PHYTOCHEMICAL SCREENING IN-VITRO ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF ALTERNANTHERA TENELLA & ALTERNANTHERA PUNGENS

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Abstract:

Synthetic chemical drugs show terrible health-related unwanted undesirable effects, microbial resistance maintained to ethanopharmacognosy obtained thousands of phytochemicals from plants with less or, no side effect, safe and mainly effective with many pharmacological activities such as analgesic, antimicrobial, wound-healing, anticancer, antidiarrheal activities. Some people insist that natural products are beneficial for health.

So, clinical trials tend to verify the claim of the bioactive part, their formulation, safety measures, and side effects before the drug is provided to the patients. Drugs derived from natural sources play a vital role in the prevention and treatment of human diseases. It is essential to explore newer drugs with lesser resistance and adverse effects.

In many developing countries, traditional medicine is one of the primary healthcare services. For the natural and minimal side effects, the plants Alternanthera tenella and Alternanthera pungens were selected. This study showed that the methanolic extract obtained from the leaves of Alternanthera tenella Colla has more antimicrobial properties when compared to ethanolic extracts of Alternanthera tenella Colla. The plant parts were directly used as such for the treatment, but now a days, the active principles are identified and isolated in pure form and also synthetically produced with the help of advanced techniques. it is observed that Alternanthera pungens Kunth have more fungal activity when compared Alternanthera tenella Colla.

Keywords: *Alternanthera tenella, Alternanthera pungens, Ethanopharmacognosy,*

1. INTRODUCTION

The majority of the world's population which means around a billion people still depends on herbal medicines on to meet it human health requirements.[1]

Over the years plants and their extracts have been applied as herbal remedies for human diseases. At present plants are still being used in numerous developing countries as a source of therapeutic agents because they believe medicinal plants are easily accessible, easily available, cost-effective, potent, and have relatively low chances of occurring adverse effects compared to modern conventional dosage forms.

Plants are still a rich source of many natural substances. Most of the plants used by rural associations have biologically active compounds that have been shown by generations to be potent against certain disorders.

According to the report of the World Health Organization, about 80% of people using traditional drugs for primary treatment of their health. In Asia, plants as medicine show a lengthy history of human involvement within the environment.

Herbal medicines contain various types of new and unique substances to treat infectious and chronic disorders. The tradition of the usage of plant products to deal with some of diseases evolved with the start of human civilization. The use of traditional medicinal drugs or natural products is oldest as with human civilization. [3]

Medicines obtained from natural sources play an important role in the prevention and treatment of human diseases. It is imperative to explore new drugs with less resistance and side effects. In some developing countries, traditional medicine is one of the primary health systems. About 61% of new drugs developed were based on natural products and they were very successful, especially in the field of infectious diseases and cancer. However, recent trends indicate that the rate of discovery of new active chemical entities is decreasing. Natural products from higher plants may provide a new source of antimicrobial drugs with potentially novel mechanisms of action.

A large number of scientists around the world have studied the effect of plant extracts on bacteria. Much work has been done on ethnomedicinal plants in India. Plants are rich in various secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have antimicrobial properties found in vitro. Herbal medicines have been known to people for centuries. Practitioners of traditional medicine have described the therapeutic efficacy of many local plants for various ailments. [4]

Antimicrobial properties of medicinal herbs are more and more reported from various parts of the world. According to the World Health Organization, 80 percent of the world's population uses plant extracts or their active ingredients in traditional medicine in folk medicine. Harmful microorganisms can be controlled by drugs, which leads to the emergence of many drug-resistant bacteria and has created alarming clinical situations in the treatment of infections.

The pharmaceutical industry produced several new antibiotics; the resistance of microorganisms to these drugs has increased. In general, bacteria have the genetic ability to transfer and acquire resistance to synthetic drugs used as therapeutic agents.

Alternanthera tenella Colla and *Alternanthera pungens Kunth* belongs to the Amaranthaceae family. Other varieties of Alternanthera are used as food and medicine in Asian and African countries and treat an amazing number of external and internal diseases. Every part of this plant has found medicinal uses. [5]

Medicines obtained from natural sources play an important role in the prevention and treatment of human diseases. It is imperative to explore new drugs with less resistance and side effects. In some developing countries, traditional medicine is one of the primary health care systems. About 61% of new drugs developed were based on natural products and they were very successful, especially in the field of infectious diseases and cancer. However, recent trends indicate that the rate of discovery of new active chemical entities is decreasing.
Natural products from higher plants may provide a new source of antimicrobial drugs with potentially novel mechanisms of action. The effects of plant extracts on bacteria have been studied by a large number of scientists around the world. Lot of work has been done on ethnomedicinal plants in India.

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The pharmaceutical industry produced several new antibiotics; the resistance of microorganisms to these drugs has increased. In general, bacteria have the genetic ability to transfer and acquire resistance to synthetic drugs used as therapeutic agents. [6]

Alternanthera tenella Colla and Alternanthera pungens Kunth belong to the Amaranthaceae family. Other varieties of Alternanthera are both food and medicine in Asian and African countries and treat an amazing number of external and internal conditions. Every part of this plant has found medicinal uses.

The approximately 200 species of Alternanthera are extremely versatile. They are colourful garden ornaments with leaves in shades of green, red, pink, purple and yellow. They have been used in formal garden design for centuries, but they also work well in containers and as houseplants. Some varieties are used as water garden and aquarium plants.

Phytochemicals are biologically active substances found in small amounts in plants. Phytochemicals are basically divided into two groups. primary and secondary components according to how they function in plant metabolism.

The main components are common sugars, amino acids, proteins and chlorophyll, while the secondary components are alkaloids, flavonoids, saponins, phenols, etc. Medicinal plants have a high content of antimicrobial agents. Plants are used in medicine in various countries and are the source of many powerful and effective medicines. Many variety parts of plants with different medicinal characteristics are used as raw materials for the extract. [7]
The various parts used include roots, stem, flower, fruit and branches as well as modified plant organs. Considering the enormous potential of plants as a source of antimicrobial agents for antibacterial and antifungal applications, a systematic study was conducted to examine the natural flora of Alternanthera tenella and Alternanthera pungens for antimicrobial and antifungal activity.

1.3 PLANT DESCRIPTION:[9]

The genus Alternanthera includes both terrestrial and aquatic species, the photosynthetic pathway is different in this genus, some species pass the carbon-fixing pathway, one clade of 17 species acquires the carbon-fixing pathway, and still other species have the carbon-carbon intermediate pathway.

They are annual perennial herbs or subshrubs, while some of the more familiar species are aquatic plants, most of which are terrestrial. They have many base shapes and vertical floating leaves are opposite each other. The inflorescence is a pointed or rounded head found in the axils of the leaves and at the tips of the branches.

Flowers have 5 petals. There are 3 to 5 stamens fused from base to margin and five pseudo-staminodes, appendages between the stamens that are not true staminodes. The fruit is a one-seeded pod.

Figure -1 *Alternanthera tenella Colla*

Figure -2 *Alternanthera Pungens Kunth*

PLANT PROFILE:

Family: Amaranthaceae

Synonym: Alternanthera ficoidea

Kingdom: Plantae

Class: Equisetopsida C.Agardh

Order: Caryophyllales

Genus: Alternanthera

Species: Tenella

Name of the plant: Alternanthera tenella Colla

PLANT PROFILE

Clade -Eudicots

Order - Caryophyllales

Family - Amaranthaceae

Genus -Alternanthera

Species -A. pungens

Name of the plant -*Alternanthera pungens Kunth*

2. MATERIALS & METHODS [13]

2.1 Plant collection and processing.

Alternanthera Tenella Colla and *Alternanthera Pungens Kunth* plants were collected from Oddipalem residential area in Venkatachalam, Nellore district, Andhra Pradesh, India. The plant sample was identified in the Department of Botany after it was air-dried on a laboratory bench at room temperature for two weeks.

Fresh leaves of Alternanthera tenella Colla and Alternanthera pungens Kunthi were collected and dried at room temperature for two weeks. The dried leaves are ground into a coarse powder and stored in a clean, sterile, and dry container.

The powder was allowed to be degreased and the selected solvents methanol and ethanol were added to these dried powders and left to stand at room temperature for about 5 days to soak and the solvent was kept in a tightly closed container for further processing.

Figure -5 (A) *Alternanthera Tenella Colla* (B) *Alternanthera Pungens Kunth*

3.2 Extraction of Plant Material

3.2.1 Methanolic Extraction

Ten grams of soil plant sample was soaked in 100 ml of methanol for 24 hours at room temperature with occasional stirring. After that, the contents were filtered with filter paper. The extract was then concentrated to a 50 mL extract and stored in an airtight container in a refrigerator at 40°C until required for analysis. [14]

3.2.2 Ethanolic extraction

Ten grams of the ground plant sample were soaked in 100 ml of ethanol for the same 24 hours at room temperature with occasional stirring. After that, the contents were filtered with filter paper. The extract was then concentrated to a 50 mL extract and stored in an airtight container in a refrigerator at 40°C until required for analysis.

Before soaking, the raw powder was washed with petroleum ether to remove fat.

3.3 PHYTOCHEMICAL SCREENING

Phytochemicals (Greek phyton = plant) are chemical compounds naturally occurring in plants that have positive or negative effects on health. Medicinal plants used for various ailments and diseases are the richest biorepositories of various phytochemicals.

The medicinal properties of plants are determined by their phytochemical components. Some important phytochemicals are alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, tannins, coumarins, terpenoids, triterpenoids, etc., which are distributed in different parts of the plant.

Phytochemicals can be isolated from plant material using various extraction techniques. The most commonly used conventional methods are soaking, percolation, infusion, digestion, boiling, hot continuous extraction, etc. Various types of solvents such as water, ethanol, methanol, acetone, ether, benzene, chloroform, etc. are used in extraction. process The extraction of phytochemicals from plant materials is influenced by pre-extraction factors (plant part used, origin and particle size, humidity, drying method, degree of processing, etc.) and extraction factors (extraction method used, solvent chosen, solvent-to-sample ratio, solvent pH and temperature, and length of extraction If previously used plant parts for processing directly, currently active ingredients are identified and isolated in pure form and also produced synthetically by advanced techniques.

These phytoconstituents can be used as models for the development of new synthetic drugs. Identification of plant components from plant material helps to predict potential pharmacological activity. [15]

3.3.1 Preliminary screening of secondary metabolites [16]

3.3.1.1 Test of Alkaloids

Iodine Test: A few drops of dilute iodine solution were added to 3 ml of the test solution. A blue color appears; it disappears during boiling and reappears during cooling.

3.3.1.2 Detection of Amino acids and Proteins

3.3.1.3 Xanthoproteic test: Plant extract + a few drops of conc. Nitric acid were added. A yellow solution was formed.

3.3.1.4 Test for Flavonoids

3.3.1.5 Ammonia test: To Filtrate + 5 ml dilution. Ammonia solution + conc. H_2SO_4 was added. A yellow colour is produced.

3.3.1.6 Test for Glycosides

To 5 ml of extract, 2 ml of glacial acetic acid, one drop of 5% $FeCl_3$, and conc. H_2SO_4 was added. The appearance of a brown ring indicates the presence of glycosides.

3.3.1.7 Test for Cardiac Glycosides

Keller-Kiliani Test: For 2 ml extract, glacial acetic acid, one drop 5% $FeCl_3$, and conc. H_2SO_4 was added. Reddish brown appears at the junction of the two liquid layers and the upper layer appears bluish green indicating the presence of glycosides.

3.3.1.8 Detection of Tannins

Bramer's test :1mL filtrated + 3mL distilled water + 3 drops 10% Ferric chloride solution were added. The appearance of Blue-green color in the presence of tannins

10% NaOH test: 0.4mL plant extract + 4mL 10% NaOH were added + shaken well. The formation of emulsion in the {Hydrolysable tannins}

3.3.1.9 Detection of Phenolic compounds

Iodine test: To 1mL extract + few drops of dil. Iodine sol were added. A transient red colour formed.

3.3.1.10 Test for Carotenoids: To 1gm extract + 10mLchloroform, vigorously shaken and filtered). Filtrate + conc. H2SO4 were added. A blue colour at the interface formed.

Fig: 4. phytochemical screening

3.3.1.9 Antimicrobial and antifungal activity[17]

Antimicrobial activity

After the growth of microbial and fungal organisms on nutrient agar medium antimicrobial activity was carried out by adding 2 ml of methanol extract of *Alternanthera tenella Colla* and *Alternanthera Pungens Kunth* to the test plates at different dilutions. The extracts used have antimicrobial and antifungal effects.

The studies were further conducted by calculating.

- Minimum inhibiting concentration (zone of inhibition)
- Minimum Bactericidal |Fungicidal concentration

It was calculated by determining the growth of bacterial and fungal colonies on the plates after incubation.

Fungal disease of stored goods is a very serious problem in warm tropical regions of the world. Contamination with storage fungi and their mycotoxins is a major problem in the plant and food industry. Fungi, particularly Aspergillus and Penicillium species, are among the most important genera with the reported ability to produce mycotoxins during storage.

These fungi-producing related mycotoxins reduce the quality of food products and the medicinal potential of herbal drugs.so *Aspergillus niger* and *Penicillium digitatum* are selected for the following study.

A. niger (commonly known as black Aspergillus) has been recorded as the most dominant fungal species associated with herbal medicines during storage. A. niger is a saprophytic and filamentous fungus found in soil, feed, organic waste and food, and causes a black color. minced onion, shallot; Dracaena stem rot; Sansevieria root rot; and cotton rot; Damage to cashews, dates, figs, vanilla beans, and plums.

The use of chemical pesticides is a very popular practice to control various plant diseases compared to pesticides made from natural plants or plant parts. However, consumers are now demanding less use of synthetic fungicides due to the biodegradability, contaminants, and residual toxicity of chemical pesticides. Several studies have shown that plant extracts are sources of natural pesticides, which make great efforts to develop new pesticides.

Since many spices and herbs have been used for centuries as food and for medicinal purposes, some of them have combined antimicrobial potential and are considered alternatives to conventional antimicrobials, especially in the current era of antimicrobial drug resistance.

The preservative effects of plants have received much attention in the literature, and studies show that some plant species can inhibit the development of mycotoxin-producing molds.

They usually produce many secondary metabolites such as alkaloids, flavonoids, tannins and phenolic compounds, which are important sources of microbicides, pesticides and many pharmaceutical drugs. [18]

Therefore, the main objective of this study was to evaluate the antifungal efficacy of the methanolic extract of Alternanthera tenella Colla and Alternanthera pungensKunth against the growth of A . niger and P. digitatum

In this study, Alternanthera tenella and Alternanthera pungens were selected as plant material to analyze their effectiveness on the growth of A. niger and P. digitatum.

Table-1: *List of plant species tested for antifungal activity*

S.NO	PLANT NAME	PART USED	NAME OF THE FAMILY
1.	*Alternanthera tenella Colla*	Leaves	Amaranthaceae
2.	*Alternanthera pungensKunth*	Leaves	Amaranthaceae

Tested organisms have antimicrobial activity: Staphylococcus thermophilus, Rhodococcus Terrae

The test organisms are antifungal: Aspergillus niger, Penicillium digitatum

Antifungal activity extraction of the plant extract To prepare the methanol and ethanol extracts, 10 g of each dried sample was ground into a fine powder with 100 mL of methanol, and the ethanol was left at room temperature (30 ± 2 °C) for 2. days the contents of the flask were then filtered through filter paper to obtain a clear infusion in a laminar airflow.

The poisoned food technique was used to evaluate the antifungal potential. Different concentrations of 10%, 15% and 20% methanol and ethanol extracts were added to the agar medium, and the medium without plant extract was used as a control.

Each concentration was maintained for five replicates. A 5 mm disk of a 3–4-day culture of experimental fungi (A. niger) was placed in the center of each Petri dish. Plates were incubated upside down at 37°C for five days and colony diameters were measured on days 3, 4 and 5.

This study deals with their antifungal activity against the growth of A. niger and P. digitatum during consumption of poisoned food. Significant variation was observed in the potency of all the methanolic and ethanolic extracts of A. niger and P.digitatum more digitized.

The antifungal activity of the methanolic and ethanolic extract of Alternanthera tenella Colla and Alternanthera pungens Kunth is described in the table below. Growth reduction in percentage was taken into account and the antifungal effect was evaluated. Increased antifungal activity of A. nigeri and P digitatum was observed with increasing concentrations of the extracts. The maximum inhibition percentage was observed at a concentration of 20%.

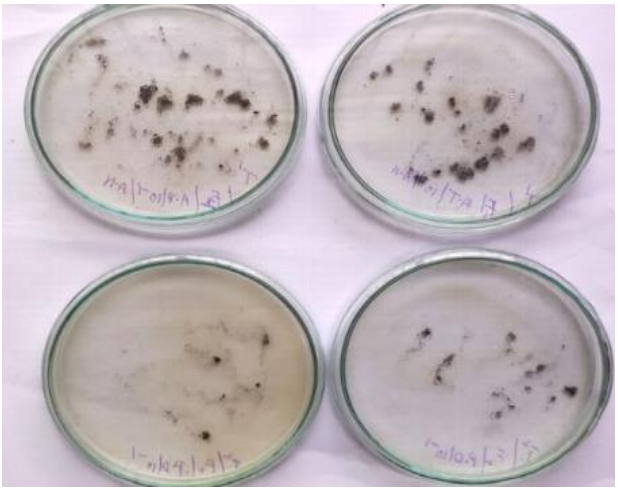

Figure 7: *Percent Inhibition of Mycelial Growth of Aspergillus Niger*

3. RESULTS&DISCUSSION

4.1 RESULTS:

Chemical constituents	***Alternanthera tenellacolla***	***Alternanthera pungenskunth***
Alkaloids	+	++
Coumarin	++	+
Glycosides	+	+
Cardiac glycosides	−	+
Saponins	++	++
Tannins	++	+
Amino acids & proteins	+	+
Flavonoids	++	++
Phenolic compounds	+	+
Terpenoides	−	−
Triterpenoides	−	−

Table 2: *Preliminary Phytochemical screening of Ethanolic extract of Alternanthera Tenella Colla &Alternanthera Pungens Kunth*

\+ = Presence − =Absence

Chemical constituents	***Alternanthera tenellacolla***	***Alternanthera pungenskunth***
Alkaloids	+	++
Coumarins	++	+
Glycosides	+	+
Cardiac glycosides	−	+
Saponins	++	++
Tannins	++	+
Amino acids & proteins	+	+
Flavonoids	++	++
Phenolic compounds	+	+
Terpenoids	−	−
Triterpenoid’s	−	−

\+ = Presence − = Absence

Table 3: *Phytochemical screening of Methanolic extract of Alternanthera Tenella Colla & Alternanthera Pungens Kunth*

Bacterial isolates	**Bacterial growth count**	**Total CFU**	**Acceptibility**
Rhodococcus terrae	Trail-1: - 10-1dilution: - Uncountable Trail 2: -10-2 dilution: - Uncountable Trail 3: -10-3dilution-198 Trail4: - 10-4dilution: -153	Trail 3+Trail 4: 198+153=351(CFU)	Acceptable
Streptococcusthermophiles	Trail-1: - 10-1 dilution: - Uncountable Trail 2: -10-2 dilution- Uncountable Trail 3: -10-3dilution-150 Trail4: - 10-4dilution-97	Trail 3+Trail 4: 150+97=247(CFU)	Acceptable

Table 4: *Bacterial Growth Count of Rhodococcus terrae and Streptococcus thermophiles*

- Bacterial growth was calculated by plate counting method depending on the concentration of dilution carried out and including the number of lanes as in Lane -1 the bacterial growth is incalculable. Experiment 2 also shows incalculable, also shows more bacterial growth at lag-3. We found that bacterial growth was countable and calculated this by carefully counting 150 colonies in a lag-4 dilution of 10-4. 97 colonies were observed, colony-forming units are considered thermophilic streptococci.
- In case of Rhodococcus terrae, bacterial growth is incalculable after -1, because 10-1 and taril-2 shows bacterial growth 323 colonies in 10-3 dilution bacterial growth shows 198 colonies finally in 10-4 dilution bacterial growth shows 154 colonies.
- The acceptable count is observed in trail -3and 4

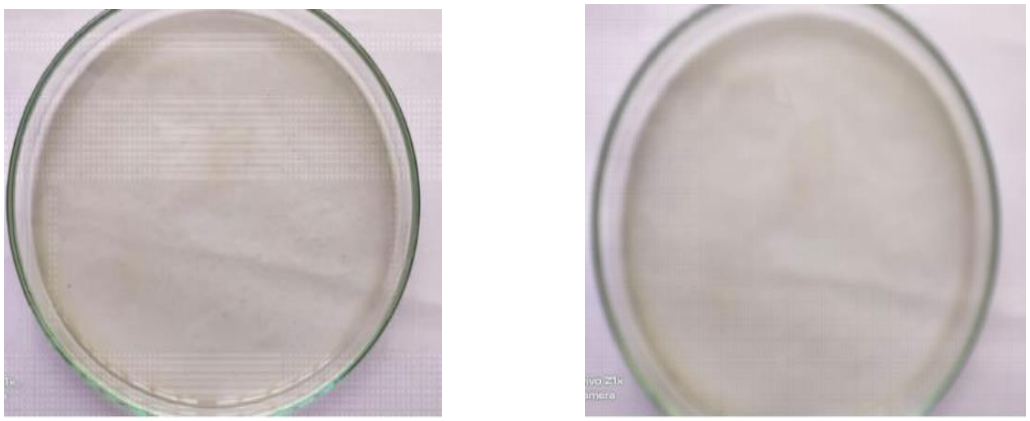

Figure 8: Antimicrobial activity of *Alternanthera tenella Colla* zone of inhibition mm

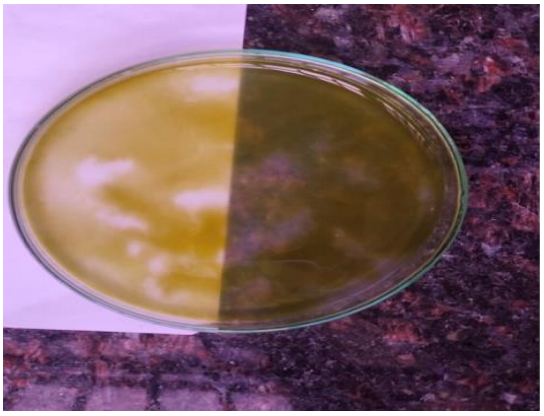

Figure 9: Antimicrobial activity of *Alternanthera pungens kunth* zone of inhibition mm

Bacterial isolates	**Aqueous extract**	**Ethanolic extract**	**Methanolic extract**
Rhodococcus terrae	) ±0.0	3.6± 0.3	4.6± 0.2
Streptococcus thermopiles	0.0± 0.0	3.1± 0.4	3 ±0.4

Table 5: *Concentration of Zone of Inhibition*

- The antimicrobial activity was calculated based on the antimicrobial zone of the methanolic extract of *Alternanthera tenella Colla*. It was observed that the aqueous extract of Rhodococcus Terrae has a zero zone of inhibition and the methanolic extract of *Alternathera tenella Colla* has a zone of inhibition of 3.3 mm and a maximum and minimum level of inhibition of 0.4 mm.
- For Streptococcus thermophiles, the microbial activity was studied by calculating the zone of microbial inhibition using the methanolic extract of *Alternanthera tenella Colla.*
- It was observed that Streptococcus thermophiles that shows nil zone of inhibition in aqueous extract and 4.6 mm zone of inhibition in maximum & minimum level of inhibition is 0.2mm in methanolic extract of *Alternathera tenella Colla* [19].

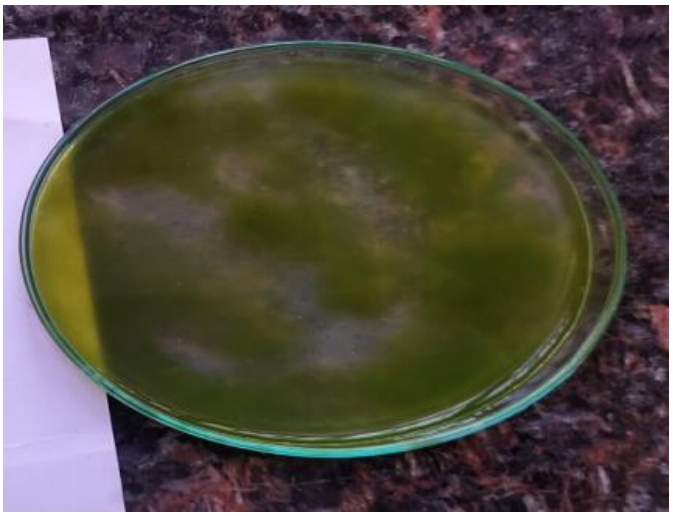

Figure -10 Antimicrobial activity of *Alternanthera pungens Kunth* zone of inhibition in mm

Bacterial isolates	Aqueous extract	Methanolic extract
Rhodococcus terrae	0.0 ± 0.0	2.4 ± 0.1
Streptococcus thermopiles	0.0 ±0.0	2.6 ±0.2

Table 6: Concentrations of Antimicrobial Activity of *Alternanthera pungens Kunth* zone of inhibition in mm

- Antimicrobial activity was calculated based on the antimicrobial zone of the methanolic extract of *Alternanthera pungens Kunth*. It was observed that Rhodococcus terrae with zero zone of inhibition in aqueous extract and 2.6 mm zone of inhibition at maximum and minimum levels is 0.2 mm in methanol extract of *Alternanthera pungens Kunth.*
- For Streptococcus thermophiles, the microbial activity was calculated based on the zone of microbial inhibition using the methanolic extract of *Alternanthera pungens Kunth.*
- The zone of inhibition of water extract of Streptococcus thermophiles was observed to be zero and the zone of inhibition of maximum and minimum inhibition level of 2.4 mm is 0.2 mm in methanol extract of *Alternanthera pungens Kunth*. [20]

Antifungal activity

Methanolic Plant extracts	Concentration (%)	Percentage of inhibition *Aspergillus niger*.in 1 to 5 days
Alternanthera tenella Colla		Day-1 Day-2 Day-3 Day-4 Day-5
	10	NE NE NE 10 15
	15	NE NE 20 26 21
	20	NE NE 15 20 18
Alternanthera pungens Kunth		Day-1 Day-2 Day-3 Day-4 Day-5
	10	10 22 30 32 35
	15	13 25 28 35 41
	20	18 22 35 40 38

Table 7: Concentration of zone of inhibition of *Alternanthera pungens Kunth & Alternanthera tenella colla*

First, mycelial growth was assessed in 60 mm Petri dishes filled with agar medium supplemented with 10% and 20% methanol and ethanol extracts of each plant. Then, 5 mm diameter mycelia collected from pure cultures were inoculated in the centre of each Petri dish, then all inoculated dishes were incubated at 25ºC for 5 days. After that, the radial growth of the mycelium after inoculation was measured.

Finally, the antifungal activity of each extract was calculated in terms of inhibition percentage of mycelia growth was evaluated by using the formula given below,

Percentage of inhibition = (dc-dt) /dc × 100

The above study shows that the inhibition percentage of *Pencillum digitatum* is more effective in methanolic extract of *Alternanthera pungens Kunth* than Alternanthera tenella Colla. To determine the percentage of inhibition, the amount of inhibition in the diameter of the control and test samples was compared.

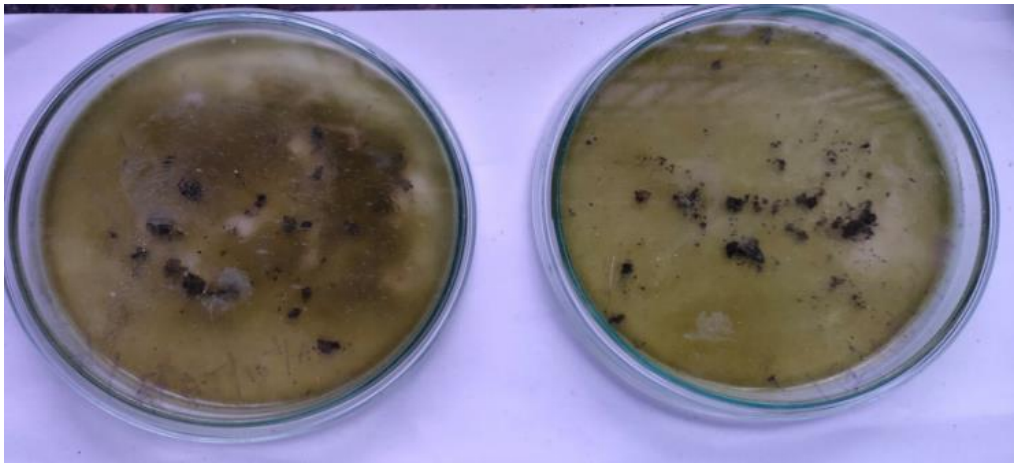

Figure 11 : Antifungal Activity of methanolic extract of *Alternanthera pungens kunth in Aspergillus niger & Pencillium digitatum*

Methanolic Plant extracts	**Concentration (%)**	**Percentage of inhibition in *Pencilliumdigitatum*in 1 to 5 days**
Alternanthera tenellacolla		Day-1 Day-2 Day-3 Day-4 Day-5
	10	NE NE NE 10 15
	15	NE NE 20 26 21
	20	NE NE 15 20 18
Alternanthera pungensKunth		Day-1 Day-2 Day-3 Day-4 Day-5
	10	10 22 30 32 35
	15	13 25 28 35 41
	20	18 22 35 40 38

***Table 8*:** Results of Antifungal activity in methanolic plant extract in *Pencillium Digitatum*

Ethanolic Plant extracts	Concentration (%)	Percentage of inhibition in Pencillium digitatum in 1 to 5 days
Alternanthera tenella Colla		Day-1 Day-2 Day-3 Day-4 Day-5
	10	NE NE NE NE 10
	15	NE NE 15 20 23
	20	NE NE 11 21 24
Alternanthera pungensKunth		Day-1 Day-2 Day-3 Day-4 Day-5
	10	10 14 20 22 25
	15	11 15 18 25 31
	20	15 18 22 28 30

Table 9: *Results of Antifungal activity in ethanolic plant extract in Pencillium Digitatum*

Ethanolic Plant extracts	Concentration (%)	Percentage of inhibition in *Aspergillus niger* in 1 to 5 days
Alternanthera tenella Colla		Day-1 Day-2 Day-3 Day-4 Day-5
	10	NE NE NE NE NE
	15	NE NE 10 13 16
	20	NE NE 15 22 26
Alternanthera pungensKunth		**Day-1 Day-2 Day-3 Day-4 Day-5**
	10	NE NE NE NE NE
	15	NE NE 10 13 16
	20	NE NE 15 22 26

Table 10: *Results of Antifungal activity in ethanolic plant extract in Aspergillus niger*

The above study shows that the inhibition percentage of Penicillium digitatum shows a more effective methanolic extract of *Alternanthera pungens Kunth* compared to *Alternanthera tenella Colla*. To determine the percentage of inhibition, the amount of inhibition in the diameter of the control and test samples was compared.

Ethanol extract shows less inhibition in *Alternanthera tenella Colla* and partial inhibition in ethanol extract of *Alternanthera pungens Kunth*. Thus, *Alternanthera pungens Kunth* is found to have more fungal activity than *Alternanthera tenella Colla*.

4.2 DISCUSSION:

This study showed that the methanol extract of *Alternanthera tenella Colla* leaves has more antimicrobial properties than the ethanol extracts of *Alternanthera tenella Colla*. Furthermore, a significant difference in the *in vitro* susceptibility of two phytopathogenic fungi, *Aspergillus niger* and *Penicillium digitatum*, treated with different plant extracts was observed.

The methanolic extract of *Alternanthera pungens Kunth* was found to be highly effective in controlling the growth of Penicillium digitatum and slightly effective in controlling the growth of *Aspergillus niger* compared to other plant extracts.

The effectiveness of plant extracts in inhibiting microbial and fungal growth shows that the methanolic extract of *Alternanthera tenella Colla* has good antimicrobial activity against Staphylococcus thermophilus and Rhodococcus Terrae. *Alternanthera pungens Kunth* methanol extract has good antifungal activity against *Aspergillus niger* and Penicillium digitatum bacteria.

These plant species can be considered to have antifungal and antimicrobial activity due to the presence of various phytochemical constituents. In addition, tannins isolated from plants have significant toxic effects against bacteria and fungi and may have pharmacological importance, and saponins represent a special class of glycosides with soapy properties and are considered to be active antifungal agents.

4. CONCLUSION

Phytochemical analysis is very important to evaluate the potential therapeutic effect of the plant and to determine the responsible biological functions of the plant. The extraction of phytochemicals from plant material largely depends on the type of solvent used. Methanol is used as a solvent due to its high polarity, which can give high extraction yields. The results of this study showed that this study was carried out and phytochemical screening showed that alkaloids, glycosides, and cardiac glycosides are present in both *Alternanthera tenella Colla* and *Alternanthera pungens Kunth*, which they used in the treatment of bacterial and fungal activity.

The methanol extract of *Alternanthera tenella Colla* as a medicinal plant source is better and more effectively used as an antibacterial agent, and *Alternanthera pungens Kunth* showed better and more effective use of antifungal drugs while the use of antibiotics increased. It is necessary to evaluate the useful and relevant features that are brought to the knowledge.

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None

Conflict of Interest:

None

5. REFERENCES

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