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R E S E A R C H A R T I C L E

Assesment of Antimicrobial Activity of Aniline and Pyridine Derivatives by Cup Plate Method

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A B S T R A C T

The discovery of antibiotics, and the subsequent development of synthetic antimicrobial compounds, altered our therapeutic approach towards infectious diseases, and improved the quality and length of life for humans and other organisms. In present study investigate the antimicrobial properties of aniline and pyridine derivatives (SAHA and MS-275). The compound were synthesized in laboratory, Muller Hinton agar media and Sabouraud dextrose agar media was used to investigate the antibacterial and antifungal activity respectively, both activities followed by gel diffusion principle in cup plate method.

Keywords: Antimicrobial properties, Cup plate method, Gel diffusion, SAHA and MS-275.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

The science dealing with the study of the prevention and treatment of diseases caused by micro-organisms is known as medical microbiology. The detection and development of antibiotics and the consequent consumption of antibiotic therapy signaled a milestone in the history of biology and medicine. Antibiotic therapy permitted for the treatment of dangerous and fatal diseases, leading to a remarkable development in health conditions for humans, as well as rising life expectancy. In addition, antibiotic therapy provided significant health benefits for animals and

vegetation, improving the production and consumption of reared organisms and human food crops. For the treatment of ailment inhibitory chemicals working to kill micro-organisms or stop their growth, are called antimicrobial agents [1,2]. These are categorized according to their use and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders [3]. The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bacteriocides (killing bacteria), algicides (killing algae) or fungicides (killing fungi). Paul Ehrlich used the word chemotherapy for curing the communicable disease without damage to the host's tissue, known as chemotherapeutic agents such as antibacterial, antiprotozoal, antiviral, antineoplastic, antitubercular and antifungal agents [4,5]. A few of the early antibiotics are at the present known to be unacceptably toxic and are therefore not used in antimicrobial therapy. Some however, such as adriamycin, bleomycin and mitomycin are presently used as cytotoxic agents in cancer therapy [6].

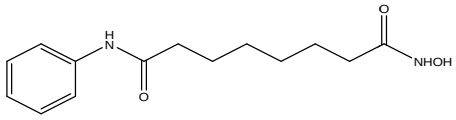

Fig 1: Phenyl amide Octanedioic acid hydroxyamide (SAHA)

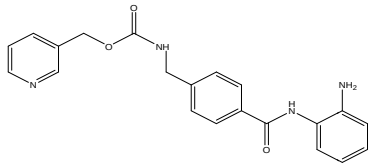

Fig 2: pyridine-3-ylmethyl{4-[aminophenyl)carbamoyl]benzyl}carbamate (MS-275)

2. Materials and Methods

Materials:

The test organisms include the following bacterisa and fungi likes *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans,* all the strains were obtained from Institute of Microbial technology, Chandigarh, India; Sabouraud dextrose agar media and Muller Hinton agar media(Himedia, Mumbai); Aniline, DMF, THF were obtained from Sisco Research Lab Pvt. Ltd., New Mumbai; Suberic acid, EDCI, HOBt.H2O, N,N-disopropyl ethylamine, Pyridine-3-methanol, CDI, were obtained from Sigma-Aldrich, Missouri, U.S; 4-amino methyl benzoic acid, DBU, Triethylamine, were obtained from TOKYO chemicals Pvt. Ltd, Chennai, India. All the Chemicals and reagents used in this experiment are of analytical grade. Both the compounds (SAHA and MS-275) are aniline and pyridine derivatives were synthesized in our laboratory [7-10]. In this experiment the synthesized compounds are used for anti-bacterial and anti-fungal activities.

Antibacterial Activity of Synthesized Compounds

Organism used - Staphylococcus aureus [Gm (+Ve)],
Pseudomonas aeruginosa[Gm (-Ve)]
Standard used - Amikacin
Incubation temperature -37°C
Incubation time - 24 hrs
Control - CH_3OH
Test concentration - 50 mcg / ml
Method - Cup- plate method
Principle - Gel diffusion

Composition of Muller Hinton agar media:

Beef extract - 30 g
Peptone - 17.5 g
Starch - 1.5 g
Agar - 17 g
NaOH - 5 g
Distilled water - 1000 ml
Final pH at 25°C-7.4 ± 0.2

Method

The beef extract, peptone, starch and agar were taken in the above proportions and dissolved in 100 ml of distilled water. The constituents were heated gently at 100°C with agitation. The pH of medium was adjusted to 7.4 using sodium hydroxide. It is transferred to boiling tube in hot condition and sealed with non-absorbent cotton and sterilized by autoclaving at 121°C (15 lbs pressure) for 15 minutes, then poured aseptically into sterile Petri dishes. Aseptic condition is maintained throughout the process.

Inoculation of microorganisms:

For the screening of antibacterial activity Cup- plate method was used. The sterilized Muller Hinton Agar media was heated on a water bath to melt the media. When media was lukewarm, the organisms were inoculated separately and poured aseptically into sterile Petri dishes and allowed to solidify. The temperature of the medium should not exceed about 50°C when organisms were inoculated. The solutions of known concentration standard drug Amikacin (50µg/ml) and corresponding concentration of synthesized compounds (50µg/ml) were prepared. Apply the solutions to the surface of the medium in sterile cavities prepared in the media. One more solution of Methanol is used as control in separate cavity. The volume of solution added to each cavity must be uniform and sufficient to fill the hole. Petri dishes were incubated at 37°C for 24 hours, after placing them in refrigerator for one hour to facilitate uniform diffusion. Observations were made for the zone of inhibition around the synthesized compounds and compared with that of standard.

Fig 3: Zone of inhibition against *S.aureus*

Fig 4: Zone of inhibition against *Pseudomonas aeruginosa*

Fig 5: Zone of inhibition against *Candida albicans*

Antifungal Activity

Organisms used - Candida albicans
Standard used - Ketoconazole (10 µg / ml)
Incubation temperature - 28°C
Incubation time - 24 hrs
Control used - CH_3OH
Test concentration - 5 µg / ml
Method - cup plate method
Principle - gel diffusion

Composition of Sabouraud dextrose agar media:

Glucose- 40 gm
Peptone - 10 g
Agar - 15 g
Distilled water - 1000 ml
Final Ph- 5.4

Method:

Glucose, peptone and agar were taken in the above proportions and dissolved up to 1000 ml of distilled water. The constituents were heated gently at 100°C with agitation. The pH of media was adjusted to 5.4. Then it was transferred to a boiling tube in hot condition and sealed with non-absorbent cotton and sterilized by autoclaving at 121°C (15 lbs pressure) for 15 mins, then poured aseptically into sterile Petri dish.

Inoculation of microorganism:

For the screening of antifungal activity disc Cup-plate method was used. Sabouraud dextrose agar plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37°C just before inoculation agar plates were incubated for about 24 hours at 37°C, which showed sufficient growth of fungi. The temperature of the medium should not exceed about 50°C when organisms were inoculated.

The solutions of known concentration standard drug Ketoconazole (10µg/ml) and corresponding concentration of synthesized compounds (10µg/ml) were prepared. Apply the solutions to the surface of the medium in sterile cavities prepared in the media. One more solution of Methanol is used as control in separate cavity. The volume of solution added to each cavity must be uniform and sufficient to fill the hole. Petri dishes were incubated at 37°C for 24 hours, after placing them in refrigerator for one hour to facilitate uniform diffusion. Observations were made for zone of inhibition around the synthesized compounds with that of standard.

3. Results and Discussion

In this present study investigated the antimicrobial activity of synthesized aniline and pyridine derivatives (SAHA and MS-275). The zone of inhibition was measured in cup plate method. According to the table: 1 the synthesized aniline derivative was potent against gram positive bacteria; pyridine derivative was potent against gram negative bacteria and its compare with standard drug. The both compounds SAHA and MS-275 also have anti-fungal activity. In this experiment the zone of inhibition was measured and reported in table: 2.

Table 1: Zone of inhibition

Compounds	Zone of inhibition against	
	Gram (+)Ve *S.aureus* in mm	**Gram(-)Ve in *P. aeruginosa* in mm**
SAHA	11	13
MS-275	14	17
Control (Methanol)	Resistant	Resistant
Standard (Amikacin)	17	18

Table 2: Zone of inhibition

Compounds	Zone of inhibition in mm by *Candida albicans*
SAHA	19
MS-275	18
Control (Methanol)	Resistant
Standard (Ketoconazole)	16

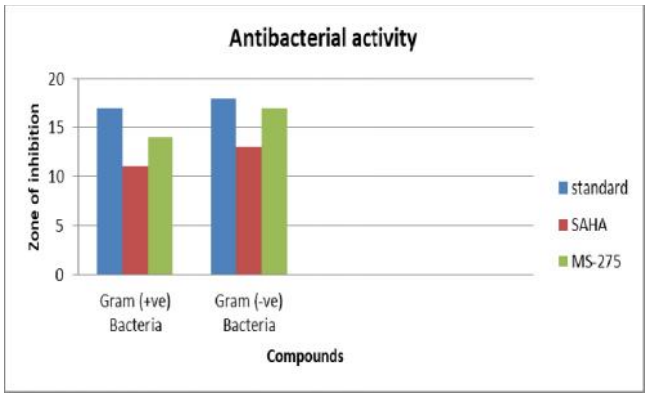

Fig 6: Antibacterial activity

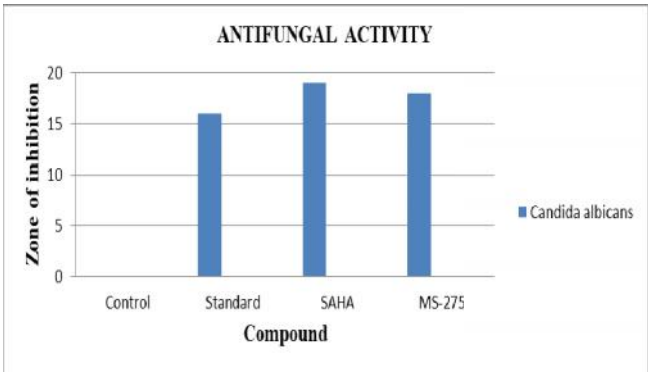

Fig 7: Anti-fungal activity

4. Conclusion

Continuous and recurrent use of antibiotics to manage human illness has caused by bacteria to obtain resistance against the frequently used therapeutic agents. As a result the difficulties of microbial resistance is rising and require to look for alternative newer molecules is increasing. The present investigation has confirmed the synthesized aniline and pyridine derivatives have potential antibacterial and antifungal properties and they need further investigations for the therapeutic value in managing diseases caused by the test bacteria based on toxicological and pharmacological study.

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