International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X
Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 2; Issue 5; September 2017; Page No. 38-43

Synthesis and characterization of anticandidal, antibacterial mannich bases

[*1] Abdel Karim M, [2] Hala I, [3] Khalid M
[1] Sudan University of Science and Technology, Faculty of Science, Sudan
[2] Jazan University, Faculty of Medicine, Sudan
[3] Omdurman Islamic University, Faculty of Science, Sudan

Abstract
Two Mannich bases were synthesized via a general synthesis strategy: Mannich base: N, N-[(5-acatamido-2-hydroxy) benzyl] acetamide (I) and N-[(5-acatamido-2-hydroxy) benzyl]-N-methylaniline (II). The structures of the target molecules were elucidated by a combination of spectral tools (IR, UV, ^{1}HNMR, and MS). The synthesized compounds were screened for their antimicrobial activity. In cup plate agar diffusion bioassay. Compounds I and II exhibited excellent activity against *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. However, compound II, unlike compound I was inactive against other test organisms.

Keywords: mannich bases, synthesis, antimicrobial activity

Introduction
The Mannich reaction is a single pot three component condensation in which ammonia, a primary or secondary amine reacts with formaldehyde and a compound containing at least one hydrogen atom of pronounced reactivity. The essential feature of the reaction is the replacement of the active hydrogen atom by an aminomethyl or substituted aminomethyl moiety. The product from acetophenone, formaldehyde, and a secondary amine is an example.
Mannich bases are known for their biological potential. Some Mannich bases possess antibacterial activity [1-7]. Some bases were identified as novel potential antimalarial agents [8-11]. Mannich bases with putative cytotoxic activity were reported [12-15]. Some Mannich bases possess anticonvulsant activity while others are claimed to exhibit antiamoebic potency [16, 17]. This study was aimed to the synthesis of two phenolic Mannich bases and evaluation of their antimicrobial potentia.

Materials and Methods
Materials
Analytical grade reagents (Sigma-Aldrich) were used. The UV spectra were recorded on a Perkin-Elmer Lambda 2 UV-Visible spectrophotometer. Infra-red spectra were run on a Perkin-Elmer 1310 Infra-red spectrophotometer.^{1}HNMR spectra were measured on EM-360 NMR spectrophotometer. Mass spectra were recorded on a Krates MS 80 RF mass spectrophotometer. The target molecules were evaluated for their antimicrobial potency against the following bacterial strains:

Table 1: Test organisms

Microorganis	Type	Source
Escherichia Coli	Gram –ve	TCC*25922
Bacillus subtilis	Gram +ve	CTC* 8236
Staphylococcus aureus	Gram +ve	TCC 25923
Pseudomonas aeroginosa	Gram –ve	NCTC6750
Aspergillus Niger	Fungus	ATCC9736
Candida albicans	Fungus	CTC10716

CTC: National Collection of type culture, Colindale England
*ATCC: American type culture collection, Rockville, Maryland, USA

Methods
Synthesis protocols
Synthesis of Mannich base: N, N-[(5-acatamido-2-hydroxy) benzyl] acetamide (I)
Formalin (3.2g, 40mmol) was added dropwise with stirring to a mixture of P-acetamidophenol (6.04g, 40mmol) and acetamide (1.18g, 20mmol) in dioxane (15ml) at 0^0C. The mixture was then stirred for four hours at 0^0C and left overnight. The solvent was removed under reduced pressure to afford the product.

Synthesis of Mannich base: N-[(5-acatamido-2-hydroxy) benzyl]-N-methylaniline (II)
Formalin (1.6g, 20mmol) was added dropwise with stirring to a mixture of P-acetamidophenol (3.02g, 20mmol) and N-methylaniline (2.14g, 20mmol) in dioxane (15ml) at 0^0C. The mixture was then stirred for four hours at 0^0C and left overnight. Removal of the solvent under reduced pressure gave the product.

Antimicrobial assay

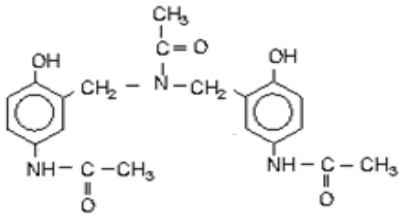
In cup plate agar diffusion bioassay, the synthesized Mannich bases were assessed for antimicrobial activity against six standard pathogenic microbes.

(1g) of the Mannich base was dissolved in 10ml of DMSO to obtain a concentration of 100mg/ml. Diffusion method was the method used for screening the antimicrobial activity. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungi respectively. The media were prepared according to the manufacturer's instructions, sterilized at 121ºC for 15 minutes, poured into sterile Petri dishes and were allowed to cool and solidify. The sterilized media were sealed with 0.1ml of the standard inoculums of the test microbe (Mueller Hinton agar was sealed with the bacteria and Sabouraud dextrose agar sealed with the fungus). The inoculums were spread over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6mm in diameters, a well was cut at the centre of each inoculated medium. (0.1ml) of the test solution was then introduced into the well on the inoculated medium. Incubation of the inoculated medium was made at 37ºC for 24 hours for the bacteria and at 30ºC and for 4 days for the fungus. After incubation each plate of the medium was observed for the growth inhibition zone. The zone was measured and the results were recorded in millimeters

Results and discussion

Two Mannich bases (I and II) were synthesized by a general synthesis protocol using p-acetamidophenol as active hydrogen component. The bases were assessed for antimicrobial activity against six standard human pathogens using the diffusion bioassay.

Mannich base: N, N-[(5-acatamido-2-hydroxy) benzyl] acetamide (I)



I

The Mannich base I was synthesized by adding formalin dropwise to amixture of P-acetamidophenol and acetamide in dioxane at 0ºC. The UV spectrum (Fig.1) showed λ_{max} (MeOH) 249nm which is a characteristic absoption of a carbonyl extended chromophore. The IR spectrum (Fig.2) showed v (KBr) 684, 802, 835 (C- H, Ar, bending), 1170(C-N), 1440, 1508, 1564 (C=C, Ar) 1654(C=O), 2792 (C-H,aliphatic), 3163(N-H), 3325cm^{-1}(OH).

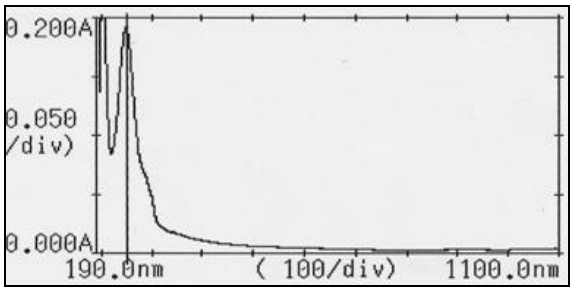


Fig 1: UV spectrum of compound I

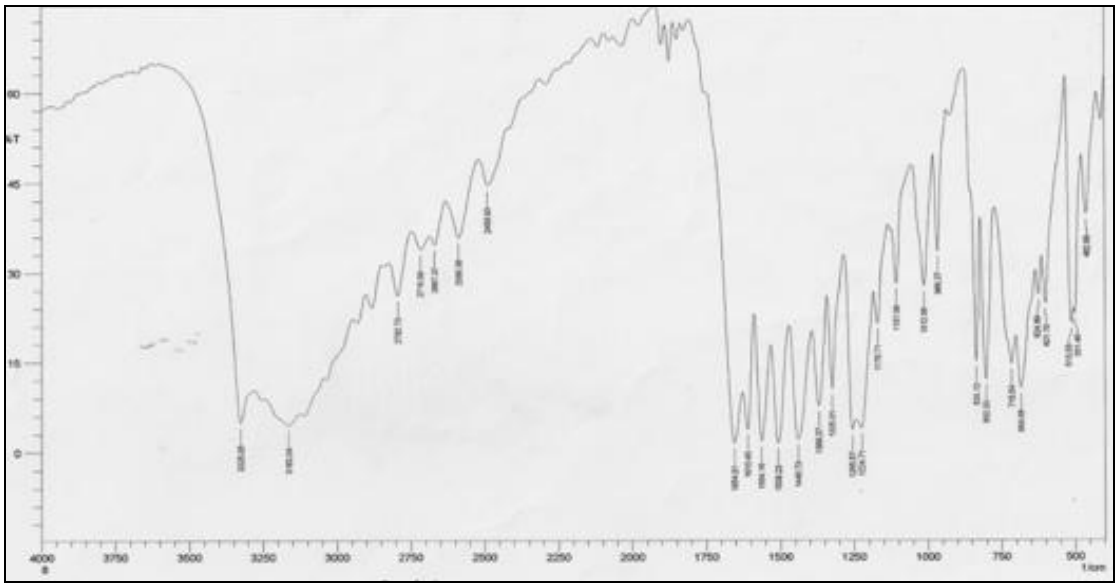


Fig 2: IR spectrum of compound I

The Mass spectrum (Fig.3) showed m/z 385 for M^+.

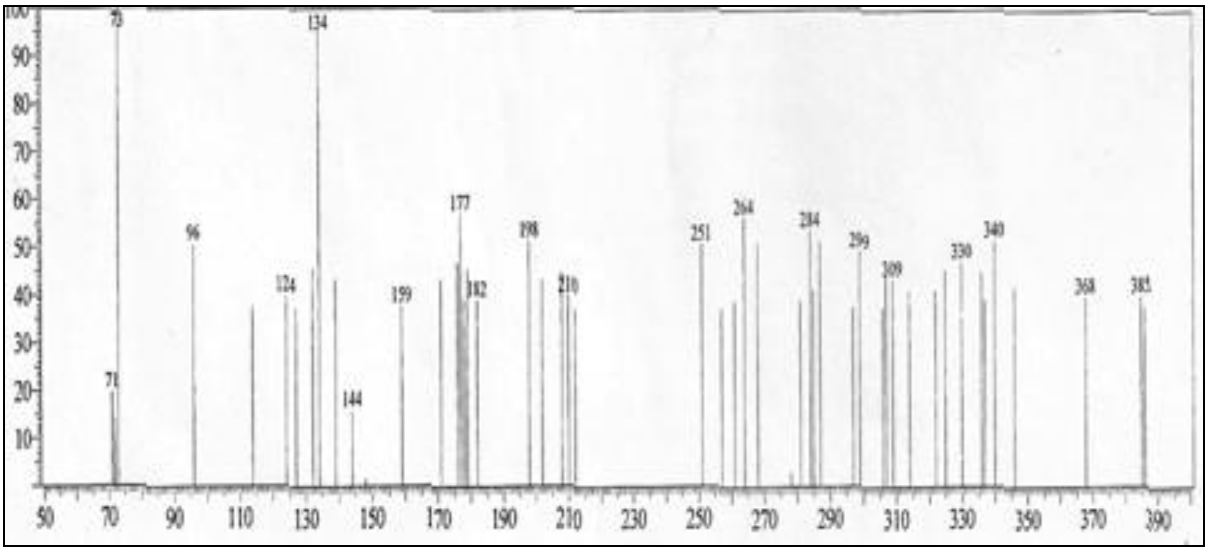


Fig 3: Mass spectrum of compound I

The ^{1}HNMR spectrum (Fig.4) revealed the following signals:

Table 2

δ 1.972	singlet	9H
δ3.40	singlet	4H
δ 6.66	doublet	4H
δ 7.32	doublet	2H
δ9.65	singlet	2H

The signal at δ1.972 (9H) was assigned for three methyl groups of the $-\overset{O}{\overset{\|}{C}}-CH_3$ shifted downfied by the electron-withdrawal influence of the neighboring carbonyl function. The singlet at δ 3.40(4H) corresponds to two methylene groups of the Mannich base being shifted downfield by neighboring nitrogen. The aromatic protons appear as a double doublet at δ6.655 (4H) and δ7.348 (2H) while the NH groups appear as singlet at δ9.65 (DMSO residual protons appear at δ2.50 ppm and its residual water appears around 3.30ppm).

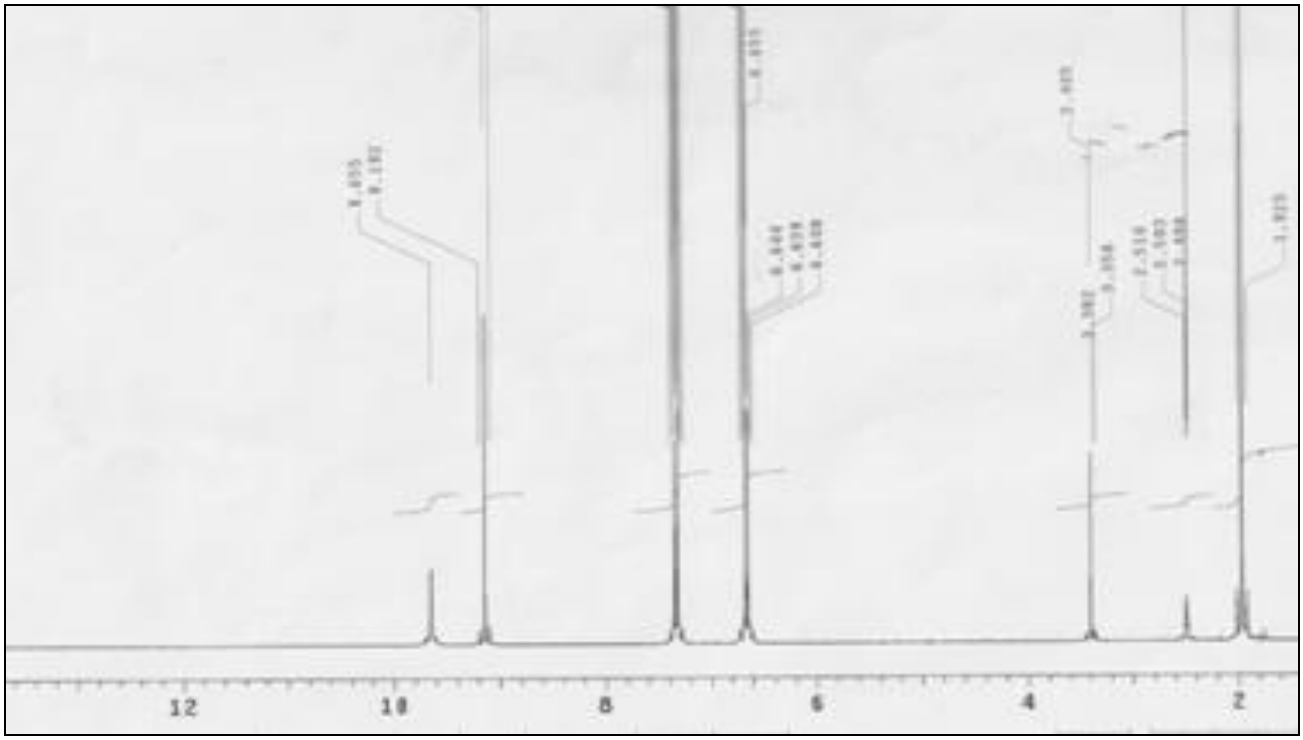
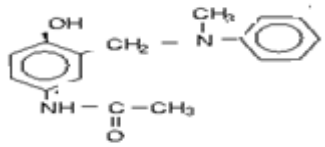


Fig 4: ^{1}H NMR spectrum of compound I

On the basis of the above argument, structure I above was assigned for Mannich base I.

Mannich base: N-[(5-acatamido-2-hydroxy) benzyl]-N-methylaniline (II)



II

The Mannich base II was synthesized by adding formalin dropwise to a mixture of P-acetamidophenol and N-methylaniline in dioxane at 0ºC. The UV sepectum of compound II (Fig.5) showed λ_{max} (MeOH) 249nm which is a characteristic absorption of an extended C=O chromophore.

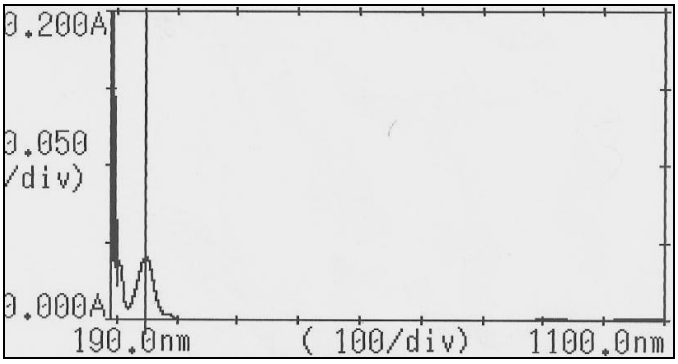


Fig 5: UV spectrum of compound II

The IR spectrum (Fig.6) showed v (KBr): 684,802,835(C-H, Ar., bending), 1172(CN), 1438, 1508, 1562 (C=C, Ar), 1654 (C=O) 2792(C-H, aliphatic), 3163(NH) and 3325cm^{-1} (OH).

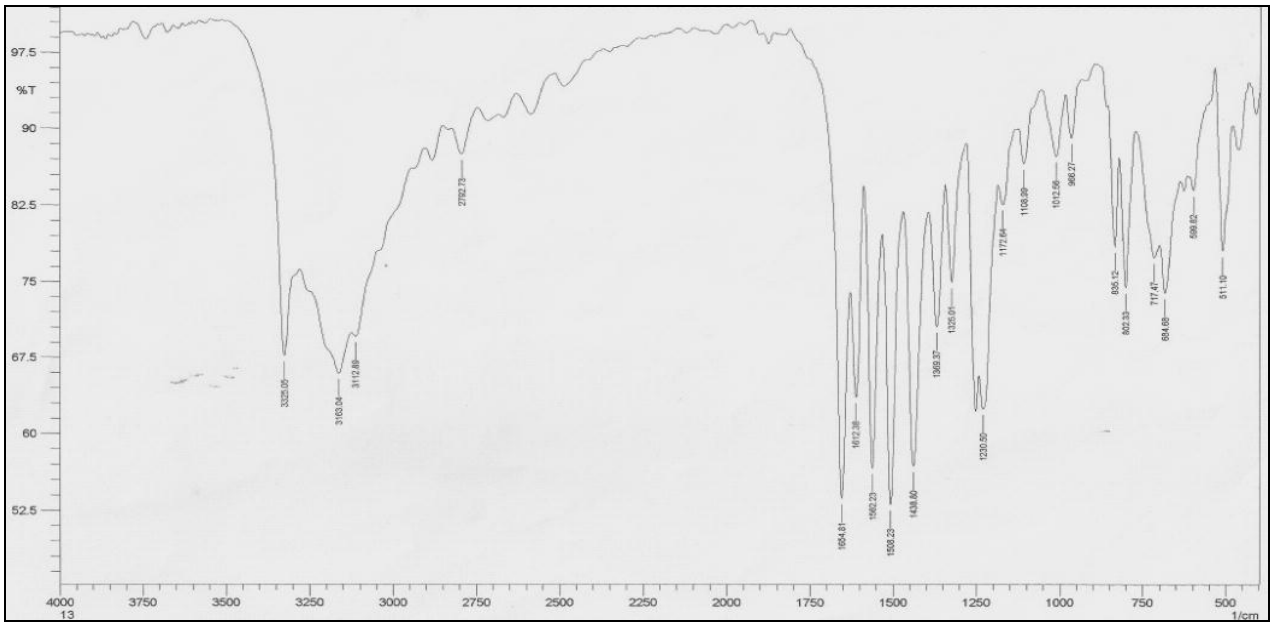


Fig 6: IR spectrum of compound II

The Mass spectrum (Fig.7) gave m/z271 for M^+ +1.

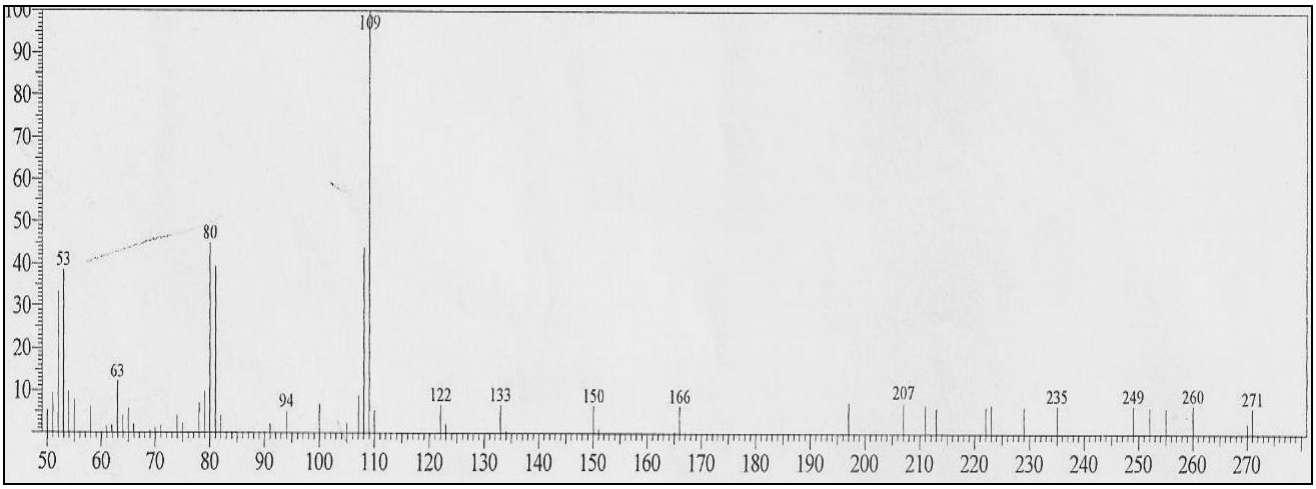


Fig7: Mass spectrum of compound II

The ^{1}H NMR spectrum (Fig.8) revealed the following signals:

Table 3

δ 1.93	singlet	6H
δ3.41	singlet	2H
δ 6.62	doublet	5H
δ7.30	doublet	3H
δ 9.10	singlet	1H
δ 9.65	singlet	1H

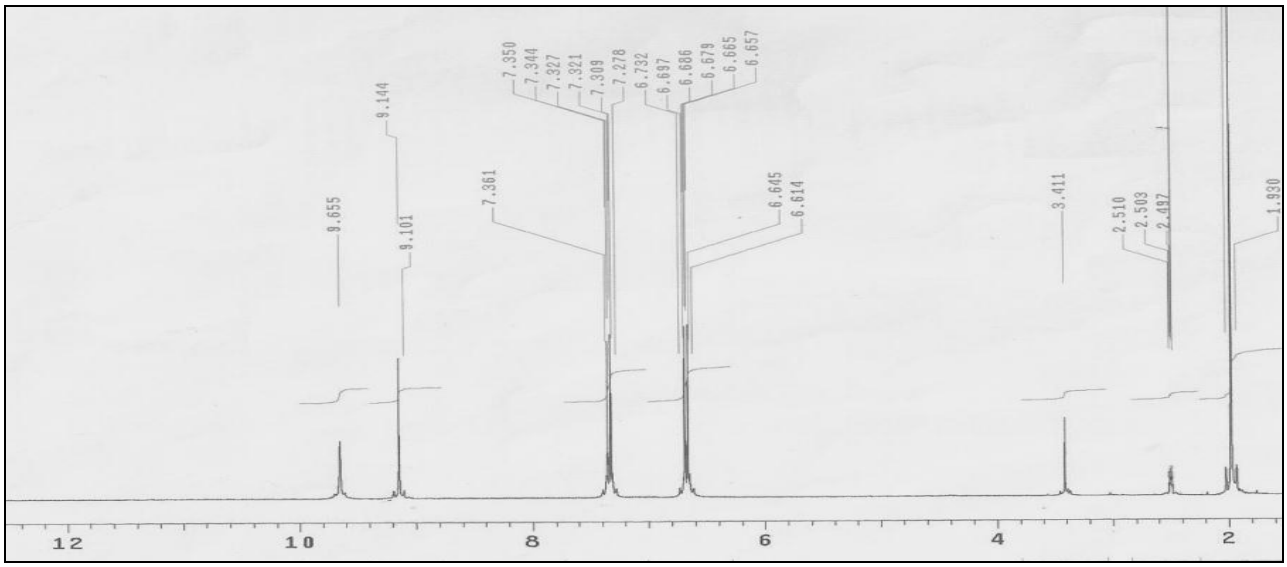
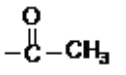
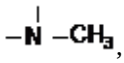


Fig 8: ^{1}H NMR spectrum of compound II

The signal at δ1.93 (6H) was assigned for the two methyl groups of  and  , while the singlet at δ3.411 (2H) is characterstic of the methylene group of the Mannich base. The aromatic protons appear as a double doublet at δ6.62 (5H) and δ7.30 (3H), The singlet at δ9.101 (1H) corresponds to NH group, while the singlet at δ9.655 (1H) corresponds to OH.

On the basis of the above cumulative data, structure II above was proposed for Mannich base II.

Antimicrobial activity

The synthesized Mannich bases were evaluated for their antimicrobial potential using the cup plate agar diffusion bioassay against six standard microbial strains: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus Niger and Candida albicans*.

M.D.I.Z: Mean diameterd of growth inhibition zone (mm), average or two replicates are shown in Table (4). Tables (5) and (6) display the antibacterial and antifungal activities of standard drugs respectively.

Compounds I and II exhibited excellent activity against *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. However,compound II was inactive against other test organisms.

Table 4: Antibacterial activity of Mannich bases I and II

Comp.	Conc.n (mg/ml)	Ec.	Sa.	Bs.	Pa.	Ca.	An.
Comp.I	100	20	15	15	15	18	19
Comp.II	100	20	-	-	-	22	20

Table 5: Antibacterial activity of standard drugs

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
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 6: Antifungal activity of standard drug

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

- Sa*: Staphylococcus aureus*
- Ec*: Escherichia coli*
- Pa: *Pseudomonas aeruginosa*
- Bs: *Bacillus subtilis*
- An: *Aspergillus niger*
- Ca: *Candida albicansd*

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