



***In-Vitro* antimicrobial screening of naphthalene acetic acid compounds**

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Abstract

Naphthalene acetic acid complexes have been prepared and characterized by elemental analysis, ¹H NMR and UV-Visible spectra and electrochemical measurements. The antimicrobial properties of these compounds have been evaluated against the test strains (*A. niger*, *St. aureus*- *Streptococcus aureus*) and thus a significant use of such compounds as antibacterial agent is reported. The biological activity data show that the present compounds are found to have greater antibacterial and antifungal activity than the respective standards.

Key-Words: Naphthalene acetic acid, *A. niger*, *St. aureus*- *Streptococcus aureus*

Introduction

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, anti bacterial (commonly known as antibiotics) are used against bacteria and antifungal are used against fungi. They can also be classed according to their function. Antimicrobials that kill microbes are called *microbicidal*; those that merely inhibit their growth are called *microbiostatic*. Disinfectants such as bleach are *non-selective antimicrobials*. Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection. More recently, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the merits of controlling these interactions in medicine. In 1928, Alexander Fleming became the first to discover a natural antimicrobial fungus known as penicillium Rubens. He named the substance extracted from the fungus penicillin and in 1942 it was successfully used to treat a streptococcus infection. Penicillin also proved successful in the treatment of many other infectious diseases such as gonorrhea, strep throat and pneumonia, which were potentially fatal to patients up until then.¹⁻³

Today, numerous antimicrobial agents exist to treat a wide range of infections. The development of new anticancer and antimicrobial therapeutic agents is one of the fundamental goals in medicinal chemistry. One newer strategy for the research on new anticancer and antimicrobial therapeutic agents has been the use of metal containing compounds. Metal complexes can also act as complementary therapeutic agents to organic compounds that are widely sought in the drug discovery efforts.³

Antimicrobial agents and modes of action

Any chemical substance which inhibits the growth or destroys microbes is referred to as an antimicrobial agent. A wide range of antimicrobial agents has been reported, however each of them has different action and purpose. The antimicrobials should be selectively toxic to the pathogenic microbes but not toxic to the host tissues. Among the antimicrobials, antibiotics (both natural and synthetic) and certain other chemical agents have a greater application in health care of humans and animals.

Microbial cells grow and divide, replicating repeatedly. To grow and divide, the microbes must synthesize or take up many of biomolecules. Antimicrobial agents interfere with the specific processes that are essential for growth or cell division. Based on the mode of action, antimicrobials are classified into (i) inhibitors of bacterial or fungal cell wall synthesis (ii) damagers of cytoplasmic membranes (iii) inhibitors of nucleic acid and protein synthesis (iv) inhibitors of ribosomal function and (v) inhibitors of specific enzyme systems by acting as antimetabolites.⁴⁻⁸

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Determination of the degree of antimicrobial activity

Different species of microorganisms have varying degrees of susceptibility to antimicrobials. Further, the pathogenic microbes may develop drug resistance to a particular type of antimicrobial agent on prolonged use. Hence, the antimicrobial sensitivity tests are very useful to determine the level of antimicrobial activity of a particular chemical compound on certain pathogenic microorganisms. The antimicrobial sensitivity testing can be performed in three different ways: Serial tube dilution technique, Disc diffusion technique and Agar well diffusion technique or ditch plate technique. Further antifungal activity may also be evaluated by spore germination method.

In any technique, the principal is the preparation and application of a concentration gradient of the drug / test compound on a nutrient medium and the observation of growth or inhibition of growth of the organism when the medium is seeded with the test organism and incubated. Many variables are involved in testing the antimicrobial activity of a test compound on a specific organism such as the size of the inoculums, the nature and composition of the culture medium, the concentration of the test compound, the pH of the medium, the temperature and duration of incubation.⁸

Antimicrobial screen of the test compounds

In the present study agar well disc diffusion technique has been used for determining the susceptibility of the microorganism viz. bacterial and fungal species on the test components namely naphthalene acetic acid and its complexes with Co (II), Ni (II), Cu (II) and Hg (II). For this purpose one fungal strain *A. niger* and one bacterial strain *Streptococcus aureus* were chosen.

Salient features of the test organisms used
Streptococcus aureus

It is Gram-positive. It forms part of the normal flora of man animals. *Streptococcus* species are susceptible to sulphonamides and many other antibiotics. Penicillin is the drug of choice and next is erythromycin. Sore throat (tonsillitis) is the commonest of the staphylococcal diseases. Scarlet fever, local infections in the superficial layers *St. aureus* is a parasite inhabiting only in human and animal intestine. *St. aureus* is a gram – negative strain. *St. aureus* is excreted in faces of man and animal in very large quantity and it contaminates the soil and water resources very widely. *St. aureus* is very sensitive to many drugs. There is a great variety of strains of *St. aureus* that cause a wide spectrum of diseases. Some of the infections caused by *St. aureus* are urinary tract infection, septic infections of wound, diarrhoeas, dysentery etc.⁹⁻¹⁰

Aspergillus

The generic name *A. niger* is derived from the Latin *aspergillum*, a mop for distributing holy water, and refers to the appearance of the mature conidiophores. *A. niger* is tolerant of low water activity, being able to grow on substrate of high osmotic potential and to sporulate in an atmosphere of low humidity. *A. niger* is the important type of fungus producing several mycotoxins, which cause many problems to animals. For example *A. niger flavus* produces Aflatoxins which cause liver damage, liver cancer etc. *A. niger* produces Ochratoxins which cause kidney damage. Presence of *A. niger* in the agricultural soil leads to denitrification and nitrogen starvation. *A. niger* results from opportunistic infection by *A. niger* species. *A. niger* is useful for commercial enzyme production, industrial alcohol production etc.

Material and Methods⁹⁻¹¹**Preparation of the media used in the study**

Two different media have been prepared and used in this study (i) nutrient agar medium for culturing bacteria and (ii) rose Bengal chloramphenicol agar medium for culturing fungal species.

Preparation of nutrient agar medium

This medium has been prepared with following composition.

Beef extract	-	1.5g
Peptone	-	5.0g
Yeast extract	-	1.5g
Sodium chloride	-	5.0g
Distilled water	-	1000ml

An amount of 28g of the ingredients of the medium (HIMEDIA) was suspended in 1000 ml of distilled water and heated to boiling to dissolve the solid completely. The nutrient agar medium was sterilized by autoclaving at 121°C for 15 Minutes. The medium was cooled to 50°C and aseptically dispensed into the already sterilized petriplates to a depth of 4mm (25ml quantity). The pH of the medium may be 7.4±0.2.

Preparation of rose bengal chloramphenicol agar medium

This medium has been prepared with following composition.

Mycological peptone	:	5.00g
Dextrose	:	10.00g
Monopotassium phosphate	:	1.00g
Magnesium sulphate	:	0.50g
Rose Bengal	:	0.10g
Chloramphenicol	:	0.10g
Agar	:	15.50g
Distilled water	:	1000 ml

A total mass of 32.2g of the ingredients of the medium (HIMEDIA) was suspended in 1000 ml of distilled

water and heated and boiling to dissolve the solid completely. The rose Bengal chloramphenicol agar medium was sterilized by autoclaving for 15 minutes at 121°C. The medium was cooled to about 50°C and dispensed aseptically into the already sterilized petriplates to a depth of 4mm (25ml quantity). The pH of the medium may be in the range 7.2 ± 0.2 .

Inoculum preparation and inoculation

The microbial concentration of the Inoculum should be approximately 10^6 cells per milliliter. The Inoculum was prepared by inoculating 4-6 selected colonies from a plate into a tube containing a broth followed by incubation for a few hours to obtain moderate turbidity. The broth cultures of actively grown organisms were diluted with a sterile broth culture to obtain turbidity equivalent to that of barium sulphate standard which may be prepared by adding 0.5ml of 1.175% BaCl₂. 7H₂O solution (w/v) to 99.5 ml of 0.36N H₂SO₄

Within 15 minutes after adjusting the turbidity of Inoculum with the standard, a sterile cotton swab was dipped into the dilute culture and the swab was rotated several times while pressing it firmly against the upper inside wall of the tube to remove excess inoculum. The dried surface of the agar plate was inoculated by streaking the swab gently three times turning the plate by 60°C to ensure even distribution of the inoculum. After replacing the lid, the agar plate was kept at room temperature for 5 to 10 minutes for the surface of the agar to dry.

Making of Wells and application of test solutions

Wells were made on the agar plate and various concentrations of the solutions were aseptically transferred into the wells. The test compounds being freely soluble in dimethyl sulphoxide (DMSO), stock solutions were prepared by dissolving 50mg of each compound in 10 ml sterile DMSO. Four different concentrations namely 25, 50, 75 and 100 µg / ml were prepared by suitably diluting the stock solution of each test compound with DMF. Then a standard antibiotic disc was placed at the centre of the agar plate.

Incubation

The nutrient agar plates inoculated with the bacterial organisms under test were incubated at 35-37°C for about 24 hours. But the plates streaked with the fungal organisms under test were incubated at the same temperature for about 48 hours.

Reading of Zones of Inhibition

Measurement of the diameter of zone of inhibition was made after 24 hours in the case of anti-bacterial susceptibility testing. But the measurement was made only after 48 hours in the case of anti-fungal screening. For each of the test compound, anti bacterial screening was carried out against *St. aureus* and anti-

fungal screening was performed against *A.niger*. The concentrations of 25, 50, 75 and 100 µg / ml were filled into the well made on the agar plate. Kanamycin was the standard antibiotic used against *St. aureus*, Amphotericin against *A.niger*. The results of the antibacterial screening and anti-fungal screening on the test compounds are furnished in Table – 1.1. Each recorded value of zone of inhibition was the mean of three replications. Vehicle (DMSO) produced no significant zone of inhibition.

Results and Discussion

Many of the Carboxylates are known to possess antimicrobial activity. Such organic compounds when complexed with metal ions exhibit enhanced antimicrobial activity due to the presence of the coordinated metal atoms in fact; some of the drugs have shown increased activity when administered in the form of their metal complexes. With this view in mind, both antibacterial and antifungal activities of naphthalene acetic acid and its cobalt (II), Nickel (II), copper (II) and mercury (II) complexes were screened.

The results of preliminary antibacterial susceptibility testing attempted on the test compounds at different concentrations (25, 50, 75 and 100 µg / ml) against the strain of *St.aureus* show that the organism is sensitive to the test compound (Table). The strains of *St. aureus* are sensitive to the standard antibiotic tetracycline. They have registered the zones of inhibition 10%. The test compounds have produced much larger zones of inhibition when tested against the bacterial strain *St. aureus* than the standard antibiotic. In other words, it is inferred that the entire compound tested are able to interfere in the metabolic process that are essential for the growth of the micro-organisms and hence they inhibit bacterial growth significantly. It is also inferred that the cobalt (II) and copper (II) complexes of NAA are slightly more active than the free ligand NAA. The effect of concentration of the test samples has also been studied. It is observed that as the concentration of the sample solution increases the diameter of the zone of inhibition increases in the cases of all the compounds tested against both of the bacterial strains.

The results of preliminary antifungal screening attempted on the test compounds at 25, 50, 75, 100 µg / ml concentrations against *A. niger* show that the fungal organism is also sensitive to the ligand NAA and its cobalt (II), Nickel (II),copper (II) and mercury (II) complexes (Table). The standard antibiotic Amphotericin (tested against *A. niger* and has registered zones of inhibition at 10, whereas when test the compounds have produced larger zones of inhibition when tested against the same fungal organism invariably at 50, 75 and 100 µg / ml

concentrations. Hence it can be concluded that the test compounds are significantly active against the fungal organisms chosen for the study at higher concentration levels of 50, 75 and 100 μg / ml. it can also be concluded that the metal complexes studied or more active against fungi than the free ligand NAA in the case of antifungal screening also it is observed that the increased concentration of the test sample has more pronounced activity on the fungal organisms studied.

Conclusion

In the present work naphthalene acetic acid compounds have been isolated and characterized. Antimicrobial susceptibility testing of naphthalene acetic acid and its complexes with a few biologically important transition metals like Co(II), Ni(II), Cu(II) and Hg(II). The basic principles of antimicrobial sensitivity testing have been discussed in this work. The bacterial strain *S. Aureus* and the fungal strain *Aspergillus* which are pathogenic were used for the antimicrobial screening. Agar well diffusion technique was employed. Nutrient agar medium was used for bacterial screening and Chloramphenicol agar medium was employed for fungal screening. Based on the values of the measured zones of inhibition all the microorganisms tested are found to be sensitive to the test compounds. The metal complexes of NAA are found to be more active than the free ligand against both the bacterial and fungal organisms tested.

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Table 1.1: Antimicrobial activity of DMSO extract of chemical in by agar Well diffusion method

Test sample	Micro organism	Ethanol Extract of <i>Tridax procumbens</i> (mm)				
		Control	25 μl	50 μl	75 μl	100 μl
NAA	<i>St. aureus</i>	25	17	20	21	23
	<i>A. niger</i>	12	23	28	30	33
NAA. Cu	<i>St. aureus</i>	25	30	34	37	40
	<i>A. niger</i>	12	25	30	35	40
NAA. Co	<i>St. aureus</i>	25	32	35	38	42
	<i>A. niger</i>	12	25	30	33	35
NAA. Ni	<i>St. aureus</i>	25	27	33	36	40
	<i>A. niger</i>	12	35	38	40	45

PLATE - 1
Antimicrobial Activity of NAA Compounds
Staphylococcus aureus

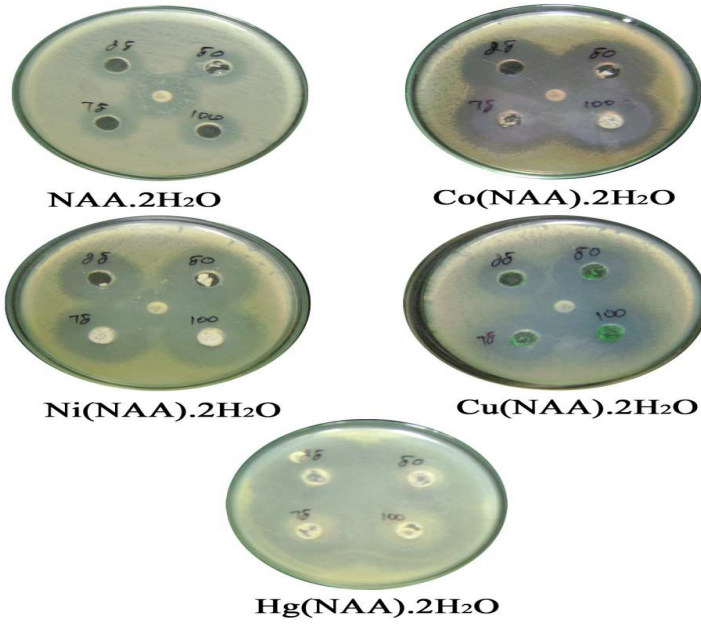


PLATE - 2
Antimicrobial Activity of NAA Compounds
A.niger

