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Synthesis, Characterization and Anti-Microbial Activity of Indole Derivatives

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ABSTRACT

Indoles are probably the most widely distributed heterocyclic compound in nature. Tryptophan and essential amino acid as such is constituent of most proteins. Starting materials were identified by physical, chromatographic and spectral analysis. For substituted isonitroso acetanilide 3.6 gm (0.05M) of chloral hydrate and 48 ml of purified water was taken in it. Then 44 gm of crystallized anhydrous sodium sulfate was added in it and a solution of substituted aniline (0.05 M) in 12 ml of water with 1.7 ml (0.052M) of concentrated hydrochloric acid was added to dissolve the amine, and finally, a solution of 4.5gm (0.158M) of hydroxylamine hydrochloride in 20 ml of water.for preparation of substituted isatin from substituted isonitroso acetanilide 32.5 ml of concentrated sulfuric acid was warmed upto 50°C in a 100 ml round bottom flask with continuous stirring, and 7.5 gram of (0.046 M) of dry substituted isonitroso acetanilide was added to such a rate that to keep the temperature 60-70 but not higher. All synthesized final products were screened for *in vitro* antibacterial activity [13-19, 20-22] against four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus, Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282). in-vitro antimicrobial activity with the zone Inhibition in mm 24±2 and activity index 0.89, against *Staphylococcus aureus*, 22±2 and 0.85 against *Staphylococcus pyogenes,* 26±2 and 0.96 against *Pseudomonas aeruginos*, 25±3 and 0.86 against *Escherichia coli* and two fungal strains shown 26±4 and 0.81 against *Candida albicans* and 14±2 & 0.88 against *Aspergilla niger* respectively.

Keywords: Indole, Isatin, Antibacterial activity, *Staphylococcus aureus.*

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1. INTRODUCTION:

Indole and its derivatives have occupied a unique place in the chemistry of nitrogen heterocyclic compounds. The indole derivatives were known for their dying properties. Many compounds of indole derivatives having the structural resemblance to the ancient dye indigo are known in the literature. A large number of naturally occurring compounds, like alkaloids, were found to possess indole nucleus [1, 2, 3-4]. This synthetic technique is based on the empirical observation that some organic reactions proceed much faster and with higher yields under microwave irradiation as compared to conventional heating. In many cases reactions that normally require many hours at reflux temperature under classical conditions can be completed within several minutes or even seconds in a microwave oven.

Isatin or 1*H*-indole-2, 3-dione is an indole derivative (figure 1). The compound was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo dye by nitric acid and chromic acids [5].

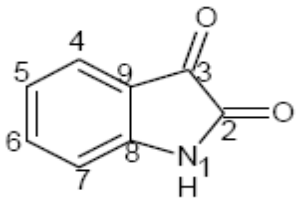


Figure 1: Isatin or 1*H*-indole-2, 3-dione or indole derivative

1.1 Indole Derivative

Protonation, nitration, sulfonation, acylation, halogenations, formation of various metal complexes etc. It gives electrophilic as well as nucleophilic reactions. Indoles are probably the most widely distributed heterocyclic compound in nature. Tryptophan and essential amino acid as such is constituent of most proteins. In animals serotonins (5-HT), melatonin are also indole derivatives. These are widely used e.g. sumatryptan, for the treatment of migraine, ondansetron for the suppression of nausea and alosteron for treatment of irritable bowel syndrome. In plant kingdom tryptophan

derived substances are also useful e.g., indole-3-yl acetic acid is a plant growth hormone. Vincristine, a dimeric indole alkaloid is still extremely important in treatment of cancer. Brassinin, isolated from turnips is a phytoalexin. It prevents plants from microbial attack. The physiological activities of LSD are well known and in synthetic field use of indomethacin in treatment of rheumatoidarthritis explains why indole and its derivatives is still a very interesting molecule since its synthesis in 1866. [6-10].

2. MATERIALS & METHOD

Starting materials were identified by physical, chromatographic and spectral analysis. The chemical structures of the synthesized compounds were established on the basis of physical, chemical, analytical data. The purification of the compounds was carried by purification methods like recrystallization. Physical constant like melting point, boiling point etc, of the new compounds were determined. The purity and progress of the reactions were monitored by TLC, and column chromatography (if needed) by using suitable solvents and UV, FTIR, NMR, CHN analysis and MASS spectral data were used for the characterization of the synthesized compounds by sending the sample to various advanced research laboratory.

2.1 Identification of Starting Materials:

***2.1.1 Physical appearance*:**

Starting materials were inspected visually for physical appearance. It was physically characterized on the basis of organoleptic properties like color and odor.

2.1.2Melting points and/or Boiling point:

Melting points of starting materials (e.g. aniline, 4 methyl aniline, 4 chloro aniline, 4 bromo aniline, 4 nitro aniline) were determined by using a digital capillary melting point apparatus (Cambell Electronics, Bombay, India) by capillary fusion method.

2.1.3 Thin layer chromatography:

All the starting materials (e.g. aniline, 4 methyl aniline, 4 chloro aniline, 4 bromo aniline, 4 nitro aniline) were further identified and confirmed by Thin Layer Chromatographic (TLC) study on readymade TLC plate in Toluene + methanol (95:5)V/V mixed solvent system and R_f values were matched with earlier reported values [11-12].

2.1.4 Ultraviolet spectrum:

20 mg of all the starting materials were dissolved individually in a 100 ml of respective solvent system. Then from this solution 10 ml was taken and volume was made up to 100 ml with respective solvent system, to make the solution concentration of 20µg/ml & the resulting solution was scanned between 200-600 nm using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan).

2.2 Synthesis of Substituted-Indole-2, 3 dione (Isatin) and Final Products:

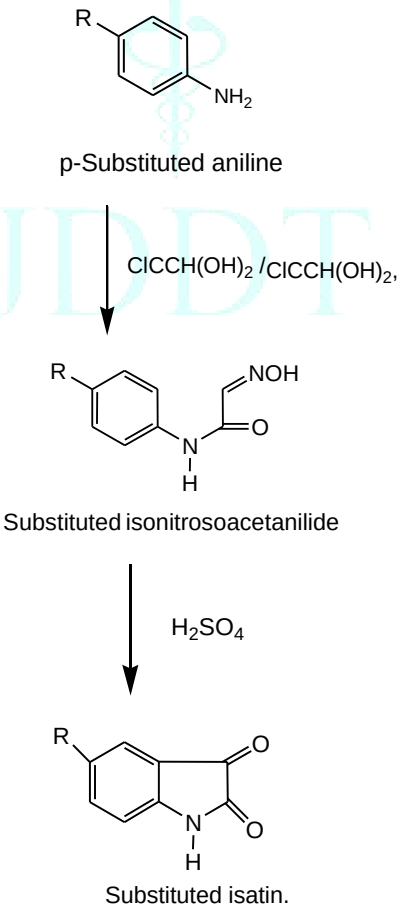


Figure 2: Substituted-1-(4-substituted benzyl) indole-2,3-dione

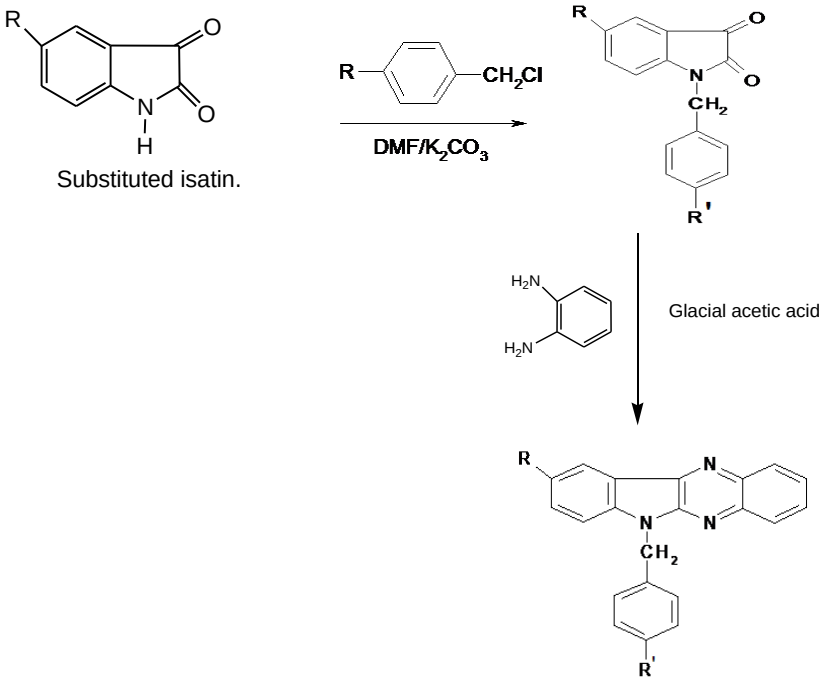


Figure 3: Scheme for synthesis of Substituted-1- (4-substituted benzyl)-1H- indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione from substituted aniline

Compound	R	R′
QX1	Br	F
QX2	CH_3	F
QX3	CH_3	CH_3
QX4	Cl	CH_3
QX5	Br	CH_3

2.3 Synthetic Procedure:

2.3.1 Preparation of substituted isonitroso acetanilide from p-substituted aniline:

100 ml round bottom flask was taken and 3.6 gm (0.05M) of chloral hydrate and 48 ml of purified water was taken in it. Then 44 gm of crystallized anhydrous sodium sulfate was added in it and a solution of substituted aniline (0.05 M) in 12 ml of water with 1.7 ml (0.052M) of concentrated hydrochloric acid was added to dissolve the amine, and finally, a solution of 4.5gm (0.158M) of hydroxylamine hydrochloride in 20 ml of water. The flask was heated over a heating mantle, so that vigorous boiling begins in about 40-45 minutes. After 1-2 minutes of vigorous boiling the reaction completes and during the heating period, some crystal of substituted isonitroso acetanilide separates. The final solution was cooled down under the running water and crystallized material was filtered on suction, and air dried.

2.3.2 Preparation of substituted isatin from substituted isonitroso acetanilide:

32.5 ml of concentrated sulfuric acid was warmed upto 50°C in a 100 ml round bottom flask with continuous stirring, and 7.5 gram of (0.046 M) of dry substituted isonitroso acetanilide was added to such a rate that to keep the temperature 60-70 but not higher. External cooling was applied at this stage to carry out the reactions more rapidly. After the addition of the substituted isonitroso acetanilide compound was finished, the solution was heated to 80°C and kept at this temperature for about 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured to 10-12 times its volume of cracked ice. After standing for about one and half hour the substituted isatin was filtered with suction, washed several times to cold water to remove the sulfuric acid, and then dried in the air. For purification the dried substituted isatin was dissolved in the 50 ml of hot water and suspension was made, to this hot reaction mixture added the solution of sodium hydroxide (5gm in 10 ml) till the complete dissolution of the substituted isatin. The resulting clear solution was neutralized slowly with dilute hydrochloric acid. Filtered the solution and made the solution acidic with the dilute hydrochloric acid. Cooled the solution and the crystals of different substituted isatins were separated. Filtered the product and dried in oven. The yield values and melting points were recorded of different substituted isatins like unsubstituted Isatin, 5-Chloro isatin, 5-Bromo isatin, 5-Methyl isatin and 5-Nitro isatin, and all the values were compared with reported values.

2.3.3 Method of preparation of substituted N-benzyl indole-2,3-dione from substituted isatin:

In the round bottom flask take indole-2,3-dione (isatin) 0.00337 M and equimolar quantity of benzyl chloride mixed with 20 ml of dimethyl formamide (DMF) and to this mixture added 2 gm of potassium carbonate. After gentle mixing of this reaction mixture, reflux for 2 hour, cooled and poured to 100 ml of ice water cold water. The resultant precipitate collected washed with water and dried and recrystallised from ethanol-water mixture. Dried and checked the melting point.

2.3.4 Method for preparation of substituted 1-benzyl-1H-indolo (2,3-b) quinoxaline FROM substituted N-benzyl indole-2,3-dione:

To the orange colored 1- (4 - substituted benzyl) - 1,3 - di hydro – indole - 2, 3-dione (1gm) equimolar quantity of orthophenylenediamine and 0.50 ml of glacial acetic acid was added and refluxed in 100ml of ethanol for two hours on water bath. The initial Colored solution slowly changes in to some fluffy solid crystals in the end of the reaction, which was verified by TLC on silica plates. Excess ethanol was removed and after drying, the compound purified by ethanol.

2.4 Microbiological Evaluation of Final Products:

All synthesized final products were screened for *in vitro* antibacterial activity [13-19, 20-22] against four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus, Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282).

All those compounds screened for antibacterial activity were also tested for their antifungal activity by using Agar diffusion cup plate method. The fungi employed for screening were: Aspergillus Niger and Candida albicans. All compounds subjected for antimicrobial activity was performed by two different concentrations and standard drugs like Ciprofloxacin and Fluconazole were used for comparison purpose respectively.

2.4.1 Preparation of Culture Media:

Nutrient broth was used as growth medium for bacteria and Saubouraud dextrose broth for fungi. Nutrient broth was prepared by dissolving 12gm of dehydrated powder (HI-media) in 100ml of purified water. Saubouraud dextrose broth was prepared by dissolving 4gm of dextrose and 1gm of peptone in 100ml of distilled water. The media were sterilized by autoclaving at 15lbs pressure for 20 minutes.

2.4.2 Preparation of Stock Culture:

Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100ml of sterile broth and incubated for 24 hours at 37^0C for bacteria and 24^0C for fungi.

2.4.3 Standardization of Stock Culture:

Stock cultures were placed in the incubator (37^0C for bacteria and 24^0C for fungi) and shaken well. 1ml of stock cultures was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed with using a cyclomixer and serially diluted from 10-1 to 10-10. From each dilution, 0.2ml was taken and spread on sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi, which were incubated for 18 hours. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum dilute tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15 × 108 cfu/ml.

2.4.4 Preparation of Working Stock Culture:

Stock culture (0.1ml) was diluted with nutrient broth (100ml) and Sabouraud dextrose broth (100ml) respectively to obtain 105 cfu/ml. This was then used for further *in vitro* screening.

2.4.5 Preparation of Drug Dilutions:

Solutions of the title compounds in DMSO (1mg/ml) were prepared and used for screening their antimicrobial activity.

2.4.6 Preparation of discs:

Discs of 5-6 mm in diameter were punched from No. 1 Whattmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C for 60 minutes. Standard and test solutions were added separately to these discs which were air dried later on.

2.4.7 Diffusion Test:

A filter-paper disk impregnated with the compound to be tested was placed on the surface of the agar carefully by using sterilized forceps. The compound diffused from the filter paper into the agar. The larger the clear area around the filter disk, the more effective was regarded the compound. These petridishes were kept up to one hour for diffusion at room temperature and then for incubation at 37°C for 24 hours in an incubator. The zones of inhibition after 24 hours were measured in millimeters. The size of the zone of inhibition was measured as a determination of compound's effectiveness.

2.5 MIC:

2.5.1 Antimicrobial Screening:

Synthesized products were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique. Test was carried out on four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus, Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282).

2.5.2 Determination of MIC:

The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in triplicate. To the first assay tube, 1.8ml of seeded broth and 0.2ml of title compound (1mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37^0C and 24^0C respectively for 24 hours for bacteria and fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC).

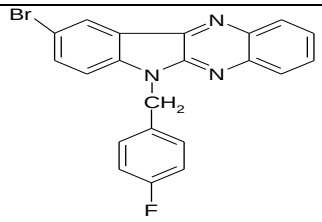
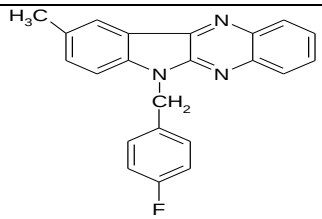
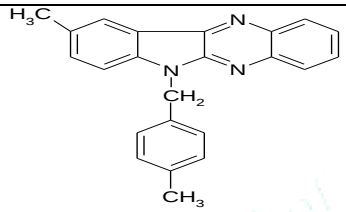
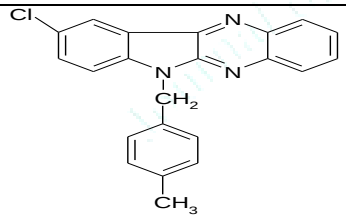
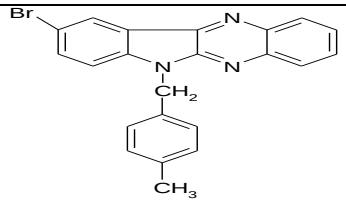
3. RESULT AND DISCUSSION:

3.1 Identification and Characterization of the Synthesized Compounds:

The identification and characterization [8-20] of the compound were carried out by the following procedure to establish the structure and chemical nature of recently synthesized compounds as Physical appearance, Melting points & boiling points, Thin layer chromatography, Ultraviolet spectrum, FTIR (FT-Infra red spectroscopy), NMR (1H NMR, 13C NMR), FAB – MS, Elemental CHN analysis.

3.2 Identification and Characterization of the Intermediate:

Table 1: Physical appearance, % yield, and molecular weight with formula of final synthesized compounds

Sl. No.	Ingredients	Compound code	Physical appearance	% Yield	Molecular Formula	Molecular Weight
1.		QX1	Yellowish solids	83±2.06	$C_{21}H_{13}BrFN_3$	406.13
2.		QX2	Yellowish solid	79±3.04	$C_{22}H_{16}FN_3$	341.25
3.		QX3	Yellowish solid	78±2.46	$C_{23}H_{19}N_3$	337.41
4.		QX4	Brownish solid	80±3.94	$C_{22}H_{16}ClN_3$	357.82
5.		QX5	Brownish solid	80±4.14	$C_{22}H_{16}N_3Br$	402.28

3.3 Microbiological Evaluation:

3.3.1 Antibacterial activity:

Table 2: Zone of inhibition of the synthesized compounds

Compound / Bacterial strain	Antibacterial Activity: Zone of inhibition (mm)							
	Gm +ve				Gm -ve			
	Staphylococcus aureus		*Staphylococcus pyogenes*		*Escherichia coli*		*Pseudomonas aeruginosa*	
Concentration (µg/ml)	100		100		100		100	
IZ and AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
QX1	24±2	0.89	22±2	0.85	25±3	0.86	26±2	0.96
QX 2	14±1	0.52	15±1	0.58	16±2	0.55	14±1	0.52
QX 3	-	-	-	-	-	-	-	-
QX 4	22±2	0.81	18±3	0.69	22±2	0.76	23±3	0.85
QX 5	20±2	0.74	18±2	0.69	21±2	0.72	22±3	0.81
Ciprofloxacin	27±3	-	26±3	-	29±4	-	27±4	-

All values are Mean±SD; n=3; IZ= Inhibition zone in mm (mean value; include 6 mm diameter of disc), AI= Activity Index (IZ developed by synthesized compound /IZ developed by standard), (-) = No activity

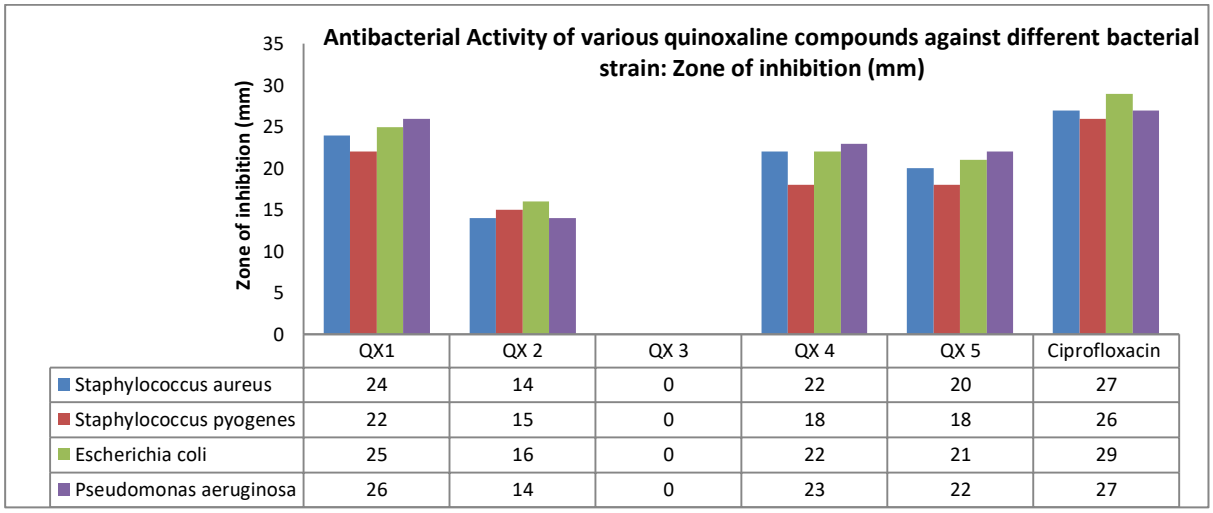
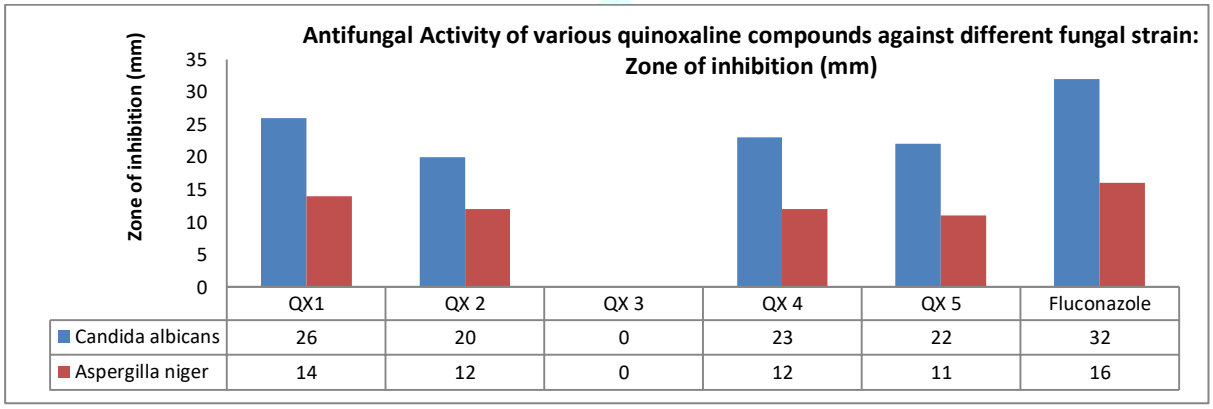


Table 3: Antifungal activity: Zone of inhibition of the synthesized compounds

Compound / Bacterial strain	Zone of inhibition: Antifungal activity			
	Candida albicans		*Aspergilla niger*	
Concentration (µg/ml)	100		100	
IZ and AI	IZ	AI	IZ	AI
QX1	26±4	0.81	14±2	0.88
QX 2	20±2	0.63	12±1	0.75
QX 3	-	-	-	-
QX 4	23±3	0.72	12±1	0.75
QX 5	22±3	0.69	11±1	0.69
Fluconazole	32±4	-	16±2	-

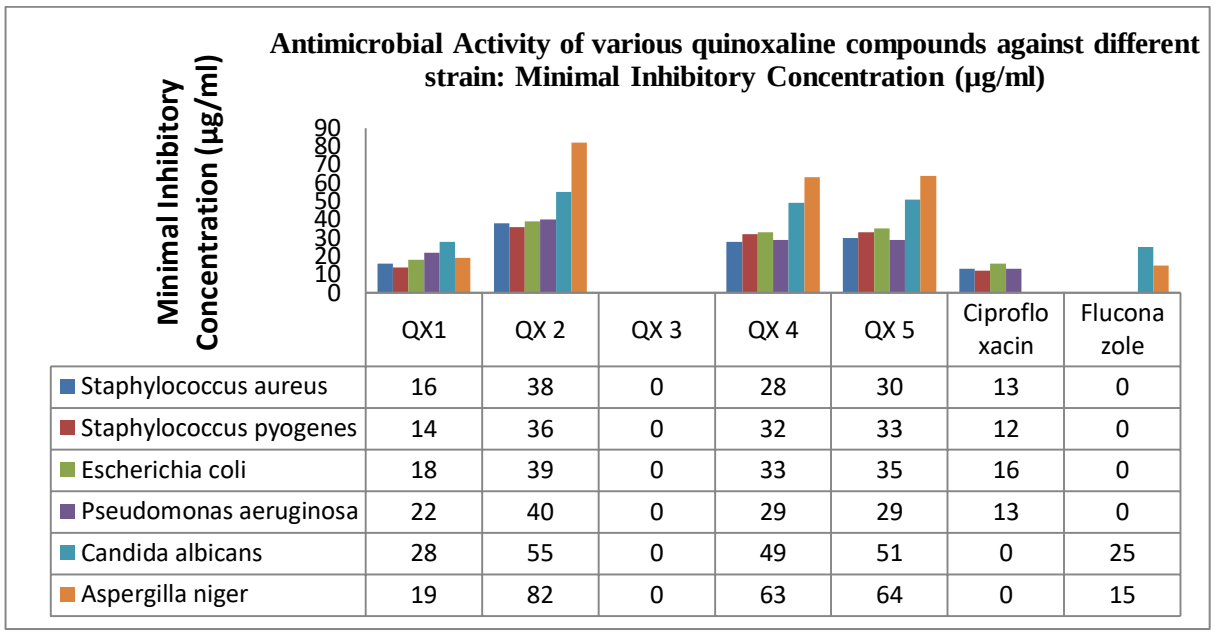
All values are Mean±SD; n=3; IZ= Inhibition zone in mm (mean value; include 6 mm diameter of disc), AI= Activity Index (IZ developed by synthesized compound /IZ developed by standard), (-) = No activity



3.3.2 Determination of MIC:

Table 4: Antimicrobial activity (MIC) of standard & synthesized compounds containing indole ring

Compound / Strain	Minimal Inhibitory Concentration (µg/ml)					
	Antibacterial Activity				Antifungal activity	
	Gm +ve		Gm -ve			
	Staphylococcus aureus	*Staphylococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergilla niger*
QX1	16	14	18	22	28	19
QX 2	38	36	39	40	55	82
QX 3	-	-	-	-	-	-
QX 4	28	32	33	29	49	63
QX 5	30	33	35	29	51	64
Ciprofloxacin	13	12	16	13	NT	NT
Fluconazole	NT	NT	NT	NT	25	15



	QX1	QX 2	QX 3	QX 4	QX 5	Ciproflo xacin	Flucona zole
Staphylococcus aureus	16	38	0	28	30	13	0
Staphylococcus pyogenes	14	36	0	32	33	12	0
Escherichia coli	18	39	0	33	35	16	0
Pseudomonas aeruginosa	22	40	0	29	29	13	0
Candida albicans	28	55	0	49	51	0	25
Aspergilla niger	19	82	0	63	64	0	15

4. CONCLUSION:

QX1 was found to exhibits the most potent in-vitro antimicrobial activity with the zone Inhibition in mm 24±2 and activity index 0.89, against *Staphylococcus aureus*, 22±2 and 0.85 against *Staphylococcus pyogenes,* 26±2 and 0.96 against *Pseudomonas aeruginos*, 25±3 and 0.86 against *Escherichia coli* and two fungal strains shown 26±4 and 0.81 against *Candida albicans* and 14±2 & 0.88 against *Aspergilla niger* respectively. These compounds were screened for their antibacterial activity. The minimum inhibitory concentrations (MICs) of the compounds were determined by agar streak or adopting serial dilution method. Among the synthesized compounds; QX1 was found to exhibits the most potent in-vitro antimicrobial activity with the MICs of 16, 14, 22 and 18 µg/ml against *Staphylococcus aureus, Staphylococcus pyogenes, Pseudomonas aeruginosa, and* Escherichia *coli* respectively. Compound Bromo-1-(4-fluro methyl benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione (QX1) was found to exhibit the most potent in-vitro anti-fungal activity with MICs value of 28 and 19 µg/ml against *Candida albicans* and *Aspergilla niger*. All substituted quinoxaline compounds have been screened for their antimicrobial activity. From the screening results it was observed that the presence of electron withdrawing group (-F, Br, Cl) made the substituted quinoxaline compounds to exhibit moderate to significant antibacterial and antifungal activity in comparison to standard drug ciprofloxacin and fluconazole respectively. Compound QX1 and QX4 exhibited promising antibacterial and antifungal activity. However other two compounds (QX5 & QX2) of the series also exhibited moderate to weak activity against the microorganisms.

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