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RESEARCH ARTICLE

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME *O*-CRESOL MANNICH BASES

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Abstract

Three N-Mannich bases have been synthesized by Mannich reaction of *o*-cresol with different secondary amines and aqueous formaldehyde. Using a general synthesis protocol the following Mannich bases were obtained: 2-(diethylaminomethyl)-6-methyl phenyl acetate**(I) ;** 4-(7-methyl-2,3-dihydro-1-benzofuran-3-yl)morpholine (II) ; 1-methyl-4-(7-methyl-2,3-dihydro-1-benzofuran-3-yl)piperazine(III).The constitution of the target molecules have been characterized by using a combination of spectral techniques (UV, IR, ^{1}HNMR and MS). The target molecules were evaluated for their antimicrobial activity against six standard human pathogens using the cup plate agar diffusion bioassay and significant activity was shown by Mannich base (I).

Introduction:-

Mannich reaction is a three-component condensation reaction involving active hydrogen compound, secondary amine and aqueous formaldehyde in ethanolic solution yielding N- Mannich bases(Dolman *et.al.* 2006; Murphy *et.al.*,2007 ; Sujith *et.al*. 2009; Arend *et.al.*1998).

The chemistry of Mannich bases has gained a great deal of attention due to the synthetic potential and the outstanding applications of this class of compounds(Tramontini,1973; Tramontini and Angiolini,1990; Tramontini, and ,Angiolini, 1994) .Two of the most remarkable features of Mannich bases are : their ability to alkylate miscellaneous substrates, and to participate in a large variety of ring closure reactions leading to numerous types of carboxylic acids and heterocyclic compounds(Roman *et.al.*,2004; Roman *et.al*.,2003; Roman *et.al*.2002a; Roman *et.al.*2002b; Roman *et.al.*2001) .

Mannich bases have gained importance due to their application in pharmaceutical chemistry. They have been encountered with antibacterial(Holla *et.al*.1998) , anticancer(Holla *et.al*.2003) , analgesic, anti-inflammatory(Gokee et.al,2005) , anticonvulsant(Dimmock *et.al*.1992) ,antimalarial (Lopes *et.al*.,2004) and antiviral activities(Sriram *et.al.*,2005) .

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The Mannich reaction involving phenols, formalin and primary amines has been used as convenient source for a large array of molecules. The course of this generally facile condensation reaction is, however, greatly influenced by a number of variables(Burke *et.al*.1952a; Burke *et.al*.,1952b). In particular, the size of the *ortho* substituent on the phenol has been shown to play an important role.
As part of our interest in biologically active Mannich bases, this study was designed to synthesize some o-cresol-derived Mannich bases and then screening the target molecules for antimicrobial acivity.To minimize potential side reactions, we initially focused on Mannich type reactions involving secondary amines.

Materials and Methods:-

Analytical grade reagents were used. They were purchased from Sigma- Aldrich Company (UK). Melting points were recorded in open capillary tubes and were uncorrected. The IR spectra were recorded on a Perkin-Elmer FT-IR spectrophotometer using (KBr) disc method .The ^{1}HNMR spectra were recorded on a Brucker AMX (400 MHZ)spectrophotometer using DMSO as a solvent and TMS as internal reference. Mass spectra were run on a Shimadzu GC.M.QP 1000 mass spectrometer at 70 ev.
The purity of the final products was checked by thin layer chromatography (TLC), using ethanol, chloroform and methanol as a mobile phase.

Synthesis of Mannich base I : 2-(diethylaminomethyl)-6-methyl phenyl acetate:-
Formalin (3.2 g, 20 mmol), o- cresol (2.16g, 20 mmol) and diethylamine (1.46g, 20mmol) in 20 ml ethanol were left at room temperature for 10 days. Removal of the solvent under reduced pressure gave the Mannich base.

(2.3g)of Mannich base I were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by (3.4 ml) of acetic anhydride. The mixture was shaked vigorously for 60 seconds. The acetate was separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

Synthesis of Mannich base II : 4-(7-methyl-2,3-dihydro-1-benzofuran-3-yl)morpholine :-
Formalin (3.2 g, 20 mmol), o- cresol (2.16g, 20 mmol) and morpholine (1.74g, 20mmol) in 20 ml ethanol were left at room temperature for 7 days. Removal of the solvent under reduced pressure gave the Mannich base.

Synthesis of Mannich base III : 1-methyl-4-(7-methyl-2,3-dihydro-1-benzofuran-3-yl)piperazine :-
Formalin (3.2 g, 20 mmol), o- cresol (2.16g, 20 mmol) and N-methyl- piperazine (2.0g, 20 mmol) in 20 ml ethanol were left at room temperature for 2 weeks. Removal of the solvent under reduced pressure gave the Mannich base.

Antimicrobial assay:-

Preparation of bacterial suspensions:-
One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^{8}-10^{9}colony forming units per ml.The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.
Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Preparation of fungal suspensions:-
Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity:-
The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antimicrobial activity of the target molecules. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were

divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for a sample. Separate Petri dishes were designed for standard antimicrobial chemotherapeutic agents. (ampicillin , gentamycin and clotrimazole).

The agar discs were removed, alternate cups were filled with (0.1) ml samples using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

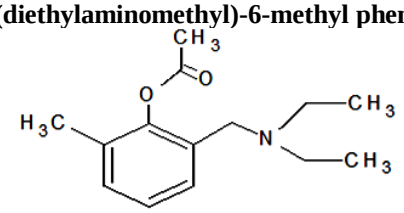
Results and Discussion:-

Herein we have described the synthesis, characterization and antimicrobial activity of the target Mannich bases. The physical data of these bases are displayed in Table 1. The formation of all the target molecules was confirmed by recording their UV, IR, ^{1}HNMR and MS spectra.

Table 1:- Some physical data of synthesized compounds

Compound	Melting point (^{0}C)	Molecular Formula	Color
I	180-181	$C_{14}H_{21}NO_2$	Yellow
II	124-126	$C_{13}H_{17}NO_2$	Pale yellow
III	170-171	$C_{14}H_{20}N_2O$	Yellow

Synthesis of the Mannich base I: 2-(diethylaminomethyl)-6-methyl phenyl acetate:-



(I)

The Mannich base I was synthesized by the reaction of formalin with o-cresol and diethylamine in absolute ethanol.The base was isolated after acetylation. The UV spectrum of base I (Fig.1) showed λ_{max} (MeOH) 236,277 nm.

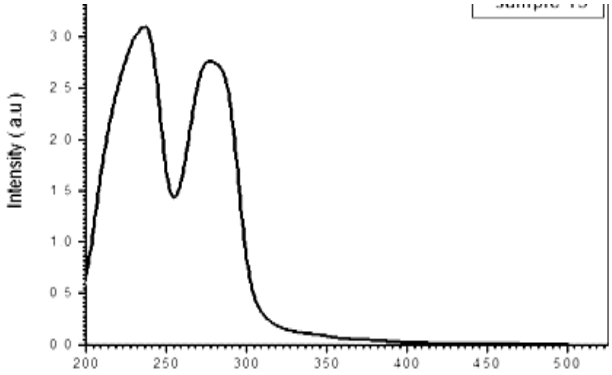


Fig.1:- The UV spectrum of Mannich base I

The IR spectrum (Fig.2) showed ν (KBr): 777, 825, 881 (C-H, Ar.,bending), 1271 (C-N), 1577 (C=C, Ar.), 2933, 2821, 2972 cm^{-1} (C-H aliphatic).

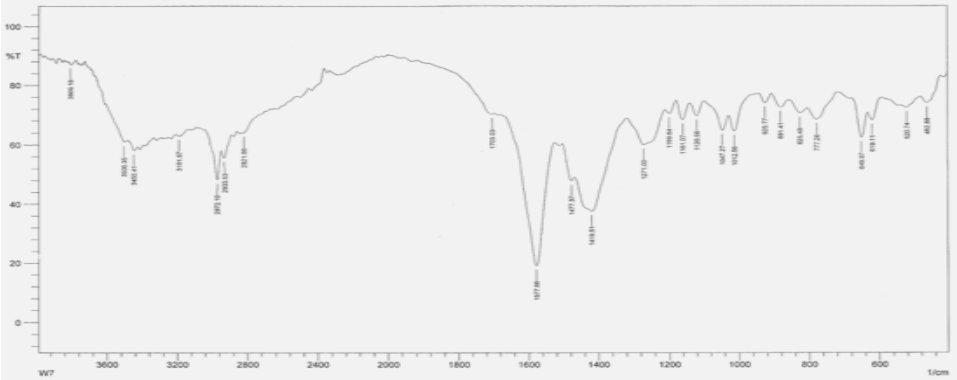


Fig .2:- The IR spectrum of Mannich base I

The ^{1}HNMR spectrum (Fig.3) showed: δ0.90(3H) attributed to a methyl function ; δ1.02(5H), δ1.64 (5H) assigned for two ethyl groups. The resonance at δ2.07 account for a methyl function(shifted downfield by electron withdrawal of a carbonyl function). The aromatic protons appeared as multiplet at : δ6.40-7.00 ppm. The Mass spectrum (Fig.4) gave m/z 235 for M^{+}.

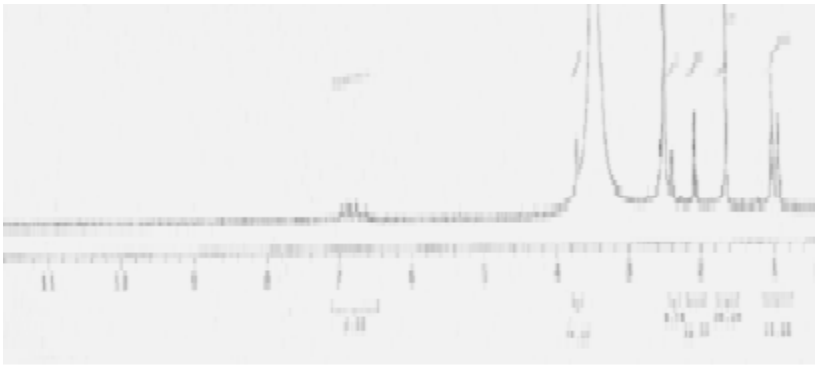


Fig 3:- ^{1}HNMR spectrum of Mannich base I

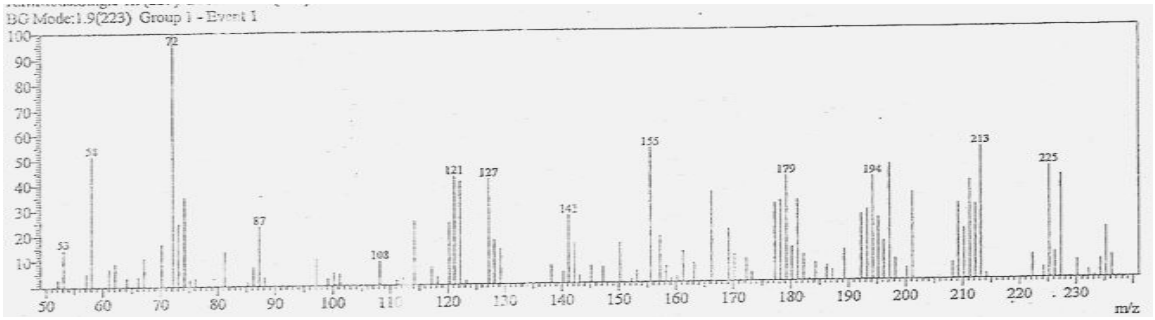
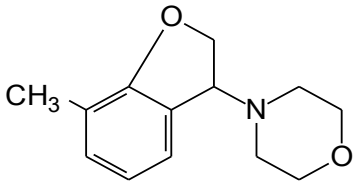


Fig 4:- The Mass spectrum of Mannich base I

On the basis of the above spectral data structure I above was assigned for this Mannich base.

Synthesis of the Mannich base II: 4-(7-methyl-2,3-dihydro-1-benzofuran-3-yl)morpholine :-



(II)

The Mannich base II was synthesized by adding formalin to a mixture of o-cresol and morpholine in absolute ethanol. The UV spectrum of base II (Fig.5) showed λ_{max} (MeOH) 230,274 nm.

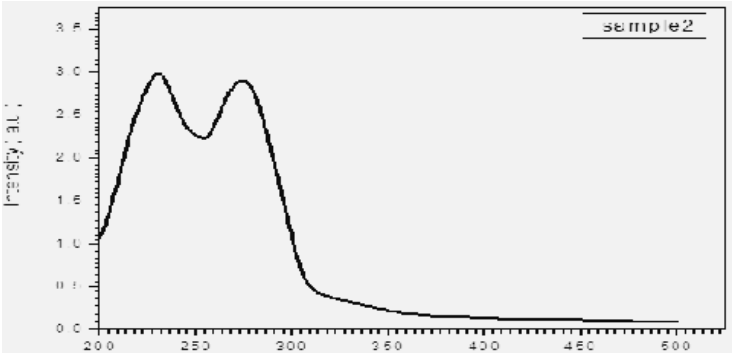


Fig 5:- The UV spectrum of Mannich base II

The IR spectrum (Fig.6) showed ν (KBr): 698, 744, 767, 800,852 (C-H, Ar., bending), 1234 (C-N) ,1515, 1577 (C=C, Ar.) , 2866, 2972 cm^{-1} (C-H aliphatic). .

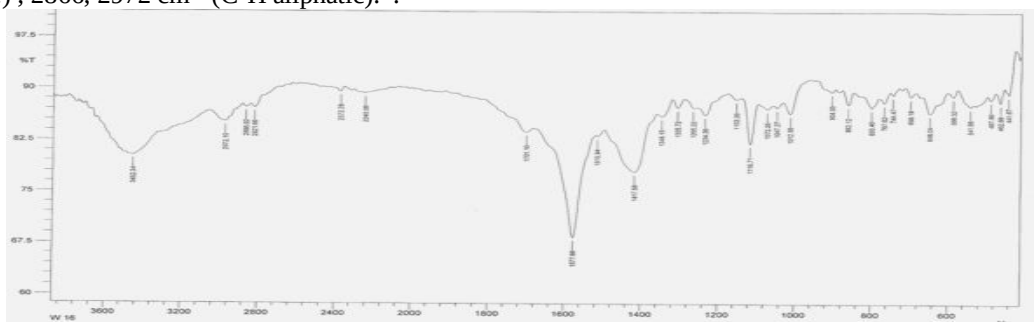


Fig 6:- The IR spectrum of Mannich base II

The ^{1}HNMR spectrum (Fig.7) showed: δ1.64 (3H) assigned for a methyl group.The resonance at δ2.07(4H) accounts for two methylene moieties, while other methylene signals were shifted more downfield(δ 3.40; 6H) due to electron-withdrawal effect of neighboring oxygen .The aromatic protons appeared as multiplet at : δ6.62-7.00 ppm. The Mass spectrum (Fig.8) gave m/z 220 for $M^{+}+H^{+}$.

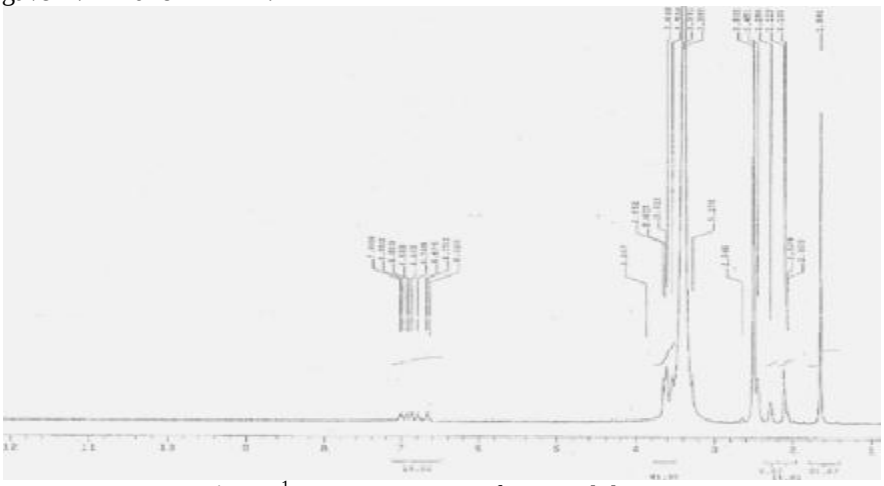


Fig 7: -^{1}HNMR spectrum of Mannich base II

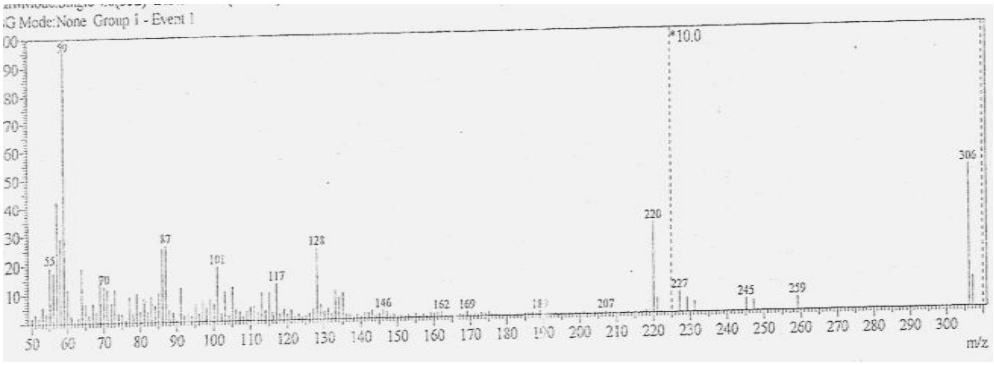
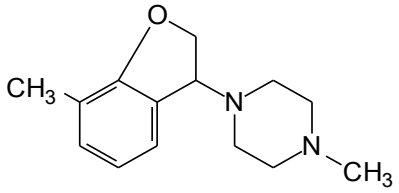


Fig 8:- The Mass spectrum of Mannich base II

On the basis of the above spectral data structure II above was assigned for this Mannich base.

Synthesis of the Mannich base III: 1-methyl-4-(7-methyl-2,3-dihydro-1-benzofuran-3-yl)piperazine :-



(III)

The Mannich base III was synthesized by adding formalin to a mixture of o-cresol and N-methylpiperazine in absolute ethanol. The UV spectrum (Fig.9) showed λ_{max} (MeOH) 203,234,281 nm.

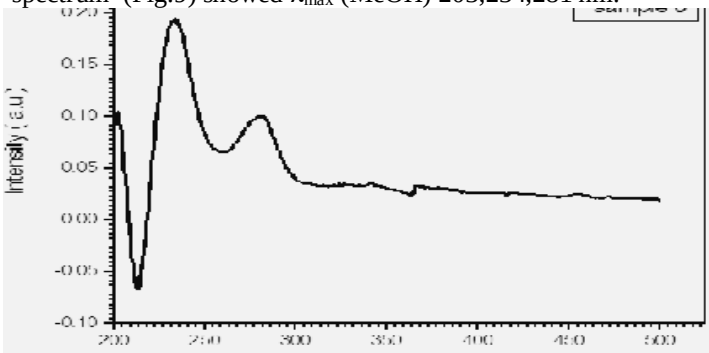


Fig 9:- The UV spectrum of Mannich base III

The IR spectrum (Fig.10) showed ν (KBr): 686,734,761,827,885,901,981(C-H,aromatic bending),1181, 1232, 1276, 1313, 1359(C-N),1467(C=C, Ar.), 2837, 2950 cm^{-1} (C-H, aliphatic).

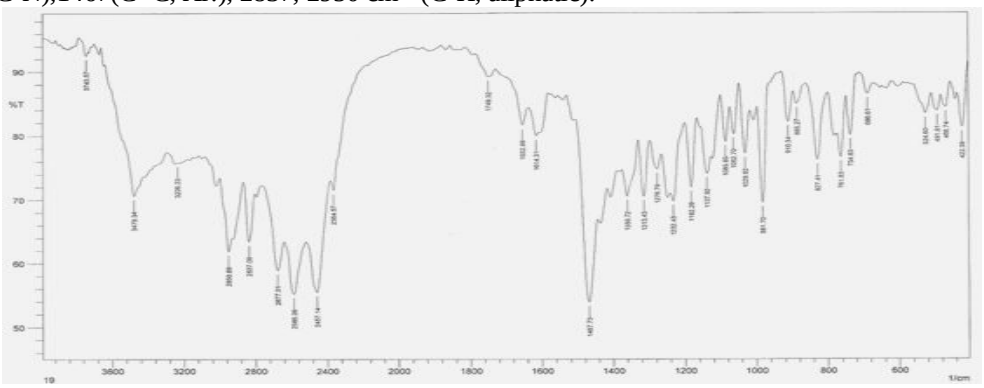


Fig 10:- The IR spectrum of Mannich base III

The ^{1}HNMR spectrum (Fig.11) showed: δ2.13 (6H) assigned for two methyl groups (being shifted downfield by electron withdrawal effects) .The multiplet at δ2.28- 2.49 and the resonance at δ3.05ppm account for five methylenes .A methine signal appeared downfield at δ3.75ppm .The aromatic protons appeared at : δ6.65 and δ7.00 ppm. The Mass spectrum (Fig.12) gave m/z 232 for M^{+}.

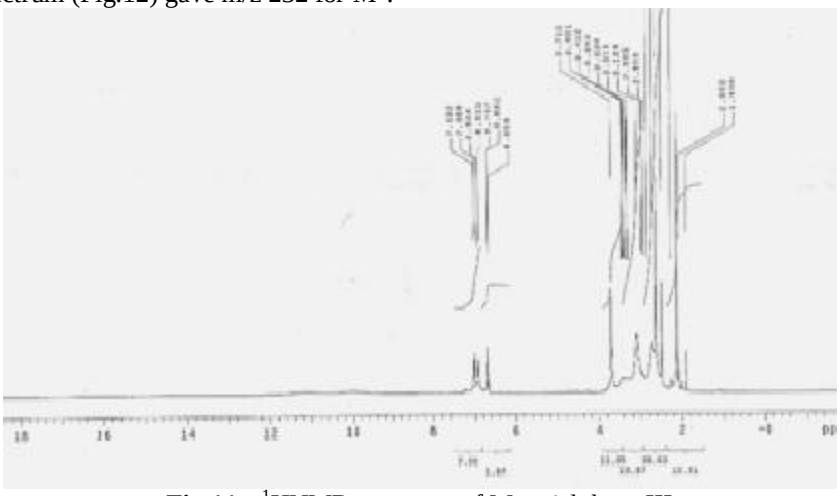


Fig 11:- ^{1}HNMR spectrum of Mannich base III

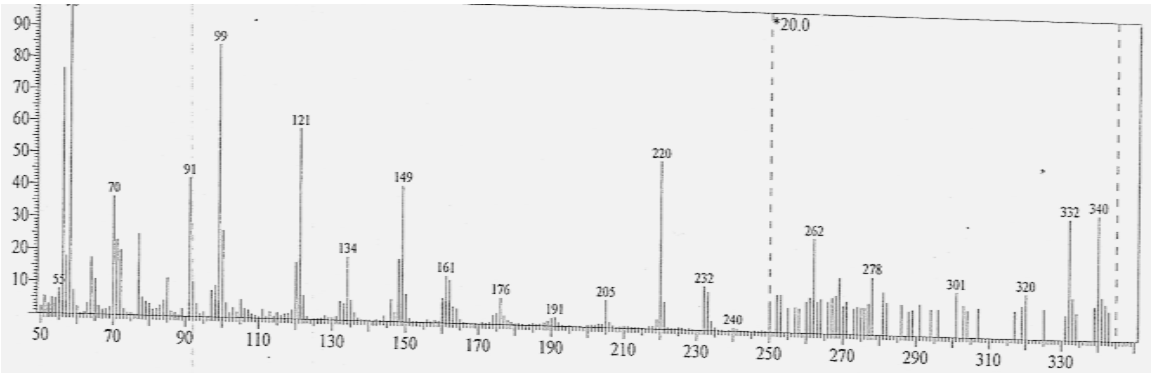


Fig 12:- The Mass spectrum of Mannich base III

On the basis of the above spectral data structure III above was assigned for this Mannich base.

Antimicrobial activity:-

The target molecules were evaluated for their antimicrobial activity using the cup plate agar diffusion method.The average of the diameters of the growth inhibition zones are shown in Table (2) .The results were interpreted in commonly used terms : 13-18mm growth inhibition zones is considered to be active; more than 18mm: very active. Values less than 9 mm indicate inactivity.Values ranging from 9-12 indicate partial activity. Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Compound I showed activity against all test organisms. It showed significant activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Compound II showed activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, but it was inactive against other test organisms. Compound III was only active against *Bacillus subtilis* and *Staphylococcus aureus* (Table 2).

Table 2:- Antibacterial activity of synthesized compounds :M.D.I.Z (mm)

Compd.	Conc.(mg/ml)	Ec	Pa	Sa	Bs	Ca	An
I	20	20	17	18	21	16	15
II	20	-	10	14	-	14	-
III	20	-	-	15	14	-	-

Table 3:- Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Pa
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 4:- Antifungal activity of standard chemotherapeutic agent against standard fungi

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- S.a*: Staphylococcus aureus*
- E.c*: Escherichia coli*
- P.a: *Pseudomonas aeruginosa*
- A.n: *Aspergillus niger*
- C.a: *Candida albicans*
- *B.a: Bacillus subtilis*

Conclusion:-

A series of phenolic Mannich bases were synthesized using *0*-cresol as a substrate. Screening of the synthesized compounds for their antimicrobial activity gave significant activity.

Acknowledgement:-

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