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Antibacterial and Antifungal Activity of 2-oxo Benzylidine (3-oxo Aniline) Cu (II)-ethylidinediamine

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Antibacterial and antifungal activity of 2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylindiamine has been tested against a number of pathogenic bacteria and fungus with different doses. At dose 25 μg $disc^{-1}$ the compound showed 6-13 mm diameter zone of inhibition against all of the tested bacteria which was found to be larger range as compared to cotrimoxazole (15-29 mm) at the same dose. Minimum inhibitory concentration was withinin the range of 64-128 μg ml^{-1} against all of the tested bacteria The compound showed 0-13 mm diameter zone of inhibition against all of the tested fungus at dose 45 μg $disc^{-1}$ which was found to be larger range as compared with nystatin. The brine shrimp lethality of the compound was found to be 5.23 μg ml^{-1} which shows a pronounced cytotoxic effect.

Key words: 2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylindiamine, antibacterial activity, antifungal activity, cytotoxic

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INTRODUCTION

2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylidinediamine complex possess significant antineoplastic activity[1]. Cu-benzohydroxamic acid (Cu-BHA) has also significant antineoplastic activity[2]. Nickel (II)[transition element] complex derived from aminosugars shows effective antifungal activity against pathogenic Yeast, *Candida albicans* with MIC. This complex act as inhibitor for chitinase (chitin-degradation enzyme) enzyme of *C. albicans*[3]. Nickel (II) complex of 5-methyl 2-furfural thiosemicarbazone have carried out *in vitro* for antifungal activity on human pathogenic fungi, *Aspergillus fumigatus* and *Candida albicans* and *in vivo* for toxicity on mice[4]. Metal complex like Copper (II)[another trasition element] complex of furan semicarbazones possess potent cytotoxic effect[5].

The above information suggested to look for the effectiveness of 2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylidinediamine against bacteria and fungus. This research paper reported for the first time, the antibacterial and antifungal effect of this complex against different bacteria and fungus. In addition, cytotoxic effect of the compound also evaluated.

MATERIALS AND METHODS

Test compound: The test compound 2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylidinediamine was synthesized according to the procedure described by Saidul Islam and Badruddoza[1].

Antibacterial screening: Five bacteria (three gram positive and two gram negative) were selected for this study. Nutrient agar was used as antibacteriological media. Antibacterial potency of the compound was measured against all of the taken bacteria according to the standard disc diffusion method[6] where air dried sterile Whatman filter paper discs (6 mm diameter) with centers of at least 24 mm apart were deposited on nutrient agar plate using aseptic technique. Bacterial inoculum containing approximately, 10^4–10^6 colony forming units (Cfu ml^{-1}) was spread on the surface of nutrient agar. The test compound at doses 80, 40 and 25 μg $disc^{-1}$ was added into three discs. The forth disc was supplemented with reference drug cotrimoxazole at dose 25 μg $disc^{-1}$ serving as positive control. The plates were incubated immediately at 37°C for 14-19 h. Activity was determined by measuring the diameter of zones (mm) showing complete inhibition. Growth inhibition was calculated with respect to positive control.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration of the compound was determined against all of the organism studied by serial dilution technique[7]. The media used in this respect was nutrient broth media. Decreasing concentration of the test compound was prepared in serial two fold dilution using the stock solution (1.024 mg ml^{-1}). Ten μl of bacterial suspension (10^7 cells ml^{-1}) was inoculated into all of the test tube. After incubation for 24 h at 37°C, the test tube were observed for no growth and growth of the used bacteria.

Antifungal Screening: Six fungus (two animal fungus and four plant fungus) were selected for this work. Seaboard dextrose agar (SDA) for animal fungus and Potato dextrose agar (PDA) for plant fungus were used for fungal growth media. Antifungal activity of the test compound was observed against all of the test microorganism according to the standard disc diffusion method[6] where air dried sterile Whatman filter paper discs (6 mm diameter) with centers of at least 24 mm apart were deposited on growth media in plate using aseptic technique. Fungal inoculum containing approximately 4-6 isolated pure spores was spread on the surface of growth media. The test compound at doses 60, 45 and 30 μg $disc^{-1}$ and known drug nystatin at dose 25 μg $disc^{-1}$ serving as positive control were applied into four discs, respectively. Plates were kept at low temperature (4°C) for 24 h to allow maximum diffusion then incubated immediately at 18-27°C for 5-7 days. Activity was confirmed by measuring the diameter of zones (mm) showing complete inhibition. Growth inhibition was calculated with respect to positive control.

Brine shrimp lethality bioassay: Twelve vials were taken (two vials for each concentration) for this study. Five ml of sea water was given to each of the vial. 1.25, 2.5, 5, 10 and 20 μg ml^{-1} solution of the test compound were transferred to 10 vials and 2 vials were used as control. With the help of Pasteur pipette 10-11 living shrimps were inoculated into each of the vial. After 24 h vials were observed and number of survival nauplii in each vial was counted[8,9].

RESULTS

Antibacterial activity: Antibacterial activity of the compound showed remarkable sensitivity against used bacteria for all doses and results were compared with known antibacterial drug cotrimoxazole (25 μg $disc^{-1}$). At dose 25 μg $disc^{-1}$ showed small diameter of zone of

Table 1: Antibacterial activity of 2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylidinediamine

	Diameter of zone of inhibition(mm)			
	Test compound (μg disc^{-1})			
Test bacteria	80	40	25	Cotrimoxazole (25)
Gram positive				
Bacillus subtilis	15	14	12	15
Streptococcus β- haemolytica	19	9	7	25
St. Bacillus haemolytica	17	12	11	19
Gram negative				
E. coli	20	10	6	26
Sarcina lutea	19	15	13	29

Table 2: MIC of the test compound against Gram positive and Gram-negative bacteria

Test bacteria	Concentration of test compound (μg ml^{-1})								
Gram positive bacteria	1024	512	256	128	64	32	16	8	4
Bacillus subtilis	-	-	-	-	+	+	+	+	+
Streptococcus β-haemolytica	-	-	-	-	+	+	+	+	+
St. Bacillus haemolytica	-	-	-	-	-	+	+	+	+
Gram negative bacteria									
E. coli	-	-	-	-	+	+	+	+	+
Sarcina lutea	-	-	-	-	+	+	+	+	+

(+) = Growth and (-) = No growth

Table 3: Antifungal activity of 2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylidinediamine

	Diameter of zone of inhibition (mm)			
	Cu (II)-complex (μg disc^{-1})			
Test fungus	60	45	30	Nystatin 45
Animal fungus				
Tniea pedis Tricophyton	0	0	0	18
Tenia subcandida	6	0	0	12
Plant fungus				
Aspergillus niger	14	9	8	28
Colitotrium sp	11	10	7	20
Alternaria sp.	15	13	12	21
Carvularia pallesens	10	9	7	25

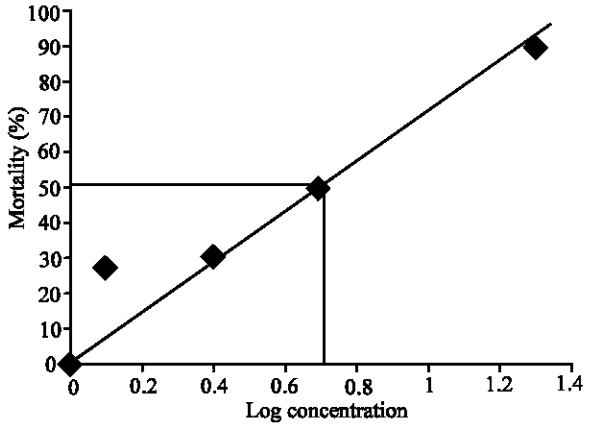

Fig. 1: Effect of 2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylidinediamine on brine shrimp mortality

inhibition 12, 7, 11, 6 and 13 mm against *Bacillus subtilis*, *Streptococcus β-haemolytica*, *St. Bacillus haemolytica*, *E. coli* and *Sarcina lutea*, respectively as compared with cotrimoxazole at dose 25 μg disc^{-1} which were 15, 25, 19, 26 and 29 mm against the same bacteria, respectively (Table 1). But at dose 80 μg disc^{-1} and 40 μg disc^{-1} of compound showed moderate activity against the same bacteria as compared with cotrimoxazole (25 μg disc^{-1}).

Minimum inhibitory concentration (MIC): MIC is the lowest amount of drug at which it is able to inhibit the growth of specified microorganism. MIC of the test compound against *Bacillus subtilis*, *Streptococcus β-haemolytica*, *St. Bacillus haemolytica*, *E. coli* and *Sarcina lutea* (Table 2) were observed no growth in the test tube at 128, 128, 64, 128 and 128 μg ml^{-1}, respectively.

Antifungal activity: The compound showed moderate activity against used fungus for all doses and compared with known antifungal drug nystatin (45 μg disc^{-1}). At the dose of 60, 45 and 30 μg disc^{-1}, the diameter of zone of inhibition were evaluated 0, 6, 14, 11, 15, 10 mm and 0, 0, 9, 10,13, 9 and 0, 0, 8, 7, 12, 7 mm against *Tniea pedis Tricophyton*, *Tenia subcandida*, *Aspergillus niger*, *Colitotrium* sp., *Alternaria* sp. and *Carvularia pallesens*, respectively, where the diameter of zone of inhibition of nystatin (45 μg disc^{-1}) was found to be 18, 12, 28, 20, 21 and 25 mm of that fungus, respectively (Table 3).

Brine shrimp lethality bioassay: To find out the effect of the test compound on the mortality of brine shrimp nauplii, median lethal concentration (LC_{50}) was calculated (Fig. 1) and it was found to be 5.23 μg ml^{-1}. The plot of mortality (%) versus log of concentration of the compound showed an approximate linear correlation between them.

DISCUSSION

The results showed the a ntibacterial and antifungal effect of 2-oxo benzylidine (3-oxo aniline) Cu (II)-ethylidinediamine against different bacteria and fungus which were responsible for large number of human, animal and plant diseases. The primary bioassay screenings such as antimicrobial assay and brine shrimp lethality bioassay were studied accordingly. Standard drug cotrimoxazole (25 μg $disc^{-1}$) showed the diameter of zone of inhibition range 15-29 mm against used bacteria. But, the diameter of zone of inhibition range 15-20 mm (equivalent to cotrimoxazole) of the compound against used bacteria was observed at the dose 80 μg $disc^{-1}$ (more than three times increased concentration of cotrimoxazole). This suggested that the antibacterial activity of the compound lower than cotrimoxazole. MIC was also obtained against the used bacteria which ranged from 64-128 μg ml^{-1}. At doses 60, 45 and 30 μg $disc^{-1}$ did not show remarkable (0-6 mm diameter of zone of inhibition) activity against animal fungus but showed moderate (9-15 mm diameter of zone of inhibition) activity against plant fungus as compared with standard antifungal drug nystatin at dose 45 μg $disc^{-1}$ where diameter of zone of inhibition was 12-28 mm.

The test compound possess cytotoxic effect revealed from the low value (5.23 μg ml^{-1}) of median lethal concentration (LC_{50}). Cytotoxicity is the backbone of bioassay for the bioactivity of the compounds as well as a wide range of pharmaceutical activities. This positive response suggested carrying out more research to modify the compound for its better activity against microbes.

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