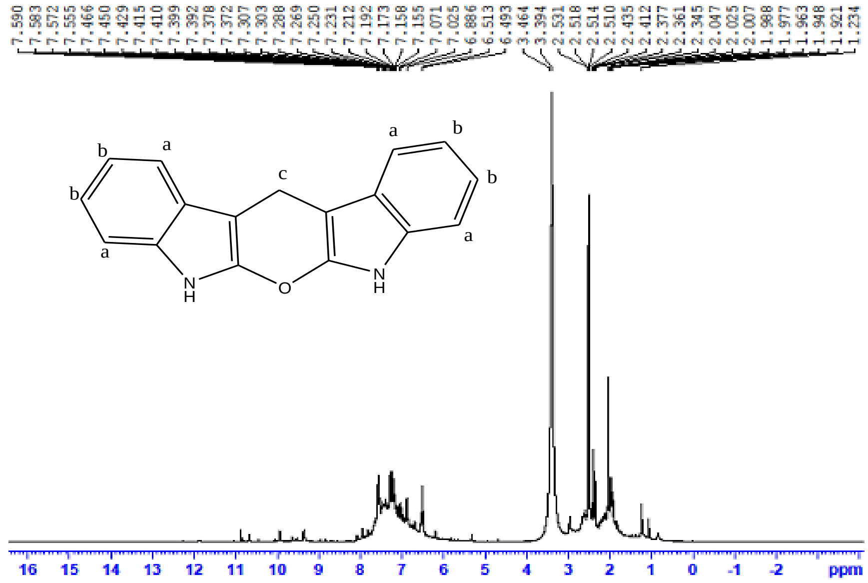


Figure 10.^{1}H NMR of 9-hydro-4H-furo-diindolo (2,3-b) carbazole.

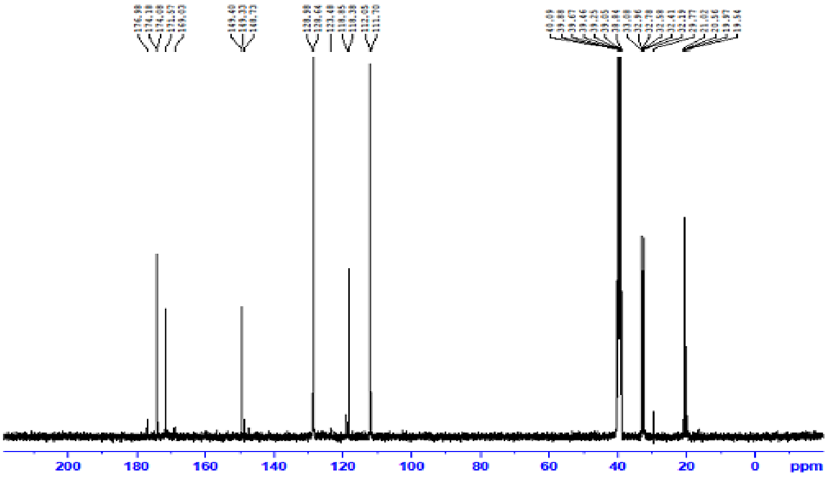


Figure 11.^{13}C NMR of 9-hydro-4H-furo-diindolo (2,3-b) carbazole.

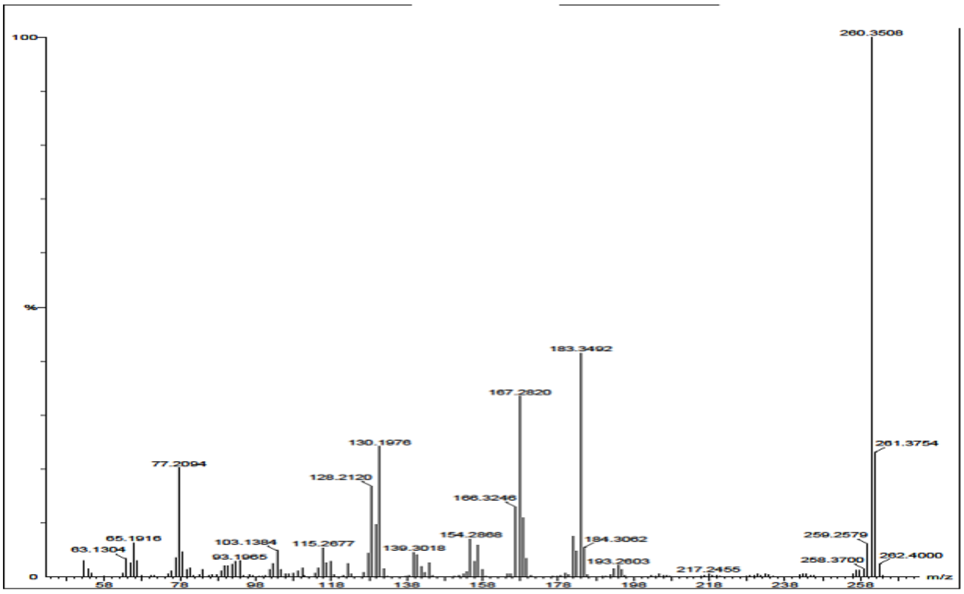


Figure 12. GC MASS spectrum of of 9-hydro-4H-furo-diindolo (2,3-b) carbazole.

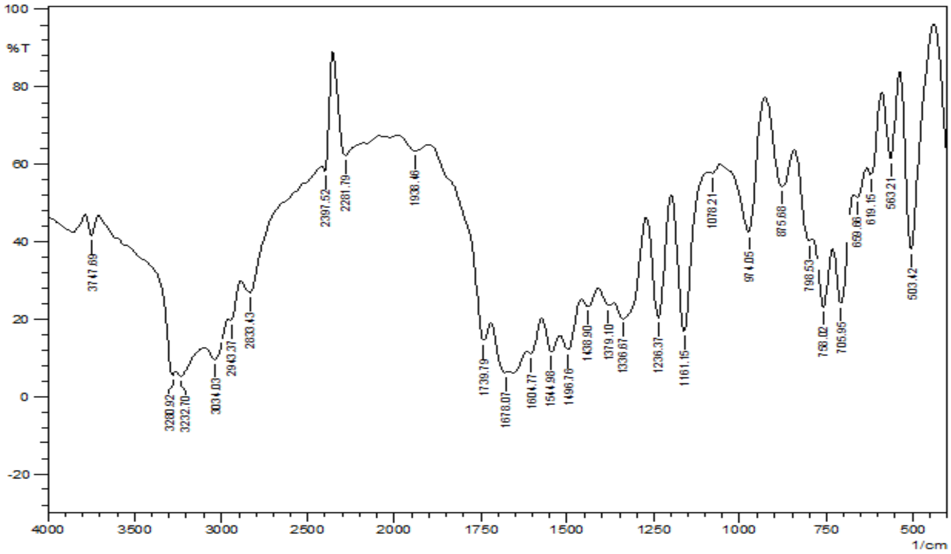


Figure 13. FT-IR spectrum of 3H-thiazolo(4,5-b) indol-2(4H)-one.

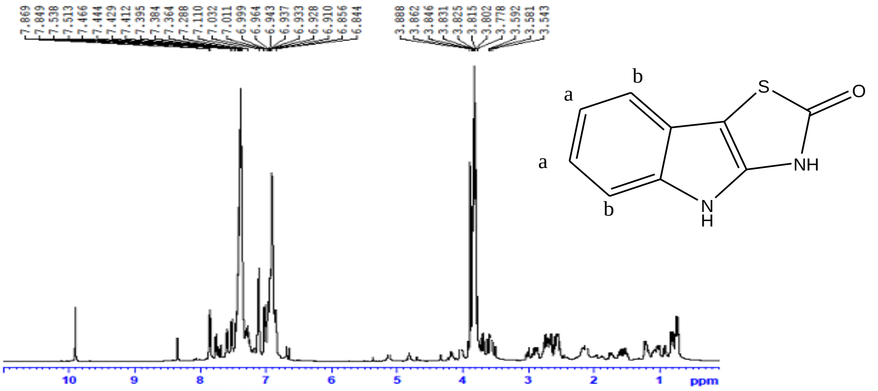


Figure 14.^{1}H NMR of 3H-thiazolo(4,5-b) indol-2(4H)-one.

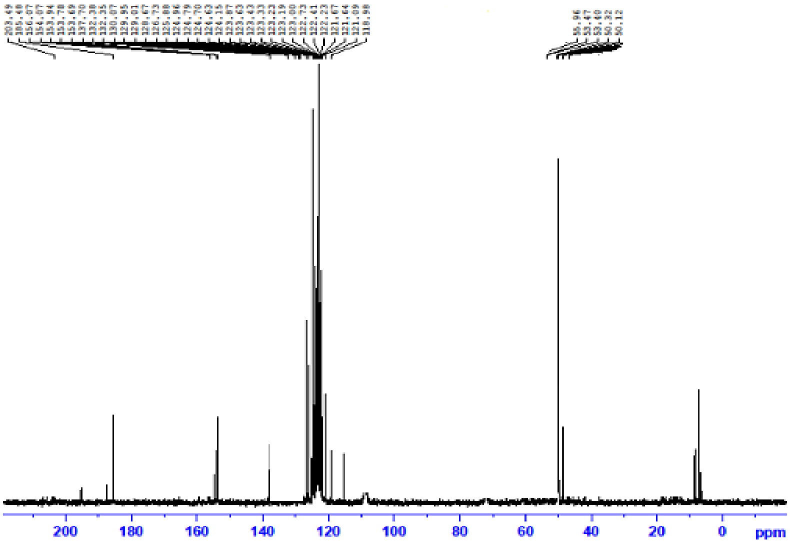


Figure 15. ^{13}CNMR of 3H-thiazolo(4,5-b) indol-2(4H)-one.

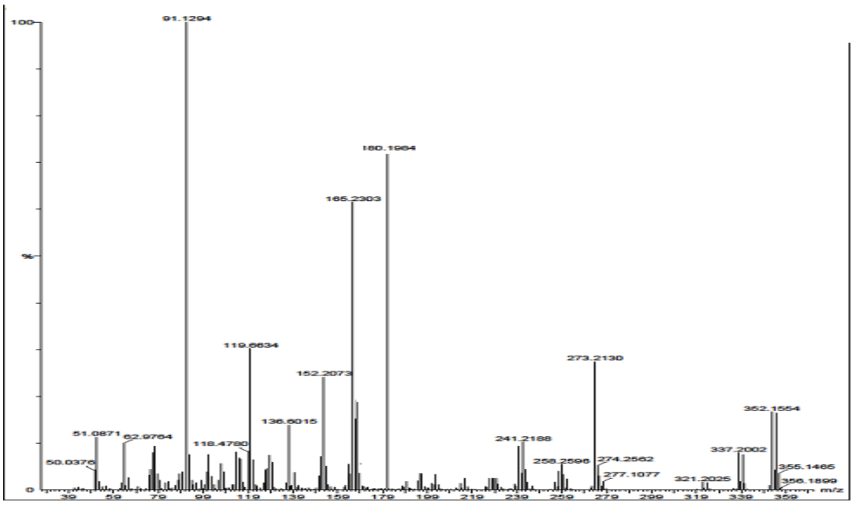


Figure 16. GC MASS spectrum of 3H-thiazolo(4,5-b) indol-2(4H)-one.

The ^{13}C NMR spectra of compound **A** have shown in Figure **3.** In ^{13}C spectra signals at around 110 ppm-129 ppm confirms the presence of aromatic carbons. The carbon present at the condensed position appeared at 133 ppm and 135 ppm. The carbon signal observed at 145 ppm and 138ppm corresponds to carbons which were near to nitrogen atom. The aliphatic carbon signals appeared at 21.9 ppm and 19.2 ppm**.** The mass spectrum of the compound was assigned to the molecular ion peak at m/z 218 have shown in Figure **4**.

COMPOUND – B

Figure **5** shows the FT-IR spectrum of Compound B. The sharp intensity band at 3745cm^{-1} was observed due to the N-H stretching. The medium band at 3062cm^{-1} were assigned to the aromatic =C-H stretching vibration. The sharp band appeared at 2927cm^{-1} and 2856cm^{-1} was associated with aliphatic C-H stretching vibration. The ^{1}H NMR spectrum of compound B have shown in Figure **6**. The chemical shift at 9.626 ppm was due to the N-H proton. The singlet peak appeared at 7.96 ppm corresponds to aromatic proton. The multiplet signal between 6.688 - 7.648 ppm due to aromatic protons.

Figure **7** shows the^{13}C NMR spectrum of compound B. The signals appeared in the range 115 -143 ppm was attributed to the eighteen aromatic carbon atoms. The chemical shift value 139 ppm and 143 ppm corresponds to the carbon neighbouring to nitrogen atom.

The molecular ion peak of the compound-3 was observed M/Z 260 (m+). The mass spectra of diindolo(3,2-b) carbazole was shown in Figure **8**.

COMPOUND C

The FTIR spectrum of compound C have shown in Figure **9**. The sharp intensity band at 3749 cm^{-1} was observed due to the N-H stretching vibration. The medium band at 3296 cm $^{-1}$ were assigned to the aromatic =C-H stretching vibration. The sharp band appeared at 2941 cm^{-1} was associated the aliphatic C-H stretching vibration. The peak at 2289cm^{-1} was associated the C=C stretching vibration. The ^{1}H NMR spectrum of compound C have shown in Figure **10**. The single appeared at 10.85 ppm was N-H proton. The four-doublet signal for four protons appeared at 9.95 ppm corresponds to '**a**' proton. The four-doublet signal for four protons appeared at 9.36 ppm was represented by '**b**' protons. The multiplet for two protons signal observed at 6.6 -7.65 ppm donated by '**c**' protons. The ^{13}C NMR spectrum of combound D have shown in Figure **11**. The signals appeared in range 111 -176 ppm was attributed to the eighteen aromatic carbons. The chemical shift value 149 ppm and 176 ppm corresponds to the carbon present to nitrogen and oxygen atom.

The molecular ion peak of the compound C was m/z 261 The mass spectrum of 9 hydro -4H furodiindolo (2,3-b) carbazole have shown in Figure **12**.

COMPOUND D

Figure **13** shows the FT-IR spectrum of Compound D. The sharp intensity band at 3280 cm^{-1} was observed due to the N-H stretching. The band at 3034cm^{-1} corresponds to the aromatic =C-H stretching vibration. The peak at 1739cm^{-1} was associated with the C=O stretching vibration. The ^{1}H NMR spectrum of compound D have shown in Figure **14**. The chemical shift at 9.9 ppm and 8.3 ppm might be due to the two N-H protons. The multiplet signal between 6.9-7.3 ppm due to aromatic **a & b** protons.

Figure **15** shows the ^{13}C NMR spectrum of compound D. The signals appeared in the range 118 - 129 ppm was attributed to the aromatic carbon atoms. The chemical shift value 156 ppm and 185 ppm corresponds to the carbon neighbouring to nitrogen atom. The molecular ion peak of the compound-F was observed m/z 181.1 (m+). The mass spectra of compound-F have shown in Figure **16**.

ANTI-MICROBIAL ACTIVITY

The synthesized compounds were tested both antibacterial and antifungal activity.

ANTIBACTERIAL ACTIVITY

The results of antibacterial activity of synthesized compounds **A, B, C & D** have shown in Table **1**. The zone of inhibition was indicated the nature of antibacterial activity. The synthesized compounds were subjected to *Escherichia coli, Bacillus subtilis and staphylococcus epidermidis.* Compound **A** and **C** shows no zone of inhibition against *Bacillus subtilis* indicates that these compounds show no antibacterial activity. Compound **A, B, C & D** found to have good antibacterial activity against all three bacteria. Compound **A** and **C** was observed no zone of inhibition which shows no antibacterial activity against *Bacillus subtilis.* Compound **B** was found to posses' excellent activity against *staphylococcus epidermidis.* Based on the survey of the antibacterial activity, among the synthesized compounds **A, B, C& D** compound **B** found to have excellent activity than the others, due to the two indole N-H present in it. The picture of zone of inhibition have shown in Figure **17**.

ANTIFUNGAL ACTIVITY

The results of antifungal activity have shown in Table **2** and Figure **18**. The synthesized compounds were subjected to *Candida albicans, Aspergillus flavus* and *Aspergillus niger*. Compound C **&** **D** found to have excellent activity against Aspergillus *flavus* and *Aspergillus niger*. Compound **C & D** shows no zone of inhibition against *Candida albicans*. Compound D was observed no zone of inhibition against three fungi.

Finally, the synthesized compounds **A, B, C& D** was found to posses' good antibacterial activity than antifungal activity.

Zone of inhibition of synthesized compounds

TABLE 1 Antibacterial activity of various synthesized compounds

Compound	**ZONE OF INHIBITION**					
	Escherichia coli		***Bacillus subtilis***		***Staphylococcus epidermidis***	
	Mm	%	mm	%	mm	%
Ciprofloxacin	20	100	20	100	28	100
A	13	65	-	-	14	50
B	17	85	10	50	115	410
C	12	60	-	-	12	42.8
D	17	85	7	35	18	64.3

(-) = No antibacterial activity

TABLE 2 Antifungal activity of various synthesized compounds

Compound	**ZONE OF INHIBITION**					
	Candida albicans		***Aspergillus flavus***		***Aspergillus niger***	
	Mm	%	mm	%	mm	%
Amphotericin-B	15	100	30	100	30	100
A	6	40	20	66.6	14	46.6
B	12	80	15	50	12	40
C	-	-	70	233.3	60	200
D	-	-	-	-	-	-

(-) = No antifungal activity

ZONE OF INHIBITION

ANTIBACTERIAL ACTIVITY

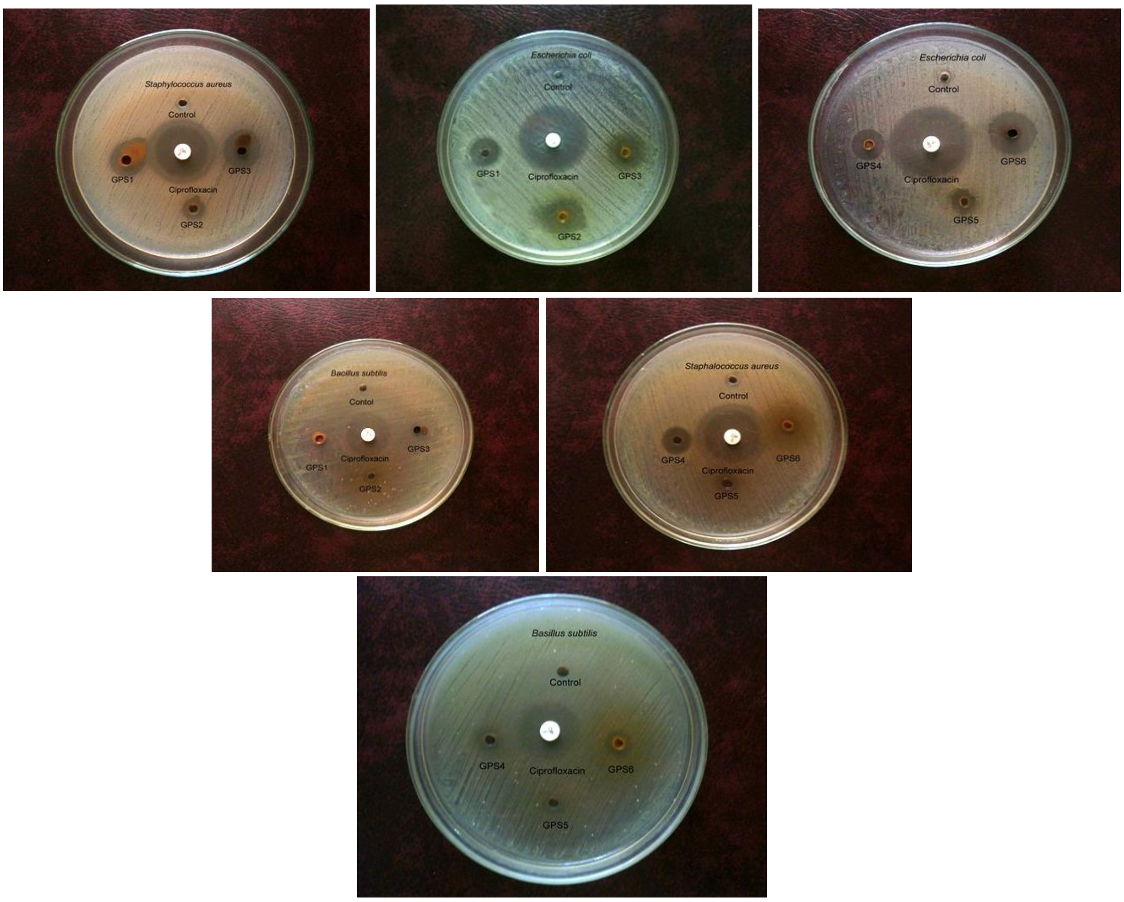


Figure 17. Antibacterial activity of the synthesized compounds A, B, C& D.

ANTIFUNGAL ACTIVITY

Figure 18. Antifungal activity of the synthesized compounds A, B, C & D.

CONCLUSION

Carbazole derivatives **A, B, C** & **D** have been synthesized by simple and easier Fischer indole synthesis with acetic acid. The formation of the various compounds was identified using thin layer chromatography and purified by column chromatography. All the synthesized compounds have been confirmed by various spectral techniques viz FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy.

The synthesized compounds found to have good antibacterial activity than antifungal activity. Among the synthesized compounds **A, B, C** & **D**, Compound **B & D** posses good antibacterial activity because the structure of **B & D** contains both N ,O & S atoms and also contains two indole N-H. Compound **A, B, C** and **D** found to have excellent antifungal activity because the structure of **A** contains benzyl substitution and **B & C** had both N & O atoms including two indole N-H.

CONFLICT OF INTEREST

The authors do not have any conflict of interest from this article.

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