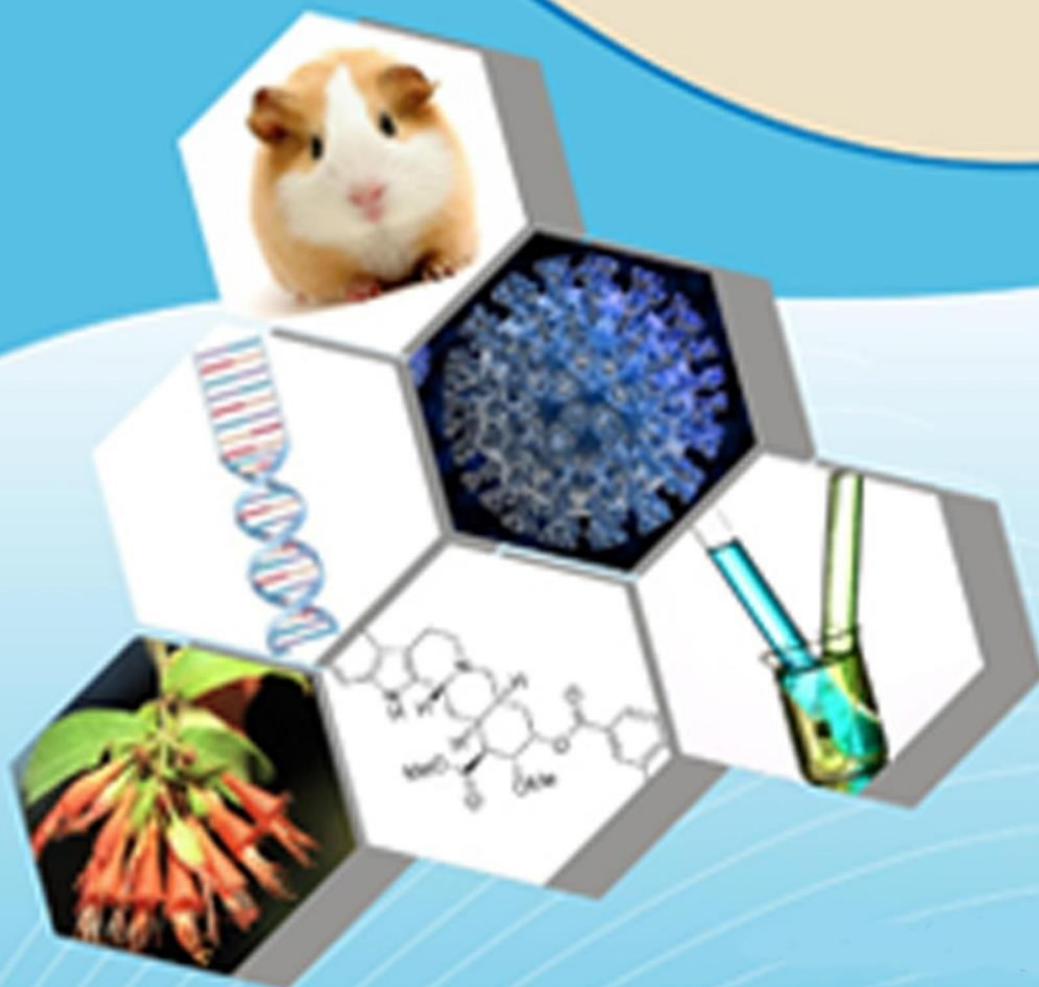




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Activity of *Alternanthera sessilis* (Linn.) R.Br.Ex DC Against Bacteria and Fungi

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Abstract: The current study set out to test the antibacterial capabilities of an extract from *Alternanthera sessilis* leaves using chloroform and methanol as solvents against a variety of bacteria, fungi, and Gram-positive and Gram-negative strains. The soxhlet apparatus was used to extract the *Alternanthera sessilis* (Linn.) R.Br.ex DC (Family Amaranthaceae) leaves thoroughly. The antimicrobial efficacy of *Alternanthera sessilis* extracts was tested using the well diffusion technique against a variety of bacterial and fungal species, including MTCC723, MTCC902, *Candida albicans*, and *Aspergillus fumigatus*. For comparison, standard antibiotics like ciprofloxacin and fluconazole were used. The methanol extract showed encouraging antibacterial activity, against *E. coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus fumigatus*, with inhibition zones of 16 mm, 9 mm, 12 mm, and 14 mm, respectively. In contrast, a zone of inhibition of 10 mm was seen against *E. coli* in chloroform extracts. Within the concentration range of 50 to 100 µg/ml, the antibacterial activity of the extract was shown by the MIC analysis against the tested strains of microorganisms. According to the ayurvedic literature, *Alternanthera sessilis* Linn. aerial parts may be helpful in treating a number of pathogenic disorders. This research supports that claim. The antibacterial action was attributed to the presence of several phytochemical elements in the leaf extracts of *Alternanthera sessilis*. Phytochemicals, chloroform, methanol, antifungal activity, and *Alternanthera sessilis* are some of the terms used to describe this plant.



1. INTRODUCTION

One of the world's oldest conventional medical systems is the traditional Indian medical system. Ayurveda, Yoga, Unani, Siddha, Naturopathy, and Homoeopathy are only a few of the internationally recognized traditional medical systems practiced there. These medical systems are significant in their own right because they share certain specializations, such as a focus on human health and the minimization of adverse consequences. Not only can traditional medicines alleviate symptoms, they also eliminate their root causes. Because it is based on natural medicine, which is less harmful, ayurveda is the greatest approach for prevention. Health is the ultimate basis of virtue, prosperity, pleasure, and salvation according to Ayurveda, which also links health to happiness. 2 There are several benefits to using herbal remedies instead of synthetic or chemical ones. Plants always have physiologically balanced active components, their effects on organisms are complex, they do not tend to build up in the body, and they may counteract the negative effects of chemical chemicals. Some examples of these remarkable medications include alkaloids, the anticancer medicine vincroleucoblastin, the HPV infection drug podophyllotoxin, the anticholinergic drug atropine, the heart failure treatment digitoxin, the cardioactive drug strophanthidin, and many more. 3 Surgery and medical treatment have a long and storied history that begins with the dawn of humanity. Medicinal plant knowledge from ancient literature, folklore, and mythology is abundant in China, India, and Egypt (Ramayana and Mahabharata). The Rig Veda, written around four thousand years ago, details the therapeutic properties of several plant species found in India. There is a thorough description of the medicinal plant's applications from ancient times in scholarly works such as the "Atharvaveda," "Charaksamhita," "Sushrutasamhita," etc. The first known mention of medicinal herbs as a treatment for illness is in the "Eber papyrus" book, which dates back to about 1600 BC. 4 Ethno-botany is a scientific discipline that draws on the traditional knowledge of a people and their environment to study plants and their applications. 5 The man often called the "father of ethnobotany" is Richard Evans Schultes. 5 The goal of ethno-botanists is to record, characterize, and explain the many cultural contexts in which plants are used. They provided an overview of the general public's perceptions, uses, and administration of plants. A wide array of drugs might be most effectively sourced from medicinal plants, says the World Health Organization (WHO). Understanding the characteristics, safety, and effectiveness of such plants need more research. 6. The term "antimicrobial activity" describes the action of eliminating or reducing disease-causing microbes. Such a substance may have antimicrobial, antifungal, or antiviral properties. 3 An increase in the number of cases when

chemotherapeutics and antibiotics fail

In response to the increasing prevalence of antibiotic-resistant microorganisms, researchers have been testing a number of medicinal plants for antibacterial properties thanks to developments in contemporary medical technology throughout the last several decades. Finding individual botanical components and evaluating their antibacterial activity has become more simpler. The medicinal properties of many plants are due to the synergistic effects of their many active ingredients. 7 Due to rising global concern about the worrisome rise in the prevalence of infection by antibiotic resistance bacteria or multi resistance microorganisms, the quest for plants with antimicrobial activity has received increasing relevance in recent years. In vitro and in vivo studies have demonstrated that *Alternanthera sessilis* has antimicrobial, wound healing, antioxidant, antipyretic, nootropic, hepato-protective, hematinic, anti-ulcer, hypoglycemic, anti-diarrheal, and anti-inflammatory properties. 8 Due to the rise of bacteria that are resistant to current antibiotics and the growing popularity of alternative treatment, scientists are studying the antibacterial properties of plant extract. A number of health issues, such as those pertaining to the skin, wound healing, the common cold, coughing, indigestion, and diarrhea, have piqued the attention of scientists in studying *Alternanthera sessilis*. These considerations led to the selection of *Alternanthera sessilis* for the antibacterial activity test. Due to its extensive usage in herbal medicine, *Alternanthera sessilis* has the potential to be a powerful antibacterial agent that can help control a broad range of infectious disorders.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Alternanthera sessilis is an annual or perennial prostrate weed, found throughout the hotter part of India, ascending to an altitude of 1200 m.⁵ The plant species was identified by following the flora of Odisha. The voucher specimen of the plant was deposited in the Herbarium of Department of Botany, Centurion University of Technology and Management. The fresh and disease free leaves of *Alternanthera sessilis* were collected from various locations of Jatni block, 20.1624° N, 85.7011° E, Odisha in the early morning. The plant is identified taxonomically and authenticated in the Department of Botany, Centurion University of Technology and Management, Bhubaneswar. The plant materials were collected in a sterile bag, washed thoroughly and transported to the laboratory. To reduce the chance of contamination, the plant materials were freshly collected and utilized for further studies.¹⁰

Taxonomic classification

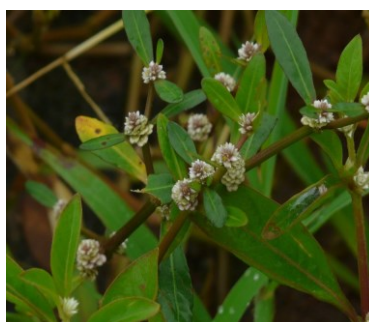


Fig I. Plant of *Alternanthera sessilis*



Synonym : *Alternanthera glabra*, *Gomphrena sessilis*

Kingdom: Plantae **Division:**

Magnoliopsida Order:

Caryophyllales Family:

Amaranthaceae

Sub Family: Gomphrenoideae

Genus: *Alternanthera* **Species:**

sessilis

2.2 Preparation Of leaves for Extraction

The plant parts were washed two to three times with tap water for removing dust and other foreign particle. Again it was thoroughly washed in double distilled water and shade dried for 2-3 weeks. Periodically the moisture levels of the leaves were observed. Once it is completely dried, using a mechanical grinder the leaves are powdered. The ground samples were stored in a sterile glass container for further process.¹¹

2.3 Extraction procedure

The dried leaves powder of *Alternanthera sessilis* was extracted by using Soxhlet apparatus using methanol and chloroform. About 30gms of leaf powder was dissolved in 250ml of methanol and chloroform separately using soxhlet apparatus at 30°C for 48-72 hours. Extracts were concentrated under reduced pressure and then stored at 4°C in an airtight container for further study.¹²

2.4 In-vitro Antibacterial Activity

2.4.1 Test Microorganisms

The test microorganisms used for the antimicrobial activity screening were 2 bacteria *Staphylococcus aureus* (MTCC902), *Escherichia coli* (MTCC723), and fungi- *Candida albicans* (MTCC4748), *Aspergillus fumigatus* (MTCC2550) obtained from Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial stock cultures were incubated for

24 hours at 37°C on nutrient agar and the fungi were incubated at 28°C for 72 hours on potato dextrose agar (PDA) medium respectively, following refrigeration storage at 4°C.¹³

2.5 Antibacterial activity assay

2.5.1 Agar Well Diffusion Method

The antibacterial activity of the leaf extracts was carried out using agar well diffusion method against the selected test microorganism. To the sterile petri plates, Mueller Hinton agar media was prepared and poured, and the plates were allowed to solidify. Using a sterile cotton swab, the bacterial strains were spread uniformly over the medium. The wells were made on the medium with the help of cork borer, 6 different concentrations (50, 60, 70, 80, 90, 100 µg/mL) of the crude extracts. To the wells, different concentration of the extracts were added, and the plates were incubated at 37 °C for 24 hours after the incubation period, the plates were

The antifungal activity of the leaf extracts was carried out

Antimicrobial Activity of Different Extracts of the Leaves of *Alternanthera sessilis* (Linn.) R.Br.ex DC

examined, and the zone of inhibition and MIC value was measured.⁴

2.6 Antifungal Activity Assay

2.6.1 Agar Well Diffusion Method

Using agar well diffusion method against the selected fungal pathogens. The fungal pathogens were maintained using sabouraud dextrose agar (SDA) [Hi-media]. SDA was prepared and poured into the Petri plates. After solidification, the fungal strain was spread onto the media plate using a sterile cotton swab. Using a cork borer the wells were punctured. For antifungal activity, (50, 60, 70, 80, 90, 100 µg/mL) concentration of the crude extract was used. And the plates were incubated at 28°C for 72 hours after the incubation period, the zone of inhibition and MIC value was measured.⁴

2.7 Mic Value Determination:

The minimum inhibitory concentration (MIC) of the extract were determined by using two fold serial dilution assay for the microorganism which are sensitive to various extract of *Alternanthera sessilis* in disc diffusion assay. The inoculums were prepared from 12 hours broth culture and suspensions were adjusted to 0.5 McFarland standard turbidity.¹⁴⁻¹⁶

3. RESULTS AND DISCUSSION

3.1. Determination of zone of inhibition by agar well diffusion method

The methanol and chloroform extracts of the plant *Alternanthera sessilis* were subjected to antibacterial and antifungal activities studies in terms of zone of inhibition. Among these two extracts methanol extract was effective on *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus fumigatus*. However among all the test organism *Escherichia coli* showed the most promising antimicrobial activity with methanol⁸. Where as chloroform did not exhibit any activity with any test microorganism except *Escherichia coli*. The zone of inhibition study reveals that methanol extract shows 16mm, 9mm, 12mm and 14mm on *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus fumigates* respectively (Table-1), Figure1. The chloroform extract seems to be effective with 10mm on *Escherichia coli* (Table-2). From the above finding it is clear that chloroform extract has no inhibition on, *Staphylococcus aureus* and *Candida albicans*.¹ The control of both methanol and Chloroform did not showed any remarkable zone of inhibition (less than 7mm) therefore their values were ignored.

3.2. MIC value determination

The MIC studies from well diffusion method exhibits in the order > 90µl/100ml, > 90µl/100ml, >100µl/100ml concentration of methanol extracts are minimum inhibitory concentration on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* respectively. Chloroform extract shows >100µl/100ml as MIC for *Escherichia coli* (Table-3).

Table 1. Methanol Extract

Sl.No.	Test Microorganism	Standard	Activity	Zone of inhibition
1	<i>Escherichia coli</i>	18.2mm	Positive	16mm ± 0.93



2	<i>Staphylococcus aureus</i>	15.1mm	Positive	9mm± 1.527525
3	<i>Candida albicans</i>	18.5mm	Positive	12mm ± 2.08166
4	<i>Aspergillus fumigatus</i>	19.9mm	Positive	14mm± 1.527525

Values are in ±S.D, n=3 , P<0.05

Sl.No.	Test Microorganism	Standard	Activity	Zone of inhibition
1	<i>Escherichia coli</i>	18.2mm	Positive	10mm±1.732051
2	<i>Staphylococcus aureus</i>	15.1mm	Nil	—
3	<i>Candida albicans</i>	18.5mm	Nil	—
4	<i>Aspergillus fumigatus</i>	19.9mm	Nil	—

Values are in ±S.D, n=3 , P<0.05

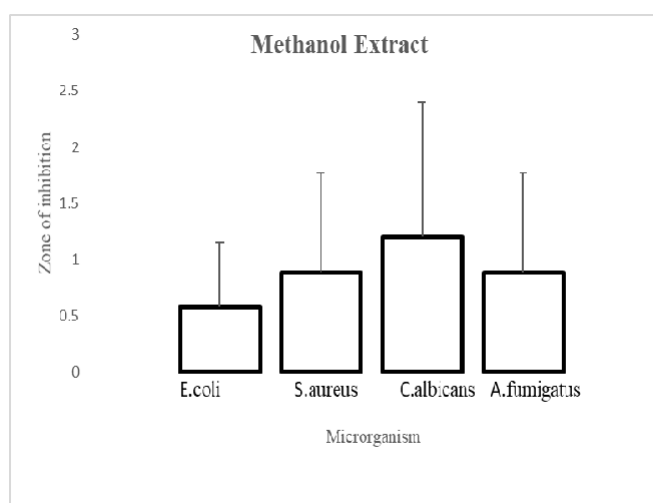


Fig 2. Zone of Inhibition of Methanolic Extract of the Leaves of *Alternanthera sessilis* on Different Microorganisms

S.No.	Extract	MIC <i>Escherichia coli</i> (µl/ml)	MIC <i>Staphylococcus aureus</i> (µl/ml)	MIC <i>Candida albicans</i> (µl/ml)	<i>Aspergillus fumigatus</i>
1	METHANOL	>90	>90	>100	>100
2	CHLOROFORM	>100	—	—	—

4. CONCLUSION

While many plants' medicinal properties have been well-documented and used in pharmaceutical formulations, many others have long been believed by indigenous and rural communities to have curative properties for a wide range of ailments. It is critical to discover and make affordable use of the untapped pharmaceutical resources and traditional knowledge. Results showed that methanol extract was more effective than chloroform extract against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*; this work was a preliminary investigation and a small step toward authenticating the age-old knowledge of some less known plant. In order to validate the plant's usefulness in pharmaceutical and everyday health demands, additional inquiry and thorough study are required.

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