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Recent Trends in Applied Zoology

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Chapter 05

Biological Activities of DHA Schiff Base Ligands

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Introduction:

Biological activities describe favorable or unfavorable effects of chemical compounds acting as drugs on living cells like microorganisms which may exist in its single-celled form or multicellular form in the biotic world. Microorganisms are microscopic in size namely bacteria, fungi, and algae. Infectious diseases are mostly caused by pathogens *i.e* microorganisms such as bacteria, fungi, etc.
Microorganisms play a vital role in human life and health in many ways. These ferment foods, treat sewage, produce fuel, enzymes and other bioactive compounds. Some microorganisms are the pathogens responsible for many infectious diseases and are major concerns of health and hygiene.

1.Bacteria: Bacteria are a type of biological cells. They constitute a large domain of prokaryotic microorganism. They are few μm in length, spiral, sphere or-rod shaped and first life form on earth.
Antibacterial activity of any chemical compound can be studied by screening the sample in vitro against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumonia, Proteus Vulgaris, Pseudomonas aeruginosa, Pseudomonas putida and Streptococcus faecalis.*
According to Davis et.al and Pervez C. Paul et.al the worldwide appearance of *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus,* and many other β-lactamase producers have become a foremost therapeutic problem. Multi-drug resistant (MDR) strains are commonly found in hospitals and are increasingly being isolated from community-acquired infections.

1.1The cells of *Escherichia coli* are gram-negative, nonspore forming, short rod-shaped, aerobic or facultative bacteria. These are commonly found in the large intestine of all warm-blooded animals including human beings. Normally the strains of E. coli are harmless and even help to keep our digestive tract healthy. These are considered opportunistic pathogens because these bacteria infect the host only when the immunity is challenged. It enters the human body through the consumption of contaminated food and water and causes food poisoning leading to diarrhea, cramps, and vomiting. Some strains are known to cause pneumonia, breathing problems, and urinary tract infections.

1.2 The cells of *Bacillus subtilis* are Gram-positive, aerobically growing, rod-shaped bacteria characterized by swarming motility. These bacteria are commonly found as the saprophytic organism in an environment such as soil, water, air, etc. These bacteria produce resistant, retractile intracellular structures under unfavorable environmental conditions called endospores that have the ability to resist

heat, radiation, and chemical treatments. These bacterial endospores are of concern to the food industry due to their ability to survive during processing, storage, and transport of food. These bacteria are non-pathogenic but can contaminate food and result in food poisoning. The enzymatic activities of *Bacillus subtilis* are responsible for the decomposition of common components of foods such as carbohydrates, proteins, and fats. This decomposition process results in various undesirable changes in food-producing defects in color, taste, consistency, flavor, nutritional value, etc. These bacterial spores can tolerate all the food preservation practices such as pasteurization, canning, radiation treatments, use of food additives.

1.3 Staphylococcus aureus is gram-positive, sphere-shaped (coccal) bacteria. These are a common member of the microflora of the body, frequently found in the upper respiratory tract and on the skin. The bacteria can spread from person to person by direct contact, through contaminated objects (such as gym equipment, telephones, doorknobs, television remote controls, or elevator buttons), or, less often, by inhalation of infected droplets dispersed by sneezing or coughing. Infections through contaminated foods are very common. *Staphylococcus aureus* infections range from mild to life-threatening. The most common staphylococcal infections are skin infections, often causing abscesses, blisters, and redness and swelling in the infected area. However, the bacteria can travel through the bloodstream and infect almost any site in the body, particularly heart valves and bones. The bacteria also tend to accumulate on medical devices in the body, such as artificial heart valves or joints, heart pacemakers, and catheters inserted through the skin into blood vessels. There are many strains of *Staphylococcus aureus* that produce toxins that can cause staphylococcal food poisoning, toxic shock syndrome, or scalded skin syndrome.

Some skin infections heal without treatment, some will require incision and drainage of the infected site and some infections may require antibiotics.

There is a possibility for longer-lasting or more severe infections with Methicillin-resistant *Staphylococcus aureus* (MRSA) if the initial antibiotic prescribed is not capable of killing the bacteria. S. aureus can also cause serious infections such as pneumonia or bacteremia (bloodstream infection). Symptoms of these infections include difficulty breathing, malaise, fever, or chills.

More serious skin infections can take longer to heal if treatment is delayed.

2. Fungi: Fungi are described as lower eukaryotes able to grow on dead and decaying organic matter. Fungi can destroy timber, cloths, foods, leathers, skin, etc. Some fungi exhibit harmful effects as pathogens of plants, animals, even human beings. The various common groups of fungi are known as Molds, Rusts, Smuts, Mushrooms, Toads, Stools, Puffballs, etc.

The physiological versatility of fungal metabolism makes them exploitable for various processes in the environment, industries, medicine, agriculture, food, nutrition, etc.

Now a day, the WHO reported the insufficiency of antifungal agents in medicine. Therefore the researchers are using fungi as an important tool in research. It is well known that Dehydro acetic acid (DHA) has good antifungal activities hence DHA Schiff base ligands and their transition metal complexes have much scope in antimicrobial studies.

Antifungal activity of sample can be studied by screening sample in vitro against

Aspergillus niger, Aspergillus flavus, Candida albicans, Tricoderma species, F.moneliforme, Penicillium notatum, Penicillium chrysogenum, Curvularia lunata, etc.

2.1 The fungus *Aspergillus* is a genus of conidial fungi commonly grown as molds (also called filamentous fungi) on the surface of organic substrates. It has a few hundred varieties of species. These are common contaminants of starchy foods (such as bread and potatoes), and grow in or on many plants and trees. *Aspergillus niger* species of genus "Aspergillus" is also common in the home. *Aspergillus niger* is a fungus whose spores are present in the air but does not normally cause illness. Only a few of these fungi can cause diseases in humans and animals. Most people are naturally immune and do not develop illness caused by *Aspergillus niger*. In those people with a weakened immune system, damaged lungs or with allergies, Aspergillus can cause disease. Common Aspergillus infections include invasive aspergillosis, ABPACPA, and aspergilloma.

2.2 Candida albicans is a single-celled fungus called yeast. It is an opportunistic pathogen that lives on your skin and in our gastrointestinal tract, Small amounts of *Candida albicans* also live in various warm, moist areas in the body, including on the skin, in the mouth and gut, and also the rectum and vagina. It even plays a part in digestion and nutrient absorption. Candidiasis is a disease condition when *Candida albicans* can multiply out of control. This is when it becomes, also known as thrush, a yeast infection, or Candida overgrowth. Candidiasis is most often noticed on the skin, mouth and vaginal infections. Candida overgrowth is also a common cause of diaper rash. These can be unpleasant infections but is not generally life-threatening except in immune-compromised individuals like diabetes, AIDS, etc. Even so, Candidiasis should be treated promptly before it can affect your overall health and quality of life. Demonstration of the antimicrobial potentiality of different DHA Schiff bases and their metal complexes against common human pathogens by doing discussion with experts is now becoming more popular. It will help the scientist to prepare drugs as antimicrobial agents. Researchers have come to know that DHA Schiff bases and their metal complexes can be used as medicine in the future against various diseases.

In concern with the results of Cohen et al., in 1992, though pharmacological industries have produced a number of new antibiotics in the last three decades, yet resistance to these drugs by microorganisms has developed. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs.

In the present work, the antibacterial activity of DHA Schiff base ligands and their metal complexes are screened in vitro against *Escherichia coli (only for ligands)*, *Bacillus subtilis* and *Staphylococcus aureus*. The antifungal activity of DHA Schiff base ligands and their metal complexes are screened in vitro against *Aspergillus niger and Candida albicans* species.

3. The biological (Antimicrobial and Antioxidant) activities were tested in our Biotechnology Research Centre, Rajarshi Shahu Mahavidyalaya, (Autonomous) Latur. Anticancer activities were tested from Biotechnology Research Centre, Savitribai Phule University, Pune.

The antimicrobial potentiality of DHA Schiff base ligands and their metal complexes was carried out by the agar well diffusion method. The minimum inhibitory concentration of DHA Schiff base

ligands and their metal complexes was determined by adopting the standard procedure of the National Committee for Clinical Laboratory standard (2004).

DHA Schiff base ligands were tested for their in vitro antibacterial and antifungal activities. The *Escherichia coli (E.coli)* (ATCC2331), *Staphylococcus aureus* (*S.aureus*) (NCIM-2079) and *Bacillus subtilis* (*B.subtilis*) (NCIM-2063) were used as bacterial strains and *Candida albicans* (*C.albicans*) (MTCC-227) and *Aspergillus niger (A.niger)* (NCIM- 545) were used as fungal strains for the study of antimicrobial activity.

3.1Agar well diffusion method:

Bacterial strains:

The 24 hour old cultures of bacterial strains propagated in peptone water were used as suspension for antibiotic susceptibility test. The bacterial suspensions were inoculated in Muller–Hinton antibiotic assay medium in Petri dishes. Wells having 6 mm diameter were punched in circular pattern and were filled with 100µl of the DHA Schiff base ligands and their metal complexes to be tested (at a concentration of 1mg/ml in ethanol/DMSO). Streptomycin was used as a reference substance. Plates were incubated at 37^0C for 24 h. The diameters of the inhibition zones produced by the test substances and reference substance around wells were measured and compared.[1,2]

3.2 Fungal strains:

For antifungal activity, the same procedure was repeated with PBS (phosphate buffered saline) for fungal suspension and YPD (Yeast Peptone Dextrose) agar as an antibiotic susceptibility testing medium. The plates were incubated at 25°C for 72hrs. For fungi Griesofulvin was used as reference. The diameters of the inhibition zones produced by the test substances and reference substance around wells were measured and compared.

Bioactivity data of DHA Schiff base ligands are summarized in Table 1 and Graphical representation is shown in Fig.1

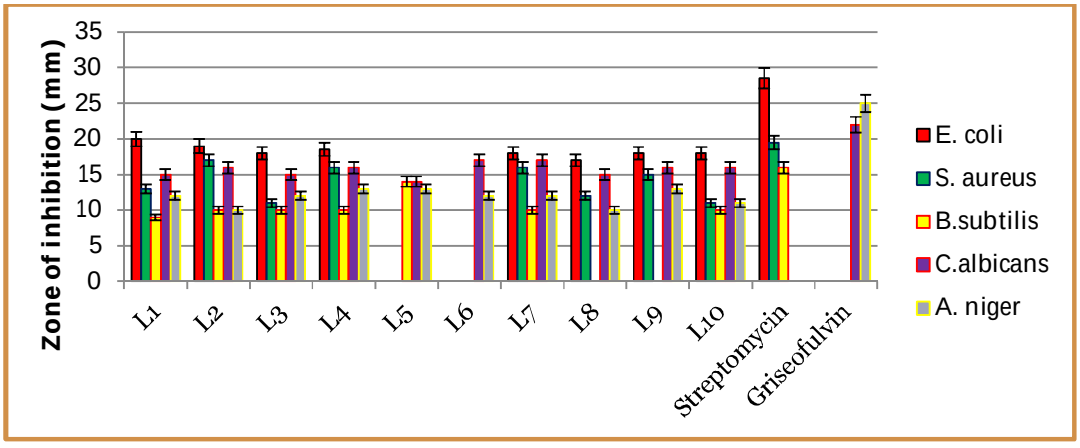

Fig.1: Graphical presentation antimicrobial activity of DHA Schiff base ligands

Table 1.1: Antimicrobial Activity data of DHA Schiff base ligands

Sr. No	Ligand symbol &Abbrriv. name	Zone of inhibition Antibacterial (mm)			Zone of inhibition Antifungal (mm)	
		E. coli	*S. aureus*	*B.subtilis*	*C.albicans*	*A. niger*
1	$L_1$3Etriz3imi4H6M2HP	20.0	13.0	09.0	15.0	12.0
2	$L_2$3Etriz4imi4H6M2HP	19.0	17.0	10.0	16.0	10.0
3	$L_3$3E3MPy2imi4H6M2HP	18.0	11.0	10.0	15.0	12.0
4	$L_4$3EPy3imi4H6M2HP	18.5	16.0	10.0	16.0	13.0
5	$L_5$3EPy2Mimi4H6M2HP	00.0	00	14	14.0	13.0
6	$L_6$3EPy3Mimi4H6M2HP	00.0	00.0	00.0	17.0	14.0
7	$L_7$3EPy4Mimi4H6M2HP	18.0	16.0	10.0	17.0	12.0
8	$L_8$4EAP4imi4H6M2HP	17.0	12.0	00.0	15.0	10.0
9	$L_9$3EF2Mimi4H6M2HP	18.0	15.0	00.0	16.0	13.0
10	L_{10}3EA34DMimi4H6M2HP	18.0	11.0	10.0	16.0	11.0
11	Streptomycin	28.5	19.5	16.0	00.0	00.0
12	Griseofulvin	00.0	00.0	00.0	22.0	25.0

All the DHA Schiff base ligands were found to be biologically active except ligands L_5 and L_6 against *E.coli* with a maximum zone of inhibition 20 mm by ligand L_1. The ligands L_5 and L_6 depicted no zone of inhibition. Ligand L_2 has shown 19 mm, L_3, L_4, L_7, L_9 and L_{10} showed 18 mm and Ligand L_8 has shown a 17 mm zone of inhibition as compared to 28.5 mm zone of inhibition exhibited by streptomycin.

Ligand L_2 has exhibited a maximum zone of inhibition of 17 mm against *S. aureus*. The ligands L_4 and L_7 have shown 16 mm, L_9 has shown 15 mm, a zone of inhibition. The ligands L_1, L_3, L_8, and L_{10} were found moderate to lower in growth inhibition (11-13 mm) while ligands L_5 and L_6 were inactive against the same species as compared to reference exhibiting 19.5 mm zone of inhibition.

The ligands L_2, L_3, L_4, L_7, and L_{10} were found moderate in growth inhibition against *B. Subtilis* with a zone of inhibition 10 mm and ligands L_6, L_8 and L_9 depicted no zone of inhibition. The ligand L_5 exhibited a 14 mm zone of inhibition as compared to a 16 mm zone of inhibition observed for streptomycin.

All the ligands have shown significant antifungal action against *Aspergillus niger* with about 10 to 14 mm zone of inhibition. Ligand L_6 has shown a maximum zone of inhibition 14 mm while ligands L_2 and L_8 have shown a minimum zone of inhibition of 10 mm in comparison to reference Griesofulvin showing a 25 mm zone of inhibition.

All ligands showed about 14 to 17 mm zone of inhibition against a human opportunistic pathogen, *C. Albicans* where ligands L_6 & L_7 depicted maximum zone of inhibition, 17 mm and ligand L_5 has shown minimum zone of inhibition of 14 mm with respect to 22 mm zone of inhibition exhibited by reference Griseofulvin.