2.Materials and Methods

2.1- Materials

2.1.1-Chemicals and Solvents

Analytical grade reagents (Table 2.1) were used. They were purchased from Sigma- Aldrich Company (UK).

Table2.1: chemicals and solvents

Ser.No.	Chemicals	Molecular formula
1	Diethylamine	$CH_{11}N$
2	Dimethylamine	C_2H_7N
3	Piperidine	$C5H_{11}N$
4	Morpholine	C_4H_9NO
5	N- Methylpiperazine	$C_5H_{12}N_2$
6	N- Ethylpiperazine	$C_6H_{14}N_2$
7	Dipropylamine	$C_6H_{15}N$
8	Dibenzylamine	$C_{14}H_{15}N$
9	Phenol	C_6H_5OH
10	O-cresol	C_7H_8O
11	B- Naphthol	$C_{10}H_7OH$
12	2-Aminophenol	C_6H_7NO
13	Formalin	CH_2O
14	Acetic anhydride	$C_4H_6O_3$
15	Absolute ethanol	CH_3CH_2OH
16	Hydrochloric acid	HCl
17	Chloroform	$CHCl_3$
18	Methanol	CH_3OH
19	Petroleum ether	C_6H_{14}
20	Ethyl acetate	$C_4H_8O_2$
21	Sodium hydroxide	NaOH

2.2 Methods

2.2.1- Synthesis of Mannich base 1

Formalin (3.2 g, 20 mmol), β- naphthol (2.88 g, 20 mmol) and piperidine (1.68 g, 20 mmol) in 20 ml ethanol were left at room temperature for 7 days. Removal of the solvent under reduced pressure gave the Mannich base.

2.2.2- Synthesis of Mannich base 2 : Formalin (3.2 g, 20 mmol), β-naphthol (2.88 g, 20 mmol) and diethylamine (1.46g, 20 mmol) in 20 ml ethanol were left at room temperature for 7 days. Removal of the solvent under reduced pressure gave the Mannich base.

1.78g of Mannich base was suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 2.48 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by the addition of hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.3- Synthesis of Mannich base 3 : Formalin (3.2 g, 20 mmol), β-naphthol (2.88 g, 20 mmol) and dimethylamine (0.9g, 20 mmol) in 20 ml ethanol was left at room temperature for 6 days. Removal of the solvent under reduced pressure gave the Mannich base.

2.2.4- Synthesis of Mannich base 4: Formalin (3.2 g, 20 mmol), β-naphthol (2.88 g, 20 mmol) andmorpholine (1.74 g, 20 mmol)in 20 ml ethanol was left at room temperature for 10 days. Removal of the solvent under reduced pressure gave the Mannich base.

(2.94g)of Mannich base were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 4.4 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.5- Synthesis of Mannich base 5: Formalin (3.2 g, 20 mmol), β- naphthol (2.88 g, 20 mmol) and N- ethylpiperazine (2.0 g, 20 mmol)in 20 ml ethanol was left at room temperature for 5 days. Removal of the solvent under reduced pressure gave the Mannich base.

(2.94g)of Mannich base were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 4.4 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.6- Synthesis of Mannich base 6: Formalin (3.2 g, 20 mmol), β- naphthol (2.88 g, 20 mmol) and dibenzyl amine (3.9g, 20 mmol) in 20 ml ethanol was left at room temperature for 7 days. Removal of the solvent under reduced pressure gave the Mannich base.

2.2.7- Synthesis of Mannich base 7: Formalin (3.2 g, 20 mmol), phenol (1.88g, 20 mmol) and diethyl amine (1.46g, 20 mmol) in 20 ml ethanol were left at room temperature for 10 days. Removal of the solvent under reduced pressure gave the Mannich base.

(3.08g)of Mannich base were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 4.4 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.8- Synthesis of Mannich base 8: Formalin (3.2 g, 20 mmol), phenol (1.88g, 20 mmol) and dimethyl amine (0.9g, 20 mmol) in 20 ml ethanol wereleft at room temperature for 7 days. Removal of the solvent under reduced pressure gave the Mannich base which treated with ethyl acetate for solidification.

2.2.9- Synthesis of Mannich base 9: Formalin (3.2 g, 20 mmol), phenol (1.88g, 20 mmol) and morpholine (1.74g, 20mmol)in 20 ml ethanol wereleft at room temperature for 7 days. Removal of the solvent under reduced pressure gave the Mannich base.

(2.21g)of Mannich base were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 4.4 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.10- Synthesis of Mannich base 10: Formalin (3.2 g, 20 mmol), phenol (1.88g, 20 mmol) and dibenzylamine (3.9g, 20mmol)in 20 ml ethanol were left at room temperature for 6 days. Removal of the solvent under reduced pressure gave the Mannich base.

2.2.11- Synthesis of Mannich base 11: Formalin (3.2 g, 20 mmol), *o*-cresol (2.16g, 20 mmol) and diethyl amine (1.46g, 20mmol)in 20 ml ethanol were left at room temperature for 10 days. Removal of the solvent under reduced pressure gave a Mannich basewhich solidified on treatment with ethyl acetate.

(2.3g)of Mannich base were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 4.4 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.12- Synthesis of Mannich base 12: 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 10 days. Removal of the solvent under reduced pressure gave the Mannich base.

(2.4g)of Mannich base were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 4.4 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.13- Synthesis ofMannich base 13: Formalin (3.2 g, 20 mmol), *o*- cresol (2.16g, 20 mmol) and N-methylpiperazine (2.0g, 20 mmol)in 20 ml ethanol wereleft at room temperature for 2 weeks. Removal of the solvent under reduced pressure gave the Mannich base.

(2.60g)of Mannich base were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 4.4 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.14- Synthesis of Mannich base 14: Formalin (3.2 g, 20 mmol), 2-amino phenol (2.2g, 20 mmol) and dimethylamine (0.9g, 20mmol)in 20 ml ethanol were left at room temperature for 2 weeks. Removal of the solvent under reduced pressure gave the Mannich base.

(3.11g)of Mannich base were suspended in 5 ml (3M) NaOH solution. Crushed ice was added followed by 4.4 ml of acetic anhydride. The mixture was vigorously shacked for 60 seconds. The acetate separated after acidification by hydrochloric acid. The acetyl derivative was collected and recrystallized from dilute ethanol.

2.2.15- Synthesis of Mnnich base 15: Formalin (3.2 g, 20 mmol), 2-amino- phenol (2.2g, 20 mmol) and dipropylamine (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.

2.2.16- Synthesis of Mannich base 16: Formalin (3.2 g, 20 mmol), 2-amino phenol (2.2g, 20 mmol) and di- benzyl amine (3.9g, 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.

2.2.17-Synthesis of Mannich base 17: Formalin (3.2 g, 20 mmol), β-naphthol (2.88 g, 20 mmol) and N- ethyl piperazine (2.28g, 20 mmol)in 20 ml ethanol were left at room temperature for 3 weeks. Removal of the solvent under reduced pressure gave the Mannich base.

2.2.18-Biological Activity

2.2.18.1 Preparation of bacterial suspension

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubation at 37˚ C for 24 hours. The bacterial broth was harvested and washed off with 100 ml sterile normal saline to opacity of matched barium chloride turbidity (standard). The suspension was stored in the refrigerator at 4˚ C till used.

2.2.18.2- Preparation of fungal suspension

The fungal cultures were maintained on sabouraud dextrose agar, incubated at 25˚C for 4 days. The fungal growth was harvested and washed off with 100 ml sterile normal saline and the suspension was stored in the refrigerator at 4˚ C till used.

2.3.18.3- Testing for antibacterial activity

The cup-plate agar diffusion method was adopted with some minor modification to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8 - 10^9 C.F.U/ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45˚ C. (20 ml aliquots of this inculcated nutrient agar were distributed into sterile petri-dishes). The agar was left to settle and in each of

these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer and agar discs were removed. Alternate cups were filled with 0.1 ml of compounds which dissolved in DMSO (0.1g of compound / 1ml of solvent) using automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37˚ Cfor 18 hours. After incubation the diameters of the resultant growth inhibition zones were measured.

2.2.18.4- Testing for antifungal activity

The same method as for bacteria was adopted. Instead of Muller Hinton agar, Sabouraud dextrose agar was used. The medium was incubated at 25 C for two days for*Candida Albicans*and three days for *Aspergillum Niger*.