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On

Applied Zoology, Profitable Animal Production, and Health: Current Status and Future
Progress (NSAZ-2022) 23rd & 24th September- 2022

Recent Trends in Applied Zoology

Dr.D.S.Rathod
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Dr. K.S.Raut
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National Edited Book

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Recent Trends in Applied Zoology

Edited by: Dr.D.S.Rathod

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Index

Chapter	Chapter/Article Title – Name of Authors	Page Number
Chapter 1	Process Upgradation of Indian Dairy Products Khojare A. S.	1-6
Chapter 02	Review on Important role of Danio rerio in Animal and human vaccination research Datta Ashok Nalle, Dnyaneshwar S. Rathod	7-13
Chapter 03	Effect of Dimethote On Biochemical Changes In Lipid Content During Lethal And Sub Lethal Exposure To The Freshwater Fish, <i>Rasbora Daniconius</i> Lokhande, M.V.¹ and Rathod, D.S.²	14-20
Chapter 04	Analysis of chromosome by Karyotyping, banding, and cryopreservation of gametes in fishes Datta Ashok Nalle, Madhuri Y. Bhande	21-28
Chapter 05	Biological Activities of DHA Schiff Base Ligands Dr. Dhananjay Palke	29-34
Chapter 06	Study of phytoplankton Diversity from Papvinash Lake Latur, in relation to Physico-Chemical Parameters Datta Ashok .Nalle	35-41
Chapter 07	A Review on Importance of DNA Bar-coding in Genomic diversity of Freshwater fish Dhanshree M. Jagtap, Dnyaneshwar S. Rathod	42-47
Chapter 08	Review-based Study on Dandelion (<i>Taraxacum Officinale</i>) biologically Effective Molecules for Animal Health with Special Reference to Diabetes Datta Ashok Nalle	48-58
Chapter 09	Study of Adulteration in common Food Items Dnyaneshwar S. Rathod, Manali Aglave , Jabeen Bagwan, and Vaishnavi bhimale	59-63
Chapter 10	Impact of Detergent Pollution on the Oxygen Consumption Capacity of the Fish <i>Cyprinus carpio</i> P. S. Shete	64-68
Chapter 11	A review of the Nutritional advantages of feeding farm animals <i>Cichorium intybus</i> as a supplement Datta A.Nalle, Abhaysinh R. Deshmukh	69-80
Chapter 12	Correlation of nutritional status of college girl students with hemoglobin level and BMI in Latur, Dist. Latur. Raut K.S., Jamale P.B1, Inamdar A.P.	81-86
Chapter-13	Importance of Mulberry plant in Sericulture Dnyanoba R. Awad	87-94
Chapter 14	Influence of four plant based carotenoids on the coloration of two ornamental fishes, Koi carp (<i>Cyprinus carpio</i>) and Molly fish (<i>Poecilia sphenops</i>). Yadav S.G.	95-100
Chapter-15	Omega -3 fatty acid and its use in fish feed formulation Madhuri Y. Bhande	101-106
Chapter 16	Potential use of <i>Spirulina platensis</i> in combating Malnutrition in India Rajkumar D.Kamble , Pratiksha Patil ,Komal Sawase , Vaishnavi U.Phulari , Aishwarya Samarth , Pranita Rathod	107-110
Chapter-17	Morphological diversity of freshwater fishes in Manjarariver, Bori, Latur, Maharashtra, India Vishal K. Moholkar, Amol S. Patil, Dhanshree M. Jagtap	111-115

Chapter 18	Ethanobotanical Studies OnPiper betle L. among the folk peoples of Vidul, Taluka Umardhed, District Yavatmal ,Maharashtra, India. Eanguwar Srinivas Reddy, Shivraj Kashinath Bembrekar Rameshwar Ramchandra Bichewar and Saiprabha Shirsat	116-120
Chapter-19	Preservation of ancestral DNA of salmon and other aquatic species with the aid of biotechnology. Datta Ashok Nalle, Swati Ganesh Swami*	121-124
Chapter -20	Bioinformatics Tools for DNA Barcoding Dnyaneshwar S. Rathod, Dhanshree M. Jagtap	125-129
Chapter -21	Analysis of Seasonal Variation in Water Quality Parameters of Manjara River (Nagzari Dam), Latur city. Waghamare Shailaja, Mushtakh Hashmi	130-139
Chapter -22	Study on Zooplankton Diversity in Manjara River (Nagzari Dam), Latur city. Shaikh Hina, Mushtakh Hashmi	140-147
Chapter -23	Use of Indian natural therapies for animals, affordable, and Eco- friendly Datta Ashok Nalle	148-151
Chapter -24	Survey of Latur fish market present status and marketing strategies. Marathwada region [M.S]. India Kakasaheb .S. Raut	152-155
Chapter -25	Phytochemical analysis of Adhatoda vasica L. Dnyanoba R. Awad, Ankita S. Suryawanshi	156-158
Chapter -26	Animal welfare Laws in India provision for use of animals in experiments and product testing in science Datta A.Nalle	159-162
Chapter -27	Effective Medicinal Plant in Cancer Treatment Dnyaneshwar S. Rathod	163-167
Chapter -28	Effective Medication for Varicella and Herpes Zoster Infection. Swati Ganesh Swami	168-171
Chapter -29	Applications of Biophysics in Animal Research Dayanand V. Raje*, Kakasaheb S. Raut**	172-173
Chapter -30	Survey of bee species, life cycle and Honey purification process at Chakur Dist. Latur Kakasaheb .S. Raut	174-177
Chapter -31	Use of Nanotechnology in fish health and aquaculture management Datta A. Nalle, Divya D.Nagapure	178-183
Chapter -32	Organic Aquaculture- the Sustainable Practice toward aquaculture development and Ecofriendly approaches Jadhav Amit, Dnyaneshwar S.Rathod	184-191
Chapter -33	Freshwater Integrated Multi-Trophic Aquaculture (FIMTA) - An Innovative Approach Jadhav Amit, Tekam Ashvini	192-206

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*, *Trichoderma 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.

3.1 Agar 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°C 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

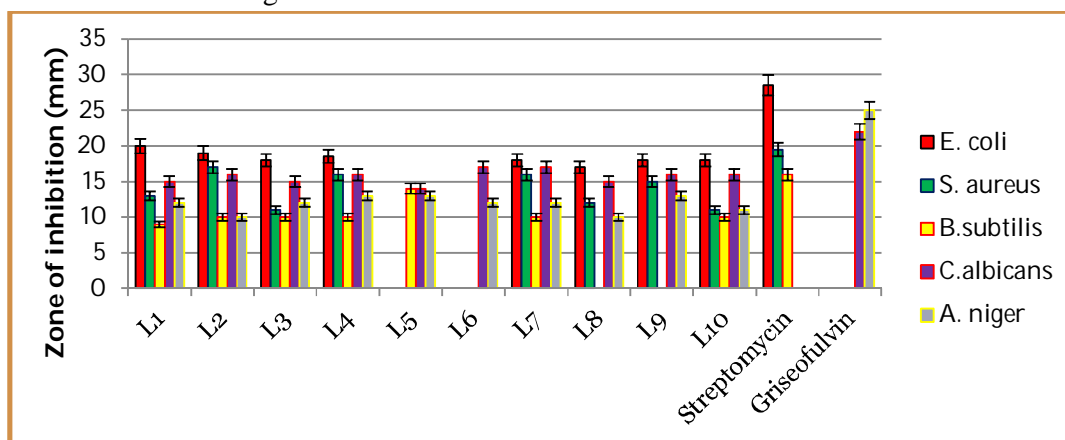


Fig.1: Graphical presentation antimicrobial activity of DHA Schiff base ligands

Table 1.1: Antimicrobial Activity data of DHA Schiff base ligands

Sr. No	Ligand symbol & Abbrev. name	Zone of inhibition Antibacterial (mm)			Zone of inhibition Antifungal (mm)	
		<i>E. coli</i>	<i>S. aureus</i>	<i>B.subtilis</i>	<i>C.albicans</i>	<i>A. niger</i>
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 L₅ and L₆ against *E.coli* with a maximum zone of inhibition 20 mm by ligand L₁. The ligands L₅ and L₆ depicted no zone of inhibition. Ligand L₂ has shown 19 mm, L₃, L₄, L₇, L₉ and L₁₀ showed 18 mm and Ligand L₈ has shown a 17 mm zone of inhibition as compared to 28.5 mm zone of inhibition exhibited by streptomycin.

Ligand L₂ has exhibited a maximum zone of inhibition of 17 mm against *S. aureus*. The ligands L₄ and L₇ have shown 16 mm, L₉ has shown 15 mm, a zone of inhibition. The ligands L₁, L₃, L₈, and L₁₀ were found moderate to lower in growth inhibition (11-13 mm) while ligands L₅ and L₆ were inactive against the same species as compared to reference exhibiting 19.5 mm zone of inhibition.

The ligands L₂, L₃, L₄, L₇, and L₁₀ were found moderate in growth inhibition against *B. Subtilis* with a zone of inhibition 10 mm and ligands L₆, L₈ and L₉ depicted no zone of inhibition. The ligand L₅ 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 L₆ has shown a maximum zone of inhibition 14 mm while ligands L₂ and L₈ 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 L₆ & L₇ depicted maximum zone of inhibition, 17 mm and ligand L₅ has shown minimum zone of inhibition of 14 mm with respect to 22 mm zone of inhibition exhibited by reference Griseofulvin.